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Black seeds, hidden diversity: Phenotypic structure in an urban landrace of amaranth (*Amaranthus* spp.) in Niger

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Abstract

Neglected crops often harbour cryptic diversity maintained through informal seed systems, particularly in urban gardens of the Sahel. This study investigates phenotypic variation within a black-seeded *Amaranthus* spp. population cultivated in Niamey, Niger, where seed colour guides local selection practices despite limited scientific documentation. A total of 180 plants were evaluated at the vegetative stage for plant height and number of leaves under a randomised complete block design, with three replicates of 60 plants each. Descriptive statistics revealed considerable intra-population variability (CV = 39.4 % for height; 27.2 % for leaf number). Robust Principal Component Analysis (rPCA) captured 93.5 % of total variance along a single axis reflecting strong trait correlation. Unsupervised clustering identified a dominant phenotypic core with peripheral structuring but no extreme outliers. This exploratory study documents the extent and internal organisation of phenotypic variation in a black-seeded landrace cultivated in Niger's urban gardens, highlighting the contribution of farmer-maintained populations to the conservation of agrobiodiversity in underutilised crops. Conducted over a single growing season, this work provides a quantitative description of morphological variation within a Nigerien black-seeded amaranth landrace and establishes a baseline for future multi-environment or molecular studies.

Keywords: Agrobiodiversity, intra-population variation, neglected and underutilised species, urban gardening

1 Introduction

Amaranths (*Amaranthus* spp.) are multifunctional crops cultivated across diverse agroecological zones for both their leafy biomass and nutrient-rich seeds. Several species, such as A. cruentus and A. dubius, are appreciated for their adaptability to low-input systems and their high nutritional value, including essential micronutrients and high-quality protein (Rastogi & Shukla, 2013; Dutta *et al.*, 2025). In West Africa, and particularly in Niger, amaranths are commonly grown in urban and peri-urban gardens, where seed colour – typically white or black – serves as a practical trait for distinguishing local seed lots. In grain amaranth, white seed colour has been identified as a domestication trait selected independently from dark-seeded wild ancestors (Stetter *et al.*, 2020). By contrast, in leafy amaranths cultivated in Niger, seed colour is not directly associated with domestication for grain, but rather serves as a proxy used by farmers to differentiate seed lots and maintain distinct landraces for leafy use. In Niger, amaranths are primarily cultivated as leafy vegetables, with harvesting usually occurring well before flowering. Seed colour, while not associated with domestication for grain, functions as a practical proxy that farmers use to distinguish and maintain seed lots, ensuring continuity of their preferred leafy types across planting cycles.

While formal breeding of amaranth remains limited in the region, smallholder farmers continue to manage and exchange seeds through informal networks (Naino Jika *et al.*,2023). This process, combined with open pollination and heterogeneous micro-environments, can generate and maintain phenotypic variability within visually uniform populations (Bellon *et al.*, 2011; Brenner *et al.*, 2010). Previous studies in Africa have reported substantial morphological diversity within farmer-managed *Amaranthus* populations (e.g. Ouedraogo *et al.*, 2021, Yeshitila *et al.*, 2023, 2024), highlighting the evolutionary potential of these landraces. However, black-seeded populations in Niger remain largely undocumented.

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The present study addresses this knowledge gap by asking a specific research question: what is the extent and internal structure of phenotypic variation within a black-seeded amaranth landrace cultivated in Niamey's urban gardens? By analysing morphological traits under standardised field conditions, we aim to provide a quantitative description of intra-population variability and to identify potential entry points for participatory selection and future germplasm characterisation. As the seed material originated from a single bulk lot maintained by an urban gardener, the study focuses on within-population variability rather than among-farm differences.

We hypothesise that this black-seeded population, although visually uniform and managed by farmers as a distinct landrace, harbours structured phenotypic diversity that reflects an underlying mixture of species and differential selection pressures arising from local management practices.

