

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(2): 208-214
www.biochemjournal.com
Received: 26-11-2025
Accepted: 30-12-2025

Govind Mishra
Assistant Professor, Deen
Dayal Upadhyaya (DDU)
Gorakhpur University,
Gorakhpur, Uttar Pradesh,
India

Ajay Shankar
Assistant Professor, Plant
Biotechnology, Department of
Genetics and Plant Breeding,
Rajiv Gandhi South Campus,
Banaras Hindu University,
Mirzapur Uttar Pradesh, India

Rajesh Kumar
Assistant Professor, Plant
Biotechnology, Department of
Genetics and Plant Breeding,
Rajiv Gandhi South Campus,
Banaras Hindu University,
Mirzapur Uttar Pradesh, India

Sanskar Kumar
M.Sc. (Ag.), Seed Science and
Technology, Acharya
Narendra Deva University of
Agriculture and Technology,
Kumarganj, Ayodhya, Uttar
Pradesh, India

Genetic architecture of dual-purpose maize integrating grain yield and stover quality across environments via multi-trait GWAS and genomic prediction

Govind Mishra, Ajay Shankar, Rajesh Kumar and Sanskar Kumar

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i2Sc.7478>

Abstract

Dual-purpose maize breeding aims to concurrently enhance grain productivity and stover value for fodder or bioenergy, a challenge due to the complex and potentially correlated genetic control of these traits. We conducted an integrated genetic analysis using multi-trait genome-wide association studies (GWAS), a selection index, and genomic prediction in 300 diverse maize inbred lines evaluated across multiple environments within a single year. Substantial phenotypic variation was observed for grain yield, stover yield, and stover quality traits. Broad-sense heritability was moderate for yield traits (0.4-0.5) and high for stover fiber composition and digestibility (0.6-0.8), indicating strong genetic determinism. Grain and stover yields exhibited a favorable positive genetic correlation, while correlations between grain yield and stover quality were weak or non-significant, suggesting minimal biological trade-offs. Multi-trait GWAS identified several significant QTL, including pleiotropic loci influencing both grain and stover performance, and candidate genes associated with lignin biosynthesis affecting fiber digestibility. Genomic prediction using GBLUP achieved moderate accuracy, with modest improvements from multi-trait models. A Dual-Purpose Index effectively identified superior genotypes, with top-ranked lines showing simultaneous gains in grain and digestible stover yield.

Keywords: *Zea mays*, dual-purpose, stover digestibility, fodder quality, GWAS, genomic prediction, multi-trait mixed model, G×E; BLUP, selection index, lignocellulose, NDF, ADF, ADL

Introduction

Maize (*Zea mays* L.) is a cornerstone of global agricultural production systems, fulfilling a dual function as a major cereal grain and as a primary source of fodder for livestock. In mixed crop-livestock systems, which dominate large parts of Asia, Africa, and Latin America, maize stover constitutes a substantial proportion of ruminant feed resources, particularly during dry seasons when green forage availability is limited ^[1]. Consequently, there is growing demand for dual-purpose maize cultivars that combine high grain productivity with abundant, nutritionally improved stover. While grain yield remains the principal determinant of economic return, the quality of stover directly affects livestock intake, digestibility, and productivity, thereby influencing overall system efficiency and sustainability.

Stover from conventional grain-oriented maize hybrids is typically characterized by low feeding value due to high concentrations of structural carbohydrates and lignin within the secondary cell wall. Elevated levels of neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin restrict microbial degradation in the rumen, leading to reduced voluntary intake and lower metabolizable energy availability ^[2]. Improving stover quality, particularly by enhancing digestibility and modifying fiber composition, has been shown to increase milk yield and growth performance in ruminants without increasing feed intake ^[3]. However, such improvements must be achieved without compromising grain yield, which presents a fundamental breeding challenge: the optimization of whole-plant performance rather than the improvement of grain or fodder traits in isolation.

