Plant and fruit trait variations among four *Capsicum* species in a Caribbean germplasm collection

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Abstract

Despite *Capsicum*'s importance in the Caribbean, comprehensive diversity studies of this species in the region are limited, especially regarding its morphological variation. This study evaluated 37 traits (seedling, vegetative and reproductive) in 201 accessions among four *Capsicum* species. Multivariate analyses revealed that (i) 54% of the quantitative (seedling and fruit) variation and (ii) 64% of the qualitative (floral and fruit) variation were explained by the first two components. The three main clusters identified did not immediately highlight geographic and species-specific separation. However, significance testing revealed some separation based on geographic subgroups and species assignment. Most Southern Caribbean accessions were considerably similar to each other (if not identical in some cases), thus providing opportunity to identify and remove duplicates from the collection. These Southern Caribbean accessions shared their greatest similarity with Upper Amazon accessions, and least similarity with Lower Amazon accessions, suggesting movement of material primarily from the Upper Amazon into the Southern Caribbean Basin. The dominant differentiating traits displayed in these Southern Caribbean accessions are probably due to strong active selection for certain morphotypes and not to founder effects. Upper and Lower Amazon accessions were largely well differentiated from each other, highlighting key underlying genetic differences between these two populations and possible ongoing barriers to germplasm exchange. Central American, Greater Antilles/Bahamas and Guiana Shield accessions shared similarities with both the Upper and Lower Amazon populations, hinting at probable introductions from both Amazon regions. Collectively, this provides essential baseline information on the morphological (and underlying genetic) relationships among these accessions to guide future characterisation and evaluation efforts on this collection.

Keywords: geographic groups; germplasm evaluation; morphological descriptors; multivariate analysis; species separation

Introduction

South and Meso America are generally considered the centres of diversity for *Capsicum* (Pickersgill, 1969; Eshbaugh *et al.*, 1983; Loaiza-Figueroa *et al.*, 1989; FAO, 1995). Additional regions of diversity can also be

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found in the Caribbean (Moses and Umaharan, 2012). Current estimates of wild species of *Capsicum* exceed 30, of which only five are domesticated (Moscone *et al.*, 2007): *C. annuum* L.; *C. frutescens* L.; *C. chinense* Jacq.; *C. baccatum* L.; *C. pubescens* Ruiz & Pav. The first three domesticated species, considered part of the same gene complex, may intercross, while the other two are generally considered reproductively isolated, although some hybridisation is possible (with difficulty) (Pickersgill, 1967, 1971, 1980). Each domesticated species is known for its wide range of diversity in plant and fruit traits.

In the Caribbean, two domesticated species are cultivated commercially: *C. annuum* (sweet peppers) and *C. chinense* (aromatic hot peppers), with the latter being commercially more important (DeWitt and Bosland, 1996) and integral to the varied cuisines of the Caribbean region. Bird peppers (*C. frutescens*) are generally planted for private consumption. *C. baccatum* and *C. pubescens* are not very well known in the region and only used on a very limited scale as ornamentals.

Taxonomic and botanical distinctions among the *Capsicum* species are generally made using floral traits (Andrews, 1995; ECPGR, 2008): *C. annuum* has very large, bright white flowers; *C. chinense* has a calyx constriction and generally more than two dull white flowers per axil; *C. frutescens* shows an unmistakably greenish-white, stiffly erect solitary flower; *C. baccatum* displays distinctive yellow-green corolla throat spots, while *C. pubescens* displays purple flowers with white throat spots. Sometimes when identity is unclear due to hybridisation, these traits may be combined with seed and leaf traits (ECPGR, 2008) to allow species assignment.

Pepper germplasm diversity is now more rapidly and effectively assessed using well-established multivariate analytical methods (Rego *et al.*, 2003; Ortiz *et al.*, 2008, 2010; Sudré *et al.*, 2010; Ibiza *et al.*, 2012). These methods conveniently allow identification of traits primarily responsible for observed variation, and offer ways to visualise and quantify the similarities/differences among accessions across multiple traits. With these analytical methods, useful complementary insight is gained regarding the structure of any germplasm collection, thereby guiding informed decision-making concerning maintenance of, and further research on, accessions within the collection.

The *Capsicum* germplasm collection (currently 487 accessions) of the University of the West Indies (UWI), Trinidad was assembled to conserve and evaluate the Caribbean's pepper genetic resources. Genetic diversity studies have recently reported on a subset of collection (Moses and Umaharan, 2012); however, there is limited morphological information on plant and fruit traits.

