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Morphological and ecological diversity of the sicklepod (*Cassia Obtusfolia*) population in Central Sudan

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Abstract
This study investigates the morphological and ecological diversity of *Cassia obtusifolia* (locally known as Kawal) populations in central Sudan. A total of 150 plant samples were collected from five geographically distinct locations within Gezira State. Seventeen morphological traits, including vegetative and reproductive characteristics, were measured and analyzed using descriptive statistics, one-way and two-way ANOVA, MANOVA, and Spearman's correlation analysis. Results revealed substantial variation in vegetative traits such as stem length (range: 23.6–165.2 cm), number of leaves (15–11,616), and biomass components, while reproductive traits like seed weight (2.5–2.6 g) remained highly conserved. South populations exhibited the tallest plants and largest shoot biomass, whereas North populations had the highest root biomass. Significant correlations (R = 0.76–0.98) between fresh and dry biomass components validate measurement consistency. These findings highlight the phenotypic plasticity of *C. obtusifolia* and its adaptation to heterogeneous environments, suggesting potential ecological and agronomic implications for resource use, genetic conservation, and climate resilience.

Keywords: *Cassia obtusifolia*, kawal, morphological variation, phenotypic plasticity, Sudan, biomass traits

Introduction

Sicklepod (*Cassia obtusifolia* L.), locally known as *Kawal* in Sudan, is an annual leguminous plant belonging to the subtribe *Cassiinae* (family *Fabaceae*), which includes numerous wild and cultivated species distributed across tropical and subtropical regions worldwide [1] In Sudan, *C. obtusifolia* naturally grows on the clay plains of the central rainlands and southern zones, where it plays ecological, nutritional, and ethnobotanical roles. Notably, its fermented leaves are consumed as a traditional protein source by rural communities, particularly during periods of food scarcity [2, 3]. In both Africa and America, sickle pods (*Cassia obtusifolia*) are extensively dispersed. It is primarily found in the southern areas known as kawal and the clay plains of the central rainlands in Sudan. Kawal leaves are made through a solid-state fermentation process and added to sauces that are meant to be eaten with porridge. It gives the sauce a meaty, savoury taste [3].

The plant's adaptive success in diverse and often challenging environments has drawn attention to its potential value in sustainable land use and agrobiodiversity conservation [4]

Despite its ecological and socio-economic importance, limited scientific attention has been given to the intraspecific morphological variation of *C. obtusifolia* populations across different environments in Sudan [5]. Most previous studies have focused on its ethnobotanical applications, seed germination, or chemical properties, with little focus on the plant’s phenotypic plasticity in response to environmental heterogeneity [6]. Understanding this variation is crucial, as morphological traits often reflect the underlying genetic diversity and environmental adaptation of species, especially in wild populations exposed to varied climatic and edaphic conditions [7]. The pot is slender and the plant has many avoid seeds [8]. General morphology of sicklepod and foetid cassia includes: a, S. tora pods and compound leaf; b & c, S. tora anthers with truncated tips; d & e, S. tora seeds with broad longitudinal areole; f, S. obtusifolia pods and compound leaf; g, S. obtusifolia - part of flower displaying the three prominent beaked anthers and the recurved style with its tip positioned over the anthers; h & i, S. obtusifolia seeds with narrow transverse areole [9]. Seed source and year affected plant height, seed per pod, number of seeds, and number of pods in field experiments. In all three years, plants grown from Tennessee seed had a 30-day shorter vegetative growth phase from emergence to the first floral buds than those grown from Alachua, FL seed. In 1983, hand-harvested seed from the 10 1982 seed sources was planted next to sicklepod seed and flowered simultaneously, indicating self-fertilization. The 10 sources' hand-harvested sicklepod seed germination ranged from 2 to 11% in 1981 and 3 to 23% in 1982 [10]. Phenotypic plasticity refers to an organism's capacity to modify its physiology, morphology, or development in reaction to environmental alterations [11].

The present study aims to assess the morphological and ecological diversity of *C. obtusifolia* populations in central Sudan using a combination of univariate and multivariate statistical approaches. By examining vegetative and reproductive traits across five distinct geographic regions, this study seeks to characterize the extent of phenotypic variation, identify key traits influenced by spatial and environmental factors, and explore potential correlations among traits to better understand the plant's growth strategies. The findings are expected to provide a scientific basis for conservation strategies, sustainable utilization, and future genetic studies of this under-researched yet valuable species.

