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# Unlocking Ugandan pumpkin landrace diversity: integrated morphological and nutritional profiling for food security and breeding innovation

Fred Bwayo Masika<sup>a,b</sup>, Godwin Anywar<sup>c,d</sup> , Mahipal Singh Kesawat<sup>e</sup> , Gabriel Ddamulira<sup>a</sup>, Caro Kawuma<sup>f</sup>, Morgan Andama<sup>b</sup>, Charity Ajoma<sup>b</sup>, Idd Ramathan<sup>a</sup>, Otuba Moses Amugoli<sup>a</sup>, Jimmy Caku<sup>b</sup>, Titus Alica<sup>g</sup>, Ephraim Nuwamanya<sup>a,g</sup> and Arthur K. Tugume<sup>c</sup>

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## ABSTRACT

Pumpkins (*Cucurbita* spp.) are vital for food and nutritional security in Uganda, yet their full potential remains underexploited due to the limited characterization of traits. To address this, 91 landraces, 21 *Cucurbita pepo* and 70 *Cucurbita moschata* were collected from 19 districts across major agroecological zones, evaluated for morphological and nutritional diversity. Results revealed wide phenotypic variation with fruit weights ranging from 0.5 to 10.0 kg and shapes varying from discoid (L/D 0.44) to highly elongated (L/D 4.00). Fruit size and shape were independent axes of variation. Regionally, Buganda and Bunyoro landraces produced larger fruits, averaging 3.84 kg and 4.07 kg, while West Nile landraces formed a distinct nutrient-rich cluster, with high dry matter (22.8%), lipids (3.75% fresh weight (FW), fiber (3.34% FW), and carbohydrates (4.07% FW). District-specific differences were also observed, with Mpigi landraces rich in phenolics content ( $0.062 \pm 0.0023$  g GAE/100 g), and Mukono landraces rich in proteins (0.00887 g/100 g). Importantly, external morphology poorly predicted internal nutritional quality, highlighting the need for direct biochemical profiling in breeding programs. This study provides Uganda's first nationally structured dataset on pumpkin diversity, offering a scientific foundation for targeted germplasm conservation, nutrient-enriched cultivar development and policy interventions to strengthen food systems across diverse agroecological zones in Uganda.

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## SUBJECTS

Food Additives & Ingredients; Food Chemistry; Botany

## 1. Background

Pumpkins and squashes (genus *Cucurbita*, family Cucurbitaceae) are globally important vegetable crops with substantial economic and nutritional value. Their edible flesh, seeds, and leaves contribute significantly to food and nutritional security in smallholder systems (Hussain et al., 2022). *Cucurbita* species provide provitamin A carotenoids, dietary fiber, essential minerals, and unsaturated lipids concentrated in seeds, alongside diverse phytochemicals with antioxidant, anti-inflammatory, and metabolic regulatory functions. Notably, intraspecific variation in proximate composition, including protein, lipid, carbohydrate, phenolic, and antioxidant profiles, underscores their potential as functional foods (Amin et al., 2019; Kulczyński & Gramza-Michałowska, 2019).

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Beyond nutrition, *Cucurbita* spp. exhibit remarkable agroecological adaptability, thriving in marginal environments and tolerating drought and poor soils (Marimo & Otieno, 2025). They also provide ecosystem services such as soil protection and fertility enhancement through organic residue return (López-Anido et al., 2021). Their resilience and multipurpose utility position pumpkins as promising components of climate-resilient agriculture (Hernández-Rosales et al., 2020; López-Anido et al., 2021). Morphological and nutritional characterization remains central to breeding, conservation, and germplasm utilization (Hosen et al., 2021). Linking phenotypic traits with farmer and consumer preferences (Tanui et al., 2023) and applying multivariate analyses such as principal component analysis (PCA) and cluster analysis, enhances trait selection efficiency (Nakazibwe et al., 2020). While molecular markers have advanced genetic diversity studies, morphological characterization remains indispensable for preliminary screening in resource-limited contexts (Ezin et al., 2022; Kazibwe et al., 2022). Integrating morphological and nutritional profiling enables identification of landraces combining agronomic and nutritional attributes, and supports indirect selection for nutrient quality (Ezin et al., 2022).

In East Africa, pumpkin landraces exhibit marked nutritional variation. Kenyan cultivars differ in protein, carbohydrate, mineral, and antioxidant content (Karanja et al., 2014), while Ugandan species (*C. moschata*, *C. maxima*, *C. pepo*) are widely cultivated and integrated into diets and markets (Marimo & Otieno, 2025). Ugandan landraces show significant pulp variation in protein, carbohydrate, mineral, and antioxidant levels, highlighting potential for value addition and functional food development (Nakazibwe et al., 2020). The Uganda Agribusiness Alliance emphasizes commercialization opportunities for pumpkin-based products, particularly in complementary foods (Uganda Agribusiness Alliance, 2018). However, production is constrained by pests and diseases (Masika et al. 2025a; 2025b), with integrated pest management strategies recommended (Masika et al., 2025b; Masika 2023; Kumar et al. 2025).

Combined morphological and molecular analyses of 66 Ugandan pumpkin landraces revealed extensive phenotypic variation across 16 traits and an average SSR polymorphism of 79.5% (Kazibwe et al., 2022; Tanui et al., 2023). However, the weak correlations observed between morphological and molecular datasets suggest that these approaches capture distinct dimensions of genetic diversity and are not interchangeable for germplasm characterization (Kazibwe et al., 2022). Despite these advances, critical knowledge gaps remain. Specifically, the spatial distribution of morphological and nutritional diversity across agroecological zones, sub-regions, and districts has not been systematically quantified. Furthermore, integration of morphological and nutritional datasets to elucidate trait interrelationships and develop indirect selection indices is limited. Importantly, there is no comprehensive information on the best-performing landraces that could be prioritized for breeding programs such as those with high dry matter content, large fruit size, or elevated antioxidant activity. The identification and harnessing of such superior landraces across different sub-regions remains largely unexplored, constraining efforts to exploit their full potential in crop improvement.

This study addressed these gaps by characterizing 91 Ugandan pumpkin landraces, 21 *Cucurbita pepo* L. and 70 *Cucurbita moschata* Duchesne using quantitative and qualitative descriptors. Objectives were to (i) assess morphological variation, (ii) identify phenotypic and nutritional clusters, and (iii) evaluate associations between morphological traits and nutrient composition to inform indirect selection for nutrient-dense cultivars. We hypothesized that Ugandan germplasm exhibits substantial, geographically structured variation in morphological and nutritional traits; that fruit size and shape represent principal dimensions of diversity; and that nutrient composition varies regionally and correlates with specific morphological attributes.

The dataset constitutes a critical resource for advancing germplasm conservation, biofortification, and targeted breeding programs aimed at enhancing food and nutritional security in Uganda. By systematically characterizing morphological and nutritional diversity, it enables the identification of superior landraces that combine desirable agronomic traits with enhanced nutrient profiles. Such landraces distinguished by attributes including high dry matter content, large fruit size, and elevated antioxidant activity can be strategically harnessed within specific agroecological zones to maximize adaptation and productivity. Moreover, the dataset provides a framework for developing indirect selection indices that link morphological descriptors with nutritional quality, thereby improving breeding efficiency in resource-constrained environments. Integration of these findings into national crop improvement pipelines not only supports the conservation of locally adapted germplasm but also facilitates the design of

climate-resilient, nutrient-dense cultivars that can address both household dietary needs and broader market demands.

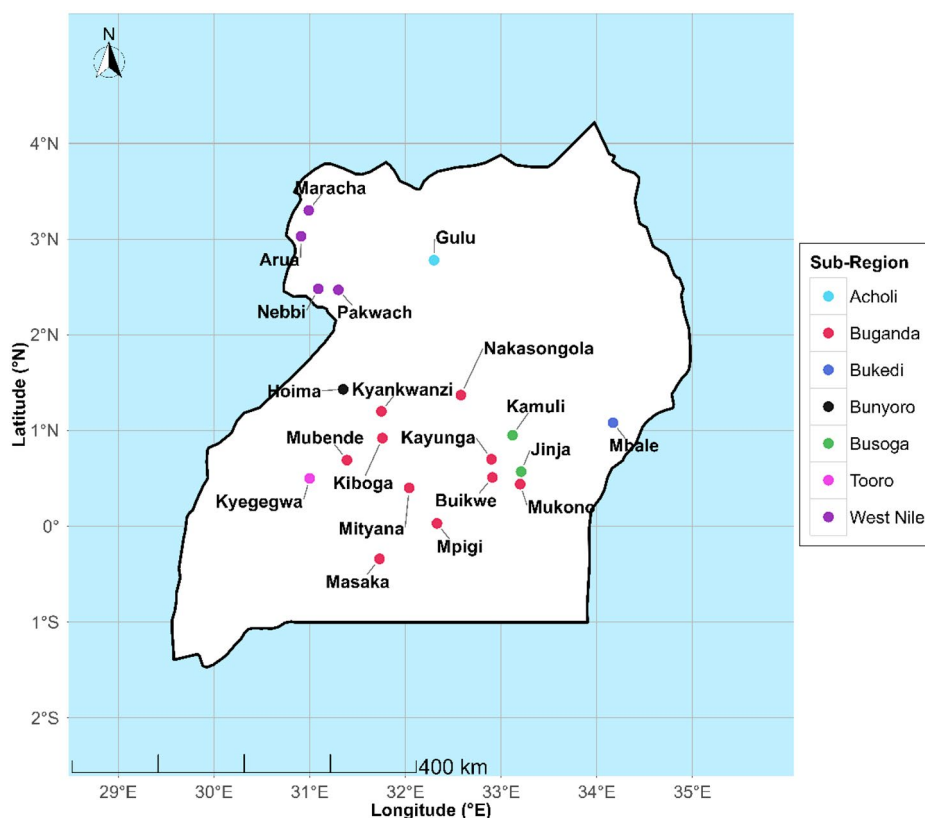
## 2. Materials and methods

### 2.1. Study area

This study was conducted across 19 districts in nine agroecological regions, including Central (Buikwe, Kiboga, Kyankwanzi, Masaka, Mityana, Mubende, Mukono, Nakasongola, Kayunga, and Mpigi districts), Elgon (Mbale district), East central (Jinja and Kamuli districts), Western (Kagadi district), South Western (Hoima district), West Nile (Arua, Maracha, Nebbi, and Pakwach districts), and Acholi (Gulu district) (Figure 1). The selected districts are nested within seven key sub-regions: Buganda (Mpigi, Masaka, Kayunga, Kampala, Kiboga, Mubende, Mityana, Mukono), Busoga (Jinja, Kamuli), Bukedi (Mbale), Bunyoro (Kagadi, Hoima), West Nile (Nebbi, Arua, Pakwach, Maracha), Tooro (Kyegegwa), and Acholi (Gulu) representing important areas for pumpkin growing as described by Masika et al. (2023b).

### 2.2. Field sampling

Pumpkin fruit sampling targeted physiologically mature fruits, either harvested directly from farmers' fields in accordance with the criteria outlined by Loy (2004) or procured from local markets within the surveyed sub-regions. Sampling was conducted in September 2024, coinciding with the peak harvest season. To minimize postharvest nutritional losses, only market fruits stored for fewer than seven days were selected. Farms with physiologically mature pumpkins were purposively chosen, and unique landraces were prioritized to ensure representation of genetic diversity. Within each sub-region, representative districts and markets were selected based on the relative importance of pumpkin production, thereby focusing on areas where pumpkins constitute a significant component of the agricultural system (Masika et al., 2017, 2023b).



**Figure 1.** Sample collection points from the different sub-regions in Uganda. Map created by author using R software version 4.5.1, using ggplot2, sf, and rnaturalearth packages.

For each landrace, three large, physiologically mature fruits were obtained either directly from farmers or from traders in local markets. Each fruit was assigned a unique identifier to enable traceability, capturing information on the farmer (where available), market, district, and sub-region, and all samples were georeferenced. Comprehensive details of the collected landraces are presented in Table 1. Following collection, fruits were transported under controlled conditions to the Food and Nutrition Laboratory at the National Crops Resources Research Institute (NaCRRI), under the National Agricultural Research Organization (NARO), Namulonge, for detailed morphological and nutritional characterization.