Using complete morphological datasets for 201 accessions (four domesticated species and 37 morphological descriptors), the following factors were investigated: (i) percentage variation explained by qualitative and quantitative traits; (ii) traits most responsible for the observed variation and the possibility of producing a more concise descriptor list; (iii) existence of any meaningful morphological groups based on geographic origin or species.

Materials and methods

Germplasm

The accessions (201) used in this study were assigned to main geographic groups and subgroups based on accession origin data. The main geographic regions used and representative countries sampled were as follows: (A) Central America (14 accessions from Costa Rica, Guatemala, Belize and Mexico); (B) Northern Caribbean [eight accessions from the Greater Antilles (Cuba, Jamaica and Puerto Rico) and the Bahamas]; (C) Southern Caribbean – Trinidad and Tobago (99 accessions), Lesser Antilles (ten accessions from St Lucia, Barbados, Guadeloupe and US Virgin Islands); (D) South America – the Guiana Shield (18 accessions from Venezuela, Guyana, Suriname and French Guiana), the Lower Amazon (14 accessions from Brazil), the Upper Amazon (16 accessions from Peru, Bolivia, Colombia and Ecuador); (E) Other regions (22 accessions which included those with unknown origin data and those from locations outside the Americas/Caribbean). Only four domesticated species could be evaluated because the accessions of *C. pubescens* failed to survive the trial: *C. annuum* (14 accessions); *C. frutescens* (eight accessions); *C. chinense* (175 accessions); *C. baccatum* (four accessions).

Field establishment

The accessions were established in a pot trial at the UWI experimental field during 2 months of the dry season (May–June) and the entire wet season (July–December). Greenhouse establishment of seedlings as well as subsequent field establishment and maintenance of the plants during the study period followed standard local pepper planting procedures (Adams *et al.*, 2007). A total of three plants were used per accession (three blocks in a randomised complete block design). Individual plants were set in plastic UV-resistant pots (20 cm diameter × 30 cm depth) at a spacing of 61 cm × 61 cm. Pot soil mixture comprised cured manure, sharp sand

and top soil (1:1:2), at a pH of 6.5 (no calcium carbonate additions were made). Average (minimum–maximum) atmospheric temperatures for the study period were 22.3 °C–31.8 °C, with a mean relative humidity of 69.6%. Plants were irrigated once per day and kept weed-free (manually) throughout the experiment.

Data collection and analysis

Data were collected using 37 descriptors according to the recommendations and scoring categories of the *Capsicum* descriptor guide (IPGRI *et al.*, 1995), unless otherwise indicated (Table 1). Cotyledon data were obtained using a minimum of 20 seedlings at the greenhouse stage, floral data were collected using ten flowers per plant, fruit data were obtained using ten fruits per plant and whole plant data were collected using each plant per block. In order to determine (a) the percentage variation explained by qualitative and quantitative traits and (b) the most variable traits, the datasets were analysed using multiple correspondence analysis (MCA for qualitative traits) and principal component analysis (PCA for quantitative traits) with Varimax rotation. Dissimilarity levels among accessions (based on species and geographic subgroups) were determined using agglomerative hierarchical clustering, employing Ward's method. All analyses were performed using the statistical package XLSTAT (version 2011.05.01).

Table 1. List of the 37 descriptor traits used in the morphological evaluation of the 201 accessions from the University of the West Indies *Capsicum* germplasm collection

