



E-ISSN: 2618-0618
P-ISSN: 2618-060X
© Agronomy
NAAS Rating (2026): 5.20
www.agronomyjournals.com
2026; 9(9): 496-506
Received: 11-07-2026
Accepted: 14-08-2026

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International Journal of Research in Agronomy

Morphological characterization and distinctness of seven pepper (*Capsicum annum* L.) varieties

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DOI: <https://www.doi.org/10.33545/2618060X.2026.v9.i9g.6548>

Abstract

Consistent morphological descriptors in crop-specific DUS guidelines support cultivar identification, yet empirical validation of their discriminating power remains scarce. This study assessed the morphological distinctness of seven *Capsicum annum* L. varieties using UPOV TG/76/8 descriptors over two growing seasons (2023-2024) at the National Agricultural Seeds Council field in Nigeria. Of 42 traits evaluated, 34 (81%) were polymorphic and used in multivariate analysis; 8 (19%) were monomorphic and excluded. Principal component analysis of the polymorphic traits showed the first three components explained 74.6% of total variation (PC1: 33.0%, PC2: 26.8%, PC3: 14.8%). Fruit apex shape, locule number, fruit length/diameter ratio, fruit shape in longitudinal and cross-section, and leaf margin undulation emerged as the primary discriminating traits on PC1. Hierarchical clustering grouped the varieties into three clusters: elongated-fruited types (Serenade, PS Pepper 1, PS Pepper 2); thick-peduncled, broad-leaved types (Commandant, Efia); and compact-fruited, sinuate-pericarp, tall types (Piquante, Spicy). PS Pepper 1 and PS Pepper 2 were the least distinct pair (Gower distance = 0.178), while Commandant and PS Pepper 1 were most distant (0.622). Seedling and floral traits contributed little discriminatory value. These results support the UPOV TG/76/8 descriptor set's effectiveness and suggest a reduced eight-trait subset could reliably determine distinctness in *Capsicum annum*.

Keywords: Polymorphic, UPOV, capsicum, DUS, descriptor

1. Introduction

Capsicum annum L. (family Solanaceae) is among the most economically and agriculturally significant vegetable crops cultivated globally, valued for its diverse fruit morphology, wide culinary application, and rich phytochemical composition. Global pepper and bell pepper production data from FAOSTAT confirms massive scale and steady growth. Spices and specialty peppers rose from 1.8 to 4.9 million tons (1994-2022), while broad *Capsicum* and bell pepper crops reached nearly 37 million tons in 2022, and global pepper production increased steadily from 1.8 million tons in 1994 to 4.9 million tons in 2022, in both fresh and dried forms, highlighting its commercial significance, positioning it among crops of greatest economic and nutritional relevance worldwide ^[1]. The crop is cultivated across tropical, subtropical, and temperate regions and serves as both a dietary staple and a source of bioactive compounds, including capsaicinoids, carotenoids, and polyphenols, which confer significant nutraceutical and pharmacological value ^[2, 3]. Depending on fruit characteristics including color, size, shape, use, pungency, and flavor peppers are classified into different types, resulting in extraordinary intraspecific morphological diversity within *C. annum* that simultaneously enriches its commercial diversity and complicates systematic variety identification ^[4, 5].

The protection and commercialization of new plant varieties within formal seed systems are governed internationally by the International Union for the Protection of New Varieties of Plants (UPOV). Distinctness, Uniformity and Stability (DUS) testing was introduced under the UPOV Convention in 1961 to safeguard investment and reward innovation in developing new plant varieties, and despite the rapid advancement in understanding of crop biology over the past 60

years, the DUS system has changed little and remains largely dependent upon a set of morphological traits for testing candidate varieties^[6, 7, 8]. Under this framework, a candidate variety must satisfy three criteria: it must be clearly distinguishable from all commonly known varieties by at least one relevant characteristic (Distinctness), it must be sufficiently uniform in its relevant characteristics (Uniformity), and its relevant characteristics must remain unchanged after repeated propagation (Stability)^[9, 10]. For *Capsicum annuum*, the UPOV Test Guideline prescribes over 66 standardized morphological descriptors spanning seedling, vegetative, floral, peduncle, and fruit characteristics as the primary tool for DUS examination^[10]. These descriptors, assessed using standardized ordinal note scales, form the basis upon which distinctness determinations are made, and variety descriptions are produced for national and international registration.

Among the three DUS criteria, distinctness the requirement that a variety be unambiguously differentiable from all known varieties using at least one expressed characteristic is the most analytically demanding^[11]. Plant morphological characters are universally accepted descriptors used to test the distinctness, uniformity, and stability of crop varieties. Yet, the collective discriminatory power of individual descriptors within the prescribed set and the efficiency with which a subset of key traits can achieve equivalent distinctness determinations remain understudied areas in *Capsicum* DUS research. DUS testing is primarily based on morphological descriptors; however, isozyme electrophoresis and molecular markers are occasionally used, and the growing demand for variety registration has highlighted the need for a more systematic evaluation of which descriptors genuinely contribute to inter-variety discrimination^[7, 8].

Multivariate statistical methods particularly principal component analysis (PCA) and hierarchical cluster analysis (HCA) have been widely adopted in the morphological characterization of *Capsicum* germplasm, offering objective, data-driven approaches to variety grouping and discrimination. PCA has been applied in the DUS-based evaluation of bell pepper to identify key components explaining the total variance, with traits such as thousand-seed weight, number of lobes per fruit, fruit diameter, fruit weight, internode length, and fruit length as major contributors to morphological variation^[4, 12, 13, 14]. Similarly, principal component analysis of fruit morphological traits in *Capsicum annuum* germplasm from North Macedonia showed that the first three principal components accounted for 83.1% of total genetic variance, with fruit weight, fruit width, fruit pedicel length, fruit wall thickness, and number of locules, as well as several qualitative traits, including fruit shape and cross-sectional corrugation, contributing most to PC1^[12]. These findings confirm that both quantitative and qualitative fruit descriptors carry substantial discriminatory information, a finding with direct implications for the efficiency of UPOV-based DUS evaluation. Hierarchical cluster analysis using Gower's distance coefficient has emerged as a particularly appropriate method for morphological characterization datasets that combine ordinal and nominal trait data as is characteristic of UPOV descriptor sets. The application of Ward's method and Gower's distance to multivariate morphological characterization of *Capsicum* accessions has been shown to identify taxonomic structure within collections and reveal relationships between groupings, making this approach well-suited to formal DUS distinctness assessment^[15, 16, 17]. Cluster analysis using DUS-based descriptors in bell pepper grouped genotypes into distinct clusters, with maximum inter-cluster distance values indicating broad genetic diversity. The study highlighted the effectiveness of DUS-based

characterization, supported by multivariate analysis, in identifying promising breeding lines and key traits^[4, 14, 18].

