

Original Research Article

Assessment of Genetic Diversity in Pepper (*Capsicum* sp.)

Accessions Using Agro-morphological Characters

ABSTRACT

Aims: To examine the genetic diversity and relatedness among pepper accessions using agro-morphological markers.

Study design: Augmented design with single rows of each genotype.

Place and Duration of Study: Research field, CSIR-Savanna Agricultural Research Institute, Nyankpala-Tamale, Ghana, May to October, 2012.

Methodology: Fifty local pepper genotypes from different agro-ecological zones in Ghana were characterized using 35 agro-morphological (11 quantitative and 24 qualitative) traits using standard descriptors for *Capsicum* sp. For quantitative traits, measurements were recorded on ten tagged plants and the mean value used while four tagged plants were scored for the qualitative traits.

Results: The first three principal components accounted for 59.61% of the total genetic variance among the genotypes with the larger part of the variance explained by fruit pedicel width, fruit weight, fruit length, plant height, stem diameter, fruit pedicel length and fruit width. For qualitative traits, the first three principal components contributed to 51.65% of the total genetic variance with leaf pubescence density, fruit position, calyx annular constriction and filament colour as the most important parameters. The study showed a great variation in fruit traits revealing six fruit shapes and four fruit colours. Fruit weight exhibited positive and significant correlations with yield components such as fruit length and fruit width.

Conclusion: The study has shown that morphological markers are effective tools in studying genetic diversity in *Capsicum* species. This observed diversity among the pepper genotypes would therefore be used for improving pepper through hybridization and selection.

Keywords: *Pepper*; correlation analysis; genetic diversity; principal components; cluster analysis

1. INTRODUCTION

Pepper, (*Capsicum* sp.), is an economically important crop grown worldwide for vegetable, spice, ornamental and medicinal uses and is a significant source of vitamins A and C [1]. It is one of the most important vegetable crops in Ghana with numerous uses. It is found within the family Solanaceae of the genus *Capsicum* and is believed to have originated from tropical South America with Brazil as the major centre of diversity where all cited levels are found [2]. The genus *Capsicum* has five domesticated species (*C. annuum*, *C. pubescens*, *C. chinense*, *C. baccatum* and *C. frutescens*) which were thought to have been domesticated through at least five independent events [3]. Natural interspecific cross compatibility among

19 these *Capsicum* species often result in complex hybrids difficult to characterize. Pepper was
20 introduced into Europe by Columbus and other early new explorers in the sixteenth century
21 and cultivation spread throughout the world with *C. annuum* being the most widely cultivated
22 [4]. The predominance of *C. annuum* among the cultivated species globally is not attributed
23 to superior agronomic or consumer traits but it was the first to be introduced into Europe [5].
24 It has therefore become one of the most important spice commodities and as well as
25 essential vegetable crop worldwide [1].

26 Pepper is a small perennial shrub characterized by white or greenish-white corolla, one or
27 more pedicels at a node with varying fruit sizes and shapes [6]. The wild ancestor of
28 domesticated *C. annuum* has erect, small fruits that are pungent, red in colour, deciduous
29 and soft-fleshed. These traits promote consumption and seed dispersal by birds rather than
30 mammals [7]. Domestication and commercialization of pepper has led to the selection of
31 traits such as compact architecture, increased efficiency of self-pollination, early flowering
32 and non-deciduous, high fruit set, pendant fruits. Pepper cultivars have been selected for
33 based on the uses of the crop and consumer-preferred fruit traits such as degree of
34 pungency, fruit shape and colour flavour, fruit wall thickness and drying ability [1].
35 Continuous selection during domestication and mono-cropping has led to the identification of
36 cultivars with bigger non-pungent fruits with greater variation in shape and remarkable
37 increases in fruit mass [8]. These large-fruited non-pungent bell type peppers were found in
38 pre-Columbian Mexico and were first described about 500 years ago. Although the pungent
39 pepper cultivars are considered the most important spice crop globally, the large non-
40 pungent bell peppers also consumed worldwide, are the most economically important
41 pepper type [1].