Materials and Methods

Study Area

The study was conducted in Gezira State, located in central Sudan between latitudes 13°36′N and 15°07′N and longitudes 32°25′E and 33°49′E. The region lies within the semi-arid agroecological zone and is characterized by clay soils, moderate to high temperatures, and a unimodal rainfall pattern during the summer months (June to September). Gezira is one of Sudan's major agricultural hubs and exhibits considerable environmental heterogeneity across its north-south and east-west gradients. Five main locations—North, South, East, West, and Middle—were selected to represent the geographic and ecological diversity of the region. Each location included three distinct sites to capture within-location variation.

Sample Collection and Morphological Characterization

Fresh plant specimens of *Cassia obtusifolia* were collected during the peak growing season from natural populations in each of the five locations. At each site, individual plants (n = 10 per site) were randomly selected and carefully uprooted to preserve root systems. Specimens were immediately placed in labeled polyethylene bags and transported to the laboratory for analysis.

A total of 150 individual plants (5 locations × 3 sites × 10 plants) were analyzed for 17 morphological traits. These included:

Vegetative traits

Leaf length (cm), Leaf width (cm), Neck leaf length (cm), Stem length (cm), Stem width (mm), Internode length (cm), Number of nodes (count), Root length (cm), Number of branches (count) and Number of leaves (count)

Biomass-related traits

Leaf weight (g), Seed weight (g), Fresh shoot weight (g), Fresh root weight (g), Dried shoot weight (g) and Dried root weight (g).

All measurements were performed using standardized protocols. Digital calipers and rulers were used for linear dimensions, and electronic precision balances were used for weight measurements. After fresh weight measurements, plant parts were dried at room temperature for two weeks before determining dry biomass.

Data Management and Statistical Analysis

All data were verified for consistency, completeness, and unit standardization. Categorical variables (location and site) were encoded appropriately to enable nested and factorial analysis. Summary statistics (mean, standard deviation, range) were computed for each trait both across and within locations.

To assess data suitability for parametric analysis, normality of trait distributions was tested using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test. Non-normally distributed variables were log-transformed to meet statistical assumptions.

Three main levels of statistical analysis were applied.

One-way ANOVA was used to assess differences in trait means among the five geographic locations. Post hoc pairwise comparisons were conducted using Tukey's HSD test to identify homogeneous groups.

Two-way ANOVA was conducted to evaluate the main and interaction effects of location, site, and location × site on each morphological trait.

Multivariate analysis of variance (MANOVA) was performed to assess simultaneous variation across multiple traits, considering location and site as fixed factors.

Additionally, Spearman's rank correlation coefficients were calculated to investigate relationships among traits, particularly between vegetative and biomass-related features. A correlation heatmap was generated to visualize inter-trait associations.

All statistical analyses were conducted using SPSS (v26) and JASP (v0.16) software, with a significance threshold of $p < 0.05$ for all tests.

Results

General Morphological Variation in *Cassia obtusifolia*

Substantial morphological diversity was observed across the *Cassia obtusifolia* population sampled in central Sudan. Descriptive statistics revealed wide variation in several vegetative traits. Leaf length ranged from 2.2 to 6.0 cm (mean ± SD = 3.7 ± 0.83 cm), while stem length showed the broadest range, from 23.6 to 165.2 cm (mean = 81.3 ± 39.8 cm). Root length ranged from 6.1 to 47.0 cm, and the number of leaves varied dramatically, from 15 to 11,616 leaves per plant (mean = 2,811.9 ± 2,804.8), indicating extensive intra-population variability. Shown as figure.1.

Traits related to biomass also showed considerable variation. Fresh shoot weight ranged from 87.5 to 2,469.5 g, and dried shoot weight ranged from 27.5 to 971.2 g. In contrast, reproductive traits such as seed weight (2.5–2.6 g; CV = 1.6%) and neck leaf length (0.1–0.5 cm; mean = 0.15 ± 0.07 cm) were remarkably conserved across samples, suggesting differential evolutionary pressures acting on vegetative versus reproductive components. Shown as figure.2 &3