**Table 1.** Information on the pumpkin landraces analysed in the study.

Landrace ID	Species	Sub-region of collection	District of collection	Place/market
P001	<i>Cucurbita moschata</i>	West Nile	Nebbi	Main market
P002	<i>Cucurbita Pepo</i>	West Nile	Nebbi	Main market
P003	<i>Cucurbita moschata</i>	West Nile	Maracha	Michu
P004	<i>Cucurbita moschata</i>	West Nile	Maracha	Rucua maracha
P005	<i>Cucurbita Pepo</i>	West Nile	Nebbi	Central market
P006	<i>Cucurbita Pepo</i>	West Nile	Arua	Main market
P007	<i>Cucurbita Pepo</i>	West Nile	Arua	Aivu
P008	<i>Cucurbita moschata</i>	West Nile	Nebbi	Main market
P009	<i>Cucurbita moschata</i>	West Nile	Nebbi	Main market
P010	<i>Cucurbita moschata</i>	West Nile	Nebbi	Puyang
P011	<i>Cucurbita moschata</i>	West Nile	Nebbi	Main Market
P012	<i>Cucurbita moschata</i>	West Nile	Pakwach	Puyang
P013	<i>Cucurbita moschata</i>	West Nile	Pakwach	Main Market
P014	<i>Cucurbita moschata</i>	West Nile	Pakwach	Main market
P015	<i>Cucurbita moschata</i>	Acholi	Gulu	Custom Corner
P016	<i>Cucurbita moschata</i>	Acholi	Gulu	Main market
P017	<i>Cucurbita moschata</i>	Bukedi	Mbale	kikundu 'Kabyanga'
P018	<i>Cucurbita Pepo</i>	Bukedi	Mbale	Main market
P019	<i>Cucurbita Pepo</i>	Bukedi	Mbale	Main market
P020	<i>Cucurbita Pepo</i>	Bukedi	Mbale	Main market
P021	<i>Cucurbita moschata</i>	Bukedi	Mbale	Main market
P022	<i>Cucurbita moschata</i>	Bukedi	Mbale	Main market
P023	<i>Cucurbita moschata</i>	Bukedi	Mbale	Kabimbiri
P024	<i>Cucurbita moschata</i>	Bukedi	Mbale	Nkoma
P025	<i>Cucurbita moschata</i>	Busoga	Jinja	Ambakot
P026	<i>Cucurbita moschata</i>	Busoga	Jinja	Ambakot
P027	<i>Cucurbita moschata</i>	Busoga	Jinja	Central
P028	<i>Cucurbita moschata</i>	Busoga	Jinja	Ambakot
P029	<i>Cucurbita moschata</i>	Busoga	Jinja	Central Market
P030	<i>Cucurbita moschata</i>	Busoga	Jinja	Ambakot
P031	<i>Cucurbita moschata</i>	Busoga	Jinja	Ambakot
P032	<i>Cucurbita moschata</i>	Busoga	Jinja	Ambakot
P033	<i>Cucurbita moschata</i>	Busoga	Kamuli	Central market
P034	<i>Cucurbita moschata</i>	Busoga	Kamuli	Central
P035	<i>Cucurbita moschata</i>	Busoga	Kamuli	Central
P036	<i>Cucurbita moschata</i>	Busoga	Kamuli	Central market
P037	<i>Cucurbita moschata</i>	Buganda	Mukono	Kabimbiri
P038	<i>Cucurbita moschata</i>	Buganda	Bukomero	Bukomero
P039	<i>Cucurbita moschata</i>	Buganda	Kampala	Nakawa Market
P040	<i>Cucurbita Pepo</i>	Buganda	Kampala	Nakawa Market
P041	<i>Cucurbita Pepo</i>	Buganda	Kampala	Kalerwe market
P042	<i>Cucurbita Pepo</i>	Buganda	Kampala	Busega market
P043	<i>Cucurbita Pepo</i>	Buganda	Kampala	Nakawa
P044	<i>Cucurbita Pepo</i>	Buganda	Kampala	Nakawa market
P045	<i>Cucurbita moschata</i>	Buganda	Kampala	Nakawa
P046	<i>Cucurbita moschata</i>	Buganda	Kayunga	Main market
P047	<i>Cucurbita moschata</i>	Buganda	Kayunga	Ntooke
P048	<i>Cucurbita moschata</i>	Buganda	Kayunga	Market
P049	<i>Cucurbita moschata</i>	Buganda	Kayunga	Market
P050	<i>Cucurbita moschata</i>	Buganda	Kiboga	Bukomero
P051	<i>Cucurbita moschata</i>	Buganda	Kiboga	Lwamata
P052	<i>Cucurbita moschata</i>	Buganda	Kiboga	Bukomero
P053	<i>Cucurbita moschata</i>	Buganda	Kiboga	Bukomero
P054	<i>Cucurbita moschata</i>	Buganda	Kiboga	Kiboga market
P055	<i>Cucurbita Pepo</i>	Buganda	Masaka	Nyendo market
P056	<i>Cucurbita Pepo</i>	Buganda	Masaka	Busega market
P057	<i>Cucurbita Pepo</i>	Buganda	Masaka	Nyendo market
P058	<i>Cucurbita Pepo</i>	Buganda	Masaka	Central
P059	<i>Cucurbita Pepo</i>	Buganda	Masaka	Central
P060	<i>Cucurbita moschata</i>	Buganda	Mityana	Market

(Continued)

Table 1. Continued.

Landrace ID	Species	Sub-region of collection	District of collection	Place/market
P061	<i>Cucurbita moschata</i>	Buganda	Mityana	Mityana market
P062	<i>Cucurbita moschata</i>	Buganda	Mityana	Nyendo
P063	<i>Cucurbita moschata</i>	Buganda	Mpigi	Nkozi, Nabyewanga
P064	<i>Cucurbita moschata</i>	Buganda	Mpigi	Nkozi
P065	<i>Cucurbita moschata</i>	Buganda	Mubende	Lusalali
P066	<i>Cucurbita moschata</i>	Buganda	Mubende	Mubende market
P067	<i>Cucurbita moschata</i>	Buganda	Mubende	Lusalia
P068	<i>Cucurbita Pepo</i>	Buganda	Mukono	Kabimbiri
P069	<i>Cucurbita moschata</i>	Buganda	Mukono	Kiko Market
P070	<i>Cucurbita moschata</i>	Buganda	Kiboga	Lwamata
P071	<i>Cucurbita moschata</i>	Buganda	Kampala	Kalerwe market
P072	<i>Cucurbita moschata</i>	Buganda	Mityana	Mityana Market
P073	<i>Cucurbita moschata</i>	Buganda	Mukono	Butternut
P074	<i>Cucurbita moschata</i>	Buganda	Kampala	Busega market
P075	<i>Cucurbita moschata</i>	Buganda	Kiboga	Cain market
P076	<i>Cucurbita moschata</i>	Buganda	Masaka	Central market
P077	<i>Cucurbita moschata</i>	Buganda	Masaka	Punyang
P078	<i>Cucurbita moschata</i>	Buganda	Mubende	Central market
P079	<i>Cucurbita moschata</i>	Buganda	Mubende	Kabimbiri
P080	<i>Cucurbita moschata</i>	Buganda	Kayunga	Ntooke
P081	<i>Cucurbita moschata</i>	Tooro	Kyegegwa	Central Market
P082	<i>Cucurbita moschata</i>	Tooro	Kyegegwa	Central market
P083	<i>Cucurbita Pepo</i>	Bunyoro	Hoima	Nyendo
P084	<i>Cucurbita Pepo</i>	Bunyoro	Hoima	Central market
P085	<i>Cucurbita moschata</i>	Bunyoro	Kagadi	Central Market
P086	<i>Cucurbita Pepo</i>	Bunyoro	Kagadi	Central market
P087	<i>Cucurbita moschata</i>	Bunyoro	Kagadi	Central market
P088	<i>Cucurbita moschata</i>	Bunyoro	Kagadi	Central market
P089	<i>Cucurbita moschata</i>	Bunyoro	Kagadi	Central market
P090	<i>Cucurbita moschata</i>	Bunyoro	Kagadi	Central market
P091	<i>Cucurbita moschata</i>	Bunyoro	Kagadi	Central market

### 2.3. Morphological characterization

Phenotypic evaluation of cultivated *Cucurbita* spp. 21 *Cucurbita pepo* L. and 70 *Cucurbita moschata* Duchesne was conducted using the standardized descriptor lists established by the European Cooperative Programme for Plant Genetic Resources (ECPGR, 2008), with minimal adjustments, as shown in Table S1. To ensure robustness, reliability, and reproducibility, each morphological trait was measured in technical triplicate or according to prescribed standards across all landraces. Individual fruits were treated as independent biological replicates, thereby capturing intra-accession variability. Variation was systematically assessed within and across landraces, sub-regions, and districts to provide a comprehensive representation of morphological diversity.

#### 2.3.1. Fruit trait measurements

The structural fruit traits analyzed included fruit length, diameter, shape ratio (length-to-diameter ratio), flesh thickness, flower scar diameter, fruit weight, fruit neck length, and fruit volume. Fruit quality traits encompassed the position of the broadest part of the fruit, overall shape, neck presence, degree of curving, stem-end and blossom-end profiles, presence of grooves, primary and secondary skin color, color intensity, waxiness, surface warts, and flesh color. Seed traits were assessed in terms of length, width, and color. To characterize phenotypic diversity across sub-regions, descriptive statistics (mean, standard deviation, range, median, and coefficient of variation) were applied, complemented by frequency distributions and proportional analyses.

Fruit length, diameter, and flesh thickness, were quantified using precision digital calipers (Mitutoyo Corp., Japan; accuracy  $\pm 0.01$  mm). Fruit weight was measured using a calibrated digital bench scale (Ohaus Corp., USA; capacity 15 kg; precision  $\pm 0.1$  g). The fruit shape index was computed as the ratio of fruit length to diameter (L/D), providing a standardized metric for shape classification. For seed phenotypic trait evaluation, a random subsample of 10 air-dried seeds per accession was selected, and seed length and width were measured using the same calipers as above as described by Kurum (2012). Colour determination was standardized using the Royal Horticultural Society (RHS) colour chart, 6<sup>th</sup> edition (Royal Horticultural Society, 2015), and descriptive colour terms were harmonized post-evaluation to ensure consistency across landraces (Ferriol et al., 2004; Murovec, 2015).

## 2.4. Sample preparation for nutritional analysis

Representative mesocarp portions, excluding the fruit cover and seed cavity, were collected for each accession for nutritional profiling. Samples were thoroughly rinsed with distilled water to eliminate surface contaminants, and then sliced into uniform segments approximately 1 cm thick. To preserve thermolabile compounds such as carotenoids and vitamin C, the slices were oven-dried at 60°C until a constant weight was achieved (Jahan et al., 2023; Rodríguez-Amaya, 2016). The dried tissues were subsequently milled into a fine, homogeneous powder using a laboratory analytical mill (IKA® A11 Basic, Germany). The powders were stored in airtight, light-impermeable polyethylene containers at -20°C to maintain chemical integrity and minimize oxidative degradation before analysis (Batool et al., 2022; Pujapanda, 2023).

### 2.4.1. Proximate nutritional composition determination

All proximate nutritional analyses were performed in triplicate, and results were expressed on a fresh weight (FW) basis unless otherwise specified. Analytical-grade reagents and solvents ( $\geq 99\%$  purity) were used throughout to ensure accuracy and reproducibility of data. The dry matter content was determined gravimetrically by weighing 2 g of fresh mesocarp and drying it in a forced-air laboratory oven (Memmert UN55, Germany) at 105°C until a constant weight was achieved, following AOAC Official Method 934.01 (AOAC, 2019). Samples were cooled in a desiccator before each weighing. The moisture content was calculated as the percentage of weight loss relative to the initial sample mass using the formula:

$$\text{percentage moisture} = \frac{(\text{initial weight} - \text{dry weight})}{\text{initial weight}} \times 100$$

This method provides a reliable estimate of water content (Pujapanda, 2023). The lipid content was determined using a Soxhlet extraction system (Buchi B-811, Switzerland), with petroleum ether (boiling range 40–60°C; Merck, Germany) as the solvent, in accordance with the Association of Official Analytical Collaboration (AOAC) official method 920.39 (AOAC, 2019). Following exhaustive extraction, the solvent was removed by rotary evaporation (Heidolph, Germany), and the lipid residue was quantitatively weighed (Rodríguez-Amaya, 2016). Total lipid content was expressed as a percentage of the fresh sample weight, providing a reliable estimate of mesocarp lipid concentration.

Crude protein content was quantified using the Kjeldahl method, employing a Kjeldahl nitrogen analyzer (VELP Scientifica, Italy) in accordance with AOAC official method 978.04 (AOAC, 2019). Total nitrogen concentration was measured and converted to protein content using a nitrogen-to-protein conversion factor of 6.25 (Jahan et al., 2023). The crude fiber content was determined using a standardized sequential acid-alkali digestion protocol described by Pujapanda (2023).

Ash content was determined by dry ashing 2 g of powdered pumpkin mesocarp at 550°C for 6 hours in a programmable muffle furnace (Carbolite ELF 11/6, UK), which ensured precise temperature control and uniform heat distribution (AOAC, 2019). Total carbohydrate content was calculated using the formula:  $[100 - (\text{water content} + \text{protein} + \text{lipid} + \text{ash} + \text{fiber})]$  (Jahan et al., 2023). The total sugar content was quantified using the Lane-Eynon titrimetric method, which exploits the reducing properties of sugars (AOAC, 2019; Jahan et al., 2023; Pujapanda, 2023; Rodríguez-Amaya, 2016).

## 2.5. Bioactive compounds and antioxidant activity

### 2.5.1. Determination of total phenolic content (TPC)

The total phenolic content (TPC) of pumpkin mesocarp extracts was quantified using the Folin–Ciocalteu colorimetric assay, following the protocol originally described by Prieto et al. (1999) and subsequently adapted by Jahan et al. (2023) and Pujapanda (2023). In brief, 0.5 mL of the 80% (v/v) methanolic extract was combined with 2.5 mL of Folin–Ciocalteu reagent (diluted 1:10 with distilled water) and 2 mL of 7.5% (w/v) sodium carbonate solution. The mixture was incubated in the dark at ambient temperature for 30 minutes to allow colour development. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu UV-1900, Japan) equipped with 1 cm path length quartz cuvettes. The TPC was

calculated from a gallic acid calibration curve ( $R^2 \geq 0.99$ ) and expressed as milligrams of gallic acid equivalents per kilogram of fresh weight (mg GAE/kg FW).

### 2.5.2. Assessment of antioxidant activity via DPPH radical scavenging assay

The antioxidant potential of the pumpkin mesocarp extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, as originally described by Braca et al. (2001) and subsequently modified by Jahan et al. (2023) and Pujapanda (2023). Absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1900, Japan). The percentage of DPPH radical inhibition was calculated using the formula:

$$\% = \frac{[A_0 - A_1]}{[A_0]} \times 100$$

where  $A_0$  and  $A_1$  are the absorbance values of the control and sample, respectively.

## 2.6. Data analysis

All statistical analyses were performed using R software version 4.5.1 (R Core Team, 2025). Data were checked for normality and homogeneity of variances using the Shapiro–Wilk and Levene’s tests, respectively, before parametric analyses.