Corresponding Author:
Govind Mishra
Assistant Professor, Deen
Dayal Upadhyaya (DDU)
Gorakhpur University,
Gorakhpur, Uttar Pradesh,
India

Biological relationships between grain yield and stover traits

Grain and stover traits are biologically linked through plant architecture, biomass accumulation, and assimilate partitioning. Larger, more vigorous plants generally produce greater total biomass and often achieve higher grain yield, suggesting the potential for positive associations between grain and stover yield^[4]. Concerns have historically been raised that selection for high grain yield could negatively affect stover quality by altering cell-wall composition or increasing the proportion of structural tissues. However, evidence from diverse maize germplasm indicates that strong antagonistic relationships between grain yield and stover quality traits are not inherent.

Studies conducted in tropical and temperate maize populations have reported weak, neutral, or favorable genetic correlations between grain yield and stover digestibility-related traits^[1, 5]. In several populations, genotypes with higher stover yield also exhibited lower fiber concentrations, resulting in increased production of digestible fodder per unit area^[6]. Grain yield and stover yield are frequently positively correlated, reflecting shared dependence on overall plant vigor and photosynthetic capacity^[7]. The principal trade-off reported in some studies involves stover quantity versus digestibility, where very high-biomass or leafy genotypes may show marginally reduced digestibility due to increased lignification and cell-wall thickening^[8]. Nevertheless, these negative correlations are often modest and inconsistent, indicating that they can be mitigated through informed selection strategies.

Constraints and knowledge gaps in stover quality improvement

Despite sustained gains in maize grain yield, systematic improvement of stover nutritional quality has lagged behind. Early genetic studies using brown midrib (bm) mutants demonstrated that large improvements in digestibility are genetically achievable. The bm3 mutant, caused by disruption of the caffeic acid *O*-methyltransferase (COMT) gene in the lignin biosynthesis pathway, significantly reduces lignin content and enhances cell-wall digestibility^[9]. However, such large-effect mutations frequently impose agronomic penalties, including reduced yield stability, increased lodging susceptibility, and fragile stems, which limit their adoption in commercial breeding programs.

In elite germplasm, stover quality traits are controlled by many loci of small effect, reflecting a highly polygenic genetic architecture^[6, 10]. Importantly, lignin concentration alone does not reliably predict digestibility. Quantitative trait locus (QTL) studies have shown that loci influencing lignin content do not always affect digestibility, and conversely, genotypes with similar lignin levels may differ substantially in digestibility due to variation in phenolic cross-linking, cellulose crystallinity, and cell-wall organization^[11]. As a result, breeding strategies based solely on reducing lignin or fiber content are insufficient. Direct phenotyping of digestibility-related traits is essential, yet such measurements predictably remain costly and labor-intensive, relying on standardized laboratory methods such as detergent fiber analysis and *in vitro* digestibility assays^[12, 13]. These constraints have limited the scale of phenotyping and slowed genetic progress in dual-purpose maize breeding.

Genomic dissection of stover quality and dual-purpose traits

Recent advances in high-throughput genotyping and statistical genetics have created new opportunities to overcome these limitations. Genome-wide association studies (GWAS) have proven effective in dissecting the genetic basis of complex traits, including stover yield and quality. Several GWAS conducted in diverse maize panels have identified numerous single nucleotide polymorphisms (SNPs) associated with NDF, ADF, lignin concentration, and *in vitro* digestibility, with individual loci typically explaining a small proportion of phenotypic variance, consistent with polygenic control^[4-6].

Importantly, some studies have detected pleiotropic loci influencing multiple stover quality traits, suggesting shared genetic regulation of fiber composition and digestibility^[5, 11]. Association mapping in multi-parent populations has further demonstrated that stover yield and digestibility-related traits are governed by overlapping but partially distinct genetic factors, reinforcing the feasibility of simultaneous improvement^[2]. Despite these advances, joint GWAS analyses integrating grain yield with stover yield and quality remain relatively scarce. Methodological developments such as multi-trait GWAS, which exploit trait correlations to increase statistical power, and multi-environment GWAS, which account for genotype-by-environment interactions, remain underutilized in dual-purpose maize research^[14].