Descriptor categories	Descriptor traits	Descriptor states
Seedling	Cotyledon leaf width[a]	After IPGRI *et al.* (1995)
	Cotyledon leaf length[a]	After IPGRI *et al.* (1995)
	Petiole length[a]	After IPGRI *et al.* (1995)
	Entire cotyledon length[a]	Measured from the blade tip to the petiole base
Floral	Corolla colour	After IPGRI *et al.* (1995)
	Corolla spot colour	After IPGRI *et al.* (1995)
	Calyx constriction	After IPGRI *et al.* (1995)
	Flowers per axil	Solitary/2 flowers/>2 flowers
	Flower position	After IPGRI *et al.* (1995)
Whole plant	Nodal anthocyanin	After IPGRI *et al.* (1995)
	Stem shape	After IPGRI *et al.* (1995)
	Stem pubescence	After IPGRI *et al.* (1995)
	Plant growth habit	After IPGRI *et al.* (1995)
	Mature leaf colour	After IPGRI *et al.* (1995)
	Tillering	After IPGRI *et al.* (1995)
	Plant height[a]	After IPGRI *et al.* (1995)
	Plant canopy width[a]	After IPGRI *et al.* (1995)
Fruit	Anthocyanin spots/stripes	After IPGRI *et al.* (1995)
	Immature fruit colour	After IPGRI *et al.* (1995)
	Mature fruit colour	After IPGRI *et al.* (1995)
	Fruit shape	After IPGRI *et al.* (1995)
	Shape at pedicel attachment	After IPGRI *et al.* (1995)
	Shape at blossom end	After IPGRI *et al.* (1995)
	Neck	After IPGRI *et al.* (1995)
	End appendage	After IPGRI *et al.* (1995)
	Cross-sectional corrugation	After IPGRI *et al.* (1995)
	Fruit surface	After IPGRI *et al.* (1995)
	Pedicel persistence with stem	After IPGRI *et al.* (1995)
	Pedicel persistence with fruit	After IPGRI *et al.* (1995)
	Fruit length[a]	After IPGRI *et al.* (1995)
	Fruit width[a]	After IPGRI *et al.* (1995)
	Pedicel length[a]	After IPGRI *et al.* (1995)
	Fruit weight[a]	After IPGRI *et al.* (1995)
	Locule number[a]	After IPGRI *et al.* (1995)
Seed	Seed colour	Tan/pale yellow/yellow
	Seed surface	After IPGRI *et al.* (1995)
	Seed diameter[a]	After IPGRI *et al.* (1995)

[a] Indicates quantitative traits.
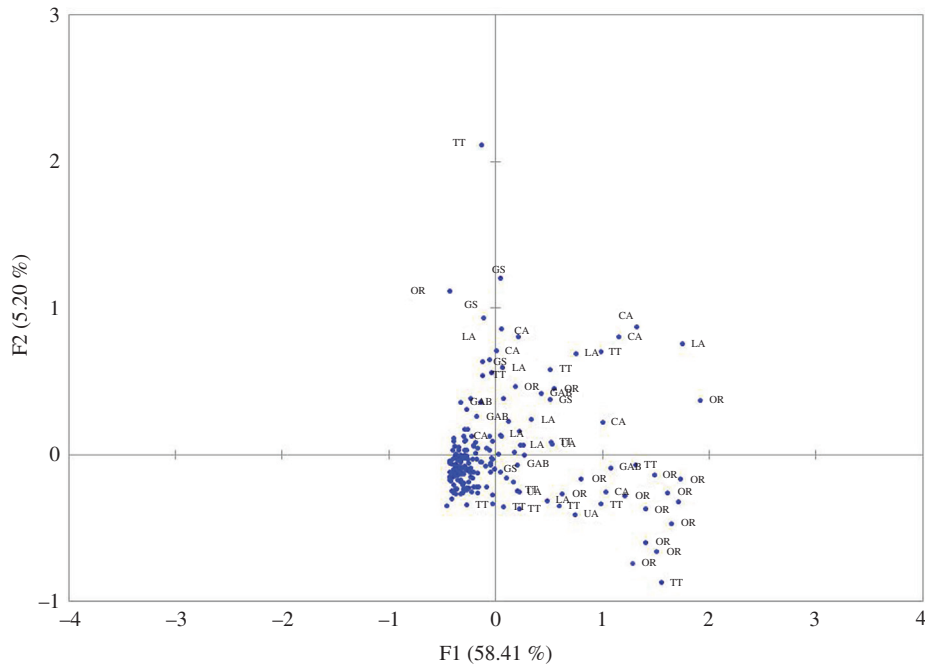
Fig. 1. (colour online). Observation plot of the 201 *Capsicum* accessions showing their distribution across the first two axes after MCA using 25 qualitative descriptor traits. Only outliers are labelled: CA, Central America; GAB, Greater Antilles/Bahamas; TT, Trinidad and Tobago; GS, Guiana Shield; LA, Lower Amazon; UA, Upper Amazon; OR, Other regions.

Results

Qualitative traits

The first two components accounted for approximately 64% of the variation among the accessions for 25 qualitative traits (Fig. 1). More than 12 components were required to account for more than 80% of the total variation observed (data not shown). The first component explained the largest percentage variance (~58%) and 15 descriptor traits were mostly associated with this component: (i) calyx constriction; (ii) corolla colour; (iii) flower position; (iv) flowers per axil; (v) stem pubescence; (vi) leaf colour; (vii) whole fruit shape; (viii) fruit shape at pedicel end; (ix) fruit shape at blossom end; (x) fruit cross-sectional corrugation; (xi) fruit surface; (xii) pedicel persistence with fruit; (xiii) pedicel persistence with stem; (xiv) seed colour; (xv) seed surface. However, only 11 of these descriptor traits were substantial contributors to the variation explained by component 1: floral traits [(i), (ii) and (iv)]; fruit traits [(vii), (viii), (x), (xi), (xii) and (xiii)]; both seed traits [(xiv) and (xv)].