### 2.6.1. Analysis of morphological traits

Descriptive statistics (mean, standard deviation, range, median, and coefficient of variation) were computed for ten quantitative fruit and seed traits, namely, fruit length, neck length, fruit diameter, fruit shape ratio (length-to-diameter), groove distance, flesh thickness, flower scar diameter, fruit weight, seed length, seed width, and fruit volume. For 16 categorical morphological fruit traits; peduncle size, position of the broadest part, fruit shape, neck presence, fruit curving, stem end profile, blossom end profile, fruit grooves, main skin colour, minor skin colour, skin colour intensity, skin waxiness, fruit warts, flesh colour, and seed coat colour frequency distributions and proportional analyses were calculated to characterize phenotypic diversity across sub-regions.

Morphological fruit variation across sub-regions was evaluated using one-way Analysis of Variance (ANOVA). For the flesh thickness, wherever ANOVA assumptions were violated, the non-parametric Kruskal–Wallis test was applied. Models showing a statistically significant effect ( $p < 0.05$ ), such as for fruit weight, post-hoc pairwise comparisons were conducted using Tukey’s Honest Significant Difference (HSD) test.

The linear relationships among all ten quantitative morphological traits were assessed using Pearson correlation analysis. To determine the underlying structure of morphological fruit variation, a Principal Component Analysis (PCA) was performed on the standardized continuous variables. Phenotypic clustering of landraces was visualized using hierarchical agglomerative clustering based on Ward’s linkage method and Euclidean distance.

### 2.6.2. Analysis of nutritional and phytochemical data

Descriptive statistics (mean, standard deviation, range, CV) were calculated for nine nutritional parameters, including total phenolic content (TPC), protein content, antioxidant activity (DPPH radical scavenging), dry matter content, water content, lipid content, fiber content, total carbohydrate content, and sugar content. Spatial variation in these nine nutritional parameters was analyzed across three hierarchical geographical levels (region, sub-region, and district) using one-way ANOVA. The interrelationships among all nine nutritional and bioactive parameters were explored using Pearson correlation analysis. The associations between three key external morphological traits (fruit length, fruit diameter, and fruit shape ratio) and three internal nutritional components (TPC, protein content, and antioxidant activity) were quantified using Pearson correlation analysis to assess the potential for indirect selection. All figures, including distribution plots (boxplots, violin plots), correlation matrices, PCA biplots, and

maps, were generated using the *ggplot2*, *corrplot*, *factoextra*, *sf*, and *rnatuarearth* packages in R to enhance data visualization, interpretability and pattern recognition.

### 3. Results

#### 3.1. Morphological variation

The collected germplasm (21 *Cucurbita pepo* L. and 70 *Cucurbita moschata* Duchesne) exhibited substantial variability in fruit morphological traits, reflecting a broad spectrum of phenotypic diversity (Table 2, Figure 2). Fruit length ranged from 10.5 to 45.6 cm (mean =  $22.14 \pm 7.96$  cm), fruit diameter from 3.50 to 55.00 cm (mean =  $19.36 \pm 6.34$  cm), and the length-to-diameter ratio (L/D) from 0.44 to 4.00 (mean =  $1.26 \pm 0.64$ ), indicating a wide array of fruit shapes from spherical to elongated forms. Fruit weight varied between 0.5 and 10.0 kg (mean =  $3.39 \pm 1.7$  kg), while flesh thickness ranged from 1.40 to 5.5 cm (mean =  $3.23 \pm 0.83$  cm), demonstrating notable differences in size and edible yield potential. Seed dimensions exhibited moderate variation, with lengths ranging from 1.00 to 3.00 cm (mean =  $1.45 \pm 0.26$  cm) and widths ranging from 0.20 to 1.70 cm (mean =  $0.80 \pm 0.16$  cm). Fruit volume exhibited the highest degree of variability (CV = 77.1%), ranging from 359.20 to 18,394.60 cm<sup>3</sup> (mean =  $5,659.00 \pm 4,360.00$  cm<sup>3</sup>).

##### 3.1.1. Fruit weight distribution across sub-regions

There was significant regional variation in size, shape, and mean fruit weight, ranging from 2.14 to 4.07 kg (Table 3; Figure 2). The heaviest fruits were recorded in Bunyoro (N=9;  $4.07 \pm 1.62$  kg), exhibiting the highest within-region variability (range: 2.0–7.0 kg; SD = 1.62 kg), and Tooro (N=2;  $4.00 \pm 1.41$  kg). Bunyoro and Buganda (each N=41) showed a comparable mean weight ( $3.84 \pm 1.97$  kg), the broadest range (0.5–10.0 kg), reflecting substantial heterogeneity. In contrast, Busoga (N=13) and West Nile (N=15) produced lighter fruits ( $2.75 \pm 0.67$  kg and  $2.14 \pm 0.89$  kg, respectively) with lower intra-regional variation ( $p=0.044$ ). Post-hoc analysis using Tukey's HSD test identified a single statistically significant pairwise difference ( $p=0.040$ ) with all other comparisons not statistically significant.

District-level distribution of individual fruit weights, ranked by median values (Figure 3), revealed clear geographical patterns. Districts within the Buganda and Bunyoro sub-regions exhibited the highest median fruit weights, while all West Nile districts clustered at the lower end of the distribution, characterised by uniformly low median weights and limited variability.

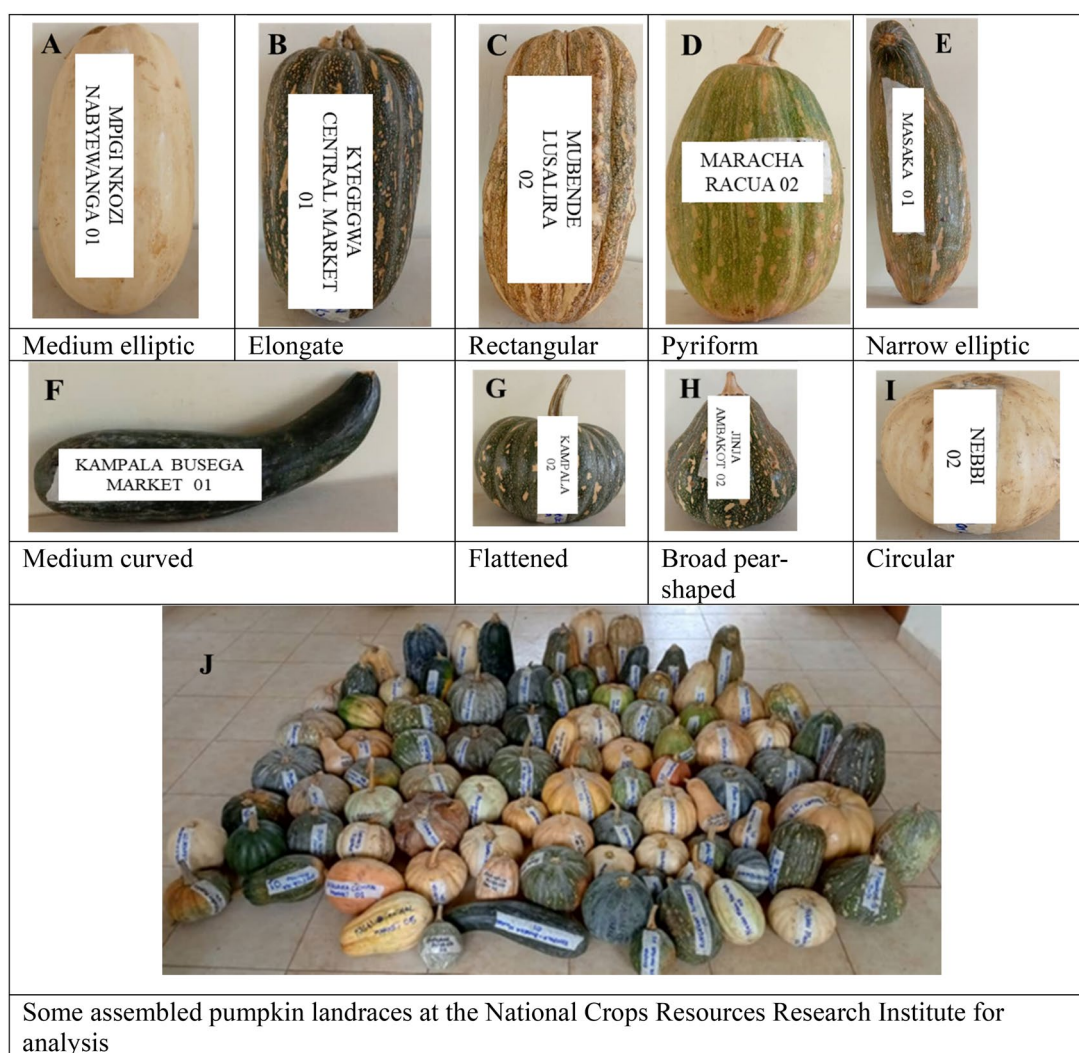
##### 3.1.2. Fruit size category proportions distribution

Generally, more than 50% of pumpkin fruits sampled from all the sub-regions were medium-sized. Fruit size-class distribution revealed marked compositional differences among sub-regions (Figure 3). West Nile (31%), and Bukedi (25%) had the highest percentage of small-sized fruits, whereas Bunyoro (33%), Tooro (50%), and Buganda (32%) yielded the highest percentage of large-sized fruits. All the pumpkin fruits sampled from Acholi were medium-sized.

Although sub-regional patterns in fruit size were generally consistent, district-level analysis revealed notable deviations. Districts such as Hoima, Mpigi, and Mityana produced exclusively large fruits, while Arua

**Table 2.** Descriptive statistics of fruit length, diameter, ratio, groove distance, flesh thickness, flower scar diameter, weight, seed length, width, and fruit volume in pumpkin landraces.

Parameter	Mean $\pm$ SD	Min	Max	Median
1. Fruit length (cm)	$22.14 \pm 7.96$	10.50	45.60	20.50
2. Fruit diameter (cm)	$19.36 \pm 6.34$	3.50	55.00	19.50
3. Fruit ratio (L/D)	$1.26 \pm 0.64$	0.44	4.00	1.07
4. Groove distance (cm)	$4.53 \pm 1.13$	2.00	7.00	4.30
5. Flesh thickness (cm)	$3.23 \pm 0.83$	1.40	5.50	3.20
6. Flower scar diameter (cm)	$1.22 \pm 0.66$	0.50	5.00	1.10
7. Fruit weight (kg)	$3.39 \pm 1.70$	0.50	10.00	3.00
8. Seed length (cm)	$1.45 \pm 0.26$	1.00	3.00	1.40
9. Seed width (cm)	$0.80 \pm 0.16$	0.20	1.70	0.80
10. Fruit volume (cm <sup>3</sup> )	$5,658.98 \pm 4,359.99$	359.19	18,394.55	4,272.99



**Figure 2.** Representative pumpkin landraces sampled from different regions during the study.

**Table 3.** Fruit weight statistics by sub-region.

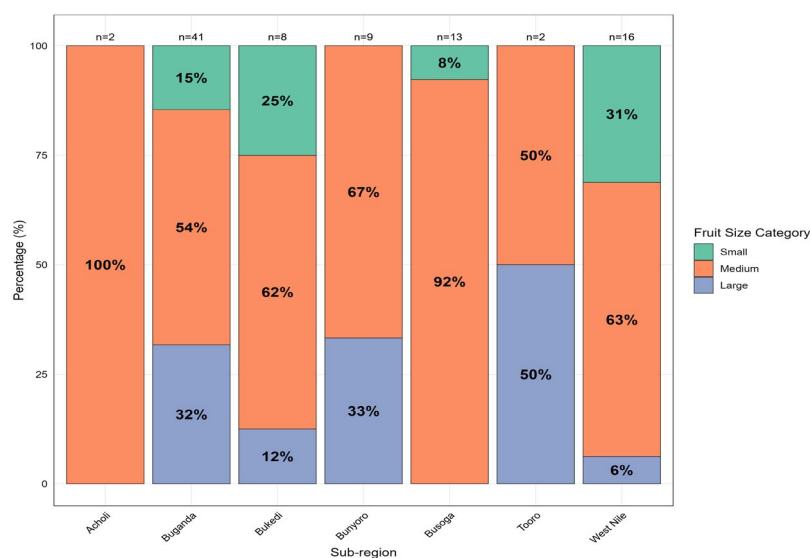
Sub-region	N	Mean (kg)	SD	SE	Min (kg)	Max (kg)	ANOVA $p$ -value	Post-hoc $p$ -values
1. Bunyoro	9	4.07	1.62	0.54	2	7	0.044	NS.
2. Tooro	2	4	1.41	1	3	5		NS.
3. Buganda	41	3.84	1.97	0.31	0.5	10		West Nile ( $p=0.040$ )
4. Acholi	2	3.75	0.35	0.25	3.5	4		NS.
5. Bukedi	8	3.24	1.53	0.54	1.5	6		NS.
6. Busoga	13	2.75	0.67	0.19	1.5	4		NS.
7. West Nile	16	2.35	1.2	0.3	0.9	5.5		Buganda ( $p=0.040$ )

Note: NS- No significant difference.

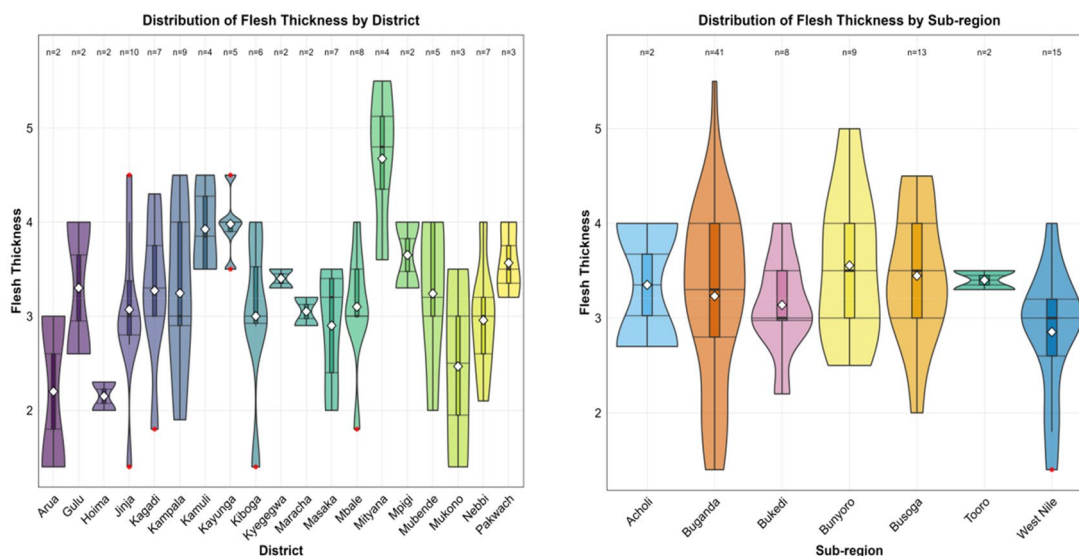
and Kamuli yielded only small-sized fruits. In contrast, Kiboga and Mubende exhibited a balanced distribution across all three size classes (Supplementary Figure SF1). These findings greatly influence district-specific factors on harvest composition, despite Buganda sub-region being a strong overall predictor of fruit size.