Despite these advances, published DUS characterization studies specifically targeting commercial pepper varieties using the UPOV guideline descriptor set and rigorously evaluating the relative discriminatory contribution of each descriptor group remain limited. Most existing studies focus on germplasm or landrace collections rather than the formally registered or candidate variety sets that constitute the primary subject of DUS examination. Research on the discriminatory power of morphological traits in *C. annuum* has indicated that leaf morphometric traits alone are insufficient for accession-level classification. That robust discrimination requires integrative phenotyping frameworks that combine multiple trait categories^[19]. Furthermore, DUS-based studies on bell pepper have reported significant variation across morphological traits, with fruit shape in longitudinal section recording the highest diversity index. At the same time, studies have also identified monomorphic traits including leaf color and fruit blossom-end appendage that show no variation within the evaluated populations, underscoring the importance of identifying non-informative descriptors and removing them from multivariate analysis to avoid dilution of the discriminatory signal^[5].

The adequacy of existing UPOV descriptor sets for unambiguous variety differentiation has been questioned in recent literature, particularly as the number of registered varieties within any crop species increases and the morphological space available for distinctness becomes progressively more crowded^[11, 20]. Genetic divergence studies in *Capsicum annuum* based on multiple morphological, growth, and chemotypic characters have demonstrated that substantial inter-variety diversity exists within the species, yet translating this diversity into reliable, reproducible, and standardized DUS descriptor notes demands systematic evaluation of which traits carry the most consistent and variety-specific morphological signal^[15, 21].

This study addresses these gaps by applying principal component analysis and hierarchical cluster analysis to the 42 morphological descriptors evaluated across seven *Capsicum annuum* varieties Commandant, PS Pepper 1, PS Pepper 2, Piquante, Efia, Serenade, and Spicy over two consecutive growing seasons (2023 and 2024) at the National Agricultural Seeds Council. The study (i) evaluated 42 UPOV descriptor set for polymorphism and discriminatory information content; (ii) identified the traits and trait combinations that account for the greatest proportion of inter-variety morphological variation; (iii) established a pairwise morphological distance matrix to quantify distinctness among varieties; and (iv) evaluated the adequacy of the UPOV TG/76/8 descriptor set for unambiguous variety differentiation in *Capsicum annuum*. The findings are expected to contribute to the evidence base for descriptor efficiency in pepper DUS testing and to provide data relevant to seed certification, plant variety protection, and variety registration protocols.

2. Materials and Methods

2.1 Plant Material

Seven *Capsicum annuum* varieties were evaluated: Commandant, PS Pepper 1, PS Pepper 2, Piquante, Efia, Serenade, and Spicy. These seven varieties were obtained from commercial certified seed companies in Nigeria. Field trials were conducted at Sheda, Federal Capital Territory, Nigeria (Latitude 8° 53' 9" N, Longitude 7° 3' 56" E), over two consecutive growing seasons (2023 and 2024) following the guidelines for plot management, plant spacing, and observation timing. Seeds of the seven varieties

were sown in greenhouse nursery trays, and seedlings were transplanted into field plots five to six weeks after sowing. The seven varieties were arranged in a Randomized Complete Block Design (RCD) with three replications. Each replication consisted of a 5 m × 5 m bed containing two rows spaced 40 cm apart, resulting in 25 plants per cultivar per replication. NPK 15:15:15 fertilizer was applied as needed, and standard agronomic practices were maintained throughout the study.

2.2 Data Collection: A total of 42 morphological descriptors were scored, covering seedling (trait 1), vegetative/plant (traits 2-17), floral (trait 18), peduncle (traits 19-21), and fruit characteristics (traits 22-42), as shown in Table 1. All traits were recorded using the standardized ordinal or nominal note scales prescribed by UPOV TG/76/8. All observations on single plants were made on 10 randomly selected plants per plot, excluding any off-plants. All observations were done when the traits were visible on the plants.