The observation plot (presented using eight geographic subgroups) shows that most of the 201 accessions were tightly clustered (Fig. 1). This suggested that irrespective of geographic origin and species assignment, morphological similarities exist for many of the accessions across most of the qualitative traits assessed. Outliers to the tight cluster were derived from all geographic subgroups except the Lesser Antilles. By species, the outliers represented each of the four species of the study.

Quantitative traits

PCA showed that the first two components explained approximately 54% of the variation. Twelve dimensions were required to explain 100% of the variation among these accessions (data not shown). Six traits were mostly associated with component 1: (i) blade length; (ii) blade width; (iii) petiole length; (iv) entire cotyledon length; (v) number of locules; (vi) seed diameter. However, the largest contributors to component 1 were cotyledon length traits [(i), (iii) and (iv)]. Component 2 was associated with (i) fruit length, (ii) fruit width, (iii) fruit pedicel length, (iv) fruit weight, (v) canopy width and (vi) plant height. However, the largest contributions to component 2 were derived from fruit parameters [(ii), (iii) and (iv)].

The observation plot shows no distinct separation of the accessions based on major geographic regions (Fig. 2). However unlike Fig. 1, there is less compact clustering of most accessions. This suggested less morphological similarity among these accessions for these quantitative parameters. When identified by species (data not shown),
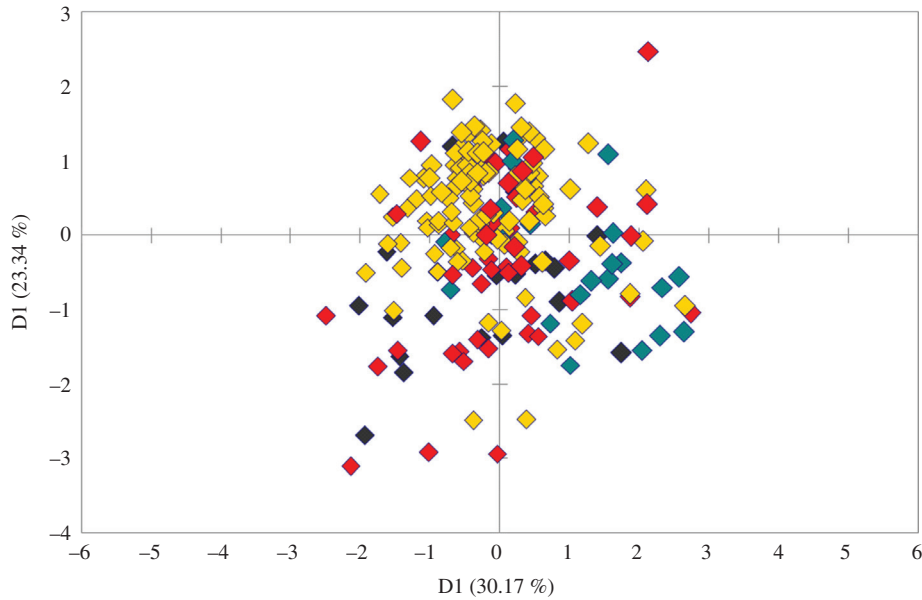
Fig. 2. (colour online). Observation plot of the 201 *Capsicum* accessions showing their distribution across the first two axes after PCA using 12 quantitative descriptor traits (with Varimax rotation). Black, Central America/Northern Caribbean; yellow, Southern Caribbean; red, South America; blue, Other regions.

most *C. chinense* accessions clustered mainly with each other and apart from the other three species.