### 3.1.3. Flesh thickness distribution among sub-regions and districts

District-level analysis of flesh thickness revealed substantial heterogeneity, with several districts exhibiting multi-modal distributions suggestive of distinct varietal groups (Figure 4). Aggregation by sub-region smoothed these patterns, revealing broader geographical trends, whereas Bunyoro and Tooro showed higher median values, while West Nile and Busoga consistently exhibited lower thickness, although they were not statistically significant.



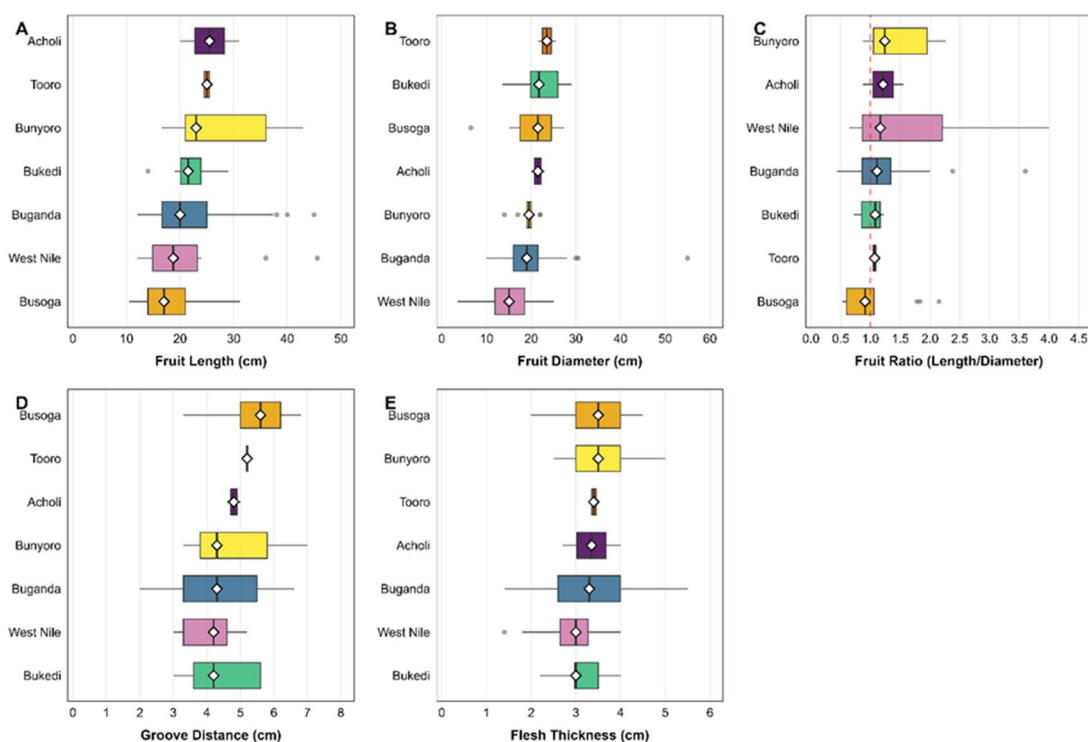
**Figure 3.** Fruit size category proportions by sub-region. Proportional representation of small (<2kg), medium (2–5kg), and large (>5kg) fruit size categories across sampled sub-regions.



**Figure 4.** Violin plots with boxplots showing flesh thickness distribution across districts and sub-regions.

### 3.1.4. Variation in pumpkin fruit length-diameter ratio, groove distance and flesh thickness across sub-regions and districts

Fruit morphology exhibited pronounced regional and district-level variation. At the sub-regional scale, Acholi consistently produced the longest fruits, whereas Busoga yielded the shortest (Figure 5(A)). Tooro and Bukedi recorded the widest fruits, contrasting with the narrowest observed in West Nile and Buganda (Figure 5(B)). Elongation ratios were highest in Bunyoro and Acholi, reflecting markedly elongated forms, while Busoga displayed the lowest ratios, indicative of flattened, oblate morphotypes (Figure 5(C)). Groove spacing was widest in Busoga and Tooro but consistently narrowest in Bukedi (Figure 5(D)). Flesh thickness was greatest in Busoga and Bunyoro, suggesting superior edible portions, whereas Bukedi and West Nile exhibited the thinnest flesh (Figure 5(E)). At the district level, fruit length varied substantially, with Mpigi and Kyegegwa producing the longest fruits, and Hoima and Pakwach the shortest. Shape ratios were highest in Mpigi and Hoima, denoting elongated morphotypes, while Kamuli and Jinja displayed the lowest ratios, consistent with discoid forms. Flesh thickness was highest in Mpigi, Kyegegwa, and Kamuli but low in Hoima and Arua. Groove spacing was widest in Kamuli and Jinja, and narrowest



**Figure 5.** Sub-regional variation in pumpkin fruit characteristics. Horizontal box plots illustrate the distribution of key fruit traits across surveyed districts: (A) fruit length, (B) fruit diameter, (C) fruit length-to-diameter ratio, (D) groove distance, and (E) flesh thickness. Each plot displays the median (central line), interquartile range (box), and overall data spread.

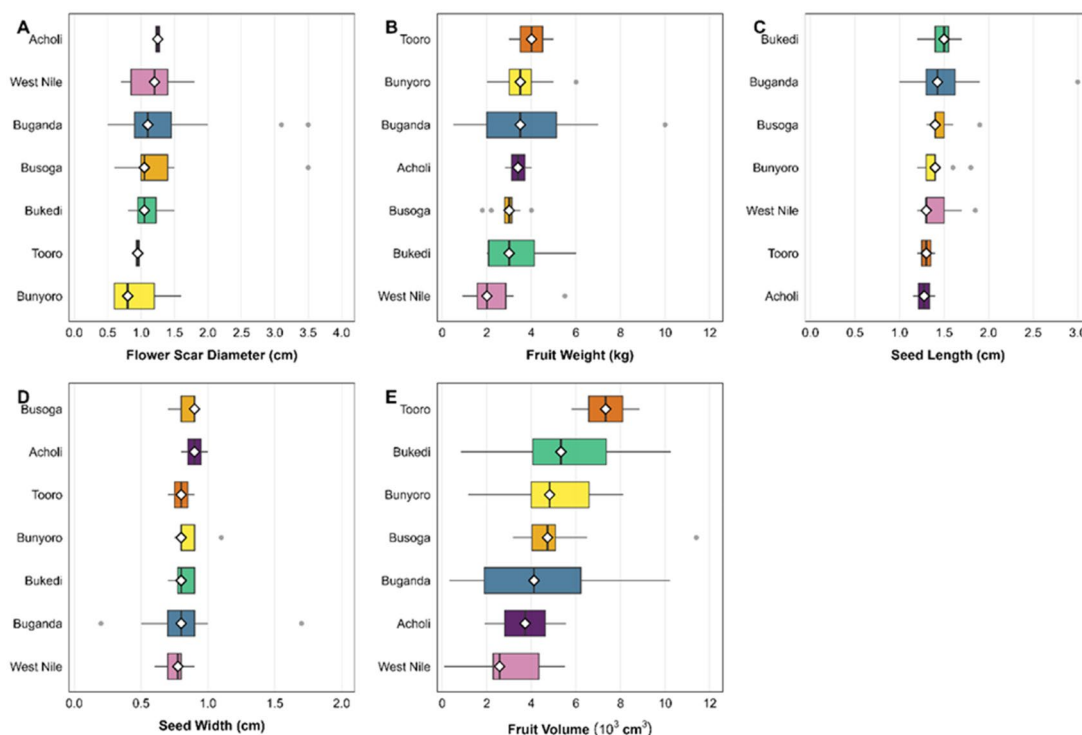
in Hoima and Kyegegwa. Collectively, these patterns highlight distinct local morphotypes, with districts such as Mpigi consistently associated with favorable traits, including increased fruit length and flesh thickness (Supplementary Figure SF2).

### 3.1.5. Inter-regional and district variation in pumpkin morphological traits: flower scar diameter, fruit weight, seed dimensions, and fruit volume

Fruit morphological traits exhibited variation across sub-regions and districts. Flower scar diameter, a trait associated with fruit attachment and pathogen ingress, was largest in Acholi and smallest in Bunyoro (Figure 6(A)). Median fruit weight was highest in Tooro and Bunyoro, highlighting their superior yield potential, whereas Bukedi and West Nile consistently produced the lowest weights (Figure 6(B)). Seed length peaked in Bukedi but was shortest in Acholi (Figure 6(C)), while seed width was greatest in Busoga and narrowest in West Nile (Figure 6(D)). Fruit volume was maximized in Tooro and Bukedi, contrasting with the smallest volumes recorded in Acholi and West Nile (Figure 6(E)). At the district level, flower scar diameter varied markedly, with Mpigi landraces showing the largest values and Hoima and Arua the smallest. Fruit weight was highest in Mpigi, Mityana, and Masaka, confirming these as high-yielding zones, while Arua, Nebbi, and Pakwach registered the lowest weights. Seed morphology also differed substantially: seed length was greatest in Mpigi and Kyegegwa but smallest in Hoima and Pakwach, whereas seed width was largest in Mpigi and Kayunga and narrowest in Arua and Gulu. Collectively, these findings underscore distinct regional and district-level morphotypes, with Mpigi and Tooro consistently associated with favorable yield and seed traits (Supplementary Figure SF3)

### 3.1.6. Identification of top-performing regions and districts by average fruit weight, mean length and mean flesh thickness

At regional level, Bunyoro sub-region emerged as the overall top performer, with the highest mean fruit weight (4.07 kg), greatest fruit length (27.5 cm), and thickest flesh (3.56 cm). Tooro ranked second, with comparable fruit



**Figure 6.** Sub-regional variation in pumpkin fruit and seed characteristics. Horizontal box plots illustrate the distribution of key harvest and seed traits across surveyed districts: (A) flower scar diameter, (B) fruit weight, (C) seed length, (D) seed width, and (E) fruit volume. Each plot displays the median (central line), interquartile range (box), and overall data spread.

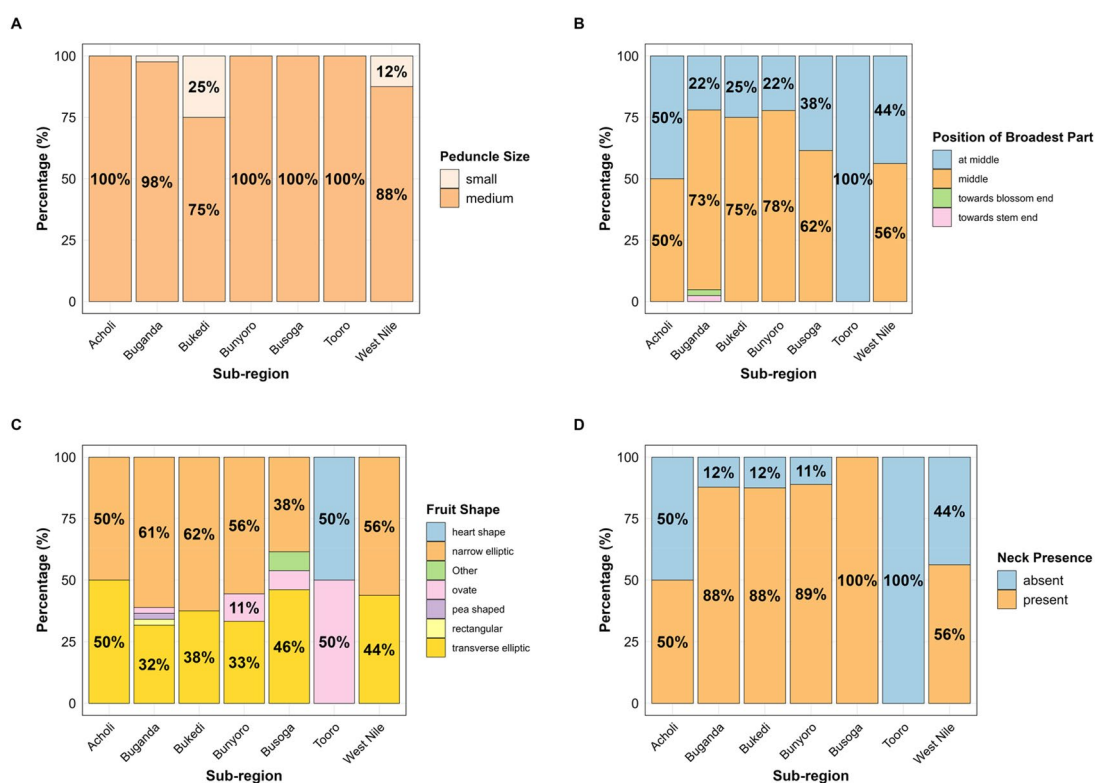
**Table 4.** Regional performance and best 10 districts by average fruit weight, mean length and mean flesh thickness.