Table 1: The 42 evaluated traits

Group	TG_NO	Traits	State of Expression and Note							
Seedling	1	Seedling: anthocyanin coloration of hypocotyl	Absent 1	Present 9						
Vegetative	2	Plant: growth type/habit	Upright 1	Semi-upright 2	Prostrate 3					
	3	Plant: length of stem	Very short 1	Very short to short 2	Short 3	Short to medium 4	Medium 5	Medium to long 6	Long 7	Long to very long 8
	4	Plant: height	Very short 1	Very short to short 2	Short 3	Short to medium 4	Medium 5	Medium to tall 6	Tall 7	Tall to very tall 8
	5	Plant: length of internodes on primary side shoot	Very short 1	Very short to short 2	Short 3	Short to medium 4	Medium 5	Medium to long 6	Long 7	Long to very long 8
	6	Stem: hairiness of nodes	Absent or very weak 1	Very weak to weak 2	Weak 3	Weak to medium 4	Medium 5	Medium to strong 6	Strong 7	Strong to very strong 8
	7	Plant: anthocyanin coloration of nodes	Absent 1	Present 2						
	8	Stem: intensity of anthocyanin coloration of node	Absent or very weak 1	Very weak to weak 2	Weak 3	Weak to medium 4	Medium 5	Medium to strong 6	Strong 7	Strong to very strong 8
	9	Stem: length	Very short 1	Very short to short 2	Short 3	Short to medium 4	Medium 5	Medium to long 6	Long 7	Long to very long 8
	10	Leaf blade: altitude	Erect 1	Semi Erect 3	Horizontal 5	Semi Drooping 7	Drooping 9			
	11	Leaf blade: length	Very short 1	Very short to short 2	Short 3	Short to medium 4	Medium 5	Medium to long 6	Long 7	Long to very long 8
	12	Leaf blade: width	Very narrow 1	Very narrow to narrow 2	Narrow 3	Narrow to medium 4	Medium 5	Medium to broad 6	Broad 7	Broad to very broad 8
	13	Leaf blade: shape	Lanceolate / ovate 1	Ovate Anaheim 2	Broad elliptic sympathy 3					
	14	Leaf: undulation of margin	Absent or very weak 1	Very weak to weak 2	Weak 3	Weak to medium 4	Medium 5	Medium to strong 6	Strong 7	Strong to very strong 8
	15	Leaf: intensity of green colour	Very light 1	Very light to light 2	Light 3	Light to medium 4	Medium 5	Medium to dark 6	Dark 7	Dark to very dark 8
	16	Leaf: glossiness	Very weak 1	Very weak to weak 2	Weak 3	Weak to medium 4	Medium 5	Medium to strong 6	Strong 7	Strong to very strong 8
	17	Leaf: blistering	Very weak 1	Very weak to weak 2	Weak 3	Weak to medium 4	Medium 5	Medium to strong 6	Strong 7	Strong to very strong 8
Flora	18	Flower: colour	White 1	Light purple 2	Medium purple 3	Dark purple 4				
Peduncle	19	Peduncle: abscission layer	Absent 1	Present 9						
	20	Peduncle: thickness	Very thin 1	Very thin to thin 2	Thin 3	Thin to medium 4	Medium 5	Medium to thick 6	Thick 7	Thick to very thick 8
	21	Peduncle: length	Very short 1	Very short to short 2	Short 3	Short to medium 4	Medium 5	Medium to long 6	Long 7	Long to very long 8
Fruit trait	22	Fruit: twisting	Absent or very weak 1	Medium 2	Strong 3					
	23	Fruit: capsaisin in placenta	Absent 1	Present 9						
	24	Fruit: intensity of green colour of shoulder (before)	Very light 1	Very light to light 2	Light 3	Light to medium 4	Medium 5	Medium to dark 6	Dark 7	Dark to very dark 8
	25	Fruit: intensity of green colour excluding shoulder	Very light 1	Very light to light 2	Light 3	Light to medium 4	Medium 5	Medium to dark 6	Dark 7	Dark to very dark 8
	26	Fruit: ratio length/diameter	Very low 1	Very low to low 2	Low 3	Low to medium 4	Medium 5	Medium to high 6	High 7	High to very high 8
	27	Fruit: Shape of apex	Strongly acute 1	Moderately acute 2	Rounded 3	Moderately depressed 4	Strongly depressed 5			
	28	Fruit: diameter	Very small 1	Very small to small 2	Small 3	Small to medium 4	Medium 5	Medium to large 6	Large 7	Large to very large 8
	29	Fruit: length	Very short 1	Very short to short 2	Short 3	Short to medium 4	Medium 5	Medium to long 6	Long 7	Long to very long 8
	30	Fruit: shape in longitudinal section	Ovate 1	Cordate 2	Elliptic 3	Circular 4	Oblate 5	Rectangular 7	Square 8	Transverse rectangular 9

31	Fruit: number of locules	Predominantly two 1	Equally two and three 2	Predominantly three 3	Equally three and four 4	Predominantly four 5			
32	Fruit: sinuation of pericarp at basal part	Absent or very weak 1	Very weak to weak 2	Weak 3	Weak to medium 4	Medium 5	Medium to strong 6	Strong 7	Strong to very strong 8
33	Fruit: sinuation of pericarp excluding basal part	Absent or very weak 1	Weak 3	Medium 5	Strong 7	Very strong 9			
34	Fruit: shape in cross section at level of placenta	Elliptic 1	Angular 2	Circular 3					
35	Fruit: thickness of flesh	Very thin 1	Very thin to thin 2	Thin 3	Thin to medium 4	Medium 5	Medium to thick 6	Thick 7	Thick to very thick 8
36	Calyx: aspect	Non enveloping 1	Semi enveloping 2	Enveloping 3					
37	Fruit: colour before maturity	Yellow 1	Orange 2	Red 3	Brown 4	Green 5			
38	Fruit: colour at maturity	Yellow 1	Orange 2	Red 3	Brown 4	Green 5			
39	Fruit: glossiness of skin	Very weak 1	Very weak to weak 2	Weak 3	Weak to medium 4	Medium 5	Medium to strong 6	Strong 7	Strong to very strong 8
40	Stalk: thickness	Very thin 1	Very thin to thin 2	Thin 3	Thin to medium 4	Medium 5	Medium to thick 6	Thick 7	Thick to very thick 8
41	Stalk: length	Very short 1	Very short to short 2	Short 3	Short to medium 4	Medium 5	Medium to long 6	Long 7	Long to very long 8
42	Fruit: stalk cavity	Absent 1							

2.3 Data analysis: Descriptive statistics were computed using the mean of all ordinal traits to summarize the range of expression across varieties. Trait polymorphism was assessed by calculating the frequency distribution of notes for each descriptor. Monomorphic traits (identical notes across all seven varieties) were identified and excluded from multivariate analysis, as they contribute no discriminatory information. Principal Component Analysis (PCA) was performed on the polymorphic trait matrix to reduce dimensionality and identify trait combinations that account for the greatest proportion of inter-variety variation. Eigenvalues, percentage variance explained, and factor loadings are reported. A biplot was also used to visualize variety groupings and trait contributions simultaneously.

Hierarchical Cluster Analysis (HCA) was conducted using Gower's distance coefficient, which accommodates mixed ordinal and nominal data types as present in the guideline. Ward's minimum variance linkage method was applied. A dendrogram was constructed to visualize morphological relationships among varieties. All analyses were performed in R (v4.x) using the *FactoMineR*, *cluster*, and *ggplot2* package.

3. Results

3.1 Descriptor Polymorphism

Figure 1 Shows which traits actually contribute to differentiating the varieties. Of all the 42 traits used for this study (Traits 1-42), 8 traits (19%) were monomorphic meaning all seven varieties received the same score for these traits in both 2023 and 2024. These are traits like anthocyanin coloration of the hypocotyl (trait 1), plant growth habit (trait 2), plant anthocyanin coloration of node (trait 7), flower color (trait 18), peduncle abscission layer (trait 19), fruit green color intensity of shoulder (traits 24 and 25), and fruit glossiness of skin-trait 39 (Table 1) because every variety received identical scores for these traits. These traits carried zero discriminatory power and were excluded from the multivariate analysis. These were non-informative descriptors for this particular variety set; suggesting that these traits are too conserved among commercially available pepper varieties to serve as useful distinctness criteria.