2 Materials and methods

2.1 Study site and planting material

The experiment was conducted during the past 2024 cropping season at the experimental garden of the Faculty of Agronomy, Abdou Moumouni University in Niamey, Niger (13°29'57.0" N; 2°05'35.6" E). The site lies within a semi-arid zone characterised by sandy loam soils and a unimodal rainfall pattern, with annual precipitation ranging between 500 and 600 mm. The seeds used in this study were obtained from a single urban gardener in Niamey, who has maintained this black-seeded population for several years through informal seed saving and exchange practices. According to farmers, this population– locally called "*Burkina tchappata*" in Zarma – is primarily valued for its leafy biomass rather than for grain.

In practice, most gardeners in Niamey obtain seed from local markets rather than self-producing it every season, with occasional on-farm seed saving; this helps explain why inflorescences are rarely observed and species identity is often blurred at farmer level.

Because harvest usually occurs before flowering, farmers rarely observe inflorescence traits, and local selection is based mainly on vegetative vigour, leaf size, and leaf abundance. To address this, during our trial we allowed a subsample of plants to flower and conducted a preliminary taxonomic check following Achigan-Dako *et al.* (2014). Based on diagnostic inflorescence traits, we identified with high confidence two species (*Amaranthus cruentus* and *A. hybridus*) in the flowering subsample, while some individuals resembled *A. dubius* but require confirmation.

The plant material consisted of seeds from a locally maintained black-seeded *Amaranthus* population, commonly cultivated in Niamey and considered a distinct landrace. We therefore report results at the population level and flag species assignment as preliminary.

2.2 Experimental design and crop management

The field layout followed a randomised complete block design with three replicates. Each replicate (block) contained 60 plants, all originating from the same bulk seed lot provided by the gardener. Thus, the design assessed within-population variability, not among-farm variation. This RCBD layout was chosen to balance statistical rigor with the practical conditions of farmer-managed production. Although an incomplete block design is sometimes recommended in the presence of strong soil heterogeneity, the site was deemed sufficiently homogeneous.

Manual soil tillage was performed to a depth of approximately 15–20 cm to ensure proper aeration and root development. Sowing was done manually on 27 July 2024, with seeds deposited into shallow holes (1–2 cm deep) spaced 30 cm apart. Initial irrigation was applied immediately after sowing to ensure uniform germination. On 9 August 2024, a thinning operation was performed to retain one healthy seedling per hole. Weed control and supplemental irrigation were carried out as needed.

2.3 Morphological measurements

Data were collected on 24 August 2024, when plants had reached the early vegetative stage. A total of 180 individual plants (60 per replicate) were randomly selected and evaluated for two key agro-morphological traits:

- Plant height (cm): measured from the soil surface to the apex of the main shoot.
- Number of leaves: counted as the total number of fully developed leaves per plant.

The two traits assessed – plant height and number of leaves – were selected for their agronomic relevance as proxies for vegetative vigour and potential edible biomass. While local farmers do not measure these traits formally, they often rely on overall visual impression of plant growth and leaf abundance when selecting individuals for consumption or seed saving. These traits also offered a practical compromise given the limited resources and the need to evaluate a large number of individuals under field conditions.

2.4 Statistical analysis

All statistical analyses were performed using the R environment (version 4.4.2). Descriptive statistics were computed using the package psych (version 2.4.3). Normality tests and correlations were performed using stats (version 4.4.2). Clustering analyses employed dbscan (version 1.2.2, Hahsler *et al.*, 2019) for DBSCAN and mclust (version 6.1.1, Fraley & Raftery, 2006) for Gaussian Mixture Modelling. Outlier detection was conducted with the Local Outlier Factor (LOF) implemented in the DMwR2 package (Version: 0.0.3, Torgo & Torgo, 2016). To investigate the internal structure of variation, a robust principal component analysis (rPCA) was applied, retaining two dimensions with the package rrcov (Version 1.7-5, Todorov & Filzmoser, 2010). This method accounts for deviations from normality and minimizes the influence of mild outliers. Unsupervised clustering was subsequently conducted using both density-based (DBSCAN) and model-based (Gaussian Mixture Modelling) approaches to detect latent phenotypic groupings. In addition, a Local Outlier Factor (LOF) analysis was performed to identify individuals with deviant morphologies relative to their local phenotypic neighborhood. The analyses focused exclusively on this black-seeded population.