Trait correlations

The correlation matrix (data not shown) revealed significant correlations ($P < 0.05$) between the following descriptor pairs: (a) cotyledon measurements – cotyledon blade length and petiole length (0.757), cotyledon entire length and petiole length (0.898) and cotyledon blade length and entire length (0.967) and (b) fruit measurements – pedicel length and fruit width (0.524) and fruit weight and fruit width (0.852). The strong significant correlations between the cotyledon measurements and between fruit weight and fruit width measurements provide a basis for the use of only one descriptor for each pair of variables. Correlations between cotyledon traits and fruit traits were significant ($P < 0.05$) but not particularly strong ($P < 0.4$), and therefore they are not considered useful for predicting fruit size parameters using seedling traits.
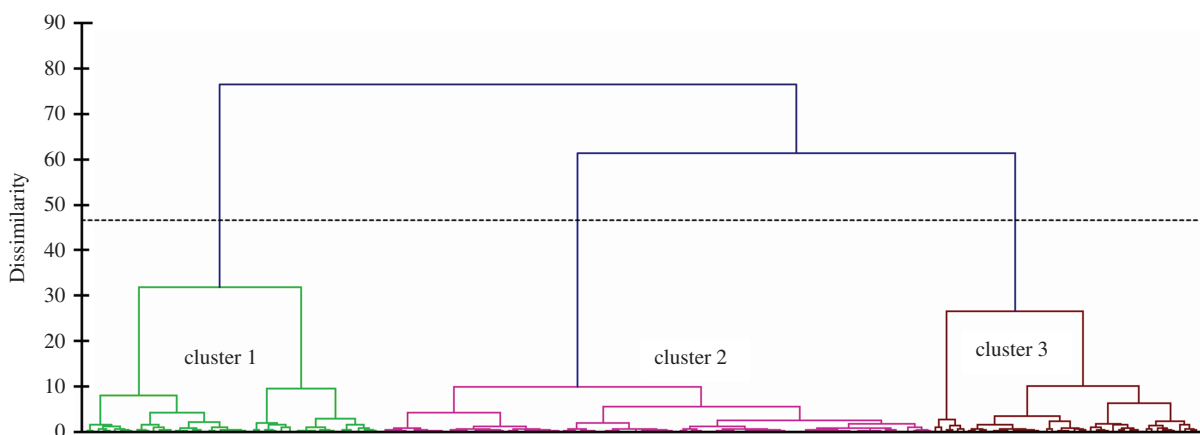
Fig. 3. (colour online). Dendrogram showing the three main clusters found after dissimilarity analysis of the 201 *Capsicum* accessions using 37 descriptor traits. Clusters in which the largest number of accessions was found (by species and geographic subgroup) include: cluster 1 = *C. annuum, C. baccatum, C. frutescens* (Central America, Greater Antilles/ Bahamas, Guiana Shield, Lower Amazon and Other regions); cluster 2 = *C. chinense* (Lesser Antilles and Trinidad and Tobago); cluster 3 = *C. chinense* (Upper Amazon). Table 2 provides a complete summary of the members of each cluster.

Cluster analysis

Three main clusters were identified (Fig. 3) and the distribution of the accessions across the clusters showed no absolute separation of the accessions by species or geographic subgroups (Table 2), but significance testing revealed that there was a link ($P < 0.0001$, $\alpha = 0.05$) between the clusters and the species and geographic subgroups used. Cluster 1 contained most of the accessions from Central America (43%), Northern Caribbean (Greater Antilles/Bahamas) (50%), Guiana Shield (39%), Lower Amazon (57%) and Other regions (45%). By species, cluster 1 contained most of the accessions of *C. annuum* (71%), *C. baccatum* (50%) and *C. frutescens* (88%). Cluster 2 comprised primarily the accessions from the geographic subgroups Trinidad and Tobago (69%) and the Lesser Antilles (90%) that represented the largest number of *C. chinense* accessions (55%). Cluster 3 contained most of the Upper Amazon accessions (56%) and the second largest number of the accessions from Trinidad and Tobago (18%). Clusters 2 and 3 were morphologically more similar to each other (than to cluster 1), indicating that the Southern Caribbean accessions (Trinidad and Tobago/Lesser Antilles) were more similar to the Upper Amazon accessions than to accessions of the other geographic subgroups (Fig. 3). The separation of most of the *C. annuum*, *C. frutescens* and *C. baccatum* accessions from most of the *C. chinense* accessions indicates some recognisable morphological differences between these species groups, thereby alluding to underlying genetic differences.

We analysed the significance of morphological differences among the cluster means for the quantitative traits (ANOVA) and the qualitative traits (χ^2 tests) in order to determine which traits significantly differentiated the clusters from each other. For the quantitative traits, the most significant differences were found in nine of the 12 traits examined: cotyledon measures (blade length, petiole length and entire length); whole plant (canopy width); fruit measures (length, width, weight, pedicel length and locule number). The analysis of the qualitative traits revealed that 11 of the 25 traits were found to have a highly significant link between the clusters and descriptor states: floral traits (calyx constriction, flower position and flowers per axil) and fruit traits (whole shape, shape at pedicel end, shape at blossom end, cross-sectional corrugation, fruit surface, pedicel persistence with fruit, pedicel persistence with stem, seed colour and seed surface).