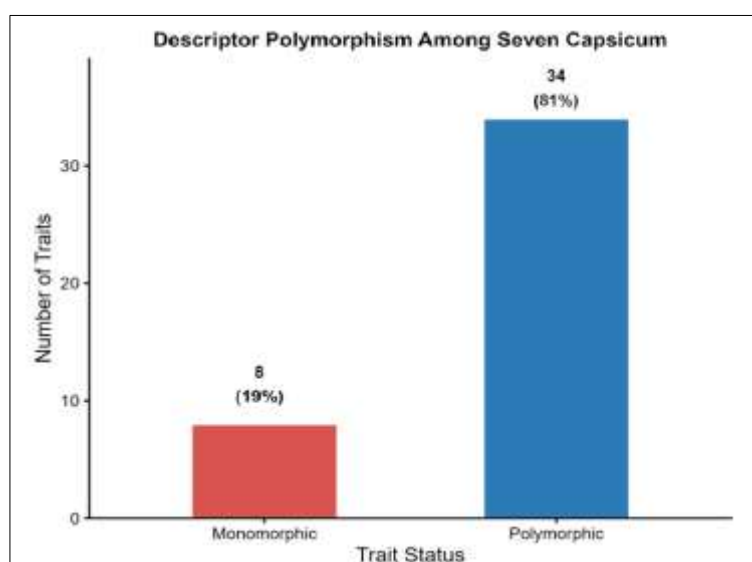


Fig 1: Descriptor Polymorphism

3.2 Scree plot

Figure 2 explains how much of the total morphological variation is captured by each principal component. The first three principal

components collectively explained 74.6% of the total morphological variation (PC1 = 33.0%, PC2 = 26.8%, PC3 = 14.8%) and were retained for further interpretation.

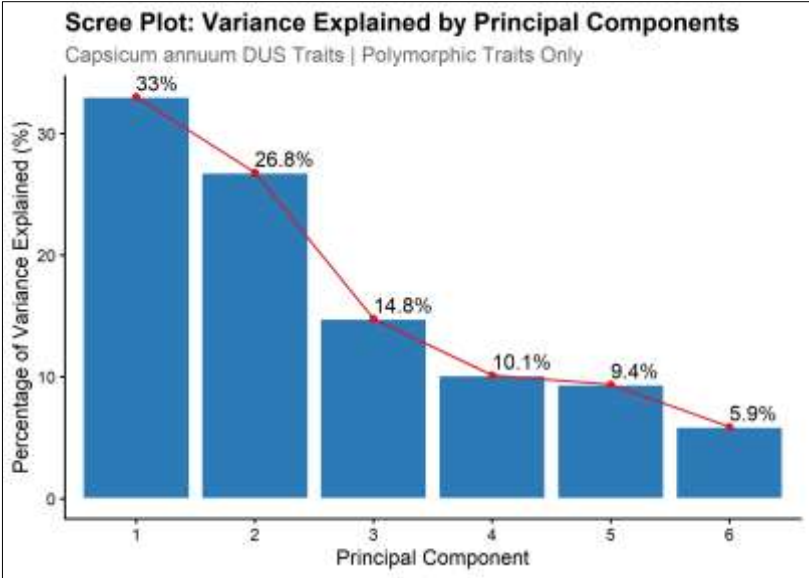


Fig 2: Scree plot

3.3 Varietal scores and trait loadings

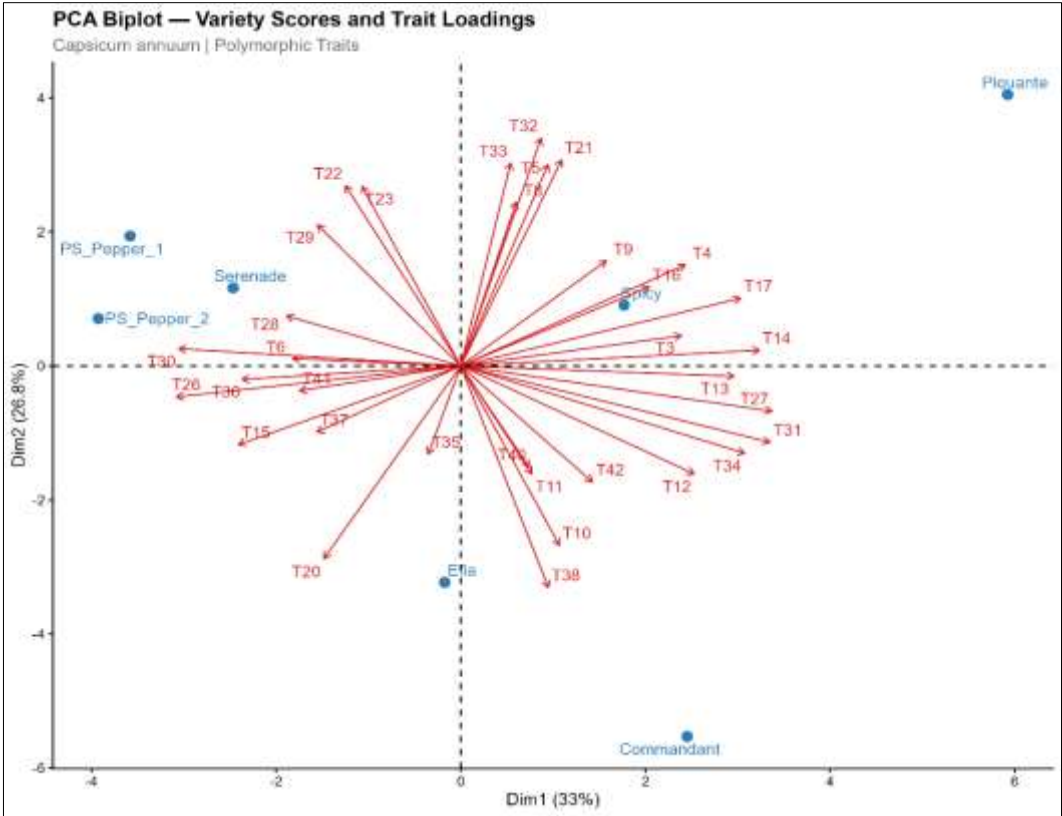


Fig 3: Principal component analysis (PCA) biplot showing the distribution of the seven *Capsicum annuum* varieties (blue dots) and the contribution of traits (red arrows). A variety positioned in the direction of a trait vector scores higher for that trait, whereas a variety positioned in the opposite direction scores lower for that trait.

PCA Biplot separated the seven varieties into distinct morphological groups along two principal axes that explained 59.8% of the total variation. Piquante was the most morphologically distinct variety, characterized by strong positive scores on PC1 and PC2, driven by fruit pericarp situation (T₃₂, T₃₃), peduncle length (T₂₁), and plant stature traits (T₃, T₄, T₅). PS Pepper 1 and PS Pepper 2 clustered closely in the negative PC1 region, distinguished by a high fruit length/diameter ratio (T₂₆), an elongated fruit shape in longitudinal section (T₃₀), and fruit

length (T₂₉). Commandant occupied the lower-right quadrant, differentiated primarily by fruit color at maturity (T₃₈), peduncle thickness (T₂₀), and leaf blade dimensions (T₁₁, T₁₂). Efia, Serenade, and Spicy occupied intermediate positions, with Efia showing the least extreme morphological expression across both axes.