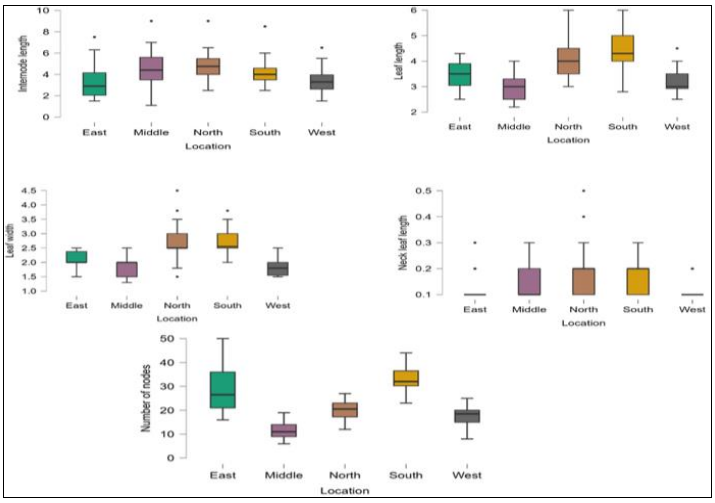

Fig 1: Vegetative Trait Plasticity in *Cassia obtusifolia*

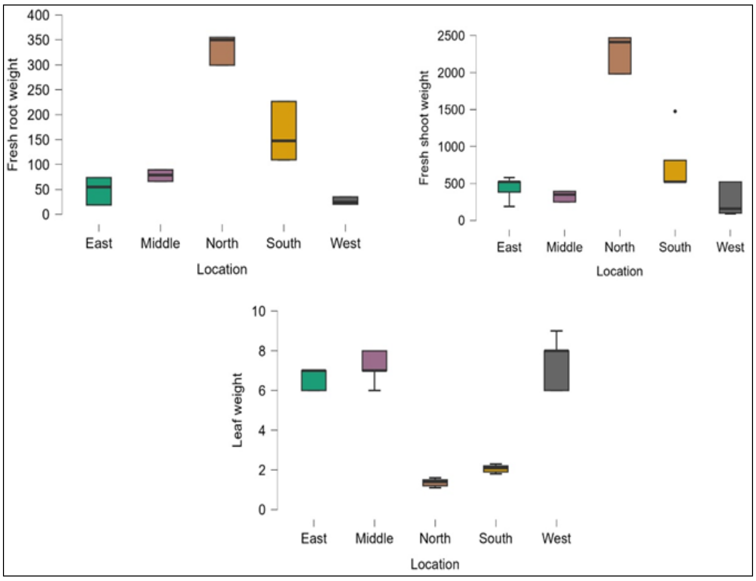

Fig 2: Biomass-related traits Fresh Wight of *Cassia obtusifolia*

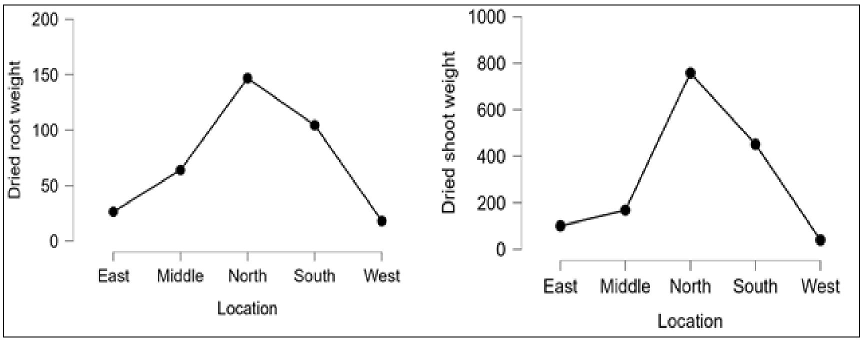

Fig 3: Biomass-related traits Dried Wight of *Cassia obtusifolia*

Geographic Patterns of Morphological Differentiation.

One-way ANOVA showed that 13 of the 15 measured traits differed significantly across the five geographic locations ($p < 0.001$). Northern and Southern populations consistently demonstrated superior vegetative growth. Plants from the South had the longest stems (mean = 132.8 cm) and highest number of nodes (32.8), while Northern populations had the widest leaves (4.21 ± 0.84 cm) and the longest roots (32.17 ± 7.03 cm).

In contrast, plants from the Western and Middle regions generally exhibited reduced vegetative vigor. For example, Western populations had the lowest fresh shoot weight (mean = 250.9 ± 205.1 g) and shortest stems. Despite low overall growth, Western populations had significantly higher leaf weight (mean = 7.44 g) than other locations, suggesting differences in biomass allocation.