Sub-region	District/Sub-region name	n	Mean weight (kg)	Mean length (cm)	Mean flesh thickness (cm)
Buganda	Mpigi	2	6.5	38.65	3.65
Buganda	Mityana	4	5.63	20.5	4.68
Buganda	Masaka	7	4.54	25.67	3.01
Buganda	Kayunga	5	4.06	24.58	3.98
Bunyoro	Kagadi	7	4	27.93	3.27
Bunyoro	Kyegegwa	2	4	25	3.4
Buganda	Kiboga	6	3.58	19.1	3.45
Buganda	Mubende	5	3.4	21.1	3.48
Acholi	Gulu	2	3.4	18.05	3.3
Bukedi	Mbale	8	3.33	21.44	3.31
Bunyoro	Bunyoro	9	4.07	27.5	3.56
Tooro	Tooro	2	4	25	3.4
Buganda	Buganda	41	3.84	22.28	3.2
Acholi	Acholi	2	3.75	25.5	3.35
Bukedi	Bukedi	8	3.24	21.75	3.14

weight (4.00kg), length (25.0cm), and flesh thickness (3.40cm). Buganda followed with moderate values (3.84kg, 22.28cm, 3.20cm), while Acholi produced the second-longest fruits (25.5cm) despite a smaller sample size, ranking fourth in weight and third in flesh thickness. Bukedi completed the top five, consistently recording the lowest values across traits. District-level analyses revealed distinct sources of promising germplasm (Table 4). Mpigi produced the heaviest fruits (6.50kg), followed by Mityana (5.63kg) and Masaka (4.54kg). Flesh thickness rankings differed, with Mityana leading (4.68cm), ahead of Kayunga (3.98cm), Kampala (3.83cm), and Mpigi (3.65cm). These findings demonstrate that while sub-regional trends are evident, superior trait performance is often district-specific, reflecting localized genetic diversity and agroecological adaptation.

### 3.1.7. Sub-regional variation in pumpkin peduncle size, position of the broadest part, fruit shape, and neck presence

Peduncle size (Figure 7(A)) was predominantly medium across all sub-regions, with complete dominance (100%) in Acholi, Bunyoro, Busoga, and Tooro. Small peduncles were observed at lower frequencies in



**Figure 7.** Sub-regional variation in pumpkin fruit morphology: (A) Peduncle size, (B) position of the broadest part, (C) fruit shape, and (D) neck presence. Bar graphs depict the percentage distribution of each trait across sub-regions.

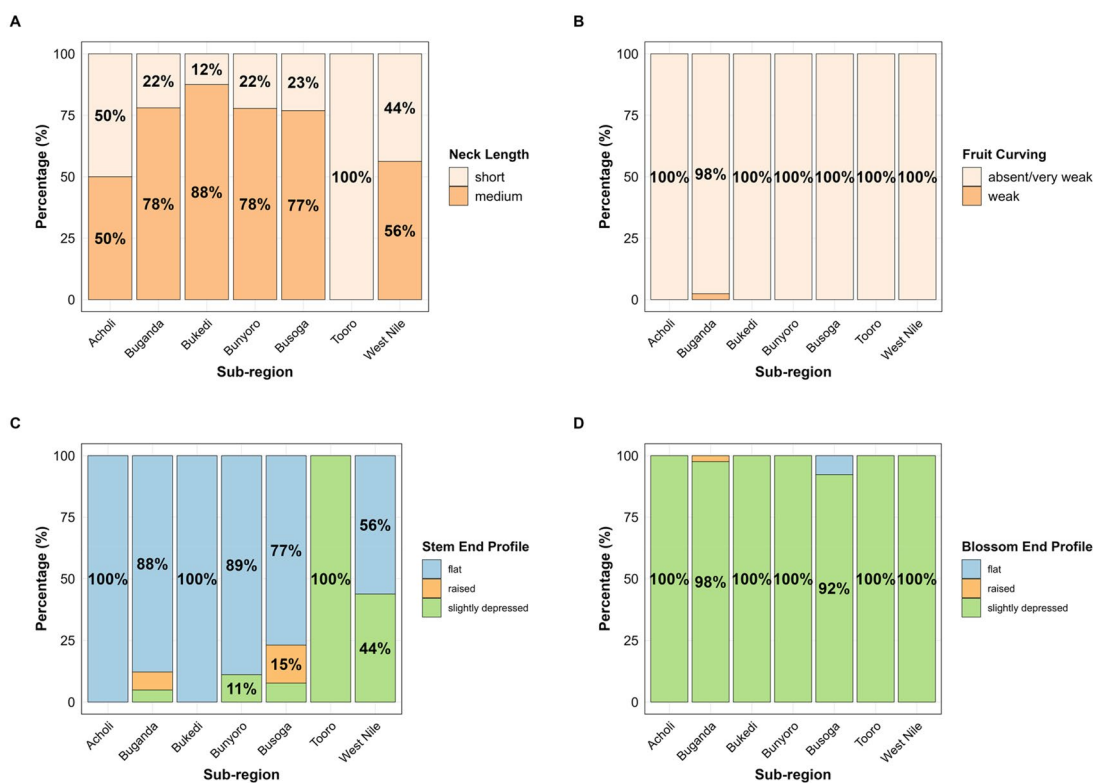
Buganda (11%), Bukedi (25%), and West Nile (13%). The broadest part of the fruit (Figure 7(B)) was located in the middle. In contrast, Acholi exhibited an even distribution between middle and mid-position placements (50% each). Blossom- or stem-end broadening was rare, occurring mainly in Buganda and Bukedi. Fruit shape (Figure 7(C)) was diverse, though narrow elliptic and ovate forms predominated. Narrow elliptic fruits were most frequent in Bukedi (62%), while ovate forms were common in Acholi (50%) and Busoga (45%). Other shapes included heart-shaped, rectangular, transverse elliptic, and trapezoidal were regionally restricted. Neck presence (Figure 7(D)) was generally weak, with over 85% of landraces in Buganda, Bukedi, Bunyoro, and Busoga exhibiting weak necks. Acholi and West Nile showed higher proportions of fruits with very weak or no necks (50% and 40%, respectively).

### 3.1.8. Variation in pumpkin neck length, fruit curving, stem end profile, and blossom end profile across sub-regions

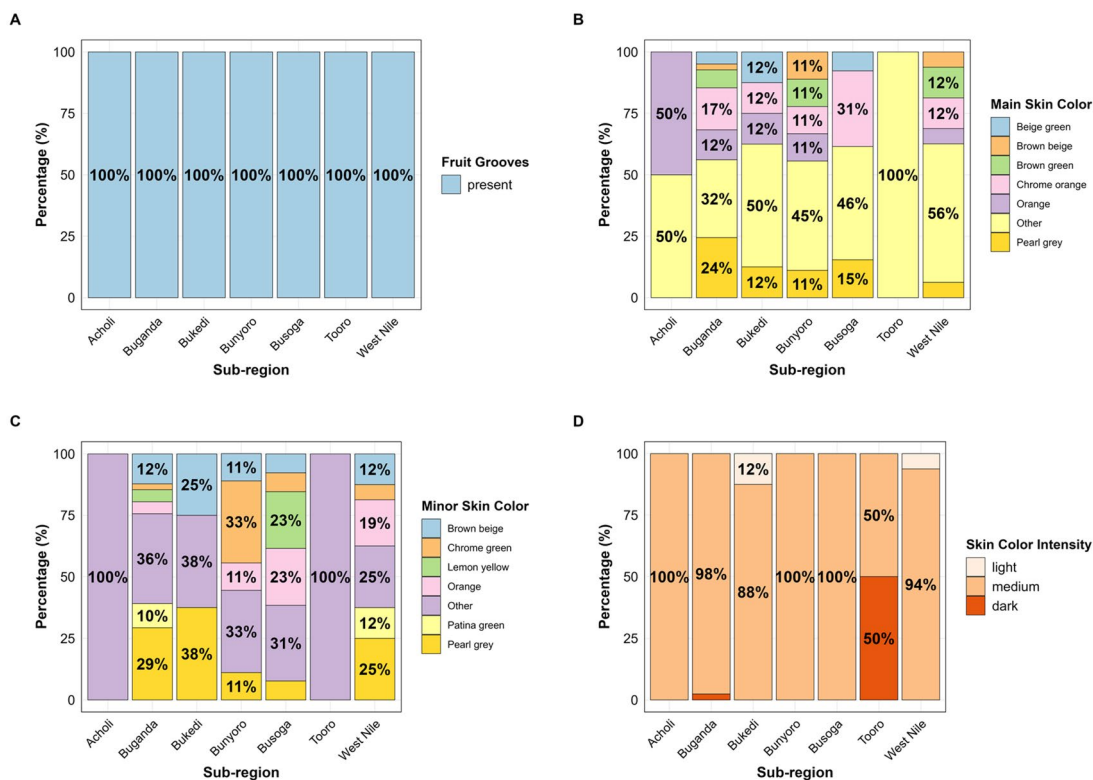
Neck length was largely medium in most sub-regions, accounting for 78–100% of landraces in Buganda, Bukedi, Bunyoro, Busoga, and Tooro sub-regions, while shorter necks were more frequent in Acholi sub-region (50%) and West Nile sub-region (40%). Fruit curving showed very limited variation across all sub-regions, with 98–100% with very weak or no curving. There were slight differences in stem end profiles, with most fruits having a flat end in Acholi, Buganda, Bukedi, and Bunyoro sub-regions ( $\geq 88\%$ ). However, slightly depressed ends were more common in Tooro (100%) and West Nile sub-regions (40%), while a small proportion of landraces in Buganda (12%) and Busoga sub-regions (15%) showed a raised profile. Similarly, the blossom end profile was predominantly slightly depressed in nearly all sub-regions ( $\geq 95\%$ ), except for a few landraces from Buganda and Tooro sub-regions that exhibited raised or flat ends (Figure 8).

### 3.1.9. Sub-regional variation in pumpkin fruit grooves, primary and secondary skin colour, and colour intensity

Fruit grooves were consistently observed across all landraces (100%), confirming fixation of this trait within the germplasm (Figure 9). In contrast, skin colouration exhibited marked sub-regional variability.



**Figure 8.** Variation in pumpkin fruit morphological traits across sub-regions: (A) neck length, (B) fruit curving, (C) stem end profile, and (D) blossom end profile. Bars represent the percentage occurrence of each trait category within sub-regions.

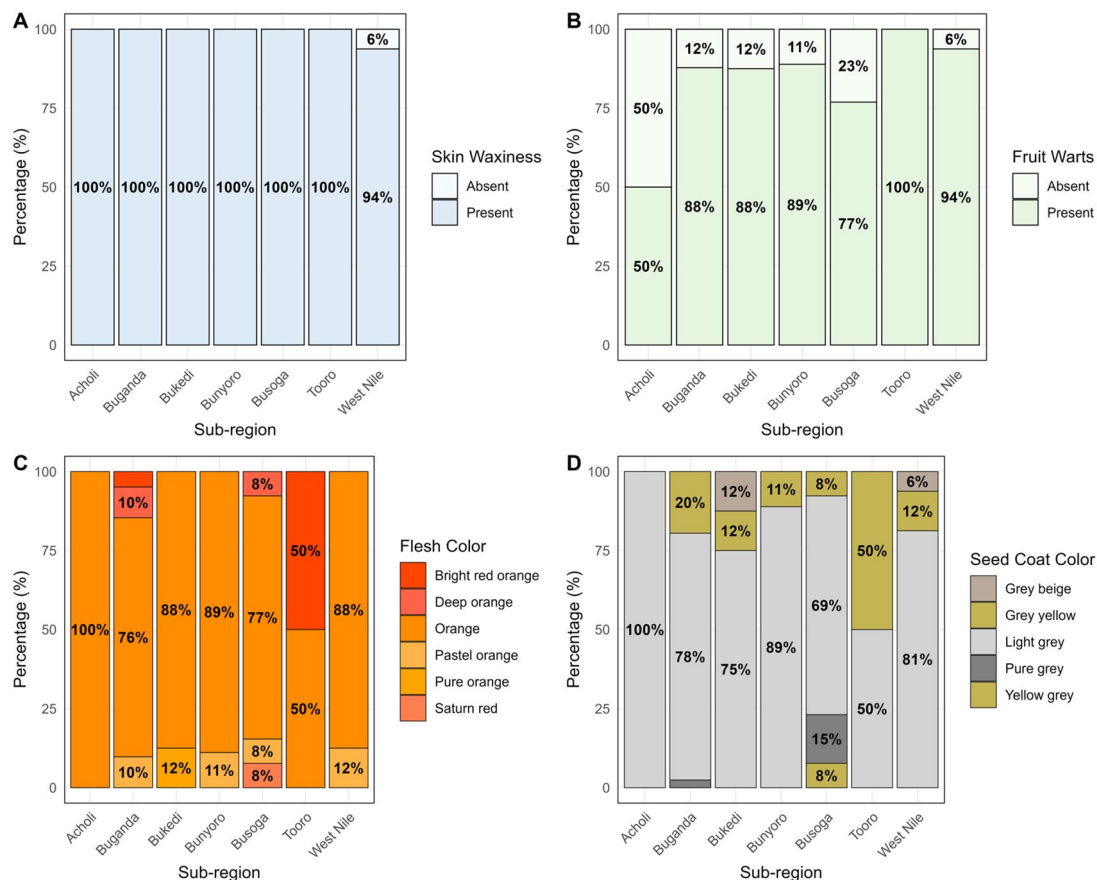


**Figure 9.** Sub-regional variation in pumpkin fruit surface and skin colouration: (A) Fruit grooves, (B) primary skin colour, (C) secondary skin colour, and (D) colour intensity. Bars indicate the percentage occurrence of each trait category across sub-regions.