3.4 Top 15 Traits Contributing to PC1

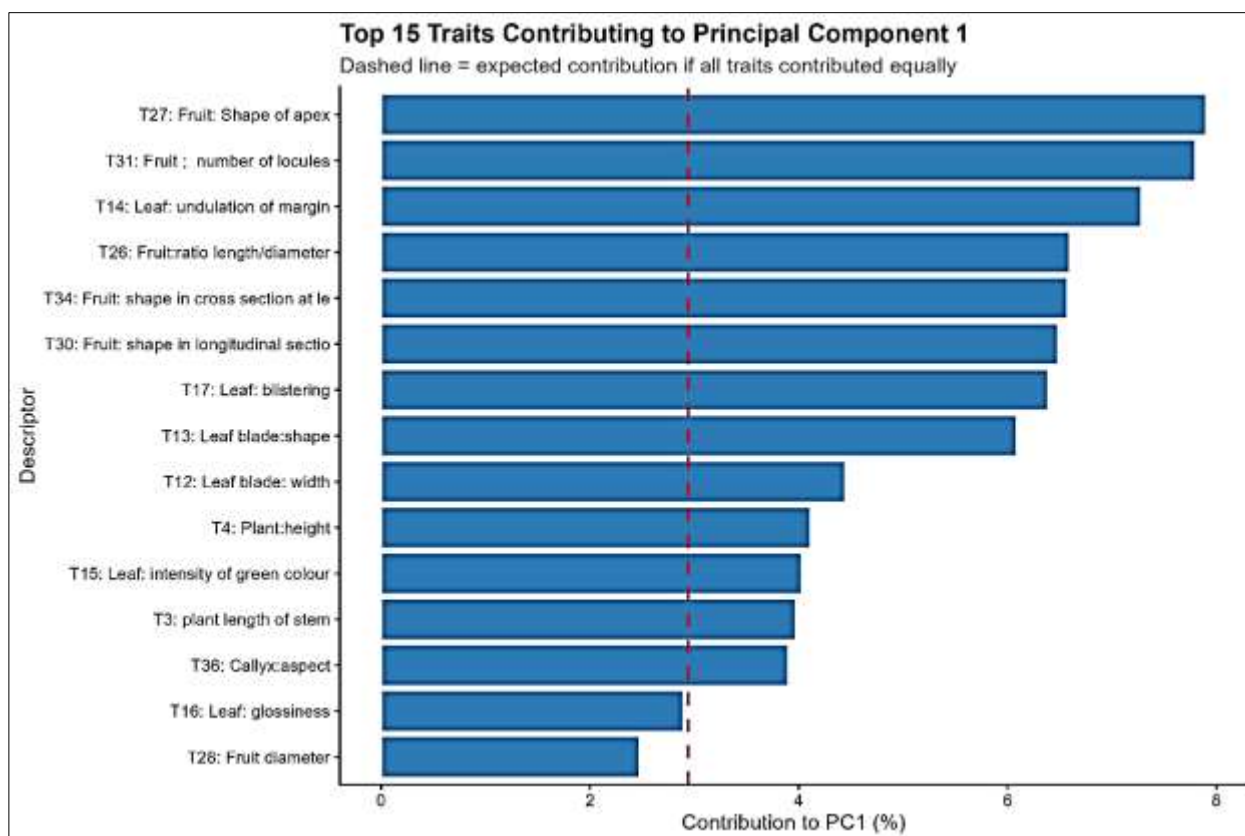


Fig 4: Top 15 UPOV Traits Contributing to PC1. The red dashed line indicates the expected contribution if all 34 polymorphic traits contributed equally ($100\%/34 = \sim 2.94\%$ per trait). Any trait whose bar crosses that line contributes more than its fair share to PC1 and is therefore a priority discriminating descriptor

The top discriminating traits for PC1 were dominated by fruit morphological descriptors apex shape, locule number, length/diameter ratio, and cross-sectional and longitudinal shape with important secondary contributions from leaf traits, particularly margin undulation, blistering, and blade shape. These

findings suggest that the eight above-threshold traits contributed disproportionately to PC1 and may therefore represent priority traits for future evaluation of streamlined DUS descriptor sets.

3.5 Cluster analysis

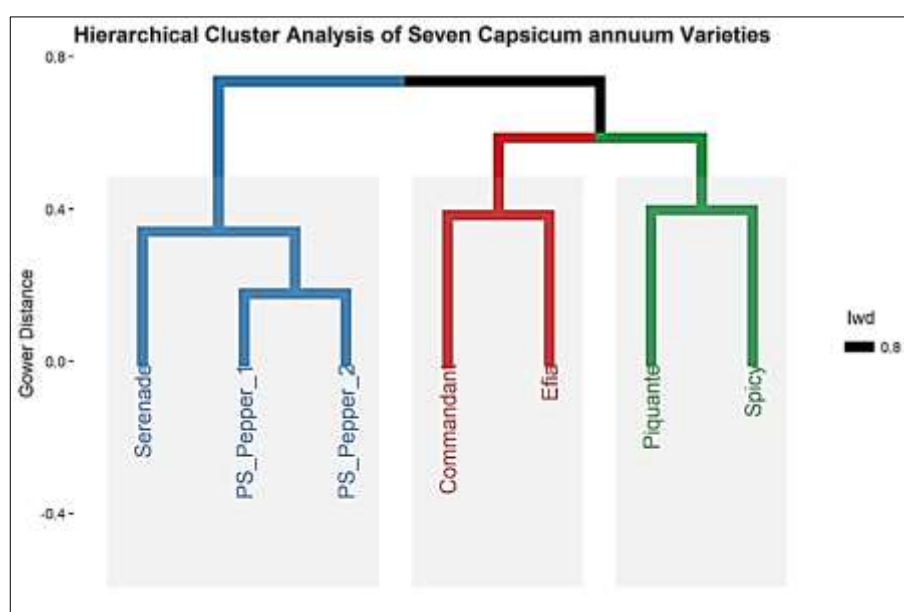


Fig 5: Hierarchical Cluster Analysis of Seven *Capsicum annum* Varieties. This shows the hierarchical grouping of the seven varieties based on their overall morphological similarity across the 34 polymorphic traits, using Gower's distance and Ward's linkage. The Y-axis is the Gower distance at which clusters merge the higher the merge point, the more different the groups being joined