Seed weight and stem width did not differ significantly among locations ($p > 0.05$), indicating strong trait conservation across environmental gradients.

Site and Microenvironmental Effects

Two-way ANOVA revealed that location had a stronger effect on most traits than site, although site-level variation was also statistically significant for 7 traits ($p < 0.05$). Significant location × site interactions were observed for key traits including stem length, number of branches, and biomass-related traits, highlighting the influence of microenvironmental heterogeneity.

Site 3, regardless of location, consistently supported more vigorous growth

- **Dried shoot weight:** Site 3 mean = 757.5 g vs Site 1 mean = 100.4 g
- **Node number:** Site 3 = 32.8 vs Site 1 = 11.3
- Root biomass was highest in Northern Site 3 (fresh: 334.8 g; dry: 146.8 g).

Multivariate Differentiation Across Locations and Sites

MANOVA confirmed the existence of statistically significant multivariate differentiation among both locations and sites

- **Location:** Wilks' $\lambda = 0.0638$, $F = 15.28$, $p < 0.001$
- **Site:** Wilks' $\lambda = 0.7540$, $F = 2.27$, $p = 0.003$.

These results demonstrate that combinations of traits—not just individual ones—differ meaningfully across environmental gradients, suggesting integrated phenotypic responses to geography and microclimate.

Trait Correlation Structure

Spearman's correlation analysis revealed strong, biologically meaningful associations among morphological traits

Stem length correlated strongly with.

- Number of nodes ($R = 0.88$)
- Number of branches ($R = 0.88$)
- Leaf number ($R = 0.61$)

Perfect correlation ($R = 1.00$) was found between number of nodes and number of branches, suggesting synchronized development of structural features.

Biomass traits were tightly interrelated.

- Fresh and dry shoot weight (R = 0.77)
- Fresh and dry root weight (R = 0.98)
- Dried shoot and root weights (R = 0.96)

Negative correlations were found between leaf weight and most vegetative traits, including.

- Leaf length (R = –0.59)
- Fresh shoot weight (R = –0.76)
- Dried root weight (R = –0.74)
- Seed weight was uncorrelated with all vegetative and biomass traits (|R| < 0.12), supporting its status as a conserved trait.

These correlations confirm the developmental integration of vegetative architecture in *C. obtusifolia*, alongside the independence of reproductive features. Figure.4.

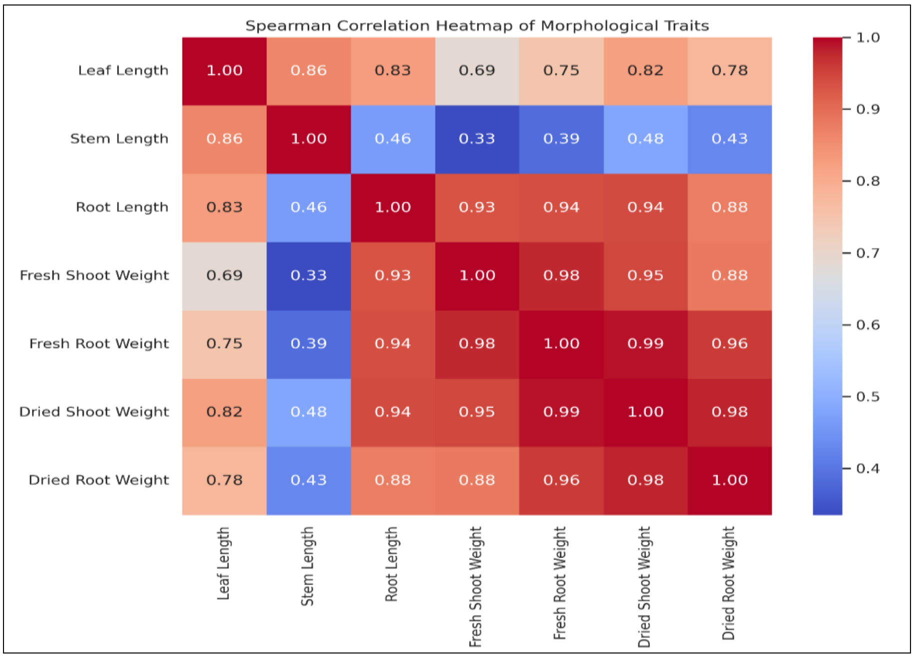

Fig 4. Heatmap of Spearman's Rank Correlations Among Morphological Traits in *Cassia obtusifolia*