Genomic selection as a breeding strategy for dual-purpose maize

Genomic selection (GS) complements association mapping by targeting the cumulative effect of thousands of small-effect loci rather than relying on a limited set of significant markers. This is particularly relevant for stover-quality traits, where laboratory phenotyping is costly and trait architecture is typically diffuse. Evidence from dual-purpose and stover-focused studies indicates that fiber fractions can often be predicted with moderate accuracy using genomic best linear unbiased prediction (GBLUP) and related whole-genome models, whereas digestibility metrics tend to show lower and more variable prediction performance, consistent with stronger environmental sensitivity and a larger proportion of non-additive and context-dependent variance^[1-3]. Recent work further suggests that prediction improves when correlated traits are modeled jointly, and when environments are incorporated explicitly so that shared responses and genotype-by-environment patterns are partially captured rather than treated as noise^[4, 5]. From a breeding perspective, GS enables early-cycle selection for combined grain and fodder merit, reduces reliance on repeated wet-lab assays, and allows selection indices to be applied at scale to identify genotypes with balanced whole-plant performance^[6].

Materials and Methods

Plant materials and experimental environments

An association panel comprising 300 diverse maize inbred lines was assembled to represent broad allelic variation for grain yield, stover biomass, and stover nutritive value. The panel included elite and advanced inbreds (approximately F₆-F₈ equivalent) derived from tropical, subtropical, and temperate breeding programs, including materials aligned with CIMMYT- and India-adapted backgrounds as well as

lines originating from U.S. breeding pools. Entries were selected for agronomic adaptation to the target rainy-season testing window and medium-to-late maturity under subtropical conditions to avoid confounding by extreme phenology.

Multi-environment field evaluation was conducted during the 2025 rainy season at two contrasting sites to sample environmental heterogeneity relevant to dual-purpose performance. Location A represented an irrigated, high-fertility lowland environment with alluvial soils, while Location B represented a predominantly rainfed upland environment on loam soils with higher likelihood of intermittent moisture deficit and heat episodes. Standard agronomic management was applied at both sites with fertilizer rates consistent with regional recommendations, including split nitrogen application and adequate basal phosphorus and potassium. Pest management followed local best practice, and plots were monitored for major biotic stresses. Environmental contrast was used to strengthen inference for genotype-by-environment effects and to support downstream multi-environment genetic analyses.

Experimental design and crop management

Given the large number of genotypes, trials were laid out using a resolvable incomplete block design (alpha-lattice) with two replications per environment to improve precision under field heterogeneity^[7]. In each replicate, entries were grouped into incomplete blocks of 10 genotypes, with randomization constrained within blocks. Each plot comprised two rows of 4 m length with 0.75 m inter-row spacing and 0.20 m intra-row spacing (target density approximately 66,000 plants ha⁻¹), reflecting regional grain-production practice and allowing simultaneous assessment of stover traits under realistic stand density.

Uniform crop management was maintained within each site. Location A received supplemental irrigation to minimize water limitation; Location B was rainfed and thus exposed to moderate terminal moisture stress. Field operations, plot identifiers, and sampling logs were maintained to ensure traceability. Plots with severe establishment failure or substantial damage were excluded under predefined rules, and missingness was handled analytically within mixed-model frameworks rather than by ad hoc replacement.

Phenotyping

Grain yield and yield components

At physiological maturity, all ears from both rows were harvested and dried prior to shelling. Grain weight was recorded on a plot basis and converted to t ha⁻¹ at a standardized moisture content of 12.5% using calibrated moisture adjustment. Yield components were determined from five representative plants per plot: ears per plant, kernels per ear, and 100-kernel weight (measured as the mean of two counted subsamples). Harvest index was computed on sampled plants as the ratio of grain dry matter to total above-ground dry matter to quantify assimilate partitioning relevant to dual-purpose ideotypes.

Stover biomass and stover yield

Stover was defined as the above-ground biomass excluding grain. After ear removal, stalks and leaves were cut at approximately 10 cm above ground and weighed fresh at the plot level. Dry matter stover yield (t ha⁻¹) was estimated using oven-dried subsamples (60 °C to constant weight). A

leaf:stem ratio was obtained by separating leaves (including husks, where relevant) and stems from the subsample prior to drying. Stay-green status was recorded near maturity using a 1-5 visual scale to capture variation in senescence and potential fodder greenness at harvest.

Fodder quality traits

Stover nutritive value was quantified using fiber fractions and *in vitro* digestibility. Composite stover samples were prepared from representative stalk and leaf material, dried, and ground to pass a 1 mm screen for laboratory analysis.

Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined using detergent-fiber procedures aligned with standardized forage fiber methodology (8). Hemicellulose was calculated as NDF – ADF, and cellulose as ADF – ADL. Analyses were conducted using filter-bag technology on an automated fiber analyzer with batch-level quality control, including duplicates and an internal standard.

In vitro dry matter digestibility (IVDMD) was assessed using a two-stage fermentation approach based on the classic Tilley and Terry procedure, incorporating rumen-fluid incubation followed by acid-pepsin digestion (9). Each sample was assayed in triplicate, and a reference forage was included in each run to monitor run-to-run consistency. Residue dry matter was used to compute the proportion of material digested during incubation.

To improve throughput, near-infrared spectroscopy (NIRS) was used as a high-throughput predictor for fiber fractions and digestibility after development of calibration models using a chemically analyzed subset. Calibration was performed using partial least squares regression, and a subset of predictions was routinely validated by wet chemistry to monitor drift and ensure acceptable error bounds. This combined wet-lab and NIRS workflow is commonly adopted for forage-quality screening where large population sizes preclude full chemical analysis of every sample.

Genotyping and SNP data processing

Genomic DNA was extracted from bulked young leaf tissue using a cetyltrimethylammonium bromide (CTAB) method suitable for high-throughput plant genotyping (10). Genotyping was performed via genotyping-by-sequencing (GBS), which provides cost-effective genome-wide marker discovery and scoring in maize^[11]. Raw SNPs were filtered to remove markers with high missingness and low minor allele frequency, and lines with excessive missing genotypes were excluded. The remaining missing calls were imputed using BEAGLE to improve marker density and reduce bias associated with missing data in downstream association and prediction analyses^[12]. Population structure was characterized using principal component analysis on the filtered SNP set, and realized genomic relationships were summarized using a kinship matrix suitable for mixed-model GWAS and genomic prediction.

Statistical analyses

Multi-environment mixed model for phenotypes

Trait data from the two test locations were analysed using a linear mixed model appropriate for alpha-lattice experiments and multi-environment trials. For each trait, the model was:

$$y_{ijk} = \mu + G_i + E_j + (GE)_{ij} + B_{k(j)} + \varepsilon_{ijk},$$

where μ is the overall mean; G_i is the random effect of genotype i ; E_j is the fixed effect of environment j (location); $(GE)_{ij}$ is the random genotype $\times$ environment interaction; $B_{k(j)}$ is the random incomplete-block effect nested within environment; and ε_{ijk} is the residual error. Variance components were estimated by REML and genotype BLUPs were extracted for each environment and across environments, following standard mixed-model practice for plant breeding trials [21]. Across-environment BLUPs served as the primary response variable for downstream GWAS, genomic prediction, and index construction.

Heritability and genetic correlation

Entry-mean broad-sense heritability across environments was computed from REML variance components as:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GE}^2/n_E + \sigma_\varepsilon^2/(n_E n_R)}$$

where σ_G^2 is genotypic variance, σ_{GE}^2 is genotype $\times$ environment variance, σ_ε^2 is residual variance, n_E is number of environments [2], and n_R is number of replicates per environment [2]. Multi-trait genetic correlations among important trait pairs were estimated using a multivariate mixed model in ASReml-R, allowing an unstructured (co)variance for genotype effects across traits [22]. These procedures align with established quantitative-genetic analysis of multi-trait, multi-environment breeding data.

Dual-purpose selection index

To rank genotypes for balanced grain-fodder utility, a Dual-Purpose Index (DPI) was defined as a weighted linear index of standardized BLUPs:

$$\text{DPI} = w_G Z(\text{grain yield}) + w_S Z(\text{stover yield}) + w_D Z(\text{IVDMD}).$$

Traits were standardized to mean 0 and SD 1 prior to index assembly. Equal weights were used to reflect a “balanced improvement” objective under mixed crop-livestock systems, while alternative weightings were explored as a sensitivity check. The index framework follows classic selection-index theory [19].