We then compared the morphological differences among the three clusters, and found that the basis for the clustering was as follows (Table 3): cluster 1 (comprising most of the Lower Amazon, Central American and Northern Caribbean accessions) contained those accessions with the largest cotyledon length measures, the second widest plant canopies and the smallest fruit measures including length, width, weight, pedicel length and number of locules. Cluster 2 contained those accessions with widest plant canopies and the heaviest fruit displaying primarily green immature fruit, red ripe fruit with campanulate fruit forms and sunken fruit bases. Most of the Southern Caribbean accessions were found in this cluster. Cluster 3 contained accessions with longest fruits, but equalled cluster 2 in terms of pedicel length and number of locules. The immature fruit was predominantly light green, ripe fruit was dark red, fruit weight was lighter than that found in cluster 2, and the fruit form was generally blocky and pointed at the fruit base. Most of the Upper Amazon accessions were found in cluster 3 together with the second largest group of the accessions from Trinidad and Tobago, highlighting some level of morphological similarity between the Upper Amazon types and some Southern Caribbean accessions.

Table 2. Summary of the three main clusters identified and the distribution of the 201 *Capsicum* accessions from the University of the West Indies germplasm collection by species and geographic subgroups

		Cluster		
	Groups	1	2	3
Geographic subgroups	Central America	6	3	5
	Greater Antilles/Bahamas	4	2	2
	Lesser Antilles	0	9	1
	Trinidad and Tobago	13	68	18
	Guiana Shield	7	5	6
	Lower Amazon	8	4	2
	Upper Amazon	5	2	9
	Other regions	10	6	6
Species groups	*C. annuum*	10	0	4
	C. frutescens	7	1	0
	C. chinense	34	97	44
	C. baccatum	2	1	1

Table 3. Descriptor traits and states contributing to the similarities and differences found among the three main clusters identified in this morphological study

Traits	Cluster 1	Cluster 2	Cluster 3
Qualitative			
Whole fruit shape	Elongate	Campanulate	Blocky
Shape at pedicel end	Obtuse	Truncate	Truncate
Shape at blossom end	Pointed	Sunken	Pointed
Cross-sectional corrugation	Intermediate	Corrugated	Corrugated
Fruit surface	Semi-wrinkled	Wrinkled	Wrinkled
Seed surface	Rough	Wrinkled	Rough
Quantitative			
Cotyledon blade length (mm)	15.61 (2.68)[a]	13.28 (1.90)	14.38 (2.98)
Cotyledon petiole length (mm)	5.82 (1.78)	4.36 (1.04)	5.13 (1.45)
Cotyledon entire length (mm)	21.42 (4.15)	17.64 (2.72)	19.52 (4.18)
Plant canopy width (cm)	68.72 (10.02)	73.67 (9.74)	56.49 (11.82)
Fruit length (mm)	30.17 (12.49)	36.21 (5.00)	43.78 (8.23)
Fruit width (mm)	14.56 (5.26)	31.81 (4.20)	24.01 (6.58)
Pedicel length (mm)	24.77 (5.11)	28.82 (2.93)	28.90 (4.87)
Fruit weight (g)	2.21 (1.51)	7.10 (1.94)	5.52 (2.13)
Number of locules	2 (0.50)	3 (0.49)	3 (0.47)

[a] Standard deviations are given in brackets.

Discussion

Trait variation and contribution to diversity

Quantitative and qualitative traits (primarily seedling, floral, fruit and seed traits) accounted for more than 50% of the observed variation. Although the variation explained by the qualitative traits (64%) exceeded that of the quantitative traits (54%), greater separation among accessions was achieved using the quantitative traits. This gives us an opportunity to use these quantitative traits (particularly the fruit traits) to create useful subsets of diverse accessions for further characterisation and breeding work on traits of commercial and agronomic importance. In a similar morphological study on Brazilian accessions, 90.5% of the variation was explained by the first two components, leading to a more distinct separation and representation of the relationships between the accessions and groups evaluated (Sudré *et al.*, 2010). Our study agreed with their finding that fruit weight and width were among the variables accounting for useful variation. However, the study by Thul *et al.* (2009) found that although fruit diameter was among the traits contributing most to accession variation, fruit weight made a minimal contribution.