Acholi and West Nile landraces were predominantly beige, green, or brown beige ( $\approx 50\%$ ), while Buganda and Bukedi showed broader mixtures, including brown beige, ochre brown, pastel green, brown grey (31%), and ochre brown (17%). Bunyoro landraces were distinguished by green ochre (19%) and cement grey (15%), whereas Busoga and Tooro displayed greater heterogeneity with chrome green, pastel orange, and pearl grey. Minor colour patterns reflected similar diversity, e.g. Acholi and Buganda ranged from light yellow to brown grey while Bukedi included brown olive and pastel green, Bunyoro was dominated by brown red (38%); Busoga and Tooro featured chrome orange and saffron yellow (23% and 100%, respectively); West Nile was largely yellow (80%). Skin colour intensity was predominantly medium ( $\geq 88\%$ ) across regions, except in Tooro, where landraces were evenly split between medium and dark. Overall, fruit grooves appeared stable, while skin colouration showed pronounced morphological variation, likely influenced by both genetic and environmental factors.

### 3.1.10. Sub-regional variation in pumpkin skin waxiness, fruit warts, flesh and seed coat colour

There was significant morphological variation among sub-regions in pumpkin traits (Figure 12). Skin waxiness (Figure 10(A)) was nearly ubiquitous, with 100% occurrence in Acholi, Buganda, Bukedi, Bunyoro, Busoga, and Tooro, and slightly lower prevalence in West Nile (93%). Fruit wartiness (Figure 10(B)) varied markedly, e.g. Acholi showed equal proportions of warted and smooth fruits (50%), while Buganda, Bukedi, and Bunyoro exhibited high wart incidence (85–89%). Busoga had moderate levels (77%), whereas Tooro and West Nile showed near-complete wartiness (100% and 93%, respectively). Flesh colour (Figure 10(C)) was dominated by orange tones across all sub-regions. Acholi displayed exclusively orange flesh (100%), while Buganda, Bukedi, and Bunyoro were dominated by orange and pastel orange (75–89%). Busoga showed a broader mix (77%), and Tooro and West Nile exhibited greater pigmentation diversity, with deep and bright red-orange hues comprising 50–87%. Seed coat color (Figure 10(D)) ranged from



**Figure 10.** Distribution of morphological traits of pumpkins across sub-regions: (A) skin waxiness; (B) fruit warts; (C) flesh colour; (D) seed coat colour. Bars represent the percentage occurrence of each trait category within sub-regions.

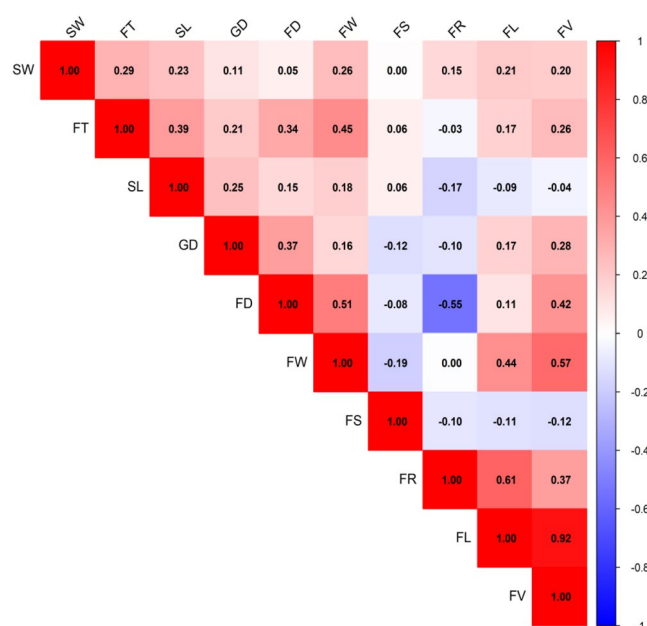
light grey to yellow-grey. Acholi was uniformly grey (100%), while Buganda, Bukedi, and Bunyoro showed mixed tones (69–85%). Busoga and Tooro had slightly higher proportions of yellow-grey seeds (15–50%), and West Nile was dominated by light-grey to grey-beige coats (80%).

### 3.1.11. Morphological trait relationships and correlations

Pearson correlation analysis (Figure 11) revealed strong interrelationships among pumpkin fruit size attributes. Fruit length (FL) was highly correlated with fruit volume (FV) ( $r=0.92^{**}$ ), while fruit ratio (FR) was positively associated with both FL ( $r=0.61^{**}$ ) and FV ( $r=0.37^{**}$ ), indicating that elongated fruits are generally larger. Fruit diameter (FD) correlated moderately with fruit weight (FW) ( $r=0.51^{**}$ ), and FW was positively related to FV ( $r=0.57^{**}$ ), underscoring size-dependent associations among key dimensions. Seed traits showed weaker relationships: seed width (SW) and seed length (SL) were modestly correlated ( $r=0.23^*$ ) and exhibited limited associations with other parameters. Flesh thickness (FT) was negatively correlated with FD ( $r = -0.55^{**}$ ), suggesting a trade-off between fruit girth and edible tissue accumulation. Groove distance (GD) displayed only weak associations ( $r \leq 0.37^{**}$ ), reflecting independence from fruit size. Collectively, these results demonstrate that fruit size traits (FD, FL, FW, FV, FR) are tightly integrated, whereas seed and structural parameters (SW, SL, FT, GD) contribute more independently to phenotypic variation.

### 3.1.12. Multivariate analysis of morphological traits

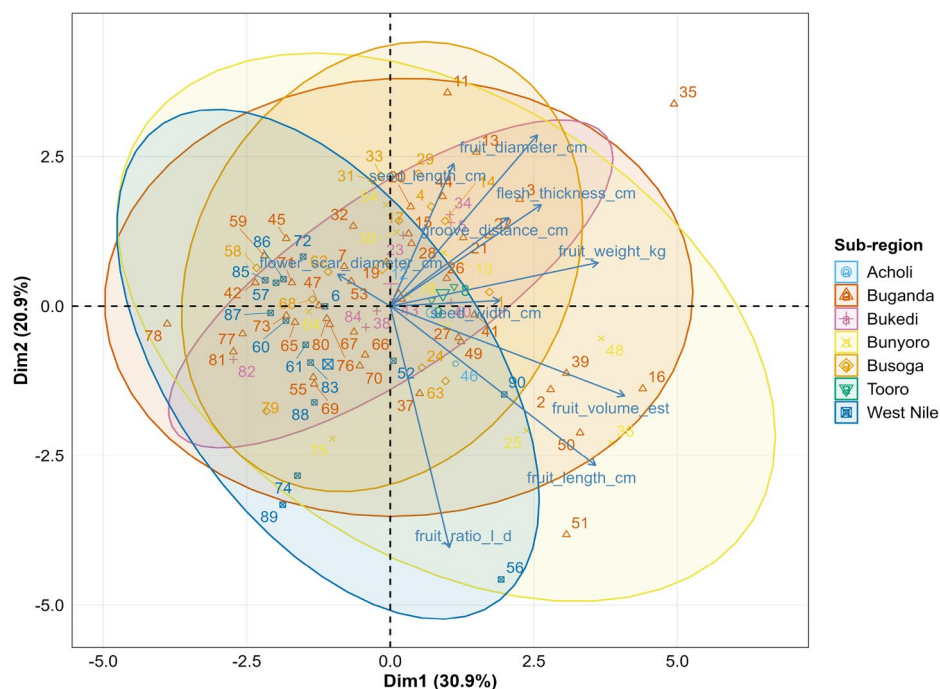
Principal component analysis (PCA) identified key traits driving morphological variation among pumpkin landraces across sub-regions. The first five components explained most of the variation, with Dim1 (30.9%) and Dim2 (20.9%) being most influential. Dim1 was dominated by fruit size traits, volume, weight, length, and diameter, highlighting overall fruit size as the primary axis of variation. Dim2 reflected a combined influence of fruit and seed dimensions, particularly diameter, length, and seed length, indicating their role in regional differentiation. Dim3 captured seed traits (width and length), Dim4 was shaped by groove distance and flesh thickness, while Dim5 was largely driven by groove distance and fruit weight. The PCA biplot illustrates these trait contributions and clustering patterns (Table 5; Figure 12) revealed overlap of landraces grouped by sub-region, indicating a lack of clear separation and that the morphological traits analysed did not provide strong discriminatory power between sub-regions because of shared variation.



**Figure 11.** Pearson correlation matrix of pumpkin morphological traits. Note: Heatmap displays correlation coefficients (R-values) among ten quantitative traits. Red indicates positive and blue negative correlations, with colour intensity reflecting strength ( $-1 \leq r \leq 1$ ). Traits include seed width (SW), fruit thickness (FT), seed length (SL), groove distance (GD), fruit diameter (FD), fruit weight (FW), flesh thickness (FS), fruit ratio (FR), fruit length (FL), and fruit volume (FV).

**Table 5.** Top eight morphological parameters contributing to the first five principal components (Dim1-Dim5) in pumpkins.

Trait	Dim1 (%)	Dim2 (%)	Dim3 (%)	Dim.4	Dim.5
1. Fruit volume (cm <sup>3</sup> )	24.8	5.0	3.0	3.1	1.3
2. Fruit weight (kg)	19.6	1.2	0.6	0.9	16.9
3. Fruit length (cm)	19.1	15.7	0.2	1.4	2.0
4. Flesh thickness (cm)	10.3	6.3	12.2	1.0	2.1
5. Fruit diameter (cm)	9.8	18	14.3	3.7	0.2
6. Groove distance (cm)	6.4	4.8	1.3	12.8	55.1
7. Seed width (cm)	5.4	0.0	26.2	2.9	5.3
8. Seed length (cm)	1.8	12.5	22.7	7.1	2.1

**Figure 12.** PCA biplot of pumpkin morphological traits, illustrating sub-regional grouping of landraces.

### 3.2. Analysis of nutritional parameters

Seventy-two pumpkin landraces were evaluated for their nutritional profiles across all sub-regions, as samples from 19 landraces were excluded due to low analytical quality (Table 1; Table S2).

#### 3.2.1. Overall nutritional composition profile

Total phenolic content (TPC) varied among landraces, ranging from 0.0184 to 0.1103g GAE/100g (mean  $\pm$ SD:  $0.0428 \pm 0.0174$ g GAE/100g). Protein content was similarly diverse (0.322–1.289g/100g (mean  $\pm$ SD:  $0.454 \pm 0.118$ g/100g). Antioxidant activity showed the highest relative variability (CV = 57.56%), with values spanning 0.69–69.30% DPPH inhibition. Dry matter content ranged widely (3.50–28.10%). Among macronutrients, fiber content displayed the greatest variability (CV = 54.11%), followed by sugar (CV = 44.04%) and total carbohydrates (CV = 37.07%) (Table 6).

#### 3.2.2. Statistical significance of nutritional parameters across regional, sub-regional, and district scales

There were significant variations among landraces at district level for protein ( $p=0.010$ ), lipid ( $p<0.001$ ), and fiber ( $p=0.004$ ), likely reflecting localized influences such as soil fertility, agronomic practices, and post-harvest handling (Table 7). The detailed TPC, antioxidant activity, and proximate composition of samples from different districts in Uganda are shown in Table S2. No parameter varied significantly at the sub-regional level ( $p>0.05$ ), indicating intra-sub-regional homogeneity despite district-level heterogeneity.

**Table 6.** Descriptive statistics of nutritional parameters in Ugandan pumpkins landraces.

Nutritional	Mean $\pm$ SD	Minimum	Maximum	CV (%)
1. Total phenolic content (mg GAE/kg)	428.49 $\pm$ 173.56	184.19	1,102.88	40.50
2. Protein content (mg/kg)	4,536.04 $\pm$ 1,181.14	3,219.28	12,888.15	26.04
3. Antioxidant activity (% DPPH inhibition)	30.31 $\pm$ 17.45	0.69	69.30	57.56
4. Dry matter (%)	11.34 $\pm$ 5.02	3.50	28.10	44.22
5. Water content (% fresh weight)	88.66 $\pm$ 5.02	71.90	96.50	5.66
6. Lipid content (% fresh weight)	2.19 $\pm$ 0.86	0.42	3.96	39.42
7. Fiber content (% fresh weight)	1.68 $\pm$ 0.91	0.08	3.59	54.11
8. Total carbohydrates (% fresh weight)	2.73 $\pm$ 1.01	0.16	4.96	37.07
9. Sugar content (% fresh weight)	0.89 $\pm$ 0.39	0.27	2.26	44.04

Note: Values represent mean  $\pm$  standard deviation. CV = coefficient of variation.

**Table 7.** Variation of nutritional parameters at the region, sub-region, and district levels.

Parameter	Geographical level	p-value	Significance
1. TPC (mg GAE/kg)	Region	0.352	NS
	Sub-region	0.387	
	District	0.892	
2. Protein (mg/kg)	Region	0.801	*
	Sub-region	0.727	
	District	0.010	
3. Antioxidant activity (% DPPH)	Region	0.099	NS
	Sub-region	0.169	
	District	0.231	
4. Dry matter (%)	Region	0.026	*
	Sub-region	0.210	
	District	0.187	
5. Water content (% FW)	Region	0.026	*
	Sub-region	0.210	
	District	0.187	
6. Lipid (% FW)	Region	0.003	**
	Sub-region	0.092	
	District	< 0.001	
7. Fiber (% FW)	Region	< 0.001	***
	Sub-region	0.081	
	District	0.004	
8. Total carbohydrate (% FW)	Region	0.011	*
	Sub-region	0.168	
	District	0.098	
9. Sugar (% FW)	Region	0.015	*
	Sub-region	0.183	
	District	0.077	

Note: Significance levels are denoted as follows:  $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*), NS- Not statistically significant. Abbreviations: FW: Fresh weight; TPC: Total phenolic content; GAE: Gallic acid Equivalents; DPPH: 2,2-Diphenyl-1-picrylhydrazyl.

The TPC and antioxidant activity showed no significant differences among landraces at either scale, suggesting potential genetic or biochemical stability across sites.