42 Crop improvement programs are aided by the availability of genetically diverse germplasm
43 within the gene pool of a crop species. However, the richness of genetic diversity in a
44 species are barely fully tapped by breeders due to scarcity of information on the extent of

45 diversity among the breeding materials [9]. Genetic diversity of landrace germplasm is
46 considered as one of the criteria for selection of parents for pepper breeding, because of
47 their competitive advantage. Tanksley and McCouch [10] indicated that the genetic base of
48 most crops were narrowed when wild species were domesticated and also when landraces
49 were replaced by modern improved cultivars. These landrace germplasm have higher
50 genetic variability among the different groups of germplasm, as well as better environmental
51 adaptation, and are an irreplaceable source of highly adapted genotypes [11]. Landraces of
52 crops constitute a dynamic and important component of agricultural biodiversity which has
53 been valued almost totally as the source of essential traits that can be used in crop breeding
54 programs and to improve the productivity of new crop varieties. However germplasm from
55 exotic sources may possess important traits that may not be found within the available
56 germplasm. Therefore assembling landraces and exotic genotypes allow breeders to
57 improve both within and between population variations which will allow these genotypes to
58 cope with variable environmental stresses [12].

59 Germplasm characterization is useful in detecting variations in different cultivars purposely
60 for identification and elimination of duplicates. It is also used for revealing distinct genotypes
61 that could be used as parental lines to establish hybridization programs. Morphological
62 markers such as plant stature, stem pubescence, fruit weight, flower colour, fruit shape,
63 plant height etc., have been used to distinguish between pepper genotypes and characterize
64 them into groups [13, 14]. The use of these agro-morphological markers in describing and
65 classifying germplasm is a fundamental step in genetic diversity assessment [15, 16]. It is
66 very useful in describing the genetic variation available within a crop species and to allow the
67 detection of sources of genetic diversity for crop improvement [17]. Estimation of the genetic
68 diversity among cultivated genotypes using agro-morphological characters has become an
69 important requirement of the crop industry for identification, elimination of duplicates and
70 crop improvement [18]. The extent of genetic variability within a species is vital for its

71 continued existence and adaptation in different agro-ecologies. The more diverse the
72 population is the better for the breeder in developing elite cultivars through careful selection
73 of superior parents. Therefore, an understanding of the extent of genetic variability of a
74 population, through the use of agro-morphological markers, is of critical importance in
75 estimating possible loss of genetic variability and developing effective strategies for
76 germplasm conservation and breeding purposes [16, 19]. The objective of this work was to
77 characterize pepper genotypes from different geographical regions of Ghana using agro-
78 morphological markers and to determine the genetic relatedness within the germplasm.

79 **2. MATERIAL AND METHODS**

80 **2.1 Study area**

81 The experiment was conducted at the experimental field of CSIR-Savanna Agricultural
82 Research Institute (CSIR-SARI), Nyankpala in the Guinea Savanna zone of Ghana (N
83 09°23'21.4"; W 001°00'13.4"). The zone is characterized by a mono-modal rainfall pattern
84 and receives an average annual rainfall of about 800-1200 mm.yr⁻¹ with the rainy season
85 falling between May and September. The mean annual temperature is recorded to be 24-
86 34°C. The soil belongs to the Kumayili series and commonly classified as Ferric Luvisols.
87 The field had a gentle slope of about 1-2% with a well-drained sandy-loam soil.

88 **2.2 Genotypes used for the study**

89 Fifty pepper genotypes comprising forty-eight landraces collected from diverse agro-climatic
90 ecologies in Ghana through regular germplasm collection as well as two improved
91 genotypes obtained from CSIR-SARI were used in the present study (Table 1). The pepper
92 seeds were nursed in seed boxes using potting mix sterilized by steam sterilization method.
93 The potting mix consisted of two parts of top soil to one part of well decomposed cow dung.
94 Nursery management practices, such as shading, forking, thinning, watering and hardening
95 off, were carried out appropriately to ensure that healthy seedlings were produced. The
96 seedlings were transplanted onto ridges 6 weeks after nursing at a spacing of 75 cm x 50

97 cm. The experimental design used was augmented design with single rows (5 m long) of
 98 each genotype. Other agronomic practices such as weeding, fertilizer application, diseases
 99 and insect pest control were carried out uniformly as required for the growth of pepper.
 100 Morphological data were collected on 35 (24 qualitative and 11 quantitative) traits from
 101 seