Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Reconciling Yield, Stability and Nutritional Quality: A BLUP-Based Multi-Trait Framework for Selecting Elite Winged Bean (Psophocarpus tetragonolobus L.) Accessions

Satish Kumar Yadav

sk_yadav06@yahoo.com

ICAR - National Bureau of Plant Genetic Resources

Raghav Garg

ICAR - National Bureau of Plant Genetic Resources

Kiran Tigga

Indira Gandhi Agricultural University

Nutan Verma

Birsa Agricultural University

Shivam Shivam

ICAR - National Bureau of Plant Genetic Resources

Nishu Gupta

ICAR - National Bureau of Plant Genetic Resources

Ravi Kumar

CCS Haryana Agricultural University

Vikram Jeet Singh

Acharya Narendra Deva University of Agriculture & Technology

Vaidurya Partap Sahi

Sam Higginbottom Institute of Agriculture

MT Bhingarde

Mahatma Phule Krishi Vidyapeeth

Article

Keywords: G × E interaction, WAASB, MGIDI, Heritability, Underutilized legume

Posted Date: November 12th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10981369/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations:
No competing interests reported.

Tables 1 to 7 are available in the Supplementary Files section.

Reconciling Yield, Stability and Nutritional Quality: A BLUP-Based Multi-Trait Framework for Selecting Elite Winged Bean (*Psophocarpus tetragonolobus* L.) Accessions

Satish Kumar Yadav[1*], Raghav Garg[1], Kiran Tigga[2], Nutan Verma[3], Shivam[1], Nishu Gupta[1], Ravi Kumar[4], Vikram Jeet Singh[5*], Vaidurya Partap Sahi[6], MT Bhingarde[7]

[1]ICAR - National Bureau of Plant Genetic Resources, New Delhi, 110012
[2]Department of Genetics and Plant Breeding, RMD, College of Agriculture and Research Station, IGKV, Ambikapur, Chhattisgarh, 497001
[3]Department of Genetics and Plant Breeding, Birsa Agricultural University, Ranchi, Jharkhand, 834006
[4]Department of Biochemistry, CCS Haryana Agricultural University, Hisar, Haryana, 125004
[5]Department of Seed Science and Technology, Acharya Narendra Deva University of Agriculture & Technology, Ayodhya, 224 229, UP, India
[6]Department of Genetics and Plant Breeding, Mahatma Phule Krishi Vidyapeeth, Rahuri-413 722, Maharashtra
[7]Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, U.P, 211007

Corresponding email: sk_yadav06@yahoo.com/vikram.rice2015@gmail.com

Abstract

Winged bean (*Psophocarpus tetragonolobus* L.) is a nutritionally rich, multipurpose legume with strong potential for food and nutritional security, yet it remains underutilized and underexploited in plant breeding programmes. In this study, 31 winged bean accessions were evaluated for agro-morphological and yield-related traits across four environments. Combined analysis of variance revealed significant genotype x environment (G x E) interaction for seed yield, pod length, number of pods per plant and 100-seed weight. To identify genotypes combining high performance with stability across environments, the Weighted Average of Absolute Scores (WAASB) was used, followed by four multi-trait selection indices, namely MGIDI, MTSI, MTMPS and FAI-BLUP, to select promising accessions based on multiple traits simultaneously. A coincidence index was used to compare the selections made by the four indices, identifying Chhattisgarh Chaudhari Sem-2 and PWB 11-2 as the most consistently selected accessions. Biochemical analysis of oil, protein, antioxidant activity and methionine content revealed significant genotypic variation for nutritional quality. Comparing the nutritional profile of the selected accessions against the panel showed that superior agronomic performance did not always correspond to superior nutritional quality. Chhattisgarh Chaudhari Sem-2 ranked below the panel average on every biochemical trait, while IC026949 and EC142654 emerged as nutritionally superior, combining above-average oil content, antioxidant activity and methionine content. These findings allow genotypes with the best combined agronomic and nutritional merit to be identified for future breeding use.

Keyword: G × E interaction, WAASB, MGIDI, Heritability, Underutilized legume

1. Introduction

Global food and nutritional security increasingly depend on diversifying the crop base beyond a handful of major staples, and underutilized legumes represent a substantial, largely untapped resource for this purpose. Winged bean (Psophocarpus tetragonolobus (L.) DC.), an orphan legume of the family Fabaceae, is among the most promising of these species. Nearly every part of the plant is edible, including the pods, seeds, leaves, flowers and tubers, a versatility that has earned it the name "one species supermarket" (Lepcha et al., 2017; Bepary et al., 2023). Its seeds are particularly notable for containing protein and oil levels comparable to soybean (Choo et al., 2004; Yanagi, 1983), along with high concentrations of minerals, vitamins, dietary fibre and antioxidant compounds (Bepary et al., 2023). Winged bean also fixes atmospheric nitrogen effectively, supporting low-input, sustainable cropping systems in the humid tropical and subtropical regions where it is traditionally grown, including South and Southeast Asia, Melanesia and parts of Africa (Lepcha et al., 2017; Tanzi et al., 2019; Eagleton, 2020). Despite this potential, cultivation of winged bean remains limited even in favourable agro-climatic zones such as India, where it lags far behind major legume crops in area and investment (Bepary et al., 2023).

This gap between winged bean's potential and its actual improvement is due in part to how it has been evaluated. Existing studies have characterized its nutritional composition, genetic diversity and agronomic variation (Kantha and Erdman, 1984; Khalili et al., 2013; Adegboyega et al., 2021; Bhadmus et al., 2023; Kamal and Rajalakshmi, 2023), but genotype performance in winged bean can shift substantially across environments, and these studies have generally evaluated agronomic and nutritional traits in isolation, without a framework capable of identifying genotypes that combine strong performance, environmental stability and nutritional quality together. Classical approaches to genotype by environment interaction, such as AMMI, do not on their own produce a single, statistically defensible ranking of genotypes by combined performance and stability (Gauch, 2013). Modern mixed model approaches address this limitation directly. The Weighted Average of Absolute Scores (WAASB) and its extension WAASBY (Olivoto et al., 2019a) use BLUP based estimates to rank genotypes on mean performance and stability together, while multi-trait selection indices, including the Multi-Trait Genotype-Ideotype Distance Index (MGIDI) (Olivoto et al., 2019b), the Multi-Trait Stability Index (MTSI), the Multi-Trait Mean Performance and Stability Index (MTMPS) and the Factor

Analysis and Ideotype-Design Index (FAI-BLUP), allow simultaneous selection across several traits. None of these tools has previously been applied to winged bean.

The present study was undertaken to provide a comprehensive agronomic and nutritional characterization of winged bean germplasm under Indian agro-climatic conditions using this BLUP-based multi-trait, multi-environment framework. The objectives were to (i) evaluate the agro-morphological performance of winged bean germplasm across diverse Indian environments, (ii) quantify genotype, environment and genotype x environment effects and estimate broad-sense heritability and related genetic parameters using a mixed-model approach, (iii) assess genotype stability and adaptability using WAASB and WAASBY, (iv) compare four complementary multi-trait selection indices, namely MGIDI, MTSI, MTMPS and FAI-BLUP, and quantify their coincidence in genotype selection, (v) characterize nutritional and biochemical variation for oil, protein, antioxidant activity and methionine, and (vi) evaluate whether the accessions selected for superior agronomic performance also show favourable nutritional profiles. The results are intended to support the identification of parents for breeding, accessions for further adaptive testing, and candidates for future varietal development, contributing to the scientific basis for bringing this underutilized, protein-rich, multipurpose legume into diversified and climate-resilient agricultural systems.

2. Materials and Methods

2.1 Genetic material, Experimental Locations and Experimental Design

The genetic material (seeds) used in this study comprised thirty-one accessions, including four checks (AKWB-1, IWB-1, PWB 11-2 and RMDWB-1) and three varieties (Chhattisgarh Chaudhari Sem-2, Indira Winged Bean-2 and Phule Shrawani) of winged bean, sourced from the National Gene Bank of the National Bureau of Plant Genetic Resources, New Delhi (Table S1). The study was conducted at four locations in India: Raj Mohini Devi College of Agriculture and Research Station (RMDCARS), Ambikapur, Chhattisgarh (23.12° N, 83.20° E; 611 m above mean sea level) in July 2025; Acharya Narendra Deva University of Agriculture & Technology (ANDUAT), Ayodhya, Uttar Pradesh (26.80° N, 82.20° E; 93 m above mean sea level) in June 2025; Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, Maharashtra (19.39° N, 74.65° E; 495 m above mean sea level) in June 2025; and Birsa Agricultural University, Ranchi, Jharkhand (23.44° N, 85.31° E; 651 m above mean sea level) in August 2025. The experiment was laid out in a randomized complete block design (RCBD) with three replications at each location. Each experimental plot measured 3 m × 6 m. Standard crop management

practices, including regular weeding, provision of twining support, and insect-pest management, were followed throughout the experiment.

2.2 Observation recorded

Data were collected on 5 random plants per plot using the descriptor of winged bean (PPV&FRA, 2025). Days to 50% flowering (D50F) was recorded as the number of days from sowing until 50% of plants in a plot reached flowering. Days to 80% maturity (D80M) was recorded when 80% of plants reached physiological maturity. Pod length (PL) was measured in centimeters, number of pods per plant (NPP) by counting pods produced by sampled plants, seed yield per plant (SYD) in grams after harvesting sampled plants, and 100-seed weight (HSW) by weighing 100 representative seeds.

Biochemical analyses were conducted at the Phytochemistry Laboratory-1, Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, Haryana, India, using seed material from the Ambikapur environment only. Field-harvested seeds were cleaned and oven-dried for 48 h at 45 degrees C, ground and milled into a fine powder using a FOSS CT 293 Cyclotec Grinding Mill, and stored in airtight containers at 4-6 degrees C until analysis. Protein content was determined by the Kjeldahl method using a VELP Automated Kjeldahl Apparatus (UDK-149) coupled with a Metrohm Eco Titrator, with a nitrogen-to-protein conversion factor of 6.25 (AOAC, 1984). Oil content was estimated using a Buchi Fat Extractor E-500 with petroleum ether as solvent following the Randall hot extraction method (AOAC International, 2019). Methionine content was estimated following the method of Horn et al. (1946). Antioxidant activity was determined using the DPPH radical-scavenging assay, measuring the inhibition of DPPH solution by a 1 mg/mL methanolic extract of the sample (Xiao et al., 2020).

2.3 Data Analyses

All statistical analyses were performed using R software (version 4.5.5). The core multi-environment trial analyses, joint analysis of variance, the BLUP/REML mixed model, WAASB and WAASBY, and the four multi-trait selection indices (MGIDI, MTSI, MTMPS, FAI-BLUP), were carried out primarily with the metan package (Olivoto and Lucio, 2020), which implements the models described in Sections 2.3.1 to 2.3.7. Principal component analysis and hierarchical cluster analysis were performed using the FactoMineR package (Le, Josse, and Husson, 2008) and the factoextra package (Kassambara and Mundt, 2020), as described in Section 2.3.9. Post-hoc comparison of environmental means for each trait was carried out using

Tukey's honestly significant difference (HSD) test, implemented with the base R stats package. Descriptive statistics and Pearson correlation analysis were computed using base R functions, and the biochemical analysis of variance was fitted using the aov function in base R. All graphics were prepared using metan's native ggplot2-based plotting functions. Statistical significance was considered at $P < 0.05$ throughout**.**

2.3.1 Joint analysis of variance

A joint (combined) analysis of variance across the four environments was fitted for each trait according to the linear model:

$$Y_{ijk} = mu + G_i + E_j + GE_{ij} + R_k(E_j) + eps_{ijk}$$

where Y_{ijk} is the observed value of the ith genotype in the jth environment and kth replication, mu is the grand mean, G_i is the effect of the ith genotype, E_j is the effect of the jth environment, GE_{ij} is the genotype x environment interaction effect, $R_k(E_j)$ is the effect of the kth replication nested within the jth environment, and eps_{ijk} is the random error. Genotype, environment, genotype x environment interaction and replication-within-environment were all treated as fixed effects for the purpose of computing F-tests and significance (Table 2), using the anova_joint() function.

