

Breeding forage sorghum for biomass productivity and reduced early- stage cyanogenic potential: Pareto analysis and HCN-constrained ranking across two rainy seasons

Ajaya Paliwal¹

ajay.paliwal@gmail.com

Veer Chandra Singh Garhwali Uttarakhand University of Horticulture & Forestry

P. K. Shrotria²

Govind ballabh pant university of agriculture and technology, india

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Abstract

This transparent secondary analysis revisited an archived forage-sorghum trial to evaluate biomass and early-stage cyanogenic potential while accounting for season interaction and selection uncertainty. Forty-five F_1 hybrids from a 9×5 line $\times$ tester set were evaluated in randomized blocks with three replications during two rainy seasons at Pantnagar, India. Green (GFY) and dry fodder yield (DFY) represented two harvests; HCN potential (HCN-P) was measured at 30–35 days after sowing. A combined block-within-season model detected hybrid $\times$ season interaction for GFY ($F_{44,176} = 3.572$, $P < .001$) and DFY ($F_{44,176} = 3.531$, $P < .001$), but not HCN-P ($F_{44,176} = 0.605$, $P = .974$). Across-season GFY–HCN-P and DFY–HCN-P associations were negative but imprecise ($r = -.264$, 95% CI $-.518$ to $.032$; $r = -.271$, CI $-.523$ to $.025$). Five hybrids were common to both seasonal and the across-season nominal fronts. A sixth, MR750A₂ $\times$ UPMC503, entered only the across-season frontier and had an exact hierarchical-bootstrap frontier frequency of 32.7%, indicating averaging sensitivity. Under the first-quartile empirical HCN-P ceiling, GFY and DFY identified different point leaders; at median and third-quartile ceilings, MR750A₂ $\times$ Pusa Chari 23 led both endpoints, with differing stability frequencies. Simultaneous comparisons retained 31 of 45 hybrids in a conservative Pareto candidate set. These historical trials do not support a positive linear biomass–HCN-P association, but significant yield interaction and limited environmental sampling preclude definitive recommendations. The identified hybrids are priorities for contemporary multi-location and multi-stage validation, not feeding-safety conclusions.

1 Introduction

Forage sorghum [*Sorghum bicolor* (L.) Moench] combines rapid C_4 biomass accumulation, heat tolerance and regrowth capacity, making it important for green fodder and conserved feed in warm production environments. Breeding targets extend beyond fresh biomass to dry-matter production, adaptation and forage-quality attributes. Multi-environment studies therefore increasingly integrate yield, stability and multiple traits when advancing forage-sorghum material (Behera et al., 2024; P. Kumari et al., 2025).

Cyanogenesis is an important constraint in vegetative sorghum. The cyanogenic glucoside dhurrin can release hydrogen cyanide after tissue disruption, and its abundance varies with genotype, environment, tissue and developmental stage (Gruss et al., 2023; Ouma et al., 2023; Wang et al., 2024). An HCN-P value measured from young tissue is consequently a stage-specific comparative breeding phenotype rather than a universal feeding-safety classification. Dhurrin-free material with maintained field biomass also shows that biomass and cyanogenesis need not be biologically inseparable, although that result does not establish the relationship in every genetic population or environment (A. Kumari et al., 2025).

Joint selection can be described through non-domination and empirical constraints. Pareto-front analysis identifies hybrids for which GFY cannot be increased without increasing HCN-P, or HCN-P reduced without reducing GFY. Threshold screening first limits eligibility to hybrids below an empirical HCN-P ceiling and then ranks GFY or DFY among the eligible set. Because fresh and dry yield can be

highly correlated, treating them as separate sensitivity endpoints avoids double-counting one biomass signal. Uncertainty and genotype $\times$ environment response must accompany these descriptive rules; otherwise pooled point estimates can imply a stability that the field design does not support.

The observations examined here originated in Paliwal's doctoral experiment (Paliwal, 2012). Heterosis from the same 9×5 crossing population was reported by Paliwal et al. (2016), including green and dry fodder yield and previously identified favourable crosses. Aruna et al. (2013) provides related literature on diverse male-sterile germplasm; common germplasm alone does not establish reuse of the present plot observations. This paper does not claim newly collected data or novel discovery of those hybrids. Its incremental objective is a plot-level secondary analysis that (i) tests hybrid $\times$ season response, (ii) estimates biomass–HCN-P associations with confidence intervals by and across season, (iii) quantifies uncertainty in Pareto membership, and (iv) evaluates HCN-constrained GFY and DFY rankings separately.