Trait reduction

Our findings indicate that the current list of descriptors can in fact be reduced by 62% (i.e. from 37 to 14 key descriptors) and still allow useful characterisation of the accessions. The PCA and MCA (variable reduction methods) and the correlation analyses identified (i) the key traits contributing to the variation and (ii) the strong significant relationships between certain traits, respectively. In so doing, we identified the high contributions of 12 qualitative traits (floral, fruit and seed) and three quantitative traits (entire cotyledon length, fruit weight and pedicel length). Based on the clustering observed among these 201 *Capsicum* accessions, many of them (derived primarily from the Southern Caribbean) are quite morphologically similar to each other (if not identical in some cases). This now gives us an opportunity to identify duplicates in the collection and facilitate proper rationalisation of resources for conservation and evaluation. This has important implications for faster data collection and processing of accessions, which in turn will reduce the total resource requirements necessary for these evaluation exercises. This is crucial as germplasm evaluation can be costly (in terms of both time and money) and with reduced financial support for evaluation work, it is beneficial to optimise data collection and evaluation where possible (Rego *et al.*, 2003).

Morphological groups based on species or geographic origin

The cluster analysis revealed no distinct separation of the accessions based on their species assignment or main geographic group, thereby suggesting a degree of genetic similarity among many accessions across all represented regions and species of this collection. However, the indication of separation at the subgroup level also suggests that there is a degree of underlying genetic differences

among some accessions, and this may prove useful for future breeding work. Some diversity studies have shown a similar geographic separation of accessions (Sudré *et al.*, 2010, Ibiza *et al.*, 2012), while others (e.g. Sreelathakumary and Rajamony, 2004; Thul *et al.*, 2009) have found no geographical distinction among the evaluated accessions. The latter was perhaps due to the fact that the accession origins were not sufficiently isolated from each other, or that active selection pressures were not strong enough to effect considerable change in form and underlying genetic structure.

Clustering based on geographical subgrouping is probably due to selection pressures (both environmental and anthropogenic). The mainland accessions (Central and South America) come from regions that are quite different in terrain and climate parameters, as well as in cultural and social uses of preferred pepper morphotypes, compared with the Caribbean region. The accessions also have possibly been physically separated for a sufficiently long time to allow such differentiation. The tight clustering seen with most of the Southern Caribbean accessions suggests considerable genetic similarity. Based on the traits responsible for these similarities (green, immature and red, mature fruit, widest plant canopy widths, campanulate fruit shapes, widest fruit widths and largest fruit weights), it appears that active selection (for these desired market characteristics) and not founder effects are responsible for the high frequency of these fruit traits. The morphological similarities of the Upper Amazon accessions and those of the Southern Caribbean types (cluster 3) could possibly be explained by the increasing ease of movement of seed/plant material (via birds and humans) across these regions (Tewksbury and Nabhan, 2001; Reid, 2009). A similar movement of material may also have influenced the similarities found between the accessions of Central America and those of the Northern Caribbean, and between accessions of the Lower Amazon and those of the Guiana Shield.

In the genetic diversity study conducted on a subset of this germplasm collection (Moses and Umaharan, 2012), three distinct phylogenetic clusters were identified (A–C); the largest cluster (B) contained most accessions from the Upper Amazon, Central America, the Southern Caribbean and the Guianas including Venezuela (collectively referred to as the Guiana Shield in the present study). A minority of the Lower Amazon accessions was included in this cluster B. The second largest cluster (C) contained most of the Lower Amazon accessions together with the minority of the Guiana Shield accessions. The third and smallest cluster (A) contained the accessions from the Greater Antilles/Bahamas. Although strict comparisons cannot be made between the individual accessions of the genetic and morphological study (due to the differences in the plant material used), some

interesting general observations can be made based on geographic (sub)groups:

(1) The largest morphological cluster (cluster 2) and largest genetic cluster (cluster B) contain most of the Southern Caribbean accessions. This highlights the high morphological and genetic similarity among the accessions of this part of the Caribbean, and supports the idea of active selection for specific traits.

(2) The morphological separation of most Upper Amazon accessions (cluster 2) from most Lower Amazon types (cluster 1) is reflected in the genetic separation into clusters B and C, respectively. As proposed by Moses and Umaharan (2012), the physical barrier between the Upper and Lower Amazon regions has possibly been quite effective in preventing the movement and exchange of germplasm between these two regions.