2.3.2 Mixed-model (BLUP/REML) analysis and genetic parameters

To obtain best linear unbiased predictors (BLUPs) and partition genotypic and genotype x environment variance components, a linear mixed model was subsequently fitted with environment and replication-within-environment as fixed effects and genotype and genotype x environment interaction as random effects, using restricted maximum likelihood (REML) via the gamem_met() function:

$$\mathrm{Y} = \mathrm{X}\beta + \mathrm{Zu} + \varepsilon$$

where y is the vector of observed phenotypic values, b is the vector of fixed effects (environment and replication-within-environment), u is the vector of random effects (genotype and genotype x environment interaction, $u \sim N(0, G)$), e is the vector of random errors ($e \sim N(0, R)$), and X and Z are the corresponding incidence matrices. Variance components ($\boldsymbol{\sigma}^2_g$ for genotype, $\boldsymbol{\sigma}^2_{ge}$ for genotype x environment interaction, $\boldsymbol{\sigma}^2_e$ for residual error) were estimated by REML, and broad-sense heritability on a genotype-mean basis was calculated as:

$$h2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_g^2}{e} + \frac{\sigma_e^2}{re}}$$

where e is the number of environments and r is the number of replications. Selective accuracy was calculated as the square root of the genotype-mean heritability, and the genotypic correlation across environments. The significance of the genotype and genotype x environment random effects was tested using a likelihood ratio test (LRT) comparing the full model against a model with the corresponding random effect removed, with the test statistic distributed as chi-square with one degree of freedom.

2.3.3 WAASB and WAASBY stability analysis

Genotype stability was quantified using the Weighted Average of Absolute Scores from the BLUP-based interaction effects matrix (WAASB; Olivoto et al., 2019a). The matrix of genotype x environment interaction BLUPs was subjected to singular value decomposition to obtain interaction principal component axis (IPCA) scores, and WAASB for the ith genotype was calculated as:

$$WAASB_i = \frac{\sum_{k=1}^{P} |IPCA_{ik} - EP_k|}{\sum_{k=1}^{P} EP_k}$$

Where, WAASBi is the weighted average of absolute scores of the i^{th} genotype; $IPCA_{ik}$ is the score of the i^{th} genotype in the k^{th} interaction principal component axis (IPCA); and EP_k is the amount of the variance explained by the kth IPCA. The WAASB index summarizes the contribution of each genotype ton GEI across all interaction component axes (IPCAs), with lower WAASB values indicating greater stability. To jointly consider mean performance and stability, the WAASBY index was computed by integrating rescaled mean performance and WAASB values, following Olivoto et al. (2019a).

where IPCA_ik is the score of the ith genotype in the kth interaction principal component axis and EP_k is the proportion of the genotype x environment interaction variance explained by the kth axis. Lower WAASB indicates greater stability. To enable simultaneous selection on mean performance and stability, mean performance (Y) and WAASB were each rescaled to a common 0-100 scale, and the simultaneous selection index WAASBY was computed as:

$$WAASBY_i = \frac{rY_i \times \theta_Y + rW_i \times \theta_S}{\theta_Y + \theta_S}$$

where rY_i and rW_i are the rescaled values of mean performance and WAASB (with the WAASB scale inverted so that higher rW_i indicates greater stability) for the ith genotype, and θ_Y *and* θ_S are user-defined weights for mean performance and stability, respectively (both set to 50, i.e. equal weighting, in this study).

2.3.4 Multi-trait genotype-ideotype distance index (MGIDI)

The MGIDI (Olivoto et al., 2019b) was computed on standardized BLUP genotypic means using exploratory factor analysis. The factor-analytic model is:

$$x_{ij} = a_{i1}F_{1j} + a_{i2}F_{2j} + \cdots + a_{ip}F_{pj} + \epsilon_{ij}$$

where x_{ij} is the standardized value of the i^{th} trait in the j^{th} genotype, a_{ip} is the factor loading of the i^{th} trait on the p^{th} common factor, F_{pj} is the score of the p^{th} factor for the j^{th} genotype, and ϵ_{ij} is the residual (unique) variance not explained by the retained factors. Factors were extracted by principal axis factoring with varimax rotation, retaining factors with eigenvalue greater than 1 (Kaiser criterion). The MGIDI for the jth genotype was then calculated as the Euclidean distance, in the rotated factor space, between the genotype and an ideotype defined by the most desirable score on every factor:

$$MGIDI_j = [\sum_{p=1}^{P}[(F_{pj} - F_{p,ideotype})^2]^{1/2}$$

whereF_{pj} is the score of the p^{th} factor for the j^{th} genotype, is the score of the p^{th} factor corresponding to the ideotype, and P is the total number of retained factors. Lower MGIDI values indicate greater proximity of a genotype to the specified ideotype.

Genotypes with the smallest MGIDI are those closest to the defined ideotype across all traits simultaneously. The relative contribution of each factor p to the MGIDI of a given genotype, used to construct the strengths-and-weaknesses view (Figures 3B, 4B, 4D, 4F), was calculated as the squared factor-distance component expressed as a percentage of the squared total distance. Selection differentials for each trait were calculated as

$$SD = X_s - X_o,$$

where Xo is the mean of the original population and Xs is the mean of the selected genotypes, with SD expressed additionally as a percentage of Xo. Because seed yield is the primary trait of applied interest but showed zero estimated BLUP genotypic variance (Section 3.2), MGIDI was computed on a mixed data matrix rather than on BLUPs alone: BLUP genotypic means

were retained for the four traits with confirmed genetic variance (days to 50% flowering, pod length, number of pods per plant, 100-seed weight), while simple phenotypic (across-environment) genotype means were substituted for seed yield and days to 80% maturity, the two traits with degenerate BLUPs. This was implemented by supplying the mixed matrix directly as a data.frame to the mgidi() function, which accepts either a fitted BLUP model or a plain data.frame of genotype means.

2.3.5 Multi-trait stability index (MTSI)

The MTSI (Olivoto et al., 2019b) follows the same factor-analytic and Euclidean-distance procedure as MGIDI (Section 2.3.4), but is computed on the WAASBY-rescaled values (Section 2.3.3) for all six traits, including seed yield, rather than on raw BLUP means; because WAASBY draws on genotype x environment interaction effects (which were significant for seed yield) rather than the genotype main effect, this index does not encounter the zero-variance issue that affects BLUP-based methods for this trait, and required no adjustment.

2.3.6 Multi-trait mean performance and stability index (MTMPS)

The MTMPS follows the same underlying factor-analytic framework as MGIDI and MTSI, but combines a mean-performance estimate with Wricke's (1965) ecovalence as the stability criterion. Ecovalence for the ith genotype was calculated as:

$$Wi = \sum_{j=1}^{E} (GE_{ij})^2$$

where GE_{ij} is the genotype x environment interaction effect of the i^{th} genotype in the j^{th} environment; genotypes with smaller ecovalence contribute less to the total genotype x environment interaction sum of squares and are considered more stable. For mean performance, the Best Linear Unbiased Estimate (BLUE, a fixed-effect genotype mean that does not shrink toward the population mean the way BLUP does) was used in place of BLUP for all six traits, computed via the mps() function with performance = "blueg". Because BLUE values retain the full observed genotype-to-genotype variation rather than shrinking it based on estimated genetic variance, this avoided the zero-variance/degenerate-BLUP issue for seed yield and days to 80% maturity without requiring a manually constructed mixed matrix, allowing all six traits including seed yield to be retained. Mean performance and ecovalence were combined and the resulting index-genotype matrix subjected to the same factor analysis and Euclidean-distance procedure described in Section 2.3.4.

2.3.7 Factor analysis and ideotype-design index (FAI-BLUP)

The FAI-BLUP index (Rocha et al., 2018, as implemented in metan) uses the same factor-analytic decomposition as MGIDI, but replaces the Euclidean genotype-ideotype distance with a spatial probability/desirability-based index computed from the estimated factor scores relative to a desired (upper or lower, according to the breeding goal for each trait) ideotype boundary in factor space, without requiring an explicit single ideotype point. Unlike mgidi(), the fai_blup() implementation in metan (v1.18.0) does not correctly support a custom mixed-data matrix supplied as a plain data.frame in combination with character-string ideotype specifications: passing a mixed BLUP/phenotypic matrix produced a degenerate result in which every genotype received an identical index value, traced to the function's internal ideotype-parsing logic, which is designed for numeric rather than "max"/"min" ideotype codes when operating outside its standard BLUP-model workflow. Rather than report this degenerate output, FAI-BLUP was retained in its original form, computed on BLUP genotypic means for the four traits with confirmed genetic variance only (days to 50% flowering, pod length, number of pods per plant, 100-seed weight); it therefore does not include seed yield, unlike MGIDI, MTSI and MTMPS in the present analysis.

2.3.8 Coincidence of selection among indices

At 15% selection intensity, five of the 31 accessions were selected by each of the four indices described above. The pairwise coincidence index between any two indices A and B was calculated as the percentage of genotypes selected by A that were also selected by B:

$$CI(A,B) = \frac{|SelectedA| \cap |Selected\ B|}{|Selected\ A|} \times 100$$

The full pattern of overlap among all four indices was visualized using a circos (chord) diagram, in which each index occupies one sector of the circle and the width of each connecting chord is proportional to the number of accessions shared between the two linked indices.

3. Results

3.1 Mean Performance and Analysis of Variance

The combined analysis of variance is presented in Table 1. Significant environmental effects were observed for SYD, D50F, D80M, NPP and HSW, whereas the environmental effect was non-significant for PL. Genotypic differences were significant only for PL, while genotype by environment interaction was significant for SYD, PL, NPP and HSW.

Average performance among accessions is presented in Table S2. Mean SYD was 53.19 g, with EC178311 recording the highest value (60.70 g per plant), followed by PWB 11-2 (59.21 g) and RMDWB-23-1 (59.18 g). The lowest values were recorded for Phule Shrawani (44.15 g) and IC095240 (44.64 g). Mean D50F was 82.73 days, with Phule Shrawani earliest (78.40 days) and EC178309 latest (87.87 days). Mean D80M was 171.33 days, with Chhattisgarh Chaudhari Sem-2 earliest (166.34 days) and EC178309 latest (176.95 days). Mean PL was 14.29 cm, with Phule Shrawani greatest (15.95 cm) and IC095239 shortest (12.18 cm). Mean NPP was 50.62, with RMDWB-23-1 greatest (62.85) and EC178304 lowest (37.17). Mean HSW was 27.20 g, with EC178275 highest (30.52 g) and EC178308 lowest (22.14 g).3.2

3.2 Variance Components and Likelihood Ratio Test

3.2 Variance Components and Likelihood Ratio Test

Likelihood ratio tests revealed a significant genotypic effect only for PL (LRT = 7.32, P = 0.007), while the genotypic effect was non-significant for SYD, D50F, D80M, NPP and HSW (P > 0.34 in all cases). Genotype by environment interaction was highly significant for SYD, D50F, PL, NPP and HSW (P < 0.001 in all cases), while it was non-significant for D80M (LRT = 0.00, P = 1.000), indicating that D80M carried essentially no detectable genotypic or genotype by environment signal in this panel, with its phenotypic variance attributable almost entirely to residual error.

Phenotypic variance (σ^2p) was highest for NPP (194.578), followed by SYD (124.113), D80M (92.457), D50F (54.144) and HSW (14.661), and lowest for PL (3.554) (Table 2). Genotypic variance (σ^2g) was highest for NPP (10.18), followed by HSW (0.831), PL (0.539) and D50F (0.407), and was zero for SYD and D80M.

Broad-sense heritability (h^2) was highest for PL (15.2%), followed by NPP (5.22%), HSW (5.65%), D50F (0.75%), and zero for SYD and D80M. Heritability on the entry-mean basis (h^2mg) followed the same ranking, highest for PL (53.4%), followed by NPP (21.8%), HSW (23.9%), D50F (5.61%), and zero for SYD and D80M. The genotype by environment interaction coefficient of determination ($GEIr^2$) was highest for SYD (0.691), followed by NPP (0.648), HSW (0.608), PL (0.371) and D50F (0.264), and zero for D80M. Accuracy (AS) ranged from 0.731 for PL to 0.000 for SYD and D80M, with intermediate values for NPP (0.467), HSW (0.489) and D50F (0.237). The genotypic correlation across environments (rge) was highest for SYD (0.691), followed by NPP (0.684), HSW (0.645), PL (0.438) and D50F (0.266), and zero for D80M. The genotypic coefficient of variation (CVg) was highest for NPP

(6.29%), followed by HSW (3.35%) and PL (5.14%), with D50F at 0.772%, and zero for SYD and D80M. The residual coefficient of variation (CVr) ranged from 5.61% for D80M to 15.1% for NPP.

3.3 Trait Associations and Distribution Patterns across Environments

The distribution of individual-plant observations for each trait varied considerably among the four environments (Figure 1A). Tukey's HSD test showed that all four environments differed significantly from one another for SYD, D50F and NPP ($P < 0.05$ for every pairwise comparison), with SYD highest in Ayodhya (mean 72.29 g) and lowest in Ranchi (mean 32.65 g), and NPP showing the largest relative environmental range among the six traits, from a mean of 89.21 in Ambikapur to only 15.00 in Ranchi. For D80M, Ambikapur and Rahuri did not differ significantly from each other ($P = 0.952$) but both differed significantly from Ayodhya and Ranchi. For HSW, Ayodhya and Rahuri did not differ significantly from each other ($P = 0.194$), while Ambikapur recorded the highest mean (32.35 g) and Ranchi the lowest (23.84 g), each differing significantly from all other environments. PL showed the least environmental differentiation among the six traits, with only the Ayodhya-Rahuri and Rahuri-Ranchi comparisons reaching significance, consistent with the non-significant environmental main effect for this trait already noted in Table 2.