2 Materials and methods

2.1 Genetic material, site and experimental design

Nine cytoplasmic male-sterile lines were used as females: 27A and 2219A in A_1 cytoplasm; C43A₂, A2N190, A2N192 and MR750A₂ in A_2 cytoplasm; and A3N191, A3N193 and A3N213 in A_3 cytoplasm. The testers were SSG59-3, Nizamabad forage sorghum, Pusa Chari 23, UPMC512 and UPMC503 (Pant Chari 6). The 9×5 crossing set produced 45 F_1 hybrids. Cytoplasmic labels identify female-line backgrounds only; causal cytoplasmic effects were not estimated because the lines were not isonuclear.

The source experiment comprised the 45 hybrids, their 14 parents and two checks. The present analysis used only the complete 45-hybrid subset. Trials were conducted at the Instructional Dairy Farm, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India (approximately 29.0° N, 79.3° E; 243.84 m above mean sea level) in the rainy seasons sown in 2009 and 2010, retained here under the archive labels 2009–10 and 2010–11. Each season was a randomized complete-block design with three replications. A plot comprised two 4-m rows spaced 0.30 m apart (2.4 m²).

2.2 Biomass and HCN-P measurements

The entire 2.4-m² plot was harvested and weighed for GFY. The first cut was taken approximately 50–55 days after sowing and the second 35 days later; the two cuts were summed. For DFY, a known quantity of chopped whole-plant material from each plot was dried at 80°C for 48 h or until constant mass, and the dry-matter percentage was used to derive total DFY.

HCN-P was estimated with the historical picrate-spectrophotometric procedure (Hogg & Ahlgren, 1942). At 30–35 days after sowing, one sample per genotype and replication was collected from basal tillers approximately 5–7 inches (13–18 cm) high, immediately from the uppermost leaf collar. Fresh green leaf tissue (0.20 g) was cut, sealed with one to two drops of chloroform and exposed for 24 h at room temperature to moist sodium-picrate paper. The reaction product was eluted into 10 mL distilled water,

optical density was read at 515 nm against KCN standards, and values were expressed as ppm on a dry-weight basis.

HCN-P sampling preceded the first biomass harvest by approximately 15–25 days and the second by 50–60 days. This temporal separation was inherent to the original breeding protocol: HCN-P represented early-stage cyanogenic potential in actively growing tissue, whereas total biomass represented later agronomic productivity. The analysis therefore concerns cross-stage hybrid co-performance, not a contemporaneous physiological association, harvest-stage HCN-P or feeding safety.

2.3 Combined model and trait associations

The analysis-ready dataset contained 270 records: 45 hybrids $\times$ two seasons $\times$ three blocks, with complete GFY, DFY and HCN-P values. Each trait was analysed as $y = \mu + S + B(S) + H + S \times H + e$, where S was season, $B(S)$ was block nested within season and H was hybrid. Season and blocks were treated as random environmental terms and hybrid as fixed. Season was tested against $B(S)$, hybrid against $S \times H$, and $S \times H$ against residual error. Because only two seasons at one location were observed, environmental inference remains narrow even though the conventional random-season denominators were used.

Balanced-design hybrid marginal means equal the arithmetic means. For across-season hybrid means, 95% linear-model intervals used the $S \times H$ mean square (44 df) for GFY and DFY. The estimated HCN-P $S \times H$ variance component was negative and was set to zero; its interval therefore used the residual mean square (176 df). These intervals describe relative hybrid performance on the observed grand-mean scale, conditional on common season and block shifts; they are not prediction intervals for new seasons. Residual normality and between-season variance homogeneity were examined with Shapiro–Wilk and median-centred Levene tests. Raw-scale GFY showed unequal residual variance, so log-GFY was fitted as a sensitivity analysis.

Pearson correlations among 45 hybrid means were calculated across seasons and separately within each season; 95% confidence intervals used Fisher's z transformation. These are associations among observed hybrid means, not estimates of genetic correlation. Spearman correlations provided a rank-based sensitivity analysis. The submitted partial correlations were removed because GFY and DFY were strongly collinear and did not provide a stable independent biomass adjustment. Correlation and contrast tests were two-sided; model F tests used the upper tail, at $\alpha = .05$. Estimates and intervals were emphasized over binary significance.