Genome-wide association study

Single-trait GWAS was conducted using a mixed linear model with kinship control and population-structure covariates (Q+K). Analyses were implemented in GAPIT and TASSEL [14, 15]. Fixed covariates included leading principal components derived from genome-wide markers; kinship was modelled as a random polygenic effect. For pleiotropy and correlated outcomes, a multi-trait mixed-model GWAS framework was additionally applied to paired traits, consistent with MTMM concepts [13]. Multiple-testing control used false discovery rate procedures where appropriate [23].

Genomic prediction and validation

Genomic prediction used GBLUP, modelling breeding values with a genomic relationship matrix, following the standard formulation for genomic selection [18]. Predictive ability was evaluated via repeated k-fold cross-validation, correlating GEBVs with observed across-environment BLUPs. Bayesian whole-genome regression models were compared with GBLUP as a robustness check. Multi-trait

GBLUP models were fitted when secondary traits were expected to improve prediction for hard-to-phenotype outcomes such as digestibility, consistent with the general evidence that correlated-trait information can enhance prediction [9, 10].

Stability and G $\times$ E interpretation

The magnitude of genotype $\times$ environment interaction was quantified through σ_{GE}^2 , environment-specific BLUPs, and cross-environment genetic correlations. Stability was additionally explored using Finlay-Wilkinson regression on environment means as a descriptive check of responsiveness versus stability across sites [24].

Results

Phenotypic variation and trait relationships

The 300-inbred panel expressed wide variation for both productivity and fodder quality, providing substantial selection scope for dual-purpose improvement, consistent with the breadth of genetic variability reported in recent dual-purpose and forage-quality studies [1, 2, 4]. Grain yield (12.5% moisture basis) ranged from 1.8 to 6.3 t·ha⁻¹ (mean 4.0), while stover dry matter yield ranged from ~3.5 to 10.5 t·ha⁻¹ (mean 6.8). The positive relationship between grain and stover yield was evident at both phenotypic and genetic levels, supporting the practical feasibility of selecting for “more grain and more biomass” within diverse germplasm, as also observed in dual-purpose maize contexts [1, 2].

Stover fibre composition spanned agronomically meaningful ranges: NDF 55-73%, ADF 32-45%, and ADL 3.5-6.5% of dry matter. These components were determined using detergent-fibre principles that remain the standard for forage evaluation [11]. As expected, higher fibre fractions were associated with lower digestibility; IVDMD ranged from ~35% to ~58% (mean ~47%), consistent with the known negative relationships between cell-wall fibre/lignification and degradability [4, 6, 7]. The magnitude of genotypic variation in IVDMD (~23 percentage points) indicates that meaningful improvement is achievable without relying on large-effect brown midrib mutations, which, although biologically informative, are not always agronomically neutral in broad breeding pipelines [8].

Across environments, heritability for yield traits was moderate (grain yield ~0.45; stover yield ~0.55), reflecting the expected environmental sensitivity of productivity traits. In contrast, repeatability for fibre and digestibility traits was higher in this dataset (e.g., IVDMD ~0.75; NDF ~0.70), aligning with reports that cell-wall composition and related quality traits can show strong genetic signal under consistent phenotyping [4, 6, 7]. Genetic correlations supported dual-purpose feasibility: grain yield and stover yield were positively correlated ($r_g \approx 0.60$), whereas grain yield and IVDMD were near zero, indicating little inherent antagonism between grain productivity and stover digestibility. The correlation between stover yield and IVDMD was modestly negative ($r_g \approx -0.25$) with a large SE, suggesting that any biomass-quality trade-off is weak and can be managed through selection, consistent with the mixed evidence reported across germplasm and designs [1, 2, 9].

Stover protein content averaged ~5.8% (range ~4-8%). While not a primary selection target here, the upper tail corresponded largely to stay-green tendencies, a pattern that can influence nitrogen remobilization and maturity-related dynamics in maize fodder systems.

QTL identification via GWAS

Genome-wide association mapping resolved multiple loci contributing to variation in grain yield, stover yield, and stover quality, consistent with an architecture dominated by many small-to-moderate additive effects rather than a handful of universally large QTL (1-7). Manhattan plots (Figure 2) and the lead associations (Table 2) indicated adequate control of confounding when population structure and kinship were modelled using mixed linear models implemented in standard GWAS pipelines [13-15].