The correlation matrix among genotype means is presented in figure 1B. SYD showed no significant correlation with any of the other five traits, with correlation coefficients of 0.016 with D50F, 0.070 with D80M, -0.004 with PL, 0.227 with NPP and 0.101 with HSW. D50F showed a significant positive correlation with D80M ($r = 0.928$, $P < 0.001$) and a significant negative correlation with HSW ($r = -0.462$, $P = 0.009$), while its associations with PL ($r = -0.340$, $P = 0.061$) and NPP ($r = 0.103$, $P = 0.582$) were not significant. D80M showed a significant negative correlation with HSW ($r = -0.471$, $P = 0.008$), in addition to its correlation with D50F already noted, while its associations with PL ($r = -0.304$, $P = 0.097$) and NPP ($r = -0.011$, $P = 0.954$) were not significant. PL showed no significant correlation with NPP ($r = 0.181$, $P = 0.330$) or HSW ($r = 0.039$, $P = 0.837$), beyond the associations with D50F and D80M already noted. NPP showed no significant correlation with HSW ($r = 0.047$, $P = 0.802$).

3.4 Multivariate Structure of the Germplasm Panel

Principal component analysis was performed on standardized genotype means. The first three principal components (PC1, PC2, PC3) explained 78.38 per cent of the total standardized variation (Table 3, Figure 2A). PC1 accounted for 39.99 per cent of the variation and was

characterized primarily by positive contributions from D50F and D80M, with negative contributions from HSW and PL. PC2 explained a further 21.36 per cent of the variation and was dominated by NPP and SYD. PC3 accounted for 17.03 per cent of the variation and was most strongly associated with PL, with HSW also contributing negatively.

Ward hierarchical clustering of the 31 accessions based on standardized agro-morphological trait means formed four principal clusters, with cluster sizes of 13, 9, 6 and 3 accessions (Figure 2B). Accessions sharing similar combinations of phenology and yield components joined at comparatively lower Ward distances, while more divergent accessions joined only at higher distances.

The genotype by trait heatmap, ordered by cluster membership, is presented in Figure 2C. EC178311, EC178288, PWB 11-2 and RMDWB-23-1 showed positive standardized SYD profiles, with RMDWB-23-1 additionally showing a positive profile for PL and NPP. Chhattisgarh Chaudhari Sem-2 showed positive NPP and HSW profiles with negative D50F and D80M profiles. EC178275 showed an early D50F and D80M profile with a high HSW profile but a negative SYD profile. Phule Shrawani showed an early D50F and D80M profile with a high PL profile but a negative SYD profile.

3.5 BLUP-Based Stability Analysis Using WAASB and WAASBY

Q-Q plots of standardized residuals from the mixed model showed reasonably close adherence to the theoretical normal line for all six traits, with only minor deviation in the extreme tails for SYD and NPP, supporting the appropriateness of the REML-based mixed model framework used throughout this study (Figure S1).

SYD showed significant genotype by environment interaction despite a non-significant genotype main effect, and zero estimated BLUP genotypic variance. WAASB and WAASBY were applied to characterize the pattern of this genotype by environment interaction (Table S3, Figure 3A), not as a criterion for selecting or recommending accessions, since there is no underlying genetic yield difference for a stability index to meaningfully rank.

The Y by WAASB biplot classified accessions into four quadrants. Eight accessions fell into the high yield, high stability quadrant, including PWB 11-2, EC178307, EC121918, EC178304 and IWB-1. Ten accessions fell into the low yield, high stability quadrant. Eight accessions fell into the high yield, low stability quadrant, including the highest raw yielding accession, EC178311. Five accessions fell into the low yield, low stability quadrant.

Ranking accessions by WAASBY placed PWB 11-2, EC178307 and EC121918 at the top (Figure 3B). The accession with the single highest raw mean SYD, EC178311, was not among the top three ranked by WAASBY. Final accession recommendations, described in Section 3.6, are based on the four multi-trait indices, which target D50F, PL, NPP and HSW, rather than on SYD.

3.6 Genotype Selection Using Multi-Trait Indices

3.6.1 Multi-Trait Genotype-Ideotype Distance Index

Factor analysis on the six-trait mixed matrix retained three factors, jointly explaining 78.4 per cent of total variance (Table 4). FA1 grouped D50F, D80M and HSW. FA2 grouped SYD and NPP. FA3 was dominated by PL. At 15 per cent selection intensity, five accessions were selected (Table S4, Figure 4A). Chhattisgarh Chaudhari Sem-2 ranked first, followed by RMDWB-23-1, IC017009, PWB 11-2 and Indira Winged bean-2.

The strengths and weaknesses view showed that Chhattisgarh Chaudhari Sem-2 and RMDWB-23-1 derived comparatively more of their advantage from FA1 and FA3 respectively, while PWB 11-2 was distinguished specifically by its favourable FA2 contribution (Figure 4B). Predicted selection differentials indicated small decreases in D50F and D80M, alongside increases in HSW, SYD, NPP and PL, with the largest gains for SYD (5.26 per cent) and PL (3.47 per cent) (Table 5).

3.6.2 Multi-Trait Stability Index

Factor analysis on the WAASBY rescaled six-trait matrix retained three factors, explaining 71.5 per cent of total variance. FA1 grouped D50F and D80M. FA2 grouped SYD and NPP. FA3 grouped PL and HSW. At 15 per cent selection intensity, the selected accessions were PWB 11-2, Chhattisgarh Chaudhari Sem-2, IWB-1, EC142654 and IC026949 (Figure 4C), sharing both Chhattisgarh Chaudhari Sem-2 and PWB 11-2 with the MGIDI selection described in Section 3.6.1. The strengths and weaknesses view showed that PWB 11-2 and IWB-1 derived comparatively more of their advantage from FA2, while Chhattisgarh Chaudhari Sem-2 derived more of its advantage from FA1 (Figure 4D).

3.6.3 Multi-Trait Mean Performance and Stability Index

Factor analysis retained three factors, jointly explaining 73.9 per cent of total variance. FA1 grouped D50F and D80M. FA2 was dominated by HSW, with a moderate contribution from

NPP. FA3 grouped SYD and PL, a different pairing from the SYD and NPP grouping observed under MGIDI and MTSI. At 15 per cent selection intensity, the selected accessions were PWB 11-2, IWB-1, Chhattisgarh Chaudhari Sem-2, EC142654 and IC026949 (Figure 4E), an identical set to the MTSI selection described in Section 3.6.2. The strengths and weaknesses view is presented in Figure 4F. This convergence between MTSI and MTMPS indicates that the two stability criteria, WAASBY-based interaction scores for MTSI and BLUE-based performance combined with Wricke's ecovalence for MTMPS, are capturing the same underlying pattern of accession performance in this dataset, despite differing in their specific mathematical formulation and despite grouping SYD with a different partner trait in their respective factor structures.

3.6.4 Factor Analysis and Ideotype Design Index

FAI-BLUP was retained on its original four-trait basis, namely D50F, PL, NPP and HSW, since it could not be extended to the six-trait mixed matrix used for MGIDI, MTSI and MTMPS. At 15 per cent selection intensity, FAI-BLUP selected Chhattisgarh Chaudhari Sem-2, Phule Shrawani, IC017009, Indira Winged bean-2 and RMDWB-23-1 (Figure 4G). Four of these five accessions, all but Phule Shrawani, are also present in the MGIDI selection described in Section 3.6.1.

3.7 Coincidence Index and Common Genotypes

The pairwise coincidence structure showed a coherent pattern once SYD was incorporated into MGIDI and MTMPS on a basis comparable to MTSI (Figure 5B). MTSI and MTMPS were in complete agreement. MGIDI and FAI-BLUP shared four of five accessions. MGIDI shared two accessions each with MTSI and with MTMPS. FAI-BLUP shared one accession each with MTSI and with MTMPS. Across all four indices, nine unique accessions were selected in total. Chhattisgarh Chaudhari Sem-2 was selected by all four indices. PWB 11-2 was selected by three of the four, namely MGIDI, MTSI and MTMPS. Predicted selection gain from the MGIDI-selected group showed the largest proportional gains for SYD and PL (Figure 5A).

3.8 Comparative Multi-Trait Profiling of Consensus Accessions

Standardized performance profiling was carried out for the eight consensus accessions selected by two or more of the four indices (Figure 5C). Chhattisgarh Chaudhari Sem-2 and PWB 11-2 combined favourable standardized scores, including for SYD, across the greatest number of traits among the consensus group.

3.9 Mean performance and descriptive statistics for biochemical traits

Descriptive statistics for the four biochemical traits are presented in Table 7. Antioxidant activity showed the highest coefficient of variation among the panel, at 23.04 per cent, more than double that of any other biochemical trait. Protein content showed the lowest coefficient of variation, at 4.18 per cent. Oil content ranged from 13.40 to 20.10 per cent, with a mean of 17.22 per cent. Protein content ranged from 23.87 to 28.73 per cent, with a mean of 25.79 per cent. Antioxidant activity ranged from 23.66 to 71.47 per cent inhibition at 1 mg per mL, with a mean of 50.59 per cent. Methionine ranged from 0.805 to 1.294 g per 16 g N, with a mean of 1.028 g per 16 g N. At the individual accession level, EC178307 recorded the lowest oil content and EC251035 the highest. IC095230 recorded the lowest protein content and EC178311 the highest. EC121918 recorded the lowest antioxidant activity and IC095230 the highest. EC178271 recorded the lowest methionine content and IC026949 the highest (Table S5, Figure 6).

3.10 Correlation between Nutritional and Agro-Morphological Traits

The correlation matrix between agro-morphological and biochemical traits is presented in and Figure 8A. SYD showed no significant correlation with oil content, protein content, antioxidant activity or methionine. PL showed a significant negative correlation with antioxidant activity ($r = -0.471$, $P = 0.007$) and a significant positive correlation with methionine ($r = 0.460$, $P = 0.009$). NPP showed a significant negative correlation with antioxidant activity ($r = -0.402$, $P = 0.025$). D50F showed a significant negative correlation with methionine ($r = -0.441$, $P = 0.013$). D80M showed a significant negative correlation with methionine ($r = -0.441$, $P = 0.013$). No significant correlation was found between any agro-morphological trait and oil content or protein content.

3.11 Nutritional Profile of Consensus Agronomically-Selected Accessions

The nutritional profile of the eight consensus accessions selected by two or more of the four multi-trait indices is presented in Figure 8B. Chhattisgarh Chaudhari Sem-2, the accession selected by all four agronomic indices, recorded below-average values for oil content, protein content, antioxidant activity and methionine relative to the 31-accession panel. IC026949 and EC142654, each selected by two of the four agronomic indices, recorded above-average values across all four biochemical traits. IC026949 recorded the highest oil content, antioxidant activity and methionine among the eight consensus accessions. PWB 11-2 recorded above-average antioxidant activity and methionine, and below-average protein content.

4. Discussion

The present study aimed at the agro-morphological and nutritional characterization of 31 winged bean accessions evaluated across four Indian environments, using a BLUP based multi-trait, multi-environment framework. Winged bean performance across environments must be understood in the context of genotype by environment interaction, a phenomenon long recognized as a central constraint on selection efficiency in crop breeding (Comstock and Moll, 1963). In light of this, the present study employed a mixed model framework combined with multivariate selection techniques, namely WAASB, WAASBY, MGIDI, MTSI, MTMPS and FAI-BLUP, to identify accessions combining favourable trait profiles with cross-environment consistency.

The combined analysis of variance revealed a significant environmental effect for SYD, D50F, D80M, NPP and HSW, and significant genotype by environment interaction for SYD, PL, NPP and HSW. The genotype effect itself was significant only for PL. This pattern indicates that, for five of the six traits studied, genotype rankings were not stable across the four environments, a common feature of multi-location legume trials and one that reflects environmental modulation of trait expression (Yan and Kang, 2003). Such interaction confounds direct phenotypic selection based on pooled means, since a genotype's apparent superiority may reflect a favourable subset of test environments rather than a stable underlying advantage. The likelihood ratio test results reinforced this pattern at the level of variance components. The genotype random effect was significant only for PL, while genotype by environment interaction was significant for SYD, D50F, PL, NPP and HSW, and non-significant for D80M, indicating that D80M carried essentially no detectable genotypic or genotype by environment signal in this panel, with its variation attributable almost entirely to residual error. PL, the only trait combining a significant genotype effect with the highest heritability values obtained, both h^2 and h^2mg, and the highest genotypic coefficient of variation, represents the trait in this panel most amenable to direct phenotypic selection, whereas SYD, despite being the primary trait of applied interest, showed zero heritability and a correspondingly high $GEIr^2$, indicating that genetic gain for yield in this germplasm will depend on exploiting genotype by environment interaction rather than on selecting for a stable genetic main effect.