Formal pairwise comparisons used the difference of across-season hybrid means, with $SE = \sqrt{(2M/6)}$, where M was the hybrid $\times$ season mean square for yields and the residual mean square for HCN-P. Common season and block effects cancel in these contrasts. Both nominal 95% intervals and Bonferroni simultaneous 95% intervals were calculated for the complete family of 990 hybrid pairs $\times$ three traits (2,970 comparisons), using 44 df for yields and 176 df for HCN-P. This family was defined over all hybrids, so the reported comparisons remain covered after inspecting ranks. The simultaneous critical

value was $t[1 - .05/(2 \times 2970), df]$; adjusted P values were $\min(1, 2970 \times P)$. These are approximate model-based inferences under the stated variance assumptions.

2.4 Quadrants, Pareto stability and HCN-constrained ranking

Four-quadrant summaries used the within-scope means of GFY and HCN-P as descriptive reference lines. A hybrid above the GFY mean and below the HCN-P mean occupied the desired quadrant; this label is population-relative and not toxicological. Pareto evaluation maximized GFY and minimized HCN-P. A hybrid was dominated when another had at least as much GFY and no more HCN-P, with strict superiority in at least one objective.

Uncertainty was evaluated by exact weighted enumeration of a hierarchical design bootstrap. Two seasons were sampled with replacement; within each selected season, three complete RBD blocks were sampled with replacement, preserving all hybrids and paired traits together. The three-block draw has ten multinomial count patterns, and the two ordered season/block draws yield 400 weighted configurations. All were enumerated with their exact probabilities, so no random seed or Monte Carlo replicate count was required. Hybrid means, HCN-P quantiles, quadrant membership, Pareto membership, eligibility and GFY/DFY winners were recalculated in every configuration; exact winner ties divided the configuration weight equally among tied hybrids. Reported frequencies measure empirical stability within this limited archive rather than calibrated selection probabilities for unobserved environments. A fixed-season 100-configuration enumeration was retained in Online Resource 1 as a sensitivity analysis.

A conservative model-based Pareto candidate set supplemented the empirical frequencies. A hybrid was excluded only when another hybrid had simultaneously supported greater GFY and lower HCN-P: the corresponding simultaneous confidence intervals had to exclude zero in both favourable directions. Hybrids not excluded form an approximate 95% outer confidence set under the model. Membership means that domination was not demonstrated, not that optimality was established. This deliberately conservative rule allows the true frontier to be wider or narrower than the observed point frontier and does not predict performance in new environments.

The first quartile, median and third quartile of across-season HCN-P means (37.09, 59.38 and 78.06 ppm) defined three empirical ceilings. Because GFY and DFY were strongly correlated, no equal-weight composite was used. GFY was the primary whole-plot yield endpoint; DFY was ranked separately as a sensitivity endpoint. Point ranks were not treated as statistically significant differences. Exact weighted 2.5th–97.5th percentile intervals describe empirical Pareto-layer ranks and yield ranks conditional on eligibility; they are not calibrated population confidence intervals. Targeted GFY differences were also summarized over the exact hierarchical configurations, alongside formal model-based comparisons.

2.5 Reproducibility, historical-data provenance and AI use

Online Resource 1 contains the analysis-ready plot dataset, data dictionary, executable Python scripts, version record, README and complete result tables. The code validates the design, fits the combined

model, calculates associations and diagnostics, enumerates all bootstrap configurations, constructs simultaneous comparisons and the conservative Pareto candidate set, and reproduces Fig. 1 and Tables 1–3. An audit script checks the analysis values against the source workbook. Analyses were run with Python 3.12.13, pandas 2.2.3, NumPy 2.3.5, SciPy 1.17.0 and Matplotlib 3.10.8. Online Resource 2 presents the additional comparison and rank-uncertainty summaries.

This is a post hoc secondary analysis of archived doctoral observations. Paliwal et al. (2016), the authors' prior journal article from this doctoral research, tabulated heterosis for yield and morphological traits; its discussion also mentioned HCN. The present study does not reproduce those heterosis tables. Its addition is the joint analysis of early-stage HCN-P and cumulative biomass with hybrid $\times$ season response, uncertainty in non-domination and endpoint-dependent ranking. The related study of Aruna et al. (2013) is cited as germplasm literature, without asserting that it reused these doctoral plot records.