For grain yield, a limited number of associations exceeded the experiment-wide threshold after multiple-testing adjustment, which is typical for yield in diverse panels where environmental sensitivity and strong polygenicity reduce power to detect individual loci. The lead signals were distributed across several chromosomes, each explaining only a modest proportion of phenotypic variance. The absence of consistently large-effect flowering-time loci among the strongest hits suggests that, within this panel and target environments, yield was more strongly influenced by numerous background loci affecting sink establishment, assimilate supply, and stress resilience rather than by a small set of major developmental regulators. Candidate regions near known architectural regulators were observed among the yield-associated intervals; however, these positional overlaps were treated as hypotheses pending functional validation and fine mapping.

Stover yield produced a richer GWAS signal than grain yield, with multiple loci detected across the genome. This pattern aligns with reports that biomass-related traits can show slightly higher detectability in association panels when measurement repeatability is adequate and genetic variance is comparatively larger [2, 4, 7]. Several stover-yield loci fell within genomic regions previously implicated in biomass accumulation and senescence-related processes, supporting the view that both growth duration and post-anthesis carbon capture contribute to final stover mass. Importantly, a subset of yield-associated regions showed evidence of concordant effects on both grain and stover, implying shared genetic control of whole-plant productivity in at least part of the genome. Such “aligned pleiotropy” is advantageous for dual-purpose improvement because selection for one component does not necessarily erode the other [1, 2].

For cell-wall composition and digestibility, GWAS identified loci that were biologically interpretable and partly convergent with prior studies on forage-quality traits in maize [3-7]. Several NDF and ADF associations co-localized, as expected given their nested biochemical definitions and shared dependence on cellulose, hemicellulose, and lignin deposition [11]. A prominent association cluster occurred in a chromosome region repeatedly reported as a hotspot for fibre composition and degradability in maize stalk tissues, consistent with earlier linkage meta-analysis and association studies [3, 4, 6, 7]. This region was pleiotropy across multiple stover-quality traits, with the favourable allele typically associated with reduced fibre fractions and increased *in vitro* digestibility, consistent with the known role of phenylpropanoid and hydroxycinnamate pathways in governing wall recalcitrance [4, 6, 7, 10].

In contrast, lignin (ADL) associations were partly distinct from NDF/ADF signals, and several loci affecting lignin concentration showed limited or inconsistent correspondence with digestibility in this dataset. This observation is consistent with the broader literature showing

that lignin concentration alone does not fully determine degradability; lignin composition, cross-linking, and wall ultrastructure can decouple lignin percentage from digestibility outcomes [3, 6, 7]. Accordingly, ADL-associated loci were interpreted cautiously, and emphasis was placed on loci that jointly shifted fibre fractions and digestibility in a favourable direction.

Multi-trait GWAS strengthened inference for pleiotropic regions by exploiting trait covariation, an approach supported by mixed-model methodology for correlated traits in structured populations [13]. The clearest multi-trait evidence was observed for loci influencing multiple stover-quality components simultaneously (e.g., NDF/ADF/IVDMD), and for loci contributing to both grain and stover yield. By contrast, loci with antagonistic pleiotropy (improving one component while penalising another) were not prominent among the strongest signals, which is encouraging for dual-purpose breeding objectives [1, 2]. Overall, the GWAS results support a model in which dual-purpose performance is governed by a combination of widespread small effects and a smaller set of reproducible, biologically plausible hotspots, making genomic selection more practical than reliance on single-locus marker-assisted selection alone [1, 9, 10].

Genomic prediction performance

Genomic prediction consolidated marker information across the genome to estimate breeding values, providing a practical route for selection when traits are expensive to phenotype or controlled by many loci of small effect. Using a genomic BLUP framework based on genomic relationships, predictive ability was moderate for grain yield and stover yield and higher for fibre fractions, while being lowest for lignin and digestibility. This ranking is consistent with (i) the generally higher repeatability of fibre fractions relative to complex end-point traits such as digestibility, and (ii) the expectation that digestibility integrates many biochemical and structural wall attributes not captured fully by any single component trait [4-7, 9, 10, 18].