The correlation analysis revealed a strong positive association between D50F and D80M, and significant negative associations of both phenology traits with HSW, while SYD showed no

significant association with any other agro-morphological trait. The tight coupling between flowering and maturity duration is expected biologically, since later flowering mechanically compresses or delays the grain filling period, and is consistent with similar phenology correlations reported in other legume multi-environment trials (Yoshida, 1981; Peng et al., 2008). The absence of a significant SYD correlation with any other trait in this panel, combined with its zero heritability, indicates that yield in this germplasm behaves largely independently of the other agro-morphological traits measured, a pattern that has direct consequences for index-based selection, since an index built purely on the correlated trait structure among D50F, D80M, PL, NPP and HSW would provide no information about SYD unless SYD is explicitly and separately incorporated, as was done here through the mixed BLUP and phenotypic matrix used for MGIDI and the BLUE based performance matrix used for MTMPS.

Multi-trait multi-environment selection indices address the challenge posed by this mixture of correlated and uncorrelated traits by incorporating trait covariance structure directly into genotype ranking, rather than relying on a single trait or a simple additive index. MGIDI, computed here on a mixed BLUP and phenotypic matrix to allow SYD to contribute despite its zero BLUP variance, grouped the six traits into three factors, D50F, D80M and HSW on FA1, SYD and NPP on FA2, and PL on FA3, jointly explaining 78.4 per cent of total variance. MTSI, built on WAASBY rescaled values, produced a similar three factor structure but grouped PL with HSW rather than with SYD. MTMPS, built on BLUE based performance combined with Wricke's ecovalence, grouped SYD with PL rather than with NPP. This shifting pattern of trait grouping across the three indices, despite all three targeting the same six traits, illustrates that the choice of underlying performance metric, raw BLUP and phenotypic values for MGIDI, WAASBY integrated values for MTSI, BLUE and ecovalence for MTMPS, meaningfully changes which traits are treated as covarying, and consequently which genotypes are identified as closest to the defined ideotype.

Despite these differences in factor structure, MTSI and MTMPS converged on an identical five-accession selection once both were placed on a comparable six-trait, yield-inclusive basis, PWB 11-2, Chhattisgarh Chaudhari Sem-2, IWB-1, EC142654 and IC026949. This convergence indicates that WAASBY based interaction scores and BLUE based ecovalence, despite their different mathematical formulations, are capturing essentially the same underlying pattern of stability and performance in this dataset. MGIDI shared four of its five selected accessions with FAI-BLUP, which remained on its original four-trait, yield-excluded basis owing to a software limitation encountered when attempting to extend FAI-BLUP to the mixed

six-trait matrix. Chhattisgarh Chaudhari Sem-2 was the only accession selected by all four indices, and PWB 11-2 was selected by three of the four, MGIDI, MTSI and MTMPS. This level of agreement, achieved only after seed yield was consistently incorporated across the BLUP-based indices, is considerably stronger than an earlier comparison in which yield was excluded from three of the four indices and no accession achieved unanimous selection, and it demonstrates that apparent disagreement among multi-trait indices can partly reflect inconsistent treatment of a trait with weak genotypic signal rather than genuine disagreement about which accessions perform well.

The WAASB and WAASBY analysis for SYD was conducted as an exploratory characterization of genotype by environment interaction rather than as a selection criterion, since SYD carried zero BLUP genotypic variance in this panel. The Y by WAASB biplot separated accessions into four quadrants, and ranking by WAASBY placed PWB 11-2, EC178307 and EC121918 at the top, distinct from EC178311, the accession with the single highest raw mean SYD. This divergence between the highest raw mean and the most WAASBY-consistent accession illustrates a broader point relevant to underutilized legume breeding programmes such as this one, where multi-environment testing infrastructure may be limited, that selecting parental material on the basis of a single season's mean yield alone carries a real risk of advancing a genotype whose apparent superiority is an artefact of favourable interaction with one or two environments rather than a stable, exploitable trait.

Biochemical evaluation revealed significant genotypic variation for oil content, protein content, antioxidant activity and methionine, consistent with the substantial nutritional variability reported for winged bean germplasm elsewhere (Lepcha et al., 2017; Bepary et al., 2023; Adegboyega et al., 2019; Bhadmus et al., 2023). Antioxidant activity showed by far the highest coefficient of variation among the four biochemical traits, indicating that this trait offers the greatest scope for genetic improvement through direct selection within this panel. Correlating the biochemical traits against the six agro-morphological traits showed that SYD had no significant relationship with any nutritional trait, indicating neither a yield penalty nor a yield benefit associated with nutritional quality in this germplasm, a finding of direct practical relevance since it means combined agronomic and nutritional improvement is not constrained by an inherent trade-off. PL, however, was significantly associated with both antioxidant activity, negatively, and methionine, positively, and D50F and D80M were both negatively associated with methionine, indicating that earlier flowering and maturity in this panel tend to accompany higher methionine content, a linkage that, to our knowledge, has not previously

been formally reported in winged bean and that may be worth further investigation using a larger, purpose-designed panel.

Directly comparing the nutritional profile of the eight consensus agronomically-selected accessions against the panel average showed that agronomic superiority and nutritional quality are not automatically aligned in this germplasm. Chhattisgarh Chaudhari Sem-2, the only accession selected by all four agronomic indices, recorded below-average values for every biochemical trait measured. IC026949 and EC142654, comparatively modest agronomic performers selected by only two of the four indices, recorded above-average values across all four biochemical traits, with IC026949 showing the strongest overall nutritional profile among the consensus group. This result indicates that a breeding programme in this germplasm seeking to combine agronomic performance with nutritional quality cannot rely on agronomic selection indices alone, and would need to incorporate biochemical traits directly into a combined multi-trait index once multi-environment biochemical data become available to support a BLUP-based treatment comparable to that used for the agro-morphological traits in the present study.

Classical approaches to genotype by environment interaction, such as AMMI and GGE biplot analysis, remain useful for identifying mega-environments and visualizing broad interaction patterns (Yan and Kang, 2003), but rely on fixed effects and do not directly produce a single, statistically defensible multi-trait ranking of genotypes. The multi-trait, multi-environment framework applied here, integrating BLUP-based genetic parameter estimation, WAASB and WAASBY, and four complementary selection indices, extends this classical foundation and has been applied successfully across a range of crops, including cotton (Raj et al., 2024), guar (Benakanahalli et al., 2021) and black bean (Ambrosio et al., 2024), but has not previously been applied to winged bean. The explicit, transparent handling of SYD, a trait with degenerate BLUP genotypic variance but clear applied importance, through mixed BLUP and phenotypic data for MGIDI and BLUE based performance for MTMPS, rather than its silent exclusion, represents a methodological contribution of the present study beyond its application to a previously unstudied crop, and provides a template for handling similarly weak-signal traits in other underutilized legume breeding programmes.

5. Conclusion

In the context of increasing climatic variability and pronounced genotype by environment interaction, the identification of accessions combining favourable multi-trait performance with

cross-environment stability has become a central objective in the improvement of underutilized legumes such as winged bean. This study demonstrates that reliance on raw mean performance alone is inadequate for capturing the true breeding value of accessions under variable environments. The integration of BLUP-based stability analysis with four complementary multi-trait, multi-environment selection indices instead provided a more defensible basis for identifying broadly adapted accessions, and the explicit, transparent handling of SYD through mixed BLUP and phenotypic data for MGIDI and BLUE based performance for MTMPS, rather than its silent exclusion, allowed this key trait to inform selection despite its weak genetic signal. From a winged bean breeding perspective, the identification of accessions combining favourable pod and seed traits with consistent performance across environments is particularly important given the limited multi-environment testing infrastructure historically available for this underutilized crop. Across all four applied indices, Chhattisgarh Chaudhari Sem-2 was consistently identified as a superior performer, selected by MGIDI, MTSI, MTMPS and FAI-BLUP alike, and PWB 11-2 was selected by three of the four indices, minimizing the risk of index-dependent selection bias in the identification of these two accessions. Chhattisgarh Chaudhari Sem-2 combined early flowering and maturity with high pod number and seed weight, while PWB 11-2 combined favourable seed yield with strong cross-environment stability. However, nutritional profiling of these-consensus accessions showed that this agronomic superiority did not extend to nutritional quality, Chhattisgarh Chaudhari Sem-2 recorded below-average values for oil content, protein content, antioxidant activity and methionine, whereas IC026949 and EC142654, comparatively modest agronomic performers, recorded above-average values across all four biochemical traits. This finding indicates that combined agronomic and nutritional improvement in winged bean cannot rely on agronomic selection indices alone. Further multi-year, multi-location evaluation of the identified accessions, together with the extension of multi-environment biochemical data collection to support a BLUP-based treatment of nutritional traits comparable to that used here for agro-morphological traits, will be required to confirm their stability and to develop a single, combined agronomic and nutritional selection index. Such a framework would accelerate the identification of parental material suited to diversified, climate-resilient cropping systems and support the wider deployment of winged bean as a protein-rich, multipurpose legume

Funding

This research received no external funding.

Ethical approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Declaration of competing interest

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

S.K.Y. contributed to the conceptualization of the study and planning of the experiments. K.T., N.V., R.V., V.J.S, V.P.S., and M.T.B., contributed to conducting the experiments in the field/lab, data collection, R.G., S., N.G and V.J.S. did analysis, and writing the first draft. S.K.Y., and V.J.S. has edited and revised the manuscript. All authors have read and agreed to the published version of this manuscript.

Institutional Permission:

Permission to conduct this study was granted by the respective host institutions - Raj Mohini Devi College of Agriculture and Research Station (RMDCARS), Ambikapur, Chhattisgarh; Acharya Narendra Deva University of Agriculture & Technology (ANDUAT), Ayodhya, Uttar Pradesh; Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, Maharashtra; and Birsa Agricultural University, Ranchi, Jharkhand - as these centres form part of the All India Coordinated Research Network (AICRN) on Potential Crops, coordinated under the Indian Council of Agricultural Research (ICAR). The field trials were conducted as part of the mandated multi-location testing programme of this project, with the requisite institutional and project-level approval at each participating centre. The genetic material used was procured through official channels from the National Gene Bank, ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi.

References

1. Adegboyega, T. T., Abberton, M. T., AbdelGadir, A. H., Dianda, M., Maziya-Dixon, B., Oyatomi, O., Ofodile, S., & Babalola, O. O. Nutrient and antinutrient composition of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) seeds and tubers. Journal of Food Quality, 2019, 3075208. (2019).

2. Adegboyega, T. T., Abberton, M. T., AbdelGadir, A. H., Mahamadi, D., Olaniyi, O. A., Ofodile, S., & Babalola, O. O. Variation in winged bean (*Psophocarpus tetragonolobus*) growth parameters, seed yield, nodulation and nitrogen fixation. Asian Journal of Agriculture, 5, 61-71. (2021).

3. Ambrosio, M., Daher, R. F., Santos, R. M., Santana, J. G. S., Vidal, A. K. F., Nascimento, M. R., et al. Multi-trait index: Selection and recommendation of superior black bean genotypes as new improved varieties. BMC Plant Biology, 24, 525. (2024).

4. AOAC International. Official Method 2003.05: Crude fat in feeds, cereal grains, and forages (21st ed.). AOAC International, Gaithersburg, MD. (2019).

5. Benakanahalli, N. K., Sridhara, S., Ramesh, N., Olivoto, T., Sreekantappa, G., Tamam, N., et al. A framework for identification of stable genotypes based on MTSI and MGIDI indexes: An example in guar (Cyamopsis tetragonoloba L.). Agronomy, 11, 1221. (2021).

6. Bepary, R. H., Roy, A., Pathak, K., & Deka, S. C. Biochemical composition, bioactivity, processing, and food applications of winged bean (*Psophocarpus tetragonolobus*): A review. Legume Science, 5(3), e187. (2023).

7. Bhadmus, A., Abberton, M., Idehen, E., Ekanem, U., Paliwal, R., & Oyatomi, O. Genetic diversity assessment of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) accessions using agronomic and seed morphometric traits. Crops, 3(2), 170-183. (2023).

8. Choo, W. S., Muhammad, K., Yusof, S., Bakar, J., & Mat Hashim, D. The optimization of conditions for the production of acid-hydrolysed winged bean and soybean proteins with reduction of 3-monochloropropane-1,2-diol (3-MCPD). International Journal of Food Science & Technology, 39(9), 947-958. (2004).

9. Comstock, R. E., & Moll, R. H. Genotype-environment interactions. In W. D. Hanson & H. F. Robinson (Eds.), Statistical genetics and plant breeding (pp. 164-196). National Academy of Sciences, National Research Council. (1963).