OpenAI ChatGPT/Codex assisted with statistical-method evaluation, code drafting and debugging, execution of analysis scripts, interpretation support, language editing, and preparation of the revision and response. Experimental observations were supplied by the authors; no observations were generated, imputed or altered by generative AI. All reported computations were executed on the supplied archive. The authors retain responsibility for the statistical choices, accuracy, citations, interpretations and final submitted text. No generative-AI images were used.

3 Results

3.1 Hybrid, season and trait-association evidence

Across 45 hybrid means, GFY averaged 27.89 kg plot⁻¹ (range 11.75–44.35), DFY 6.69 kg plot⁻¹ (2.67–10.42), and HCN-P 60.32 ppm (5.79–129.50). Season means were 30.57 and 25.21 kg plot⁻¹ for GFY, 7.37 and 6.01 kg plot⁻¹ for DFY, and 61.40 and 59.24 ppm for HCN-P in 2009–10 and 2010–11, respectively.

The season effect was supported for GFY and DFY but not HCN-P (Table 1). More importantly, hybrid $\times$ season interaction was supported for GFY ($F_{44,176} = 3.572$, $P < .001$) and DFY ($F_{44,176} = 3.531$, $P < .001$), but not HCN-P ($F_{44,176} = 0.605$, $P = .974$). Consequently, across-season yield means are summaries of these two trials and require season-specific qualification. Residual Shapiro–Wilk P values were .467, .630 and .130 for GFY, DFY and HCN-P. GFY residual variance differed by season (Levene $P = .005$), whereas DFY ($P = .399$) and HCN-P ($P = .240$) did not. Log-GFY removed that heterogeneity (Levene $P = .295$) and retained the hybrid $\times$ season conclusion ($F_{44,176} = 3.542$, $P < .001$).

GFY and DFY were strongly correlated across seasons ($r = .930$, 95% CI .875 to .961). GFY–HCN-P ($r = -.264$, 95% CI $-.518$ to $.032$, $P = .079$) and DFY–HCN-P ($r = -.271$, 95% CI $-.523$ to $.025$, $P = .072$) were negative but imprecise. The same direction occurred within both seasons, and every yield–HCN-P Pearson interval included zero (Table 1). Rank-based sensitivity estimates were also negative (Spearman

$\rho = -.361$ for GFY–HCN-P and $-.297$ for DFY–HCN-P). These data therefore did not support a positive linear association, but neither established biological independence.

Table 1
Combined randomized-block model and associations among hybrid means

Effect or association	df / scope	MS or estimate	F or 95% CI	P
Panel A. Combined model				
Season: GFY	1, 4	MS = 1937.908	F = 29.431	P .006
Season: DFY	1, 4	MS = 124.943	F = 12.776	P .023
Season: HCN-P	1, 4	MS = 316.228	F = 1.776	P .253
Block within season: GFY	4, 176	MS = 65.846	F = 7.478	P < 0.001
Block within season: DFY	4, 176	MS = 9.779	F = 11.517	P < 0.001
Block within season: HCN-P	4, 176	MS = 178.011	F = 3.057	P .018
Hybrid: GFY	44, 44	MS = 237.615	F = 7.555	P < 0.001
Hybrid: DFY	44, 44	MS = 16.623	F = 5.544	P < 0.001
Hybrid: HCN-P	44, 44	MS = 5806.658	F = 164.926	P < 0.001
Season x Hybrid: GFY	44, 176	MS = 31.451	F = 3.572	P < 0.001
Season x Hybrid: DFY	44, 176	MS = 2.999	F = 3.531	P < 0.001
Season x Hybrid: HCN-P	44, 176	MS = 35.208	F = 0.605	P .974
Error: GFY	176	MS = 8.805	—	—
Error: DFY	176	MS = 0.849	—	—
Error: HCN-P	176	MS = 58.236	—	—
Panel B. Trait associations				
GFY–HCN-P	Across-season	r = -0.264	-0.518 to 0.032	P .079
GFY–HCN-P	2009-10	r = -0.272	-0.523 to 0.024	P .071
GFY–HCN-P	2010-11	r = -0.221	-0.483 to 0.077	P .144
DFY–HCN-P	Across-season	r = -0.271	-0.523 to 0.025	P .072
DFY–HCN-P	2009-10	r = -0.246	-0.503 to 0.052	P .104
DFY–HCN-P	2010-11	r = -0.256	-0.512 to 0.040	P .089
GFY–DFY	Across-season	r = 0.930	0.875 to 0.961	P < 0.001

For tested model effects, numerator and denominator degrees of freedom are shown; the error row gives its source df. Season was tested against block within season, block within season against error, hybrid against season $\times$ hybrid, and season $\times$ hybrid against error. Association intervals use Fisher's z transformation; $n = 45$ hybrid means per scope.