3 Results

3.1 Descriptive morphological variation

The black-seeded *Amaranthus* population exhibited considerable phenotypic variability across the 180 individuals analysed under a randomised field design. Consistent with our initial hypothesis stated at the end of the Introduction, we expected high morphological variance within this farmer-maintained seed lot, given its open-pollinated reproduction and informal management. Two quantitative traits were recorded at the early vegetative stage: plant height (cm) and number of leaves.

Descriptive statistics indicated a broad range of expression for both traits (Table 1). Plant height ranged from 5.0 to 32.5 cm, with a mean of 12.37 cm and a coefficient of variation (CV) of 39.4 %. The number of leaves ranged from 4 to 18, with a mean of 8.97 and a lower CV of 27.2 %, suggesting relatively less dispersion in foliar development compared to plant stature.

Distributional analysis showed mild to moderate asymmetry. Height exhibited a skewness of 1.13, indicating a longer tail on the right side of the distribution, while leaf number was slightly right-skewed (0.72). Both traits displayed platykurtic distributions, with kurtosis values of 1.52 and 1.03 respectively, suggesting flatter distributions with less concentration around the mean than would be expected under normality. These observations were confirmed by Shapiro–Wilk tests ($p < 0.001$ for both traits), supporting the choice of robust statistical methods in subsequent analyses. The interquartile ranges (IQR) were 6 cm for height and 4 leaves for leaf number, further reinforcing the intra-population dispersion observed. The strong correlation typically observed between height and foliar development in amaranth was also evident here, with a Pearson correlation between plant height and leaf number was $r = 0.78$ ($n = 180$, $p < 0.001$).

Taken together, these statistics indicated a continuous morphological spectrum within this farmer-maintained

Table 1: *Morphological trait summary statistics.*

Variable	*Plant height (cm)*	*Number of leaves (count)*
mean	12.371	8.967
median	11	9
mode	9.5	7
sd	4.869	2.442
mad	4.003	2.965
iqr	6	4
cv	39.361	27.239
min	5	4
q1.25 %	9	7
q2.50 %	11	9
q3.75 %	15	11
max	32.5	18
range	27.5	14
trimmed_mean	11.835	8.833
skewness	1.134	0.716
kurtosis	1.524	1.026
shapiro_p	$4.6e^{-8}$	$1.01e^{-5}$

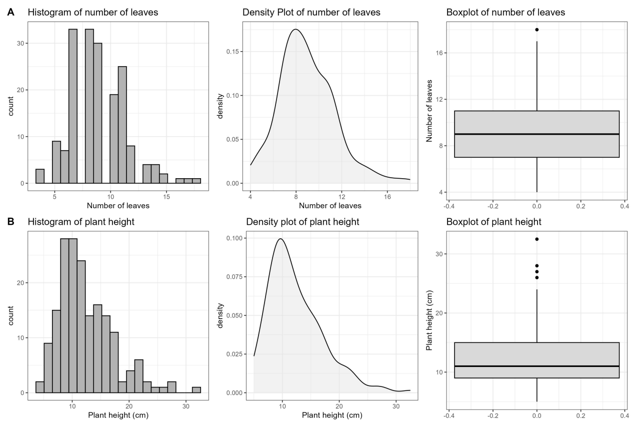

Fig. 1: *Distribution of early vegetative traits in a black-seeded amaranth landrace. Frequency distributions (histograms with density curves) and boxplots for plant height (cm) and number of leaves per plant (count). The boxplots show the median (center line), interquartile range (IQR, box bounds), and 1.5*IQR (whiskers).*

landrace, with no indication of bimodal distribution or abrupt trait discontinuities.