689 10. Eagleton, G. E. Review: Winged bean (*Psophocarpus tetragonolobus*) cropping systems.
690 Biodiversitas, 21, 5927-5946. (2020).

691 11. Gauch, H. G. A simple protocol for AMMI analysis of yield trials. Crop Science, 53(5),
692 1860-1869. (2013).

693 12. Kantha, S. S., & Erdman, J. W. The winged bean as an oil and protein source: A review.
694 Journal of the American Oil Chemists' Society, 61(3), 515-525. (1984).

695 13. Kassambara, A., & Mundt, F. factoextra: Extract and visualize the results of multivariate
696 data analyses (R package version 1.0.7). (2020).

697 14. Khan, T. N. Papua New Guinea: A centre of genetic diversity in winged bean. Euphytica,
698 25(1), 693-706. (1976).

699 15. Kumar, V. K., & Rajalakshmi, R. Population diversity analysis of an underutilized legume,
700 winged bean (*Psophocarpus tetragonolobus* (L.) DC.) using ISSR markers. *Plant Gene,*
701 *36*, 100436. (2023).

702 16. Lepcha, P., Egan, A. N., Doyle, J. J., & Sathyanarayana, N. A review on current status and
703 future prospects of winged bean (*Psophocarpus tetragonolobus*) in tropical agriculture.
704 Plant Foods for Human Nutrition, 72(3), 225-235. (2017).

705 17. Olivoto, T., & Lucio, A. D. C. metan: An R package for multi-environment trial analysis.
706 Methods in Ecology and Evolution, 11(6), 783-789. (2020).

707 18. Olivoto, T., Lucio, A. D. C., da Silva, J. A. G., Marchioro, V. S., de Souza, V. Q., & Jost,
708 E. Mean performance and stability in multi-environment trials I: Combining features of
709 AMMI and BLUP techniques. Agronomy Journal, 111(6), 2949-2960. (2019a).

710 19. Olivoto, T., Lucio, A. D. C., da Silva, J. A. G., Sari, B. G., & Diel, M. I. Mean performance
711 and stability in multi-environment trials II: Selection based on multiple traits. Agronomy
712 Journal, 111(6), 2961-2969. (2019b).

713 20. Peng, S., Khush, G. S., Virk, P., Tang, Q., & Zou, Y. Progress in ideotype breeding to
714 increase rice yield potential. Field Crops Research, 108, 32-38. (2008).

715 21. Protection of Plant Varieties and Farmers' Rights Authority. Plant Variety Journal of India,
716 19(12). Government of India. (2025).

22. Raj, D. S., Patil, R. S., Patil, B. R., Nayak, S. N., & Pawar, K. N. Characterization of early maturing elite genotypes based on MTSI and MGIDI indexes: An illustration in upland cotton (Gossypium hirsutum L.). Journal of Cotton Research, 7, 1-12. (2024).

23. Rocha, J. R. A. S. C., Machado, J. C., & Carneiro, P. C. S. Multi-trait index based on factor analysis and ideotype-design: Proposal and application on elephant grass breeding for bioenergy. GCB Bioenergy, 10(1), 52-60. (2018).

24. Tanzi, A. S., Eagleton, G. E., Ho, W. K., Wong, Q. N., Mayes, S., & Massawe, F. Winged bean (*Psophocarpus tetragonolobus* (L.) DC.) for food and nutritional security: Synthesis of past research and future direction. Planta, 250(3), 911-931. (2019).

25. Ward, J. H., Jr. Hierarchical grouping to optimize an objective function. Journal of the American Statistical Association, 58(301), 236-244. (1963).

26. Wricke, G. Zur Berechnung der Okovalenz bei Sommerweizen und Hafer *Zeitschrift für Pflanzenzüchtung, 52*, 127–138. (1965).

27. Xiao, F., Xu, T., Lu, B., & Liu, R. Guidelines for antioxidant assays for food components. Food Frontiers, 1, 60-69. (2020).

28. Yan, W., & Kang, M. S. GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists. CRC Press. (2003).

29. Yanagi, S. O. (Properties of winged bean (*Psophocarpus tetragonolobus*) protein in comparison with soybean (Glycine max) and common bean (Phaseolus vulgaris) protein. Agricultural and Biological Chemistry, 47(10), 2273-2280. 1983).

30. Yoshida, S. Fundamentals of rice crop science. International Rice Research Institute. (1981).

Figure 1. Trait distribution and correlation structure among 31 winged bean accessions across four environments. (A) Distribution of six agro-morphological traits across the four environments, with individual plant observations shown as jittered points overlaid on boxplots. Environmental means sharing the same lowercase letter within a panel are not significantly different according to Tukey's HSD test at P less than or equal to 0.05. (B) Correlation and distribution matrix among the six traits based on genotype means. The diagonal shows trait

distribution, the lower triangle shows pairwise scatter plots with a fitted trend line, and the upper triangle shows the Pearson correlation coefficient with significance stars.

Figure 2. Multivariate structure of the 31 accession winged bean germplasm panel. (A) Principal component biplot showing PC1 against PC2, based on six standardized agro-morphological traits, with trait loading vectors overlaid. (B) Ward hierarchical clustering dendrogram of the 31 accessions. (C) Standardized genotype by trait performance heatmap, ordered by cluster membership.

Figure 3. WAASB based stability analysis for seed yield. (A) Y by WAASB biplot classifying accessions into four quadrants of mean performance and stability. (B) Genotypes ranked by WAASBY. This analysis is presented as an exploratory characterization of genotype by environment interaction, not as a selection criterion, given the absence of genotypic variance for seed yield in this panel.

Figure 4. Genotype ranking and strengths and weaknesses profile for the four multi-trait selection indices at 15 per cent selection intensity, with selected accessions shown in red. (A) MGIDI ranking. (B) MGIDI strengths and weaknesses. (C) MTSI ranking. (D) MTSI strengths and weaknesses. (E) MTMPS ranking. (F) MTMPS strengths and weaknesses. (G) FAI-BLUP ranking.

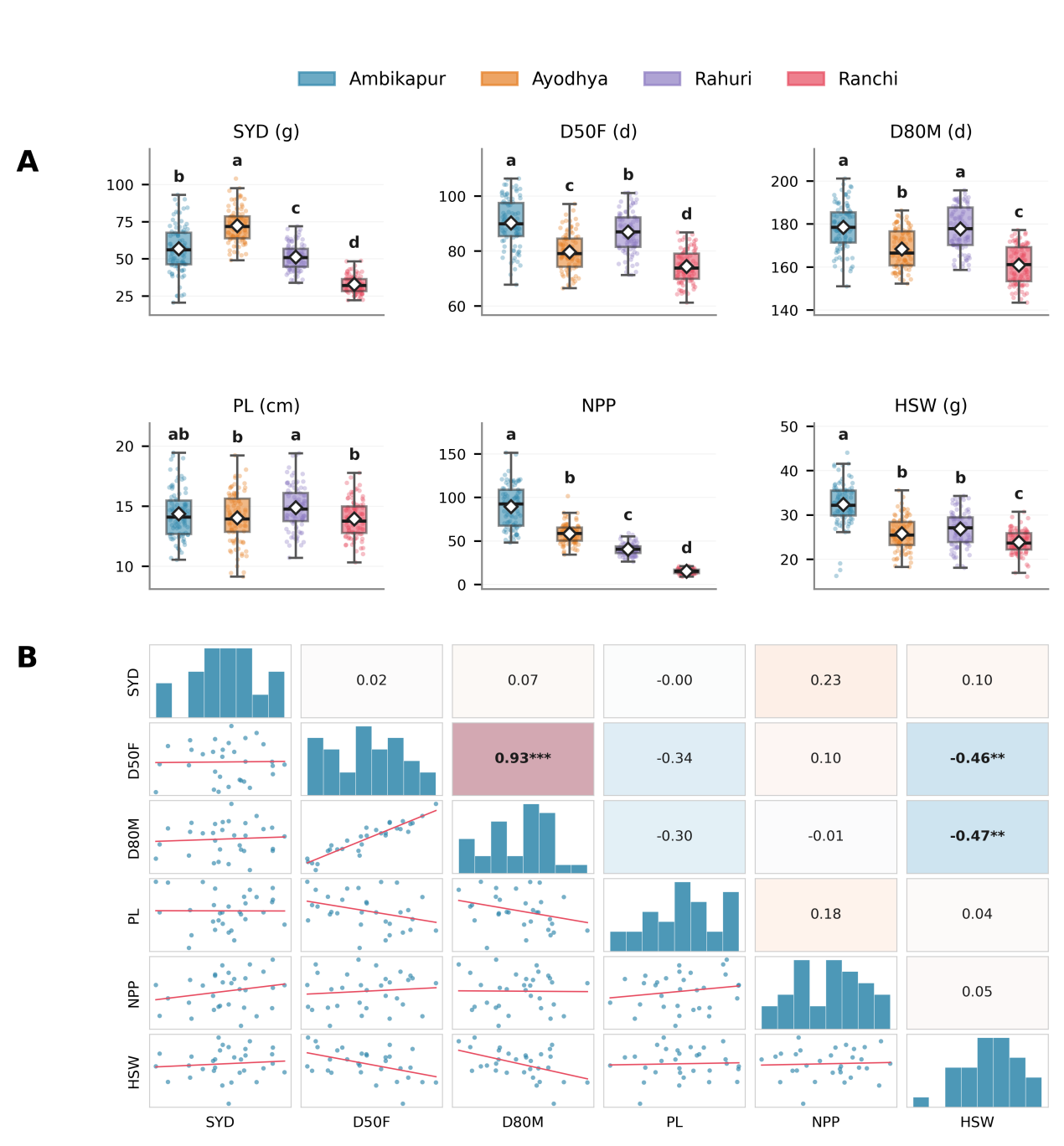
Figure 5. Coincidence of selection among the four multi-trait indices. (A) Predicted selection gain from the mixed-data MGIDI selected group. (B) Circos plot of genotype overlap among MGIDI, MTSI, MTMPS and FAI-BLUP at 15 per cent selection intensity, with chord width proportional to the number of accessions shared between each pair of indices. (C) Standardized performance heatmap of the eight consensus accessions selected by two or more indices.

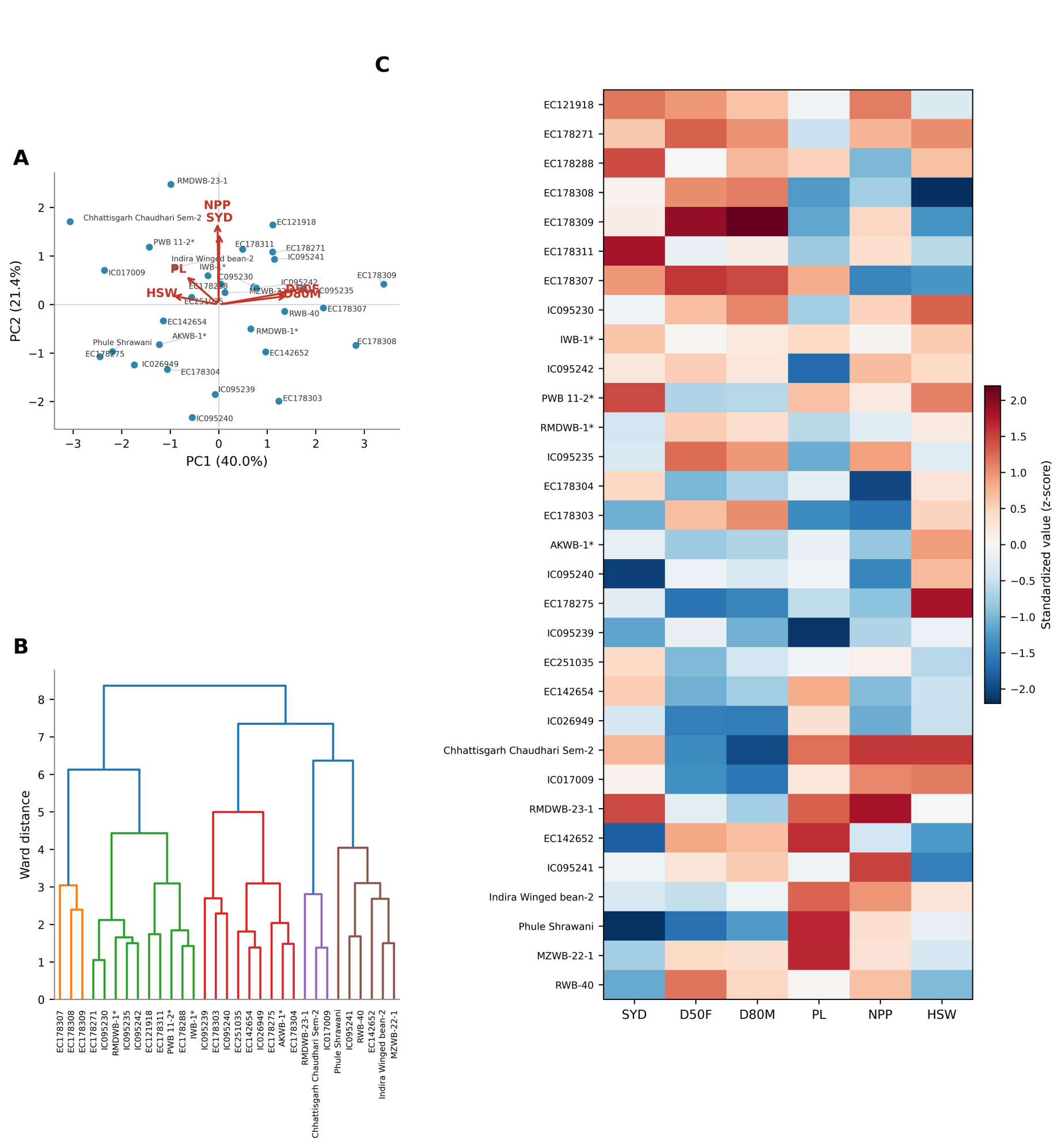
Figure 6. Distribution of biochemical traits among 31 winged bean accessions, based on genotype means. Oil content, protein content, antioxidant activity, and methionine content are shown.