3.2 Descriptive quadrants

Across-season means placed 14 hybrids in the above-mean-GFY/below-mean-HCN-P quadrant, five in above/above, nine in below/below and 17 in below-GFY/above-HCN-P (Fig. 1c). The desired-quadrant count was 15 in 2009–10 and 14 in 2010–11. These counts describe the evaluated population; membership was recalculated in every exact bootstrap outcome and the full hybrid frequencies are reported in Online Resource 1.

Several high-GFY/lower-HCN-P combinations remained notable as point summaries. A3N193 $\times$ UPMC503 averaged 38.87 kg GFY, 8.88 kg DFY and 19.38 ppm HCN-P. MR750A₂ $\times$ UPMC503 averaged 34.65, 8.50 and 18.63, respectively. MR750A₂ $\times$ Pusa Chari 23 had the greatest across-season GFY (44.35 kg plot⁻¹) and DFY (10.42 kg plot⁻¹), with HCN-P of 58.32 ppm. These values do not constitute formal pairwise superiority or feeding-safety evidence.

3.3 Pareto membership depends on season and uncertainty

Six hybrids formed the nominal across-season GFY–HCN-P frontier (Table 2). Five—MR750A₂ $\times$ SSG59-3, MR750A₂ $\times$ Pusa Chari 23, A3N191 $\times$ UPMC503, A3N193 $\times$ UPMC503 and A3N213 $\times$ SSG59-3—were also nominal frontier members in both individual seasons. Their exact hierarchical frontier frequencies ranged from 53.4% to 100.0%, showing that even season-consistent point membership varied under resampling.

MR750A₂ $\times$ UPMC503 was a pooled-only frontier member: it was dominated within each individual season and its hierarchical frontier frequency was 32.7%. Its across-season non-domination is therefore an averaging-sensitive result, not evidence of stable efficiency. A3N213 $\times$ UPMC503 was season-2-only and had a 16.3% hierarchical frontier frequency (Online Resource 1). These findings replace the submitted description of six unqualified frontier hybrids.

The simultaneous-comparison rule excluded 14 hybrids and retained 31 in the conservative candidate set, including all six nominal frontier members (Online Resource 2). The wide set indicates unresolved classification rather than 31 proven optimal hybrids. Empirical 2.5th–97.5th percentile Pareto-layer intervals were 1–3 for MR750A₂ $\times$ UPMC503, 1–2 for MR750A₂ $\times$ SSG59-3 and A3N213 $\times$ SSG59-3, and 1–1 for the other three nominal members. These empirical rank intervals describe the enumerated archive only.

Table 2
Nominal across-season Pareto-front members, model intervals and empirical stability

Hybrid	GFY mean (95% CI)	DFY mean (95% CI)	HCN-P mean (95% CI)	Front 2009–10	Front 2010–11	Stability (%)
A3N191 × UPMC503	26.43 (21.82, 31.04)	6.53 (5.10, 7.95)	5.79 (-0.36, 11.94)	Yes	Yes	100.0
MR750A ₂ × SSG59-3	27.75 (23.14, 32.36)	6.06 (4.64, 7.49)	9.23 (3.09, 15.38)	Yes	Yes	76.2
A3N213 × SSG59-3	28.57 (23.95, 33.18)	6.57 (5.14, 7.99)	15.08 (8.93, 21.23)	Yes	Yes	53.4
MR750A ₂ × UPMC503	34.65 (30.04, 39.27)	8.50 (7.07, 9.92)	18.63 (12.48, 24.78)	No	No	32.7
A3N193 × UPMC503	38.87 (34.25, 43.48)	8.88 (7.46, 10.31)	19.38 (13.23, 25.53)	Yes	Yes	98.9
MR750A ₂ × Pusa Chari 23	44.35 (39.74, 48.97)	10.42 (8.99, 11.84)	58.32 (52.17, 64.47)	Yes	Yes	99.9

GFY and DFY are kg plot⁻¹; HCN-P is ppm on a dry-weight basis. Intervals are conditional hybrid-mean intervals from the combined linear model, not simultaneous pairwise intervals or predictions for new seasons. Stability is the exact weighted frequency of frontier membership over all 400 hierarchical season-and-complete-block configurations. These six nominal members are contained in a larger conservative candidate set of 31 hybrids (Online Resource 2). A negative lower linear-model bound for HCN-P is a statistical interval limit and has no physical interpretation.