To explore the internal structure of phenotypic variation within the black-seeded *Amaranthus* population, a robust principal component analysis (rPCA) was performed on two agro-morphological traits: plant height and number of leaves. This method was selected due to the moderate skewness and non-normality of the data, as confirmed by Shapiro–Wilk tests (see section 3.1), and aims to reduce the influence of outliers while providing a stable multivariate representation.

The rPCA retained two dimensions. The first principal component (PC1) accounted for 93.5 % of the total variance, and the second component (PC2) explained the remaining 6.5 %. Both traits contributed strongly to PC1, with loadings of –0.91 for plant height and ˘0.41 for number of leaves, indicating a dominant axis of co-varying vegetative vigor. PC2 captured a weaker contrast, primarily driven by divergence in leaf number (loading = 0.91) relative to height (˘0.41).

The biplot of individual scores revealed a continuous phenotypic spread along PC1, with no evidence of discrete subgroups or strong clustering (Fig. 2). This gradient reflects internal heterogeneity in plant morphology, consistent with farmer-managed seed lots subject to open pollination and informal selection. A few individuals occupied peripheral positions on the biplot, but no extreme outliers were detected at this stage of analysis.

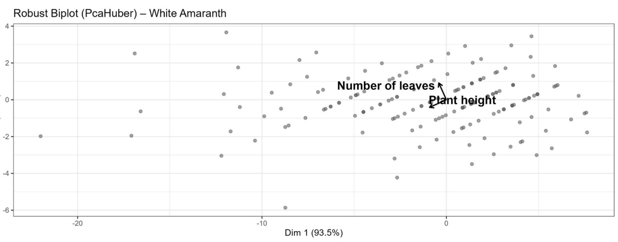

Fig. 2: *Robust PCA of early vegetative traits in a black-seeded amaranth population: Scores for n = 180 plants based on height (cm) and leaf number (count). PC1 = 93.5 % of variance (loadings: height ˘0.91; leaves ˘0.41), PC2 = 6.5 % (leaves 0.91; height ˘0.41). Continuous spread along PC1 indicates no discrete subgroups.*

Overall, the robust PCA confirmed the existence of structured yet continuous phenotypic variation within the population. No evidence was found for distinct subgroups, supporting the view of a cohesive but heterogeneous landrace.

3.2 *Pattern recognition and outlier analysis in morphological space*

To further investigate the internal structure of phenotypic variation, unsupervised learning methods were applied to the rPCA scores (PC1 and PC2). These included density-based clustering (DBSCAN), model-based clustering (GMM), and local outlier factor (LOF) analysis.

DBSCAN identified a dominant cluster (n = 175) encompassing the majority of individuals, while five points (2.8 %) were classified as noise due to their peripheral position in the morphological space. These scattered individuals did not form coherent subgroups and appeared marginal relative to the dense central population.

Gaussian Mixture Modelling (GMM) provided a finer decomposition of the phenotypic structure, suggesting the presence of nine overlapping clusters (VEV model, k = 9). Cluster sizes ranged from 3 to 33 individuals. These clusters represent statistical substructures within the main morphological gradient, but without clear biological or farm-level interpretation in the absence of additional taxonomic or origin data (Fig. 3).