3.6 Mean trait scores

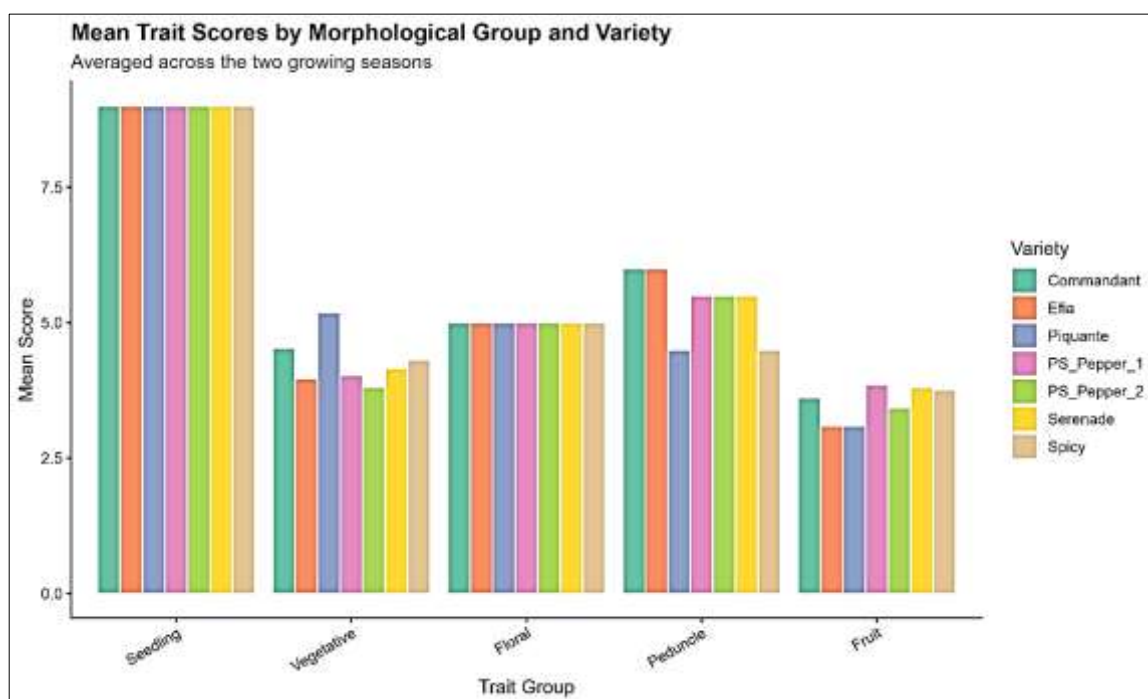


Fig 6: Mean trait scores by Morphological Group and Variety. Each group of bars represents one morphological category, and each coloured bar within the group is one variety.

Analysis of mean trait scores by morphological group (Figure 7) confirmed that fruit traits (T_{22} - T_{42}) and vegetative traits (T_2 - T_{17}) provided the greatest inter-variety differentiation, while seedling and floral trait groups were uninformative across the variety set. Peduncle traits, though comprising only two descriptors, contributed meaningfully to variety separation, particularly for Commandant and Efi

4. Discussion

4.1 Descriptor polymorphism

The polymorphism analysis revealed that 34 of the 42 evaluated morphological descriptors (81%) were polymorphic across the seven *Capsicum annuum* varieties in at least one of the two growing seasons, whereas 8 descriptors (19%) were monomorphic and therefore excluded from the multivariate analysis. This finding is consistent with reports from other DUS-based characterization studies in pepper and related solanaceous crops [8, 22]. DUS-based evaluation of bell pepper genotypes has similarly identified monomorphic traits including leaf color and fruit blossom end appendage that showed no variation within evaluated populations, while morphological characterization of 48 chilli genotypes by Sonanya R *et al.* under DUS guidelines reported maximum variation for fruit shape in longitudinal section, plant habit, leaf shape, and leaf undulation of margin, with green leaf colour among the traits recorded as non-variable [23]. The identification of monomorphic traits is not merely a statistical cleaning step; it carries practical implications for DUS protocol design. Descriptors that are invariant within a defined variety set contribute no discriminatory information to distinctness determination and may inflate the apparent comprehensiveness of the descriptor list without improving its functional performance. The removal of these eight descriptors prior to multivariate analysis follows the methodological precedent established by Kaur G, *et al.* (2026) who excluded monomorphic DUS descriptors from diversity analysis of bell pepper accessions to avoid dilution of the discriminatory signal [19,

24]. The retention of 81% polymorphic descriptors in the present study indicates that the UPOV TG/76/8 guideline is broadly informative for the evaluated variety set, with the majority of the evaluated descriptors showing polymorphism.

4.2 Principal component analysis: Variance partitioning and key discriminating traits

The first three principal components collectively explained 74.6% of total morphological variation (PC1 = 33.0%, PC2 = 26.8%, PC3 = 14.8%), a level of cumulative explained variance that is broadly consistent with published PCA studies in *Capsicum*. PCA of DUS-based bell pepper morphological data identified six components explaining 83.08% of total variance, with fruit diameter, fruit weight, internodal length, and fruit length among the major contributors. PCA analysis of fruit morphological traits in *Capsicum annuum* germplasm from North Macedonia identified that two principal components explained more than 77% of variability based on quantitative fruit parameters, with fruit width, fruit weight, fruit pedicel length, fruit wall thickness, and number of locules [12]. The slightly lower cumulative variance explained by the first two components in this study (59.8%) compared to some germplasm studies may reflect the broader trait scope encompassing vegetative, floral, peduncle, and fruit descriptors simultaneously which can distribute variation across multiple principal components. The elbow in the scree plot after PC3, where individual component contributions dropped below 15%, validates the retention of three components as the threshold for meaningful interpretation, in agreement with the widely applied Kaiser-Guttman criterion of retaining components with eigenvalues greater than one [25].