3.4 HCN-constrained GFY and DFY rankings

Separating the two biomass endpoints changed the strict-screen conclusion (Table 3). At HCN-P ≤ 37.09 ppm, A3N193 × UPMC503 had the greatest point GFY and was the GFY winner in 83.1% of exact hierarchical configurations. A3N213 × Pusa Chari 23 had the greatest point DFY but a DFY-winner frequency of only 45.5%; A3N193 × UPMC503 was the leading DFY alternative (36.0%). The GFY difference between A3N193 × UPMC503 and A3N213 × UPMC503 was 2.80 kg plot⁻¹ (nominal model 95% CI – 3.73 to 9.33; simultaneous 95% CI – 12.84 to 18.44; unadjusted P = .392). The empirical bootstrap interval was – 1.50 to 8.50 kg plot⁻¹. The DFY difference between the strict-screen point leaders was only 0.038 kg plot⁻¹ (unadjusted P = .970). Thus neither close ordering establishes superiority; all pairwise contrasts and conditional rank intervals are supplied in Online Resources 1 and 2.

At the median ceiling, MR750A₂ × Pusa Chari 23 led both GFY and DFY, but its eligibility changed when the empirical HCN-P median was recalculated: eligibility and GFY-winner frequency were 74.5%, and DFY-winner frequency was 70.8%. At the third-quartile ceiling it remained the point leader for both endpoints; GFY-winner frequency was 100.0% and DFY-winner frequency 58.2%. The separate rankings therefore expose endpoint and threshold sensitivity that the submitted equal-weight index obscured.

Table 3
HCN-constrained GFY and DFY point leaders and stability frequencies

HCN-P ceiling	Cut-off ppm (n)	GFY point leader	Eligible / winner (%)	DFY point leader	Eligible / winner (%)	Season-specific GFY leaders
First quartile	≤ 37.09 (12)	A3N193 × UPMC503	100.0 / 83.1	A3N213 × Pusa Chari 23	100.0 / 45.5	2009-10: A3N193 × UPMC503; 2010-11: A3N213 × UPMC503
Median	≤ 59.38 (23)	MR750A ₂ × Pusa Chari 23	74.5 / 74.5	MR750A ₂ × Pusa Chari 23	74.5 / 70.8	2009-10: MR750A ₂ × Pusa Chari 23; 2010-11: MR750A ₂ × Pusa Chari 23
Third quartile	≤ 78.06 (34)	MR750A ₂ × Pusa Chari 23	100.0 / 100.0	MR750A ₂ × Pusa Chari 23	100.0 / 58.2	2009-10: MR750A ₂ × Pusa Chari 23; 2010-11: MR750A ₂ × Pusa Chari 23

Cut-offs are empirical distributional values and not toxicological limits. Eligibility and winner percentages are exact hierarchical stability frequencies. HCN-P ceilings were recalculated in every outcome. GFY and DFY were ranked separately; no equal-weight biomass score was used.

4 Discussion

4.1 Limited evidence for a positive biomass–HCN-P association

Across- and within-season Pearson estimates were negative, and the across-season rank correlations were also negative. These results do not support the hypothesized positive biomass–HCN-P relationship in the archived population. However, all Pearson intervals for yield–HCN-P included zero, so the data cannot establish independence, exclude modest relationships or identify a causal mechanism. The inference is further narrowed by the measurement lag between early HCN-P and later cumulative biomass.

That qualified pattern is biologically plausible. Dhurrin is responsive to genotype, tissue, stage and environment (Gruss et al., 2023; Ouma et al., 2023; Wang et al., 2024), and dhurrin suppression can occur without an obvious biomass penalty in other material (A. Kumari et al., 2025). Those external findings

support testing both traits directly; they do not convert the present nonsignificant correlations into proof that trade-offs are absent.