### 3.2.3. Total phenolic content

Total phenolic content (TPC) varied widely (0.0184–0.0620 g GAE/100 g), reflecting substantial phytochemical diversity. The landraces from Buganda sub-region exhibited the highest concentrations, with Mpigi (0.0620  $\pm$  0.0229 g GAE/100 g), Mityana (0.0550  $\pm$  0.0249 g GAE/100 g), and Kiboga (0.0520  $\pm$  0.0156 g GAE/100 g) leading. Landraces from Kayunga also showed elevated TPC (0.0478  $\pm$  0.0369 g GAE/100 g) but with high variability (CV = 77.15%), indicating sample heterogeneity. In West Nile, landraces from Maracha recorded a uniformly high TPC (0.0535 g GAE/100 g; CV = 0.00%), contrasting sharply with landraces from Gulu (Acholi sub-region), which had the lowest nationwide value (184 mg GAE/kg). Busoga districts, such as Jinja (0.0444  $\pm$  0.0115 g GAE/100 g) exhibited intermediate levels (Table S2).

### 3.2.4. Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity in landraces showed no direct correlation with TPC across districts. Mpigi, despite the highest TPC (0.0620  $\pm$  0.0229 g GAE/100 g), exhibited

low antioxidant activity ( $9.57 \pm 4.45\%$ ). In contrast, Mubende ( $0.0423 \pm 0.0121$  g GAE/100 g) and Nakasongola ( $0.0385 \pm 0.0089$  g GAE/100 g) recorded high antioxidant activities ( $42.77 \pm 32.73\%$  and  $46.50 \pm 14.90\%$ , respectively). Similarly, Pakwach ( $0.0477 \pm 0.0253$  g GAE/100 g) demonstrated strong antioxidant capacity ( $52.87 \pm 7.70\%$ ) despite moderate TPC levels.

### 3.2.5. Protein content

The protein content varied markedly across districts. Mukono district recorded the highest mean ( $8,867 \pm 5,687$  mg/kg), though high variability (CV = 64.14%) suggests sample heterogeneity and potential for genotype selection. A consistent high-protein accession cluster was observed in West Nile, with Nebbi ( $0.475 \pm 0.085$  g/100 g), Pakwach ( $0.471 \pm 0.048$  g/100 g), and Arua ( $0.463 \pm 0.034$  g/100 g) all exceeding 4,600 mg/kg. Mbale (Bukedi sub-region) also showed elevated levels ( $0.482 \pm 0.079$  g/100 g). In contrast, Maracha ( $0.364$  g/100 g) and Nakasongola ( $0.398$  g/100 g) had the lowest mean protein contents.

### 3.2.6. Dry matter

Dry matter content varied substantially by district with a distinct geographical gradient among landraces. The West Nile sub-region recorded the highest values, led by Maracha (22.80%) and Pakwach ( $18.07 \pm 5.23\%$ ). In contrast, Gulu (Acholi sub-region) had the lowest content (6.90%), while districts in Buganda (e.g. Mukono: 7.20%; Kyankwanzi: 7.80%) and Bukedi (e.g. Kamuli: 8.80%) also showed consistently low levels among landraces. Kayunga (CV = 80.77%), Jinja (CV = 74.38%), and Mukono (CV = 72.67%) landraces exhibited high heterogeneity, whereas Nakasongola (CV = 0.54%) and Mityana (CV = 1.36%) showed uniformity in dry matter content.

### 3.2.7. Water content

Water content varied significantly among landraces across districts. The highest levels were recorded in Buganda sub-region in Mukono ( $92.80 \pm 5.23\%$ ), Kyankwanzi ( $92.20 \pm 0.00\%$ ), and Kiboga ( $91.75 \pm 2.71\%$ ). In contrast, the lowest values occurred in West Nile. Districts in other sub-regions exhibited moderate water content (Table S2; Figure 15).

### 3.2.8. Lipid content

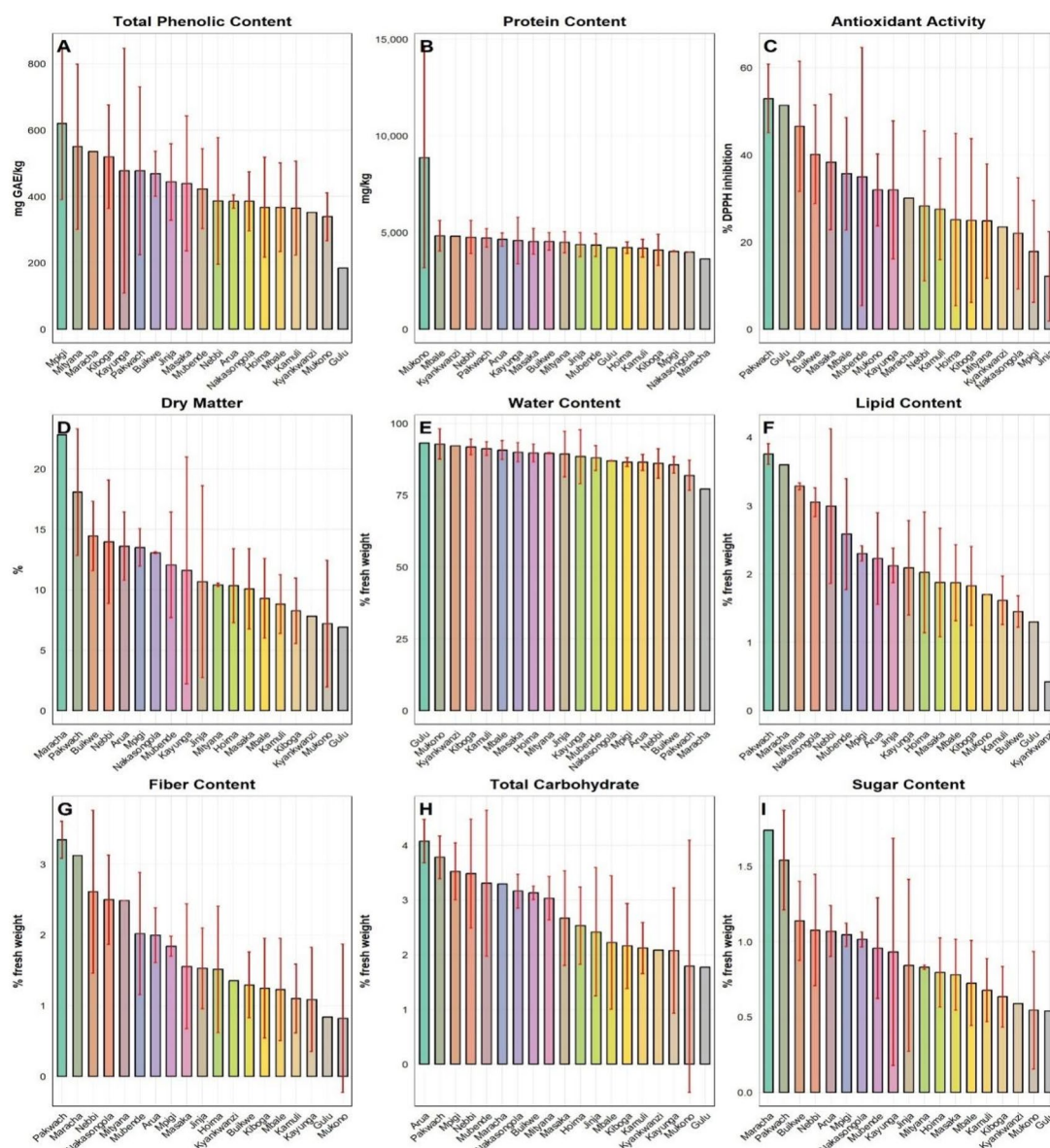
Lipid content among landraces exhibited distinct geographical patterns and marked inter-district variability among landraces (Table S2). The highest mean values were in the West Nile, notably Pakwach ( $3.75 \pm 0.15\%$  FW) and Maracha (3.60% FW). The landraces from Central region (Buganda) showed the widest range, from elevated levels in Mityana ( $3.29 \pm 0.05\%$  FW) to minimal content in Kyankwanzi (0.42% FW). Landraces from Eastern districts (Busoga and Bukedi) displayed moderate lipid levels (1.61–2.12% FW), those from Hoima (Bunyoro sub-region) recorded low lipid content ( $2.02 \pm 0.88\%$  FW). Lipid homogeneity was highest in Mityana (CV = 1.51%), Nakasongola (CV = 6.96%), and Pakwach (CV = 4.01%). In contrast, landraces from Hoima (CV = 43.67%), Masaka (CV = 42.37%), and Kayunga (CV = 33.16%) exhibited substantial intra-district variation, indicating diverse lipid accumulation among landraces (Table S2).

### 3.2.9. Fiber content

Fiber content ranged from 0.82% to 3.34% FW, with a clear geographical gradient among landraces. The highest levels were concentrated in the West Nile sub-region, notably Pakwach ( $3.34 \pm 0.26\%$  FW) and Maracha (3.12% FW). Buganda districts showed wide variation, while Eastern (e.g. Jinja:  $1.53 \pm 0.57\%$  FW) and Western (e.g. Hoima:  $1.51 \pm 0.89\%$  FW) regions generally exhibited landraces with lower fiber content. Intra-district variability, assessed via coefficient of variation (CV), was minimal in Mityana (CV = 0%) and Pakwach (CV = 7.83%), but pronounced in Mukono (CV = 127.62%), Kayunga (CV = 67.74%), and Hoima (CV = 59.01%), indicating heterogeneous fiber accumulation (Table S2).

### 3.2.10. Total carbohydrate content

Total carbohydrate content among landraces ranged from 1.77% to 4.07% FW, with substantial inter-district variation. The highest levels were recorded in the West Nile sub-region, notably Arua ( $4.07 \pm 0.40\%$  FW) and



Data source: Nutritional analysis | Total samples: 72 | Districts: 19

**Figure 13.** District variation in nutritional composition among landraces. Box plots illustrate the distribution of (A) total phenolic content (TPC), (B) protein, (C) antioxidant activity, (D) dry matter, (E) water, (F) lipid, (G) fiber, (H) total carbohydrates, and (I) sugar content across districts. Districts are ranked by ascending mean values per parameter. Bars and error bars denote the coefficient of variation.

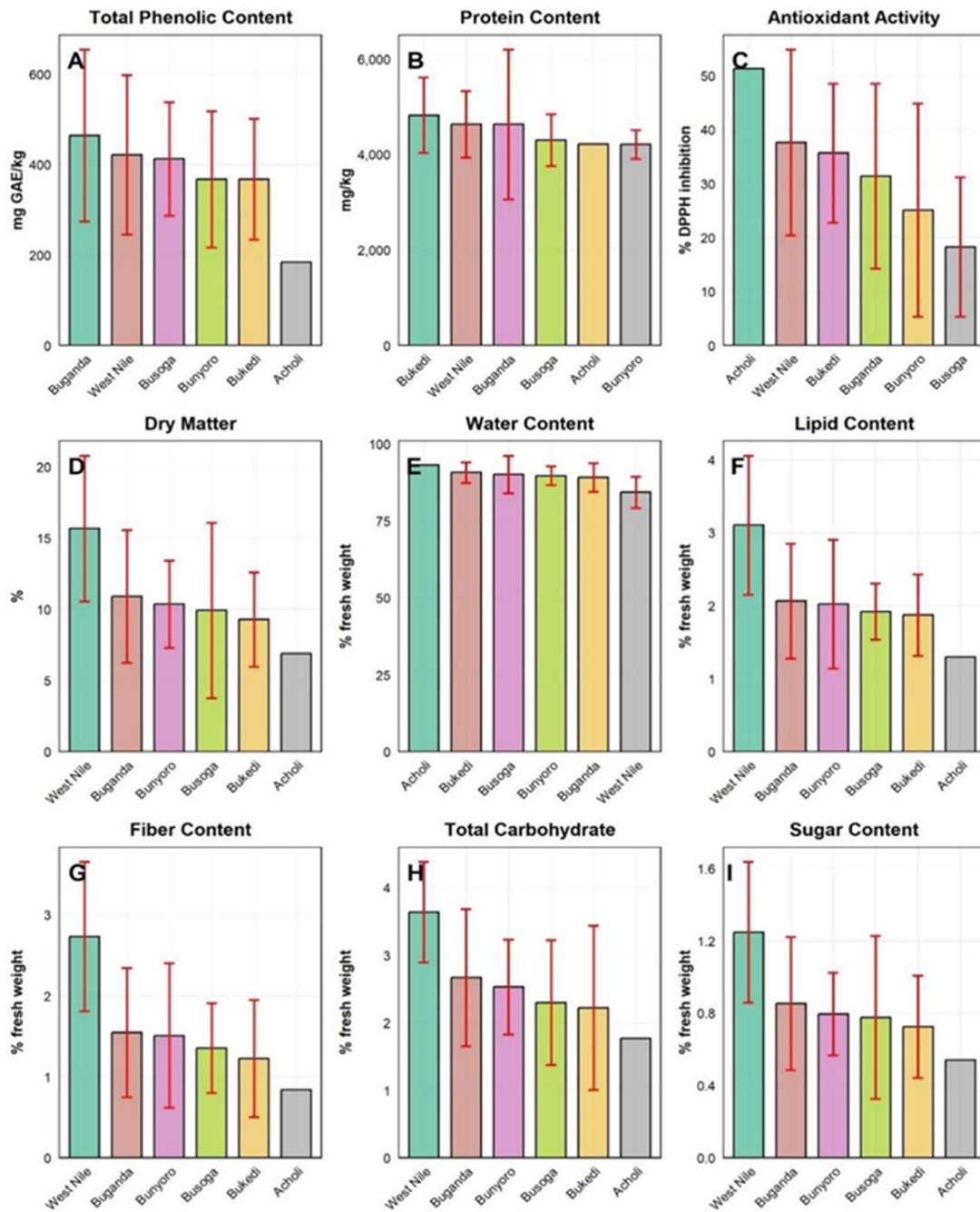
Pakwach ( $3.78 \pm 0.39\%$  FW). Landraces from Buganda districts showed a broad spectrum, from high in Mpigi ( $3.52 \pm 0.52\%$  FW) to low in Mukono ( $1.79 \pm 2.31\%$  FW). Carbohydrate homogeneity among landraces was highest in Buikwe (CV = 4.02%) and Nakasongola (CV = 9.85%), while Mukono (CV = 128.78%), Kayunga (CV = 55.13%), and Mbale (CV = 54.74%) showed pronounced intra-district variability (Table S2).