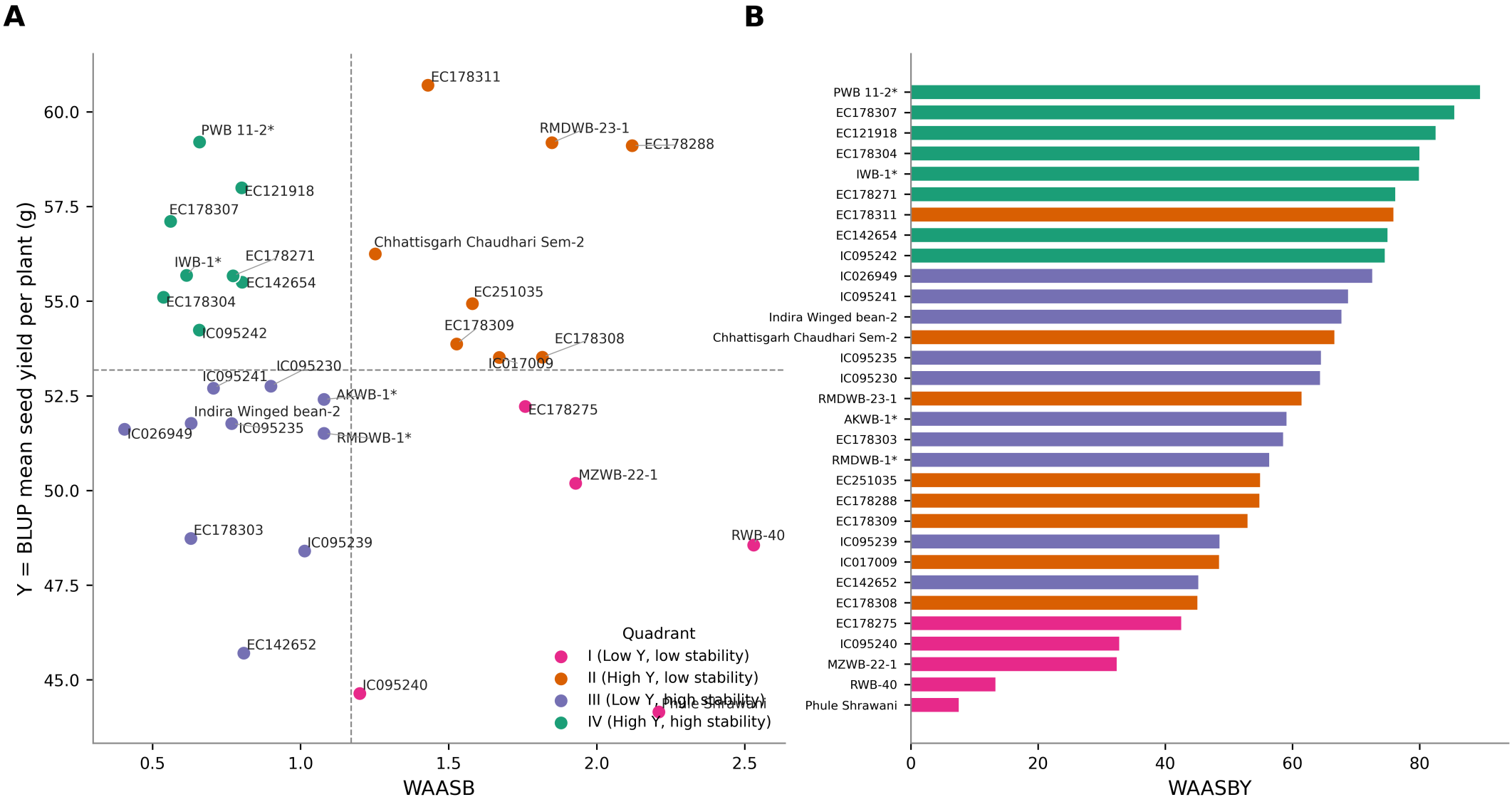
Figure 7. Standardized nutritional trait profile of 31 winged bean accessions, hierarchically clustered.

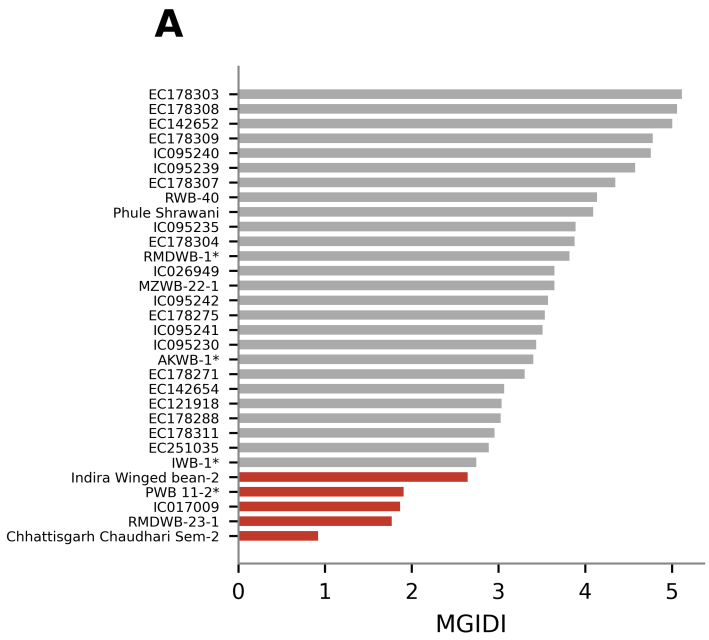
Figure 8. Relationship between agro-morphological and nutritional traits. (A) Correlation matrix combining the six agro-morphological traits and the four biochemical traits. (B) Standardized nutritional profile of the consensus agronomically selected accessions relative to the 31 accession panel.

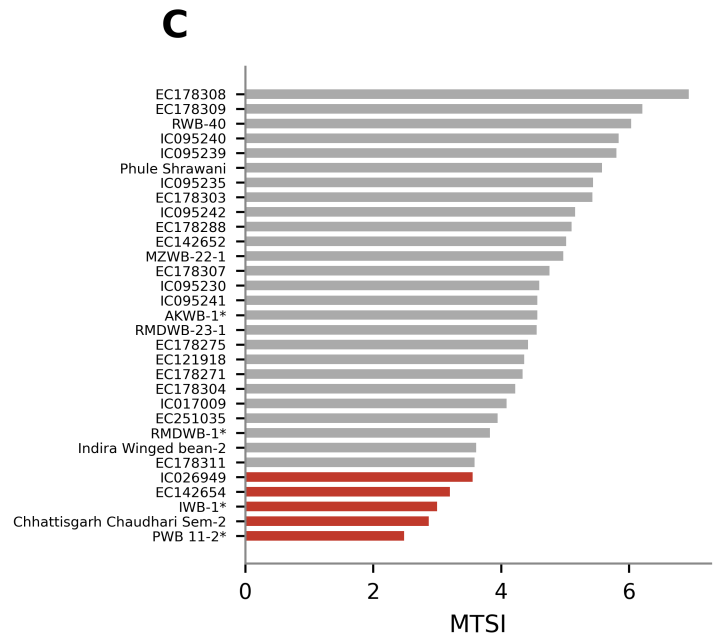
Supplementary Figure 1. Q-Q plots of standardized residuals from the BLUP/REML mixed model for all six agro-morphological traits.

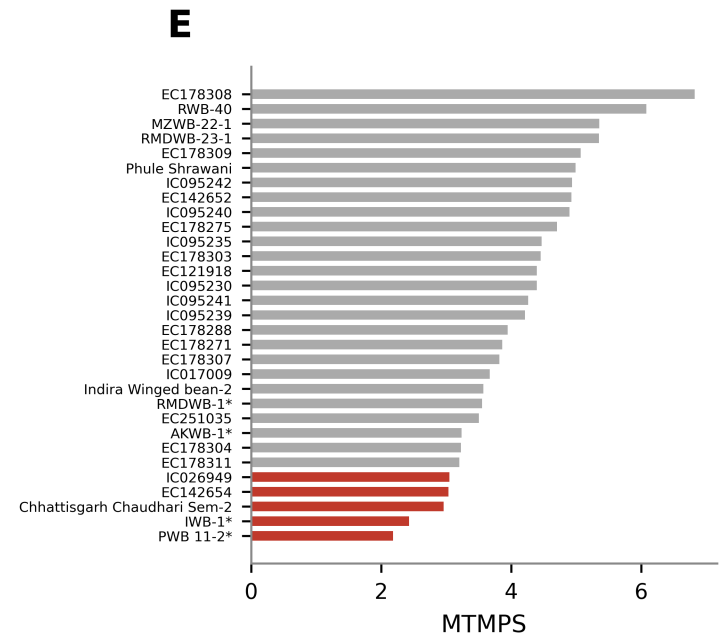

A
Ambikapur
Ayodhya
Rahuri
Ranchi
SYD (g)
D50F (d)
D80M (d)
PL (cm)
NPP
HSW (g)
B
SYD
D50F
D80M
PL
NPP
HSW
0.02
0.07
-0.00
0.23
0.10
0.93***
-0.34
0.10
-0.46**
-0.30
-0.01
-0.47**
0.18
0.04
0.05

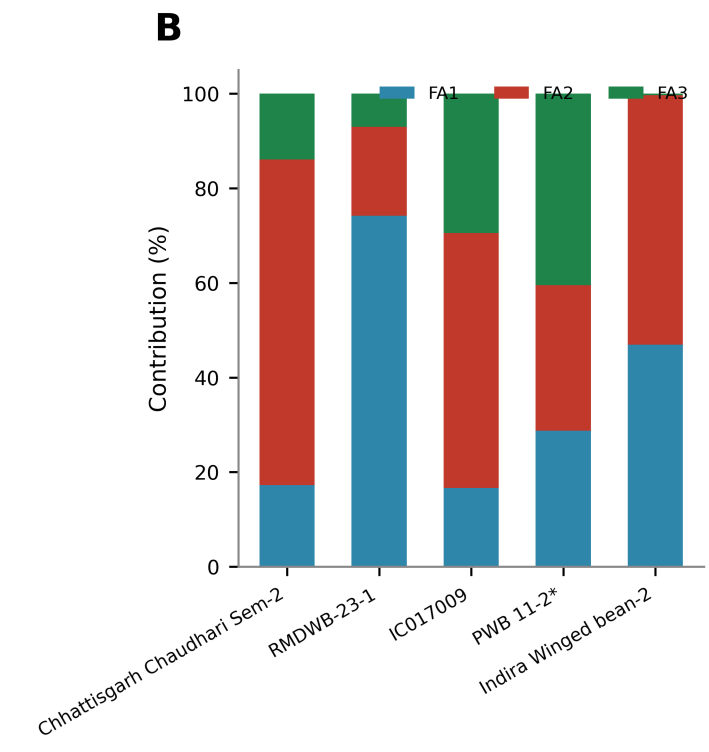

A
RMDWB-23-1
NPP
SYD
Chhattisgarh Chaudhari Sem-2
EC121918
PWB 11-2*
EC178311
EC178271
IC095241
Indira Winged bean-2
IWB-1*
PL
IC017009
IC095230
EC178309
IC095242
EC178288
D50F
HSW
D80M
IC095235
EC251035
RWB-40
EC178307
EC142654
RMDWB-1*
AKWB-1*
Phule Shrawani
EC178308
EC178275
EC142652
IC026949
EC178304
IC095239
EC178303
IC095240
PC2 (21.4%)
PC1 (40.0%)
B
Ward distance
EC178307
EC178308
EC178309
EC178271
IC095230
RMDWB-1*
IC095235
IC095242
EC121918
EC178311
PWB 11-2*
EC178288
IWB-1*
IC095239
EC178303
IC095240
EC251035
EC142654
IC026949
EC178275
AKWB-1*
EC178304
RMDWB-23-1
Chhattisgarh Chaudhari Sem-2
IC017009
Phule Shrawani
IC095241
RWB-40
EC142652
Indira Winged bean-2
MZWB-22-1
C
EC121918
EC178271
EC178288
EC178308
EC178309
EC178311
EC178307
IC095230
IWB-1*
IC095242
PWB 11-2*
RMDWB-1*
IC095235
EC178304
EC178303
AKWB-1*
IC095240
EC178275
IC095239
EC251035
EC142654
IC026949
Chhattisgarh Chaudhari Sem-2
IC017009
RMDWB-23-1
EC142652
IC095241
Indira Winged bean-2
Phule Shrawani
MZWB-22-1
RWB-40
SYD
D50F
D80M
PL
NPP
HSW
Standardized value (z-score)