4.2 Pareto membership is conditional on season and sampling

The revised analysis changes the interpretation of the original pooled frontier. Five point-frontier hybrids were common to both seasons, but their hierarchical stability frequencies still differed. MR750A₂ × UPMC503 was especially informative: its pooled mean was non-dominated although it was dominated within both individual seasons. Treating it as a stable Pareto candidate would therefore be an averaging artifact. Reporting seasonal status and exact stability frequency distinguishes descriptive non-domination from repeatable field performance. The model-based candidate set retained 31 hybrids, emphasizing that a six-member nominal frontier is not a statistically exclusive group.

Significant hybrid × season interaction for both yield endpoints explains why this distinction matters. The interaction does not invalidate across-season means, but it prevents them from serving as unqualified predictions. Contemporary multi-environment data are needed before any genotype is described as broadly adapted or superior.

4.3 Separate endpoints clarify HCN-constrained priorities

GFY and DFY were strongly correlated ($r = .930$), so their equal-weight standardized average largely counted the same biomass dimension twice. Removing that score makes the decision rule easier to audit. GFY is the direct whole-plot harvest endpoint; DFY asks whether the candidate choice is robust to dry-matter production. The strict-ceiling winners differed, and DFY winner frequencies were more diffuse than GFY frequencies at every ceiling. Thus no single hybrid should be labelled the universally preferred biomass genotype.

The empirical ceilings remain sensitivity devices, not safety gates. Recalculation of each ceiling in every exact outcome also showed why a hybrid close to a cut-off, notably MR750A₂ × Pusa Chari 23 at the median, may alternate between eligibility and exclusion. Release or feeding decisions require validated HCN or dhurrin measurements at the intended harvest and use stage.

4.4 Cross specificity supports direct phenotyping

Hybrids sharing a parent varied widely in early HCN-P, so parental recurrence on a frontier cannot replace direct screening of new crosses. The presence of candidates from A₂- and A₃-associated female backgrounds may guide validation, but the design does not separate nuclear background from cytoplasm. The analytical unit remains the observed line × tester combination, not a causal cytoplasmic class (Aruna et al., 2013).

4.5 Implications for forage-sorghum evaluation

The practical implication is procedural rather than a release recommendation: measure HCN-P at developmentally relevant stages during multi-environment biomass testing, apply a biologically justified eligibility rule, and examine both green and dry yield among eligible entries. Environment-specific and uncertainty-qualified summaries should accompany any across-environment mean (Behera et al., 2024; P. Kumari et al., 2025). The historically favourable combinations in Tables 2 and 3 are priorities for renewed seed increase and validation, not established cultivars or feeding recommendations.

4.6 Limitations and data-audit boundaries

The archive represents one location and two seasons, so neither the random-season model nor the bootstrap supplies broad environmental generalization. HCN-P and biomass were measured at different developmental stages; the associations are cross-stage and cannot describe a contemporaneous physiological trade-off or HCN-P at feeding. The historical picrate assay should be cross-validated with a contemporary method.

The supplied plot archive contains total GFY, derived DFY and final HCN-P values, but not cut-specific GFY, dry-matter subsample calculations, absorbance readings or the KCN standard curve. Upstream transformations therefore could not be independently reconstructed. One quality-control flag remains: A3N191 × UPMC503 has exactly the same three DFY replicate values in both seasons. These values were retained as supplied; the original calculation or field sheets are needed to determine whether this repetition is valid or reflects a transcription error. The source-to-analysis audit verified all 810 focal-trait cells but cannot resolve that upstream question.

Finally, the field experiment was documented in a doctoral thesis and heterosis from the same crossing population was reported by Paliwal et al. (2016). The novelty claim is limited to the season-aware and uncertainty-aware secondary analysis. Model-based confidence statements rely on the stated variance assumptions and the zero-boundary treatment of the HCN-P interaction; the conservative Pareto candidate set is deliberately broader than the nominal frontier. These boundaries narrow the inference but make the contribution and evidence trail explicit.

5 Conclusions

Within these two historical Pantnagar trials, association estimates did not support a positive linear relationship between early-stage HCN-P and later cumulative biomass. Significant hybrid × season interaction for GFY and DFY, a pooled-only frontier member and endpoint-dependent strict-screen leaders show why pooled point rankings require qualification. Five hybrids were common to both seasonal nominal fronts, but their empirical stability frequencies varied. These entries should be treated as priorities for contemporary multi-location, multi-stage HCN/dhurrin and forage-quality validation rather than as definitive selections, release candidates or feeding-safety recommendations.