The identification of fruit shape of apex (T_{27}) and fruit number of locules (T_{31}) as the two highest-contributing traits to PC1 each exceeding 7.5% contribution is a particularly significant finding with direct relevance to DUS practice. Sood *et al.* observed considerable variation among bell pepper DUS descriptors, with fruit shape at the apex recording among the lower Shannon

Diversity Index values, yet fruit shape in longitudinal section recording the highest diversity index of 1.29, suggesting that different fruit shape descriptors carry variable discriminatory power depending on the variety set evaluated ^[5]. In the present study, apex shape emerged as the primary discriminator, reflecting the marked contrast between the depressed apex of Piquante and Commandant and the acutely pointed apex of the PS Pepper varieties a distinction that is visually. Locule number (T_{31}) as the second most important descriptor corroborates findings from Biljana Drvosanova *et al.* (2022) ^[12], who identified locule number as a key variable in fruit morphological diversity among North Macedonian *Capsicum annuum* landraces, and from Kaur *et al.* (2026) ^[8], whose DUS-based bell pepper characterization identified number of lobes per fruit among the principal contributors to PCA-derived variation.

The high contribution of leaf margin undulation (T_{14}) the third most important trait at approximately 6.9% confirms that variety discrimination in *C. annuum* is not exclusively a fruit-based phenomenon. Morphological characterization of chilli germplasm under DUS guidelines has reported maximum variation for leaf undulation of margin alongside fruit shape traits, identifying it as among the most variable and therefore most informative descriptors in the UPOV chilli/pepper descriptor set ^[26, 27, 28]. Studies on Greek traditional pepper landraces using 39 UPOV-based characteristics have similarly evaluated leaf blade undulation of margin and blistering as part of a comprehensive trait assessment framework ^[29], confirming their standardized scoring and relevance to variety typification. The co-contribution of leaf blistering (T_{17}) and leaf blade shape (T_{13}) as above-threshold PC1 contributors in the present study suggests that the leaf morphological complex encompassing shape, surface texture, and margin characteristics constitutes a secondary but substantive axis of inter-variety variation that should not be overlooked in streamlined DUS protocols. This finding supports the recommendation by Yang *C. et al.* (2021) ^[20] that DUS descriptor selection should be evidence-based, retaining traits that consistently demonstrate above-expected discriminatory contribution rather than maintaining descriptor lists by convention.

The fruit length/diameter ratio (T_{26}), fruit shape in longitudinal section (T_{30}), and fruit shape in cross section at the level of the placenta (T_{34}) each contributed between 6.5% and 6.7% to PC1, forming a coherent cluster of correlated fruit geometry descriptors. Fruit shape is recognized as one of the most important morphological characteristics considered in the selection and classification of plants from different *Capsicum* cultivars, and the convergence of multiple fruit shape descriptors as PC1 drivers confirms that the primary axis of morphological differentiation among commercially cultivated *C. annuum* varieties is organized around fruit geometry rather than any single trait ^[13, 30, 31]. Wide variability in fruit morphology including shapes, sizes, and colors has been documented across diverse *Capsicum* germplasm, with substantial differences among accessions in traits such as fruit width, length, weight, and number of seeds per fruit, and the present study confirms that this diversity is expressed, though more narrowly, even within a set of seven commercially available varieties. Below the equal-contribution threshold, traits such as plant height (T_4), stem length (T_3), leaf blade width (T_{12}), and fruit diameter (T_{28}) contributed modestly to PC1, functioning as secondary discriminators that reinforce but do not independently drive variety separation.

4.3 Variety distribution in PCA space and morphological characterization of individual varieties: The biplot overlay of

variety scores and trait loadings provided a unified interpretation of the morphological relationships among the seven varieties. Piquante occupied the most extreme position in the PC1-PC2 space, driven by its strong positive scores on pericarp sinuation at the basal part (T_{32}), pericarp sinuation excluding the basal part (T_{33}), peduncle length (T_{21}), internode length (T_5), and anthocyanin coloration of nodes (T_8). This combination of traits is characteristic of the Piquante pepper market type a compact, highly sinuated, small-fruited pepper with an elongated peduncle relative to fruit size and confirmed that the variety is morphologically the most distinct within the evaluated set. Comprehensive phenotypic characterization of *Capsicum* accessions has demonstrated that fruit sinuation, cross-sectional corrugation, and pericarp-related traits are important discriminators of *C. annuum* variety types across diverse germplasm collections, ^[5, 12, 30, 31] and this study confirmed the primacy of these traits for Piquante characterization at the commercial variety level.

PS Pepper 1 and PS Pepper 2 clustered in the negative PC1 region, defined by high fruit length/diameter ratio (T_{26}), distinct longitudinal section shape (T_{30}), and elevated fruit twisting (T_{22}). This positioning is consistent with an elongated-fruit morphology associated with cayenne-type or chili-type commercial pepper classes. The close proximity of these two varieties in PCA space corroborated by the lowest Gower distance of 0.178 in the morphological distance matrix necessitates careful interpretation, as it raises questions about their distinctness for DUS purposes. Morphological marker-based evaluation of *C. annuum* accessions using PCA found that fruit-related traits, including fruit apex shape, fruit curvature, and locule number, were among the traits showing greatest divergence among accessions, suggesting that a targeted assessment of precisely these traits where PS Pepper 1 and PS Pepper 2 differ is critical for establishing their distinctness ^[32, 33, 34, 35]. The intermediate positions of Commandant (lower-right quadrant), Spicy (upper-right quadrant), Efia (centre-bottom), and Serenade (upper-left, adjacent to the PS Peppers) reflect their position as morphologically intermediate types, each distinguishable from the extremes but differentiated by specific trait combinations rather than overall size or shape dominance.

4.4 Hierarchical cluster analysis and morphological groupings:

The hierarchical cluster analysis based on Gower distance and Ward's minimum variance linkage ^[16] grouped the seven varieties into three distinct clusters, a finding that is consistent with the prevailing pattern of three to six clusters reported in comparable *Capsicum* characterization studies. Cluster analysis of *Capsicum annuum* accessions from North Macedonia classified the accessions into three clusters, where the third cluster grouped local landraces with the highest values for fruit width, fruit weight, and pericarp thickness as major yield components, mirroring the structure observed here where each cluster was defined by a distinct fruit morphological profile ^[12]. The application of Gower's dissimilarity matrix with Ward's clustering method to *Capsicum* accessions has been shown to produce biologically meaningful groupings, with separation of accessions aligned to their morphological and commercial type, validating the methodological choice made in the present study ^[16, 36].