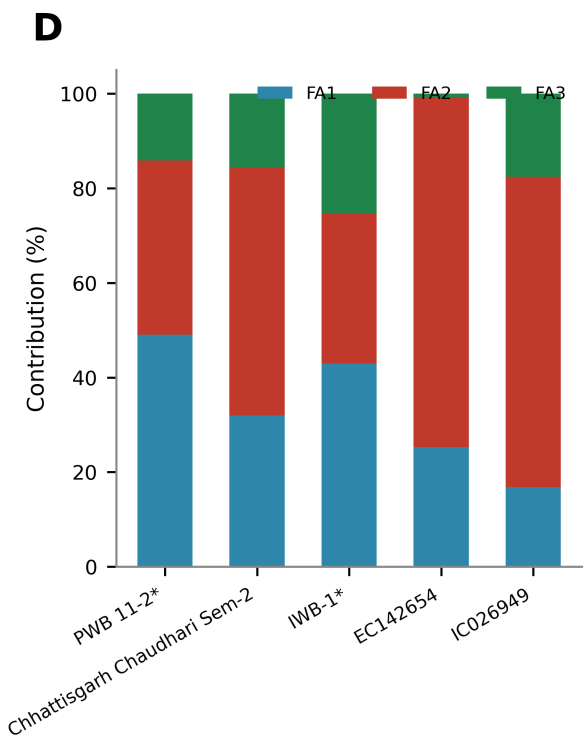

A
B
Y = BLUP mean seed yield per plant (g)
WAASB
WAASBY
Quadrant
I (Low Y, low stability)
II (High Y, low stability)
III (Low Y, high stability)
IV (High Y, high stability)
EC178311
PWB 11-2*
RMDWB-23-1
EC178288
EC121918
EC178307
Chhattisgarh Chaudhari Sem-2
IWB-1*
EC178271
EC142654
EC178304
EC251035
EC178309
EC178308
IC095242
IC017009
IC095230
IC095241
AKWB-1*
EC178275
Indira Winged bean-2
IC026949
IC095235
RMDWB-1*
MZWB-22-1
EC178303
IC095239
RWB-40
EC142652
IC095240
Phule Shrawani

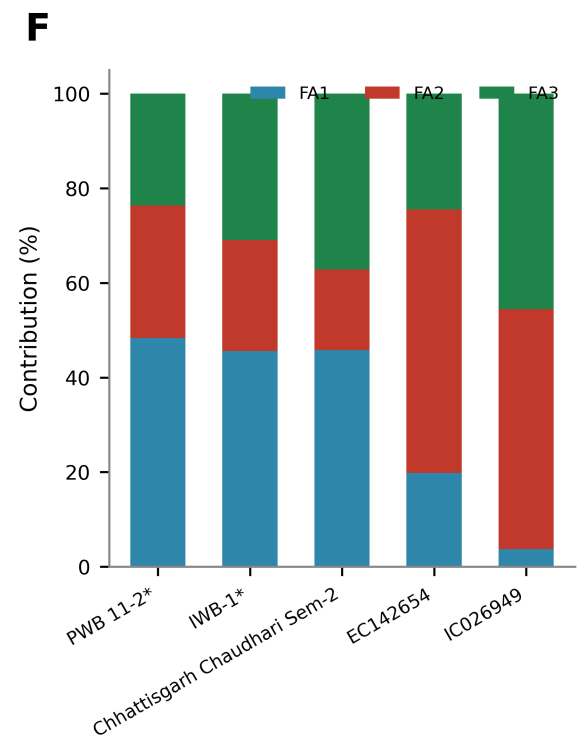

A
EC178303
EC178308
EC142652
EC178309
IC095240
IC095239
EC178307
RWB-40
Phule Shrawani
IC095235
EC178304
RMDWB-1*
IC026949
MZWB-22-1
IC095242
EC178275
IC095241
IC095230
AKWB-1*
EC178271
EC142654
EC121918
EC178288
EC178311
EC251035
IWB-1*
Indira Winged bean-2
PWB 11-2*
IC017009
RMDWB-23-1
Chhattisgarh Chaudhari Sem-2
0
1
2
3
4
5
MGIDI

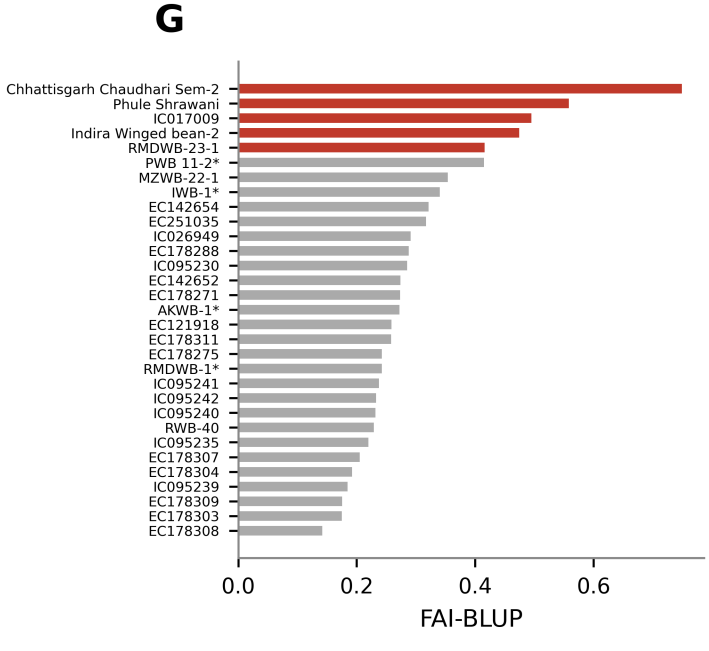

C
EC178308
EC178309
RWB-40
IC095240
IC095239
Phule Shrawani
IC095235
EC178303
IC095242
EC178288
EC142652
MZWB-22-1
EC178307
IC095230
IC095241
AKWB-1*
RMDWB-23-1
EC178275
EC121918
EC178271
EC178304
IC017009
EC251035
RMDWB-1*
Indira Winged bean-2
EC178311
IC026949
EC142654
IWB-1*
Chhattisgarh Chaudhari Sem-2
PWB 11-2*
0
2
4
6
MTSI

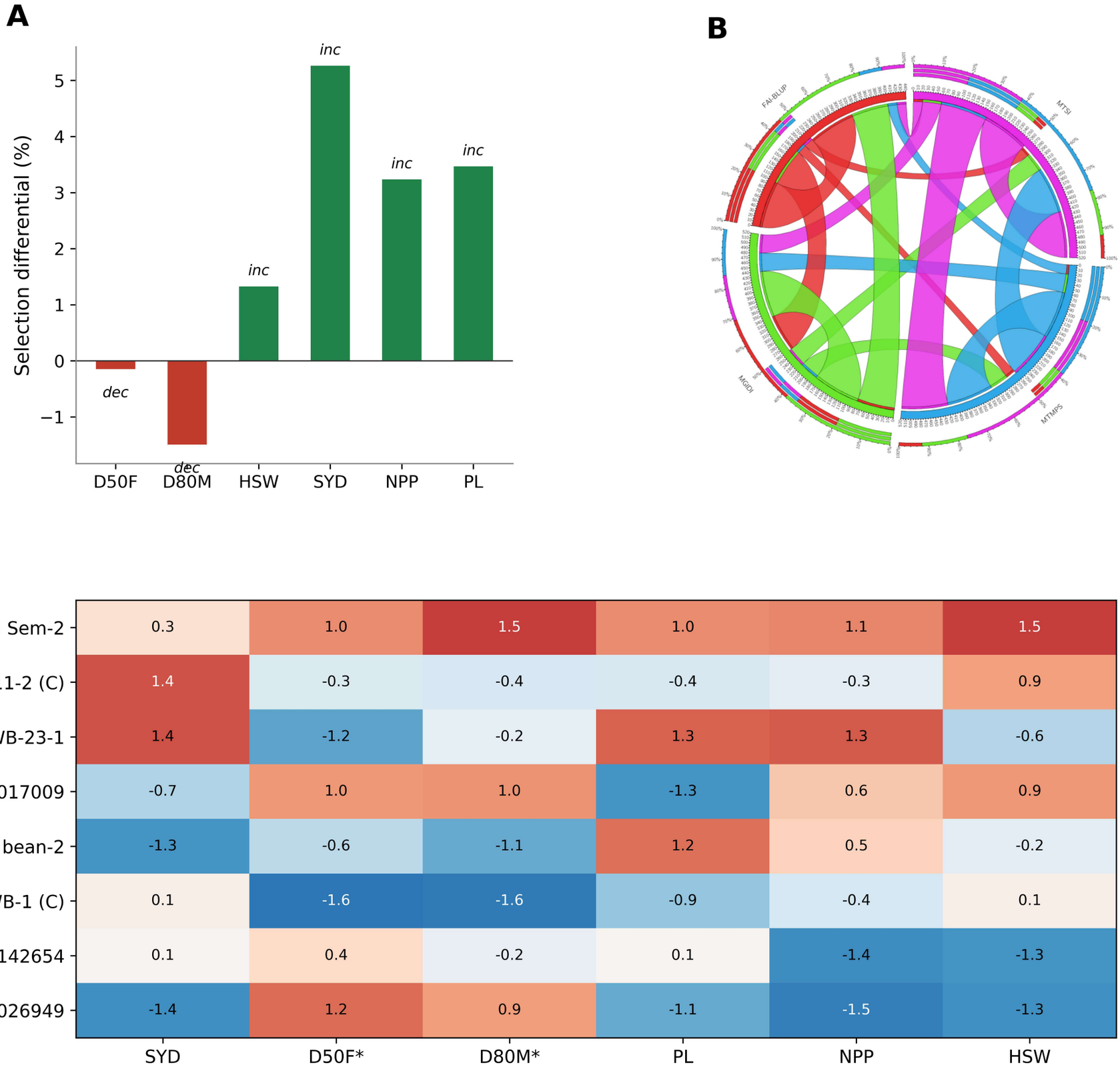

E
EC178308
RWB-40
MZWB-22-1
RMDWB-23-1
EC178309
Phule Shrawani
IC095242
EC142652
IC095240
EC178275
IC095235
EC178303
EC121918
IC095230
IC095241
IC095239
EC178288
EC178271
EC178307
IC017009
Indira Winged bean-2
RMDWB-1*
EC251035
AKWB-1*
EC178304
EC178311
IC026949
EC142654
Chhattisgarh Chaudhari Sem-2
IWB-1*
PWB 11-2*
0
2
4
6
MTMPS

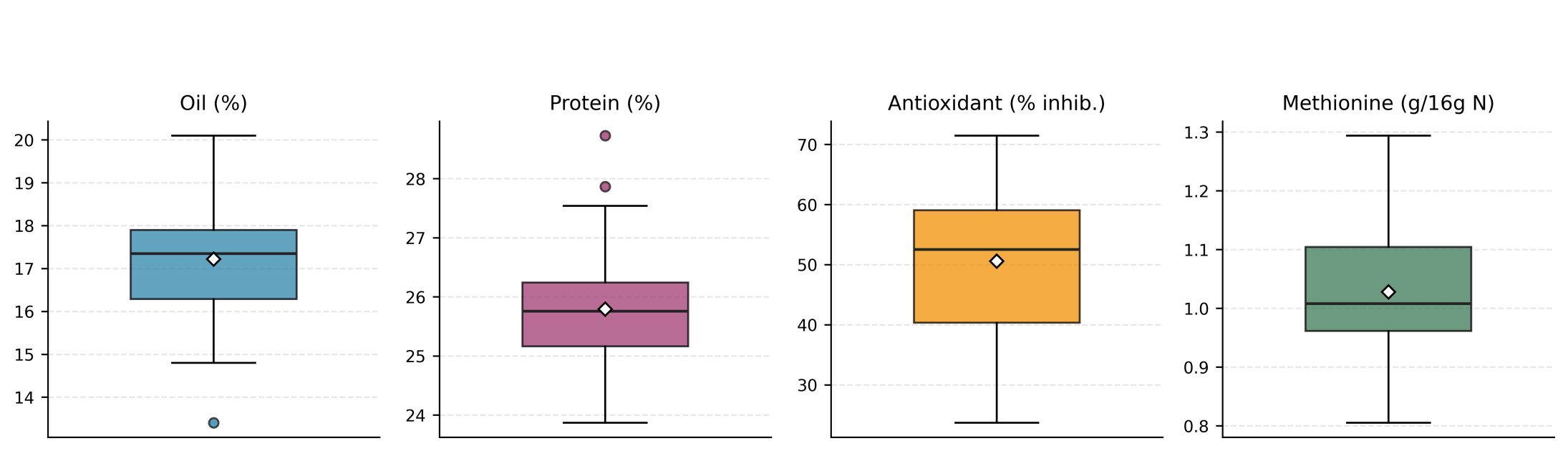

B
100
80
60
40
20
0
Contribution (%)
FA1
FA2
FA3
Chhattisgarh Chaudhari Sem-2
RMDWB-23-1
IC017009
PWB 11-2*
Indira Winged bean-2