Declarations

Prior dissemination and related work: The experiment formed part of Paliwal's doctoral thesis (2012). Paliwal et al. (2016) reported heterosis from the same crossing population and is the authors' prior journal article from this research. The present secondary analysis addresses hybrid × season response, uncertainty in non-domination and separate HCN-constrained GFY/DFY rankings; it reports no new field experiment or repeated heterosis tables.

Competing interests:

The authors declare no financial or non-financial competing interests directly or indirectly related to this work.

Ethics approval:

This secondary analysis uses historical plant-breeding field and laboratory observations and involves no humans or animals. Animal-based observations from the wider doctoral programme are not analysed here.

Consent to Participate declaration

not applicable

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Author Contribution

A.P. conducted the field and laboratory experiments, collected and analysed the data, interpreted the results, prepared the tables and figures, and wrote the original manuscript. P.K.S. supervised the research, contributed to the conceptualization and methodology of the study, provided scientific guidance in interpretation of the results, and critically reviewed and revised the manuscript. Both authors read and approved the final manuscript.

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Data Availability

The data generated and analysed during the current study are available from the corresponding author upon reasonable request.

Code availability

Executable code, a data dictionary, version record, README and complete machine-readable outputs are supplied in Online Resource 1. The exact weighted bootstrap enumerates every outcome and therefore does not require a random seed.

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Figures

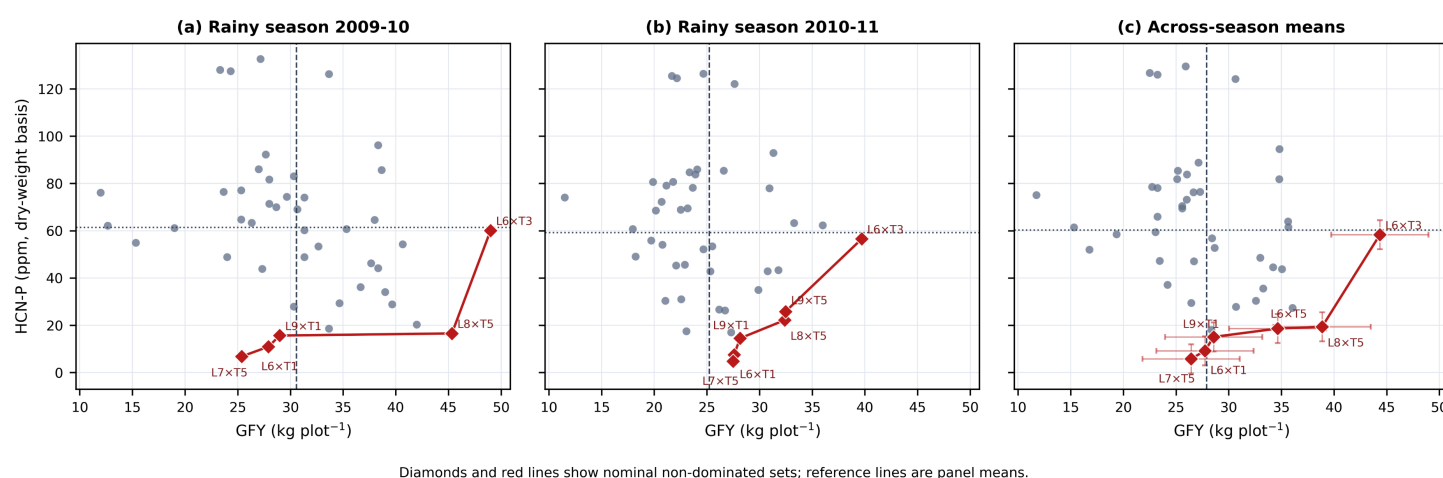


Figure 1

Green fodder yield (GFY) and early-stage HCN potential (HCN-P) for 45 forage-sorghum hybrids in (a) 2009–10, (b) 2010–11 and (c) across-season means. Diamonds and red connecting lines show the nominal GFY-maximizing/HCN-P-minimizing non-dominated set; dashed and dotted lines show within-panel means. Error bars in panel c are 95% conditional hybrid-mean intervals for nominal frontier members. These are not simultaneous pairwise intervals. Hybrid codes are defined in Online Resource 1. HCN-P values are population-comparative measurements, not feeding-safety thresholds.

Supplementary Files

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