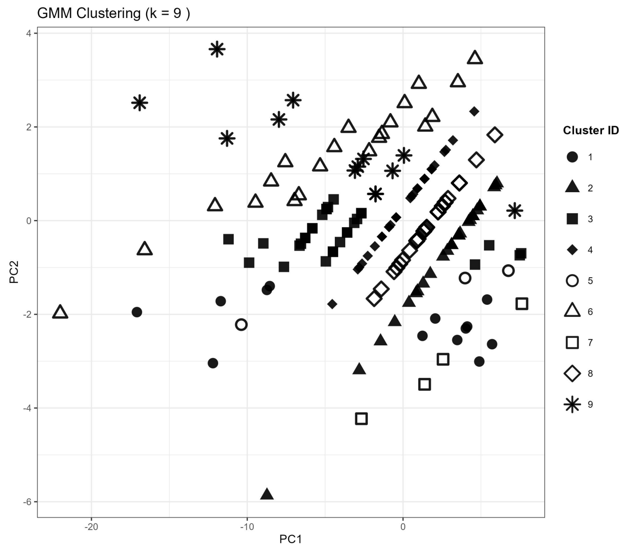

Fig. 3: *Gaussian Mixture Model clustering in morphological space: VEV model (variable volume, equal shape, variable orientation) with k = 9 fitted to (PC1, PC2); cluster sizes ranged from 3 to 33 individuals. Structure reflects statistical subgroups within a continuous gradient; no LOF (Local Outlier Factor) outliers were detected at the 95th percentile.*

Local Outlier Factor (LOF) scores confirmed the robustness of the dataset: no individuals exceeded the 95th percentile threshold typically used to flag extreme deviations. This supports the absence of strong phenotypic outliers, consistent with the continuous nature of the variation observed in the PCA.

These findings reinforce the view of a morphologically cohesive yet internally differentiated population, maintained under farmer conditions without formal varietal control. The clustering patterns should therefore be interpreted as indicative of intra-population differentiation, rather than evidence of specific selection pressures or resilience mechanisms.

4 Discussion

This exploratory study, conducted over a single growing season, documents for the first time the cryptic diversity of a Nigerien amaranth landrace. While limited in scope, it provides a preliminary baseline that should be expanded in future research by including additional traits such as leaf size, total biomass, and seed quality, together with data on seed origin and taxonomic identification across multiple environments.

Despite being managed as a single landrace, the population exhibits continuous morphological dispersion, particularly in plant height and leaf number. Coefficients of variation for these traits (39.4 % and 27.2 %, respectively) are consistent with previous findings on farmer-managed *Amaranthus* populations in West Africa (Ortiz *et al.*, 2023; Ouedraogo *et al.*, 2024) and mirror the high levels of phenotypic diversity found in landraces from other regions, such as Eastern Africa (Mbwambo *et al.*, 2015).

The strong correlation observed between height and leaf number ($r = 0.78$, $p < 0.001$) suggests coordinated vegetative growth, a pattern frequently observed in *Amaranthus* under non-restrictive environmental conditions (Jamalluddin *et al.*, 2022). This association, while informative, does not by itself imply genetic linkage, as it may also reflect uniform microclimatic conditions or consistent management practices across replicates. Moreover, without comparative data from multiple farmers or seed origins, it is not possible to determine whether this correlation is consistent across different batches or specific to the lot studied here.

Robust Principal Component Analysis (rPCA) captured over 93 % of total variance along the first axis alone, confirming a dominant morphological gradient within the population. This result highlights the structured yet continuous nature of variability within the landrace.

Although the black-seeded population showed a compact multivariate distribution, the biological meaning of this tighter clustering remains uncertain in the absence of broader genetic or environmental data. Clustering analyses reinforced this interpretation. DBSCAN isolated a single dense core with only 2.8 % of individuals classified as marginal points, while GMM suggested the existence of overlapping subgroups. These subgroups represent statistical decompositions of the continuous morphological gradient, but without information on farm origin, environment, or species identity, they cannot be interpreted as distinct biological clusters. Notably, the absence of extreme outliers (as confirmed by LOF) points to a stable population structure, with variability occurring within agronomically acceptable bounds.

From a conservation and crop improvement perspective, these results are relevant for both *in situ* management and participatory selection. Although our trial does not allow direct testing of climatic stress buffering, the documented morphological heterogeneity suggests a potential for resilience that warrants further evaluation under multi-environment trials.