Cluster I (Serenade, PS Pepper 1, PS Pepper 2) represents elongated-fruit varieties with a shared vegetative profile. The sub-clustering of PS Pepper 1 and PS Pepper 2 within this group at a notably lower Gower distance (~0.18) than either merges with Serenade (~0.35) clearly identified these two as the closest variety

pair in the dataset. Ward's clustering algorithm applied to ornamental pepper genotypes grouped 16 genotypes into three groups based on joint quantitative and qualitative data, demonstrating that qualitative traits are important in identifying genetic divergence among closely related accessions, a finding that underscores the importance of examining the specific qualitative descriptors^[37, 38] particularly fruit apex shape and cross-sectional corrugation that differentiate PS Pepper 1 from PS Pepper 2 in this dataset. Cluster II (Commandant and Efi) represents a morphologically heterogeneous pairing, united not by close similarity but by their combined distance from both the elongated-fruit cluster and the Piquante/Spicy cluster. Cluster III (Piquante and Spicy) unites the two most commercially distinct types, a grouping that may initially appear counterintuitive given Piquante's extreme PCA position, but reflects that across the full 34-trait polymorphic space, Spicy shares more overall trait overlap with Piquante than with any other variety. Hierarchical cluster analysis of bell pepper genetic diversity studies have shown that while PCA and HCA generally corroborate each other's groupings, hierarchical methods can provide clearer differentiation among genotype groups by integrating all trait dimensions simultaneously, explaining why Piquante and Spicy co-cluster in HCA despite their visual separation in the two-dimensional PCA biplot.

4.5 Trait group analysis and descriptor adequacy

The analysis of mean trait scores by morphological group revealed important differences in the discriminatory contribution of each descriptor category. The seedling group (trait 1 only anthocyanin coloration of hypocotyl) was uniformly scored at 9 (present) across all varieties, confirming its monomorphic status and the complete absence of discriminatory value for this descriptor in this varietal set. DUS characterization studies of *Capsicum annuum* have similarly identified anthocyanin-related and other seedling-stage descriptors among those requiring careful evaluation for their utility, with only specific qualitative and quantitative characters proving important morphological discriminators across the full descriptor set^[23, 24]. The floral group (trait 18) was equally monomorphic, with all varieties scoring identically for flower color (white) and peduncle abscission layer (present). This result is consistent with the relatively conserved floral morphology of *C. annuum* compared with other *Capsicum* species and raises the question of whether floral traits should be retained in a streamlined DUS descriptor set for commercial variety registration, as opposed to germplasm-level characterization, where interspecies comparisons may reveal greater floral diversity.

The vegetative group showed the most meaningful within-group variation across varieties, with Piquante recording the highest mean vegetative score (~5.3) driven by its strong anthocyanin coloration, long internodes, and distinctive leaf characteristics. These findings align with Wang Y *et al.*'s evaluation of 217 sweet pepper germplasm samples for 25 horticultural traits, which identified plant height, leaf shape, and anther color as essential traits for understanding phenotypic diversity and agronomic potential, confirming the role of vegetative descriptors in comprehensive variety characterization even when fruit traits dominate the primary axes of variation^[39]. The peduncle group comprising only three descriptors (Peduncle abscission T₁₉, peduncle thickness T₂₀ and peduncle length T₂₁) showed notable inter-variety differentiation, particularly separating Commandant and Efi from the remaining varieties on the basis of peduncle thickness. This finding highlighted that even a small number of well-chosen descriptors can carry substantial discriminatory

weight, supporting the broader argument for descriptor streamlining.

The fruit group's low mean scores but high variance reflected in its strong contribution to PC1 despite relatively modest group means illustrates an important statistical principle: it is the *variance* in trait expression, not the *level* of expression, that drives discrimination. This distinction is critical for DUS descriptor evaluation, where a trait that scores moderately on average across varieties may still be highly discriminating if different varieties consistently express different scores. Morphological characterization of chili under DUS guidelines has confirmed that fruit shape in longitudinal section, fruit sinuation of pericarp, fruit shape at the base, and fruit shape of apex are among the most variable and therefore most useful descriptors for variety discrimination^[29, 40], and the present study confirms this pattern, with the concentration of top PC1 contributors in the fruit shape and geometry descriptor categories validating their primacy for DUS-based distinctness determination in *C. annuum*.

4.6 implications for UPOV descriptor efficiency and DUS protocol design:

The collective findings of this study support several conclusions with practical implications for the DUS examination of *Capsicum annuum*. First, the identification of eight monomorphic descriptors representing 19% of the evaluated set provides empirical evidence that a meaningful proportion of the UPOV TG/76/8 list does not contribute to inter-variety discrimination in commercial variety sets of this size. Second, the concentration of PC1 discriminatory power in eight above-threshold traits (T₂₈, T₃₃, T₁₄, T₂₇, T₃₆, T₃₁, T₁₇, T₁₃) suggests that these traits are candidates for further evaluation as a reduced descriptor subset to the full 34-trait polymorphic set, provided that the key fruit geometry and leaf morphology descriptors are retained. The DUS system has changed little over the past 60 years and remains largely dependent on a full set of morphological traits to test candidate varieties. As the demand for more plant varieties increases, the barriers to registration become more acute and thus require urgent review, making evidence-based descriptor reduction an increasingly important research priority^[20]. Third, the PS Pepper 1/PS Pepper 2 distance of 0.178 the only pair falling below 0.3 in the matrix identifies a specific distinctness challenge that requires targeted trait documentation across additional growing environments to confirm whether this distance is consistent or whether environmental conditions may reduce it further to the point where clear distinctness becomes difficult to establish. Taken together, these findings contribute a quantitative, DUS-specific evidence base for descriptor efficiency in *Capsicum annuum* and provide a methodological template applicable to DUS evaluation of other horticultural crops. Conclusively, the seven *Capsicum annuum* varieties exhibit sufficient morphological distinctness to be clearly differentiated, with fruit morphology traits contributing disproportionately to inter-variety discrimination relative to vegetative and floral traits.

5. Author contributions: Conceptualization, methodology, writing original draft preparation, T.O.; investigation, T.K. and T.O.; resources, writing review and editing, visualization, B.O., T.A. and A.O; supervision, project administration, funding acquisition, O.S. All authors have read and agreed to the published version of the manuscript.

6. Funding statement

This research was funded by Alliance for a Green Revolution in Africa (AGRA) under AGRA Grant Reference Number 2023 NG 003-01 and the Nigeria Government

7. Institutional review board statement

Not applicable.

8. Informed consent statement

Not applicable.

9. Data availability statement

The data will be made available upon request.

10. Acknowledgments: We sincerely appreciate all members of the DUS team in National Agricultural Seeds Council, Abuja, Nigeria and all the Seed Companies that supplied their seeds for this study.

11. Conflicts of interest

The authors declare no conflicts of interest.

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