(3) Central American and the Greater Antilles/Bahamas accessions are almost equally represented in clusters 1 and 3, which is not reflected in the genetic findings of a completely separate cluster (A) for the Greater Antilles/Bahamas accessions. This contrasting result with the genetic data may be due primarily to differences in accessions used for that study. The morphological similarity observed here hints at underlying genetic similarity of the Greater Antilles/Bahamas and Central American accessions with both the Lower and Upper Amazon populations.

(4) The almost equal distribution of the Guiana Shield accessions between clusters 1 and 3 suggests similarities with both Lower and Upper Amazon regions, thereby implying the probable introduction of genetic material from both Amazon regions. The genetic data also show some similarity of the Guiana Shield accessions with both Lower and Upper Amazon regions and so lend some support to our morphological findings.

(5) Clusters 2 and 3 of this morphological study (more similar to each other than to cluster 1) may be considered as one large morphological group. In this way, we begin to see that most accessions of Central America (8), Upper Amazon (11), Southern Caribbean (96) and Guiana Shield (11) are separated from most accessions of Lower Amazon (8) of cluster 1. This pattern is also seen in the genetic separation of most Lower Amazon accessions into cluster C and apart from the accessions of the four aforementioned regions found in cluster B.

Taken together, this information highlights that although the morphological findings did not completely mirror the genetic findings, they still provide some useful

preliminary insight into a possible genetic basis for the observations made in the morphologies of clusters 1–3.

Species separation

The absence of complete separation based on species was not unexpected because species separation is often primarily used for taxonomic purposes and is not always reflected in morphological/agronomic traits (Thul *et al.*, 2009). A similar species overlap across clusters was observed by Thul *et al.* (2009) and Sudré *et al.* (2010), and cross-fertilisation was put forward as one explanation of these observations. However, our finding that there was in fact some significant difference in species clustering is also reflected in the study by Sudré *et al.* (2010). Despite the known genetic relatedness of *C. frutescens* to *C. chinense* (Ibiza *et al.*, 2012) and the morphological clustering of these two species in the study by Sudré *et al.* (2010), our study showed that most of the *C. frutescens* accessions clustered only with a few *C. chinense* accessions as well as with the majority of *C. annuum* and *C. baccatum* accessions in cluster 1. One of the reasons for this may be due to the strong active selection of certain *C. chinense* morphotypes in the Southern Caribbean, leading to a substantial change in some (particularly fruit) traits.

Two key limitations to this study were (i) the small number of plant replicates used per accession (three) and (ii) representative accessions from (a) the species other than *C. chinense* and (b) the mainland regions of Central and South America. At the time this study was conducted, it was necessary to simultaneously evaluate as many accessions as was feasible. Due to space and resource constraints, only three plants could be accommodated per accession. This low level of repeatability (although still statistically acceptable) may in fact potentially bias the (measured) morphological variation found. Therefore, this limitation must be borne in mind when evaluating our findings and their implications. It must, however, also be noted that this study sought to evaluate mainly the inter-accession (and not intra-accession) variation in an effort to provide preliminary baseline data and perspective on the collection of accessions successfully evaluated.

Concerning the assessment of species variation, it is now known that potential intraspecific variation can be inadequately represented when small numbers of accessions are used (Zuriaga *et al.*, 2009), and this can bias the wider interspecific comparisons. It is therefore important to have good accession representation since interspecific variation can be very important in any study on variation among species and their genetic relatedness (Ibiza *et al.*, 2012). It is therefore proposed here that with a more balanced representation of all the species and geographic groups, as well as increased numbers of plant replicates used, the separation between and among them may be more distinct and offer even more comprehensive insight into the accession, geographic and species-specific differences. Despite the limitations in this regard, however, our study still allowed some useful comparison among accessions, species and geographic (sub)groups, and has provided an important first view and explanation of the morphological structure (based on plant and fruit traits) in this subset of the UWI collection.

Overall, the study has shown that with the aid of multivariate analyses, we have obtained useful explanations of the variation present, as well as some indication of differentiation among accessions based on species and geographic origin. The variation present in this collection is one that may be used for improvement in required plant and fruit traits. The high similarity among the Southern Caribbean accessions provides an opportunity to optimise the number of accessions currently maintained in the collection. The diversity found in accessions of the other geographic regions offers important possibilities for developing a more diverse genetic base (of particularly the Southern Caribbean varieties), in order to facilitate future research, breeding and industry needs.

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