Discussion

This study reveals substantial morphological and ecological diversity in *Cassia obtusifolia* populations across central Sudan, reflecting strong phenotypic plasticity and potential local adaptation. The pronounced variability in vegetative traits—particularly stem length, number of leaves, and biomass components—contrasted sharply with the stability of reproductive traits such as seed weight. These patterns suggest differential selective pressures shaping distinct functional modules within the species. Traits associated with plant architecture and resource acquisition (e.g., stem length, root length, number of branches) displayed significant variation across both geographic locations and microsites. Southern populations, for example, produced taller plants with more nodes and larger shoot biomass, likely reflecting favorable edaphic or microclimatic conditions. In contrast, Western and Middle populations exhibited more conservative growth forms, possibly due to resource limitations or environmental stressors. The significant site effects and location × site interactions observed in two-way ANOVA support the role of microenvironmental heterogeneity in shaping phenotypic expression. These findings align with broader ecological theories that emphasize environmental filtering and local adaptation as key drivers of intraspecific trait variation in wild plant populations (Valladares *et al*., 2006; Sultan, 2000) [12, 13]. Seed weight and stem width showed minimal variation across locations and sites, forming a single homogeneous group. This pattern suggests strong stabilizing selection on reproductive traits, which are often more canalized due to their direct link to fitness (Ackerly *et al*., 2000 14) [14]. The lack of correlation between seed weight and vegetative traits further supports the idea that reproductive output in *C. obtusifolia* is developmentally and functionally decoupled from vegetative growth. Such modular independence has been documented in other leguminous species and may represent an adaptive strategy under unpredictable environmental conditions, allowing the plant to maintain reproductive assurance even when vegetative performance is compromised [15].

The strong positive correlations between stem length, node number, branch number, and biomass traits (e.g., R = 0.88–0.98) reflect high degrees of developmental integration in the vegetative module. These coordinated traits likely operate under shared hormonal and physiological regulation, facilitating efficient resource allocation and mechanical stability [16]. The perfect correlation (R = 1.00) between node and branch number suggests that these features may represent a single developmental axis rather than independent traits, and thus should be considered jointly in ecological modeling and breeding programs.

Interestingly, leaf weight was negatively correlated with both leaf size and biomass traits, indicating potential trade-offs in carbon allocation between leaf density and total leaf area. Such trade-offs are common in plants occupying variable environments and reflect the balance between photosynthetic efficiency and structural investment [17]. The MANOVA results confirmed that *C. obtusifolia* populations differ not only in individual traits but in overall morphological profiles. These multivariate patterns support the use of *C. obtusifolia* as a model for studying intraspecific functional diversity, with implications for agrobiodiversity conservation, particularly in marginal or climate-sensitive environments [18, 19, 20, 21, 22].

Given its nutritional and ethnobotanical significance in Sudan, preserving the morphological diversity of *C. obtusifolia* may contribute to local food security and resilience under climate change, especially as the plant shows signs of adaptation to a range of environmental conditions. This underlines the need for further studies integrating genetic, phenological, and ecological data to guide conservation and potential domestication efforts [23, 24, 25].

Conclusion

This study provides compelling evidence of significant morphological and ecological diversity within *Cassia obtusifolia* populations across central Sudan. Vegetative traits, particularly those associated with growth form and biomass allocation, displayed high levels of variability, while reproductive traits such as seed weight remained largely conserved. This contrast underscores the dual influence of phenotypic plasticity and stabilizing selection in shaping the plant's functional morphology.

The pronounced regional and site-specific variation in traits such as stem length, node number, and shoot/root biomass reflect the influence of both macro- and micro-environmental conditions. The high degree of trait integration within vegetative structures, and the independence of reproductive characteristics, suggests modular developmental organization—a feature with important ecological and evolutionary implications.

These findings highlight the adaptive potential of *C. obtusifolia* under diverse and shifting environmental conditions. As a species of nutritional, cultural, and ecological importance in Sudan, conserving its morphological diversity is critical. Future work should aim to integrate molecular, physiological, and phenological data to deepen our understanding of this species' adaptive strategies and support its potential use in sustainable agriculture and climate-resilient systems.

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Data availability: The data supporting the results of this study are included in the manuscript

Declarations

Conflict of interest the author has declared no conflicts of interest.

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