We additionally note that predominantly vegetative use in Niger (harvesting before flowering) focuses farmer attention on leaf abundance and vigour rather than reproductive traits, in contrast to grain-oriented systems where panicle characters are under direct selection. This management context helps explain reliance on seed colour as a practical proxy and, together with market-based seed sourcing, may allow multiple *Amaranthus* species to co-persist within a "black-seeded" lot, thereby inflating within-population variation. Preliminary taxonomic checks in our trial support the presence of *A. cruentus* and *A. hybridus* (with some *A. dubius*-like individuals pending confirmation), underscoring the value of integrating reproductive descriptors in future work.

Similar findings on the adaptive value of intra-population diversity have been reported in other underutilised crops (Ceccarelli & Grando, 2007; Aderibigbe *et al.*, 2022). In the absence of formal breeding programs, the ability of farmers to identify and multiply preferred phenotypes from within such diverse populations may prove critical for future resilience strategies.

Finally, this work confirms that visible homogeneity at the seed level does not preclude meaningful phenotypic variation. These findings reaffirm the importance of documenting intra-population diversity in underutilised crops. Rather than assuming phenotypic uniformity based on seed traits, a more nuanced understanding of internal structure can inform both conservation efforts and farmer-led innovation. Future research should integrate genotypic markers, multi-location trials, and a broader set of morphological and agronomic traits to better link phenotypic structure with adaptive function.

4.1 Practical implications of the result

The structured yet moderate phenotypic diversity observed in this black-seeded *Amaranthus* population offers practical entry points for decentralised crop improvement. Key vegetative traits – early plant height (CV = 39.4 %) and leaf number (CV = 27.2 %) – emerge as effective targets for farmer-led selection, given their strong correlation ($r = 0.78$) and direct relevance to leafy vegetable yields. The absence of extreme outliers and the presence of a continuous phenotypic gradient indicate that gardeners can reliably select high-performing individuals without compromising overall population resilience. The relatively compact multivariate structure observed here may reflect local seed circulation patterns and farmer preferences, but this interpretation remains hypothetical and requires confirmation through genetic and multi-site analyses. To harness this diversity, participatory trials integrated into urban gardening schemes could enable community-level identification and multiplication of desirable accessions (e.g., individuals exceeding 25 cm height by 30 days after sowing). Such farmer-driven strategies, supported by urban agroecology policies, would reinforce the adaptive value of farmer-maintained landraces in the face of climatic uncertainty. In this sense, we present this study explicitly as a baseline designed to motivate and guide subsequent multi-environment and genetic work with urban gardeners.

5 Conclusion

This exploratory study, although limited to a single growing season and one experimental site, reveals previously undocumented cryptic diversity within a black-seeded amaranth landrace. The focus on early vegetative traits provides an initial characterisation, but future research should expand to include: (a) reproductive traits, (b) multi-location trials, and (c) genetic markers, in order to better understand the heritable basis and adaptive significance of the observed variation.

The present study highlights the existence of significant phenotypic diversity within a black-seeded *Amaranthus* population cultivated in Niger under smallholder conditions. Despite visual uniformity at sowing, individual plants displayed wide variation in vegetative traits, with no evidence of discrete morphotypes or strong outliers. The dominant gradient structure captured by robust multivariate analysis reflects a cohesive but internally differentiated population.

Such intra-population heterogeneity, when maintained by farmers through informal seed systems, represents both a conservation reservoir and a practical basis for decentralised crop improvement. In contexts where formal breeding efforts are limited or absent, documenting and utilizing this diversity can support selection strategies adapted to local needs. While this study focused on a single season and two traits, its findings underscore the evolutionary potential embedded within farmer-managed landraces. Future work should aim to integrate genetic markers, environmental replication, and trait-based selection indices to better link phenotypic structure with adaptive function. Recognising and supporting these dynamics will be essential to sustaining agrobiodiversity in neglected but nutritionally valuable species like *Amaranthus*.

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Conflict of interest

The authors declare no conflict of interest.

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