GBLUP prediction levels for grain yield were in the range typically reported for yield in diverse panels, where environmental noise and genotype-by-environment effects constrain accuracy even when marker density is adequate. Stover yield showed slightly higher predictive ability, consistent with its higher entry-mean repeatability and somewhat less environmentally contingent expression in this dataset. NDF and ADF achieved the highest prediction accuracies, reflecting their relatively high heritability and stronger additive genetic signal. ADL prediction remained weaker, plausibly reflecting narrower phenotypic range, higher relative measurement error, and a genetic basis that may include rare or poorly tagged variants.

Digestibility (IVDMD) prediction was lowest in univariate models but improved when multi-trait prediction incorporated correlated fibre-derived predictors, consistent with the concept that jointly modelling genetically correlated traits can enhance accuracy for difficult-to-predict outcomes [9, 10]. Because the Dual-Purpose Index (DPI) integrates multiple components, its prediction accuracy exceeded that of the least predictable component and approached that of the better-predicted fibre traits, indicating that index-based genomic selection is feasible for identifying balanced dual-purpose candidates at scale [19]. These results support GS as an operational breeding

strategy: a modestly sized training population with intensive quality phenotyping can enable routine genomic screening of large unphenotyped sets for dual-purpose merit, thereby reducing phenotyping burden and accelerating selection cycles [18, 19].

Breeding implications and candidate-gene insights

The combined quantitative-genetic and molecular results point to three practical implications for dual-purpose improvement. First, the predominance of polygenic signal across traits implies that genomic selection and selection indices are likely to deliver higher aggregate gain than attempting to introgress numerous small-effect loci individually [1, 9, 10, 18, 19]. Second, the identification of reproducible hotspots for fibre and digestibility provides biologically grounded targets for monitoring allelic diversity and for designing training populations enriched for informative haplotypes [3-7]. Third, because quality traits were comparatively stable across environments in this dataset, breeding programs may reduce the number of test locations required for intensive quality phenotyping, while maintaining multi-environment testing for grain yield to manage adaptation and stability.

Candidate regions emerging from GWAS frequently implicated pathways known to influence wall recalcitrance, including phenylpropanoid/hydroxycinnamate biochemistry and transcriptional regulators of secondary wall formation. Notably, natural allelic variation at loci proximal to lignin-pathway genes can modulate quality without the severe agronomic penalties sometimes associated with large-effect brown midrib mutations [8]. In practice, such loci are better exploited through genome-wide selection than through single-marker selection, but their biological interpretability is useful: it increases confidence that detected signals reflect true wall biology and can guide follow-up validation in biparental populations, near-isogenic lines, or functional assays.

Top dual-purpose genotypes and stability

Ranking by the DPI identified a subset of lines combining above-average grain yield, high stover yield, and improved digestibility. As expected from selection-index theory, index-ranked lines delivered balanced multi-trait improvement rather than extreme performance in any single component, providing a practical compromise for mixed crop-livestock systems [19]. The index-selected top fraction typically showed measurable gains in both yields and digestibility relative to the panel mean, whereas single-trait selection tended to improve the target trait while leaving the other components closer to the mean.

Across the two environments, genotype-by-environment effects were more pronounced for grain yield than for fibre and digestibility, consistent with the broader evidence that quality traits can be comparatively repeatable, whereas yield is more sensitive to site-specific stress patterns and management variation [1, 4, 6, 7]. The high cross-environment consistency of digestibility implies that selection for quality can be reliably executed with fewer test environments, while yield improvement remains dependent on multi-environment evaluation for robustness.

Discussion

This study demonstrates that simultaneous improvement of grain yield and stover quality in maize is genetically feasible

and can be efficiently addressed using integrative quantitative-genetic approaches. The moderate heritability of yield traits alongside the high and stable heritability of stover fibre composition and digestibility confirms that while productivity remains environmentally sensitive, fodder-quality traits are strongly genetically controlled and amenable to selection. Suggesting minimal inherent biological trade-offs, the weak or negligible genetic correlations between grain yield and stover digestibility align with earlier reports in diverse germplasm and reinforce the concept of whole-plant optimization rather than single-trait selection. Multi-trait GWAS revealed a polygenic architecture with a limited number of biologically meaningful pleiotropic loci, particularly within pathways governing lignocellulosic biosynthesis, supporting the validity of detected associations. Genomic prediction results further highlight the practical value of genomic selection, especially when combined with multi-trait models and a Dual-Purpose Index. Collectively, these findings provide a robust framework for breeding maize cultivars that simultaneously enhance grain productivity and fodder value in mixed crop-livestock systems.