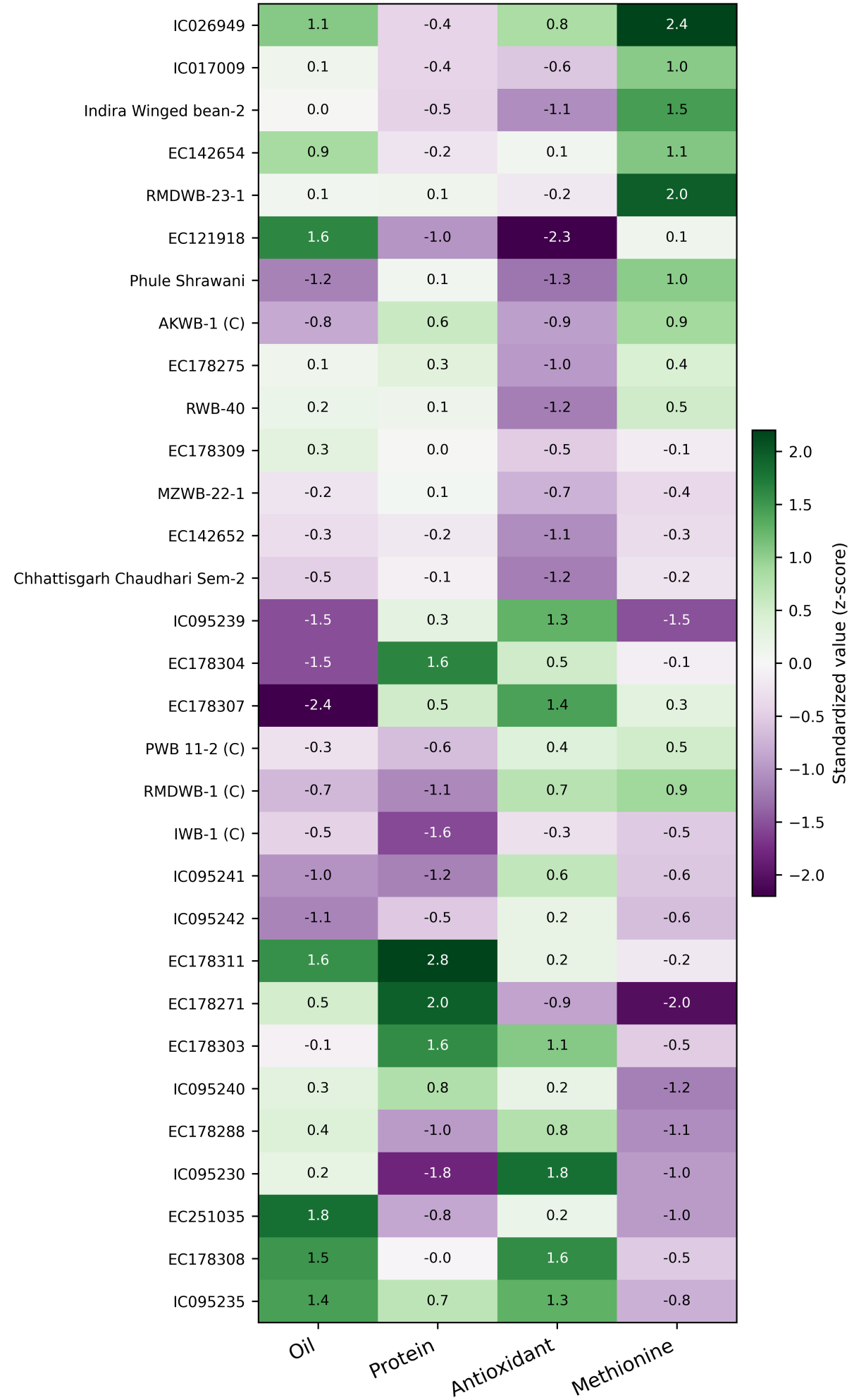

D
100
80
60
40
20
0
Contribution (%)
FA1
FA2
FA3
PWB 11-2*
Chhattisgarh Chaudhari Sem-2
IWB-1*
EC142654
IC026949

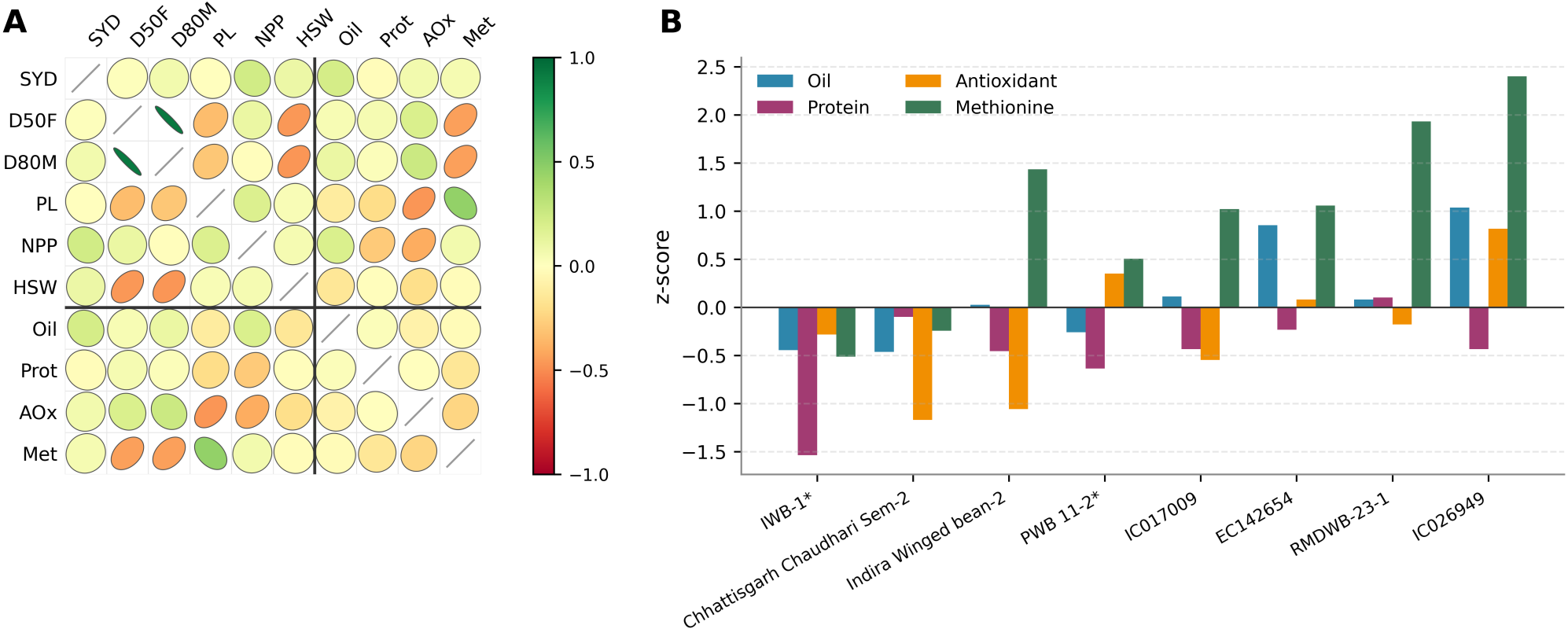

F
100
80
60
40
20
0
Contribution (%)
FA1
FA2
FA3
PWB 11-2*
IWB-1*
Chhattisgarh Chaudhari Sem-2
EC142654
IC026949


G
Chhattisgarh Chaudhari Sem-2
Phule Shrawani
IC017009
Indira Winged bean-2
RMDWB-23-1
PWB 11-2*
MZWB-22-1
IWB-1*
EC142654
EC251035
IC026949
EC178288
IC095230
EC142652
EC178271
AKWB-1*
EC121918
EC178311
EC178275
RMDWB-1*
IC095241
IC095242
IC095240
RWB-40
IC095235
EC178307
EC178304
IC095239
EC178309
EC178303
EC178308
0.0
0.2
0.4
0.6
FAI-BLUP


A
Selection differential (%)
5
4
3
2
1
0
−1
inc
inc
inc
inc
dec
dec
D50F
D80M
HSW
SYD
NPP
PL
B
FAI-BLUP
MTSI
MGIDI
MTMPS
C
Chhattisgarh Chaudhari Sem-2
PWB 11-2 (C)
RMDWB-23-1
IC017009
Indira Winged bean-2
IWB-1 (C)
EC142654
IC026949
0.3 1.0 1.5 1.0 1.1 1.5
1.4 -0.3 -0.4 -0.4 -0.3 0.9
1.4 -1.2 -0.2 1.3 1.3 -0.6
-0.7 1.0 1.0 -1.3 0.6 0.9
-1.3 -0.6 -1.1 1.2 0.5 -0.2
0.1 -1.6 -1.6 -0.9 -0.4 0.1
0.1 0.4 -0.2 0.1 -1.4 -1.3
-1.4 1.2 0.9 -1.1 -1.5 -1.3
SYD
D50F*
D80M*
PL
NPP
HSW
Standardized value (z-score)
2.0
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5
−2.0


Oil (%)
20
19
18
17
16
15
14
Protein (%)
28
27
26
25
24
Antioxidant (% inhib.)
70
60
50
40
30
Methionine (g/16g N)
1.3
1.2
1.1
1.0
0.9
0.8


IC026949 1.1 -0.4 0.8 2.4
IC017009 0.1 -0.4 -0.6 1.0
Indira Winged bean-2 0.0 -0.5 -1.1 1.5
EC142654 0.9 -0.2 0.1 1.1
RMDWB-23-1 0.1 0.1 -0.2 2.0
EC121918 1.6 -1.0 -2.3 0.1
Phule Shrawani -1.2 0.1 -1.3 1.0
AKWB-1 (C) -0.8 0.6 -0.9 0.9
EC178275 0.1 0.3 -1.0 0.4
RWB-40 0.2 0.1 -1.2 0.5
EC178309 0.3 0.0 -0.5 -0.1
MZWB-22-1 -0.2 0.1 -0.7 -0.4
EC142652 -0.3 -0.2 -1.1 -0.3
Chhattisgarh Chaudhari Sem-2 -0.5 -0.1 -1.2 -0.2
IC095239 -1.5 0.3 1.3 -1.5
EC178304 -1.5 1.6 0.5 -0.1
EC178307 -2.4 0.5 1.4 0.3
PWB 11-2 (C) -0.3 -0.6 0.4 0.5
RMDWB-1 (C) -0.7 -1.1 0.7 0.9
IWB-1 (C) -0.5 -1.6 -0.3 -0.5
IC095241 -1.0 -1.2 0.6 -0.6
IC095242 -1.1 -0.5 0.2 -0.6
EC178311 1.6 2.8 0.2 -0.2
EC178271 0.5 2.0 -0.9 -2.0
EC178303 -0.1 1.6 1.1 -0.5
IC095240 0.3 0.8 0.2 -1.2
EC178288 0.4 -1.0 0.8 -1.1
IC095230 0.2 -1.8 1.8 -1.0
EC251035 1.8 -0.8 0.2 -1.0
EC178308 1.5 -0.0 1.6 -0.5
IC095235 1.4 0.7 1.3 -0.8
Oil
Protein
Antioxidant
Methionine
Standardized value (z-score)
2.0
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5
−2.0


A
SYD
D50F
D80M
PL
NPP
HSW
Oil
Prot
AOx
Met
1.0
0.5
0.0
−0.5
−1.0
B
Oil
Protein
Antioxidant
Methionine
z-score
2.5
2.0
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5
IWB-1*
Chhattisgarh Chaudhari Sem-2
Indira Winged bean-2
PWB 11-2*
IC017009
EC142654
RMDWB-23-1
IC026949

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tables.docx
- SupplementaryFigure1QQplots.tif