### 3.2.11. Sugar content

Sugar content ranged from 0.54 to 1.74% FW, with a clear geographical gradient. The highest concentrations were observed in the West Nile sub-region, notably Maracha (1.74% FW) and Pakwach ( $1.54 \pm 0.33\%$  FW). While landraces from Eastern (e.g. Jinja:  $0.84 \pm 0.57\%$  FW) and Western (e.g. Hoima:  $0.80 \pm 0.23\%$  FW) regions recorded lower values. Sample homogeneity varied markedly among landraces, for example, in Mityana (CV = 1.70%) and Nakasongola (CV = 4.88%) exhibited high consistency, whereas Kayunga (CV = 80.82%), Mukono (CV = 71.36%), and Jinja (CV = 67.70%) showed substantial intra-district variability (Table S2; Figure 13).

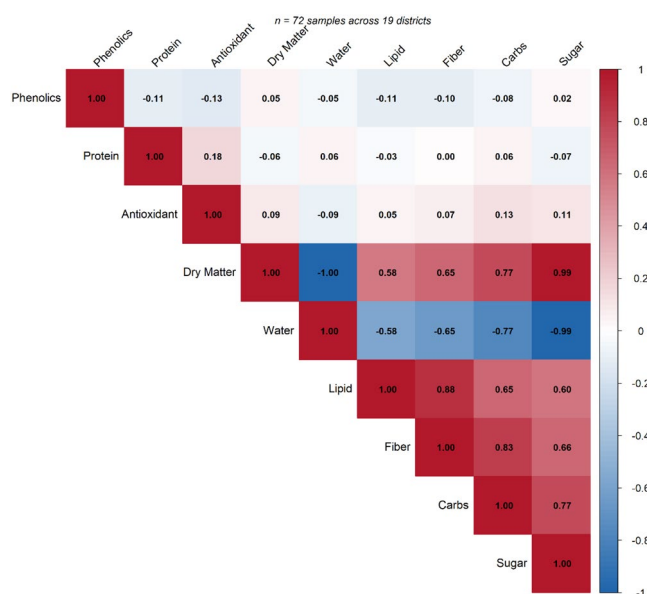
### 3.2.12. Sub-regional level nutritional variation

The nutritional profiles across six Ugandan sub-regions showed that landraces from West Nile exhibited elevated levels of total phenolics (GAE), lipids (g/100g FW), fiber (g/100g FW), carbohydrates (g/100g FW), and sugars (percentage sucrose equivalents), while Acholi showed consistently low values except for antioxidant activity, which was highest. Protein content peaked in Bukedi with notable variability. Water content remained uniformly high across regions, inversely correlated with dry matter among landraces (Figure 14).



Data source: Nutritional analysis | Total samples: 72 | Sub-regions: 6

**Figure 14.** Sub-regional variation in nutritional composition. Box plots illustrate the distribution of (A) total phenolic content (TPC), (B) protein, (C) antioxidant activity, (D) dry matter, (E) water, (F) lipid, (G) fiber, (H) total carbohydrates, and (I) sugar content across districts, colour-coded by region (Central, Eastern, Northern, Western). Districts are ranked by ascending mean per parameter. Bars and error bars represent the coefficient of variation.



**Figure 15.** Correlation matrix of nutritional and bioactive parameters ( $n=72$  samples from 19 districts). Values represent Pearson correlation coefficients ( $r$ ). Key: Phenolics – total phenolic content; Carbs – total carbohydrates.

**Table 8.** Pearson correlations among pumpkin morphological traits and nutritional components across landraces.

Morphological trait	Nutritional component	R	$p$ -value
1. Fruit length (cm)	Total phenolic content	-0.12	0.322
2. Fruit length (cm)	Protein content	0.27	0.024
3. Fruit length (cm)	Antioxidant activity	0.065	0.595
4. Fruit diameter (cm)	Total phenolic content	-0.133	0.273
5. Fruit diameter (cm)	Protein content	-0.065	0.595
6. Fruit diameter (cm)	Antioxidant activity	0.069	0.569
7. Fruit shape ratio	Total phenolic content	-0.008	0.946
8. Fruit shape ratio	Protein content	0.377	0.001
9. Fruit shape ratio	Antioxidant activity	-0.007	0.956

### 3.2.13. Correlation matrix of nutritional and bioactive parameters

Correlation analysis ( $n=72$  samples across 19 districts) revealed strong, statistically significant associations among macronutrient parameters (Figure 15). Dry matter content was inversely correlated with water content ( $r = -1.00^{**}$ ) and positively associated with total carbohydrates ( $r=0.77^{**}$ ), fiber ( $r=0.65^{**}$ ), and lipids ( $r=0.58^{**}$ ). Lipid, fiber, and carbohydrate contents were mutually correlated, with the strongest relationship between lipid and fiber ( $r=0.88^{**}$ ) across landraces. In contrast, total phenolic content and antioxidant activity showed no significant correlations with macronutrients or with each other ( $|r| < 0.13$ ). Protein content was similarly independent, lacking significant associations with other parameters.

### 3.2.14. Relationships between pumpkin morphology and nutritional composition

Pearson correlation analysis revealed predominantly weak and non-significant associations between morphological traits and nutritional components among landraces. The exception was fruit shape ratio, which showed a significant positive correlation with protein content ( $r=0.377^{**}$ ). All other trait–nutrient relationships were negligible across landraces (Table 8).

## 4. Discussion

This study demonstrates that Ugandan pumpkin (*Cucurbita* spp.) germplasm exhibits extensive morphological and nutritional diversity, geographically structured across sub-regions and districts. Fruit weight significantly varied, accompanied by a broad spectrum of fruit shapes, reflecting the influence of farmer selection for culinary use, storage, market preferences, and local adaptations. The differences were hallmarks of landrace populations maintained under smallholder systems (Ezin et al., 2022; Ferriol & Picó, 2008). Comparable patterns have been reported in other *Cucurbita* spp. collections globally (Murovec, 2015; Pan et al., 2020).

A key finding was the spatial structuring of phenotypic diversity, with large-fruited landraces predominantly concentrated in the Buganda and Bunyoro sub-regions. We hypothesize that this pattern is influenced by the prevalence of commercial agriculture and the proximity of these areas to urban markets, which exert selective pressure favoring high-yield, high-value phenotypes (Hernández et al., 2023).

In contrast, smaller-fruited landraces predominated in West Nile and Busoga, suggesting adaptation to subsistence-oriented systems, limited inputs, and selection for traits such as early maturity and ease of transport, critical for household food security (Ezin et al., 2022; Nyabera et al., 2021). District-level analyses further identified Mpigi, Mityana, and Masaka districts as consistent sources of large, heavy fruits, offering strategic entry points for trait-targeted breeding initiatives.

Principal component and correlation analyses revealed that fruit size and shape represent largely independent axes of variation within Ugandan *Cucurbita* spp. germplasm. The negligible correlation between fruit length and diameter underscores this distinction, indicating that selection for elongated, bottle-shaped fruits can proceed without concomitant increases in girth or weight. This finding supports the development of specialized cultivars tailored to niche market demands while preserving other agronomic traits (Öztürk et al., 2022). The decoupling of fruit shape from size has been documented in other *Cucurbita* species (Pan et al., 2020; Paris, 2016), and is reaffirmed here within the Ugandan context.

This investigation uncovered a uniquely defined identification of a distinct, nutrient-dense cluster of pumpkin landraces originating from the Northern region, particularly the West Nile sub-region. This finding corroborates previous work by Kazibwe et al. (2022), which highlighted that Arua pumpkin landraces are genetically distinct and highly variable, emphasizing strong regional differentiation within Ugandan pumpkin germplasm. Landraces from West Nile consistently exhibited elevated levels of dry matter, lipids, fiber, total carbohydrates, and sugars. The dry-flesh profile is particularly beneficial in the Ugandan context, where elevated dry matter content extends shelf life and reduces post-harvest losses in regions lacking adequate cold storage facilities (Rosales et al., 2023; Xu et al., 2024). In addition, the enhanced nutritional density makes these landraces strong candidates for addressing malnutrition and for use in value-added products such as flours and concentrates (Bemfeito et al., 2020; Xu et al., 2024). The nutritional attributes of these pumpkin landraces play a critical role in enhancing household food and nutritional security (Aziz et al., 2023; Kulczyński & Gramza-Michałowska, 2019). The consistent macronutrient superiority of West Nile landraces suggests the presence of a unique, locally adapted germplasm base, likely maintained through farmer selection for storability and energy-rich traits.

At a finer resolution, district-level analysis revealed distinct hotspots for key phytochemical and nutritional traits within Ugandan pumpkin germplasm (Nakazibwe et al., 2020). Mpigi district emerged as a consistent source of phenolic-rich landraces, while Mukono district harbored landraces with markedly elevated protein content. This level of granularity represents a novel contribution, advancing beyond broad regional classifications to inform targeted conservation and breeding strategies. Specifically, it enables conservationists to prioritize districts for in-situ preservation and equips breeders with precise geographic origins for sourcing parental lines in biofortification programs (Amin et al., 2019). Notably, the substantial intra-district variability observed in areas such as Kayunga and Mukono should be viewed not as a constraint but as a breeding advantage, reflecting a rich reservoir of allelic diversity within locally adapted genetic backgrounds (Aliu et al. 2011).

A critical insight for breeding efficiency is the weak overall correlation between external morphological traits and internal nutritional composition. With the exception of a moderate association between fruit shape ratio and protein content, external appearance proved to be a poor proxy for nutritional value. This finding highlights a key limitation of phenotype-based selection commonly employed by farmers and breeders (Öztürk et al., 2022). It underscores the necessity of integrating direct biochemical profiling into breeding pipelines, as selection for size or visual appeal does not reliably confer nutritional enhancement (Aziz et al., 2023; Ezin et al., 2022). The observed disconnect implies that morphological and nutritional traits are governed by distinct genetic loci, reinforcing the need for trait-specific selection strategies in cultivar development (Aliu et al. 2011).

Nutrient partitioning across pumpkin fruit tissues warrants closer examination. Empirical evidence indicates that peel and seed tissues possess elevated concentrations of bioactive compounds and antioxidant activity, often surpassing those found in the flesh (Aziz et al., 2023; Kar et al., 2023). These findings underscore the potential of postharvest processing and byproduct utilization to enhance value addition and minimize waste within local production systems (Aziz et al., 2023). Consequently, breeding programs should incorporate direct biochemical assays rather than relying exclusively on morphological traits (Kar et al., 2023).

Furthermore, the observed lack of correlation between total phenolic content (TPC) and antioxidant activity in certain districts suggests that other phytochemicals such as carotenoids, vitamin C, and specific phenolic sub-classes contribute substantially to antioxidant capacity (Aziz et al., 2023; Jahan et al., 2023; Kulczyński & Gramza-Michałowska, 2019). This highlights the importance of non-phenolic antioxidants, including  $\beta$ -carotene, ascorbic acid, and tocopherols, in determining the nutritional quality of Ugandan pumpkins. Reliance on a single assay such as TPC may therefore yield incomplete assessments. Future evaluations should adopt compound-specific profiling to accurately capture the full spectrum of health-promoting attributes.

The study faced certain limitations, notably the few number of fruits sampled per landrace and the potential risk of nutritional deterioration in fruits sourced from large markets, where extended storage durations are common. To mitigate this challenge and ensure data reliability, only market fruits stored for fewer than seven days post-harvest were selected, thereby minimizing the likelihood of nutrient loss and preserving the integrity of the nutritional analyses.

## 5. Conclusion

This study presents the first nationally integrated morphological and nutritional characterization of Ugandan pumpkin germplasm, revealing extensive, geographically structured diversity. Key findings include: (i) substantial morphological variation, with fruit size and shape can be distinct breeding targets; (ii) the West Nile sub-region as a reservoir of nutrient-dense, high dry-matter landraces; and (iii) poor correlation between external morphology and nutritional quality, underscoring the need for direct biochemical screening. For immediate breeding impact, West Nile germplasm can be prioritized to enhance dietary energy and postharvest longevity, while Mpigi and Mukono offer valuable sources of phenolics and protein, respectively. To fully exploit this diversity, integration of molecular markers with phenotypic and nutritional data is essential. We recommend Genome-Wide Association Studies (GWAS) to identify loci linked to key nutritional traits, enabling marker-assisted selection for superior, nutrient-rich cultivars that advance food and nutritional security in Uganda and beyond.

## Author contributions

CRedit: **Fred Bwayo Masika**: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing; **Godwin Anywar**: Conceptualization, Funding acquisition, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing; **Mahipal Singh Kesawat**: Formal analysis, Methodology, Supervision, Writing – original draft, Writing – review & editing; **Gabriel Ddamulira**: Conceptualization, Formal analysis, Funding acquisition, Methodology, Supervision, Writing – original draft, Writing – review & editing; **Caro Kawuma**: Conceptualization, Funding acquisition, Methodology, Project administration, Writing – original draft, Writing – review & editing; **Morgan Andama**: Conceptualization, Investigation, Writing – original draft, Writing – review & editing; **Charity Ajoma**: Investigation, Methodology, Writing – original draft; **Idd Ramathan**: Formal analysis, Methodology, Writing – original draft, Writing – review & editing; **Otuba Moses Amugoli**: Investigation, Methodology, Writing – original draft; **Jimmy Caku**: Data curation, Formal analysis, Investigation, Writing – original draft; **Titus Alicai**: Investigation, Supervision, Writing – review & editing; **Ephraim Nuwamanya**: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft; **Arthur K. Tugume**: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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No potential conflict of interest was reported by the authors

## Open scholarship



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## Data availability statement

All data supporting the findings of this study are available from the corresponding author on reasonable request.

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