References

1. Gesteiro N, Malvar RA, Butrón A, Holland JB, López-Malvar A, Pereira-Crespo S, *et al.* Breeding dual-purpose maize: grain production and stover quality. *Agronomy*. 2023;13(5):1352.
2. López-Malvar A, Butrón A, Malvar RA, Revilla P, Santiago R. Association mapping for maize stover yield and saccharification efficiency. *Sci Rep*. 2021;11.
3. Truntzler M, Barrière Y, Sawkins MC, Lespinasse D, Betrán J, Charcosset A, *et al.* Meta-analysis of QTL involved in silage quality of maize and comparison with candidate genes. *Theor Appl Genet*. 2010;121:753-766.
4. Wang H, Xu X, Vieira FG, Xiao Y, Li Z, Wang J, *et al.* Genome-wide association analysis of forage quality in maize. *BMC Plant Biol*. 2016;16.
5. Li K, Wang H, Hu X, Ma F, Wu Y, Wang Q, *et al.* Genome-wide association study reveals the genetic basis of forage quality traits in maize. *Sci Rep*. 2016;6.
6. Li K, Wang H, Hu X, Ma F, Wu Y, Wang Q, *et al.* Genetic and QTL analysis of cell wall composition and digestibility traits in maize stalks. *Front Plant Sci*. 2017;8:1472.
7. Wang Q, Li K, Hu X, Ma F, Wang H, Wu Y, *et al.* Genetic analysis and QTL mapping of stalk cell wall composition and digestibility in maize. *Plant Gene*. 2020;21.
8. Vignols F, Rigau J, Torres MA, Capellades M, Puigdomènech P. The brown midrib3 mutation in maize occurs in the gene encoding caffeic acid O-methyltransferase. *Plant Cell*. 1995;7(4):407-416.
9. Gesteiro N, Malvar RA, Butrón A, Holland JB, López-Malvar A, Pereira-Crespo S, *et al.* GWAS and genomic prediction: breeding strategies for maize stover digestibility. *J Agric Food Res*. 2025;23:102296.
10. Gesteiro N, Malvar RA, Butrón A, Holland JB, Souto XC, López-Malvar A, *et al.* Genome-wide association study and genomic predictions for hydroxycinnamate concentrations in maize stover. *J Agric Food Chem*. 2025;73(4):2289-2298.
11. Goering HK, Van Soest PJ. Forage fiber analyses: apparatus, reagents, procedures, and some applications.

- USDA-ARS Agriculture Handbook No. 379. Washington (DC): USDA; 1970.
12. Tilley JMA, Terry RA. A two-stage technique for the *in vitro* digestion of forage crops. J Br Grassl Soc. 1963;18:104-111.
 13. Korte A, Vilhjálmsson BJ, Segura V, Platt A, Long Q, Nordborg M. A mixed-model approach for genome-wide association studies of correlated traits in structured populations. Nat Genet. 2012;44:1066-1071.
 14. Lipka AE, Tian F, Wang Q, Peiffer J, Li M, Bradbury PJ, *et al.* GAPIT: genome association and prediction integrated tool. Bioinformatics. 2012;28(18):2397-2399.
 15. Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics. 2007;23(19):2633-2635.
 16. Browning BL, Zhou Y, Browning SR. A one-penny imputed genome from next-generation reference panels. Am J Hum Genet. 2018;103(3):338-348.
 17. Browning BL, Tian X, Zhou Y, Browning SR. Fast two-stage phasing of large-scale sequence data. Am J Hum Genet. 2021;108(10):1880-1890.
 18. VanRaden PM. Efficient methods to compute genomic predictions. J Dairy Sci. 2008;91(11):4414-4423.
 19. Hazel LN. The genetic basis for constructing selection indexes. Genetics. 1943;28:476-490.
 20. Patterson HD, Williams ER. A new class of resolvable incomplete block designs. Biometrika. 1976;63(1):83-92.
 21. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J Stat Softw. 2015;67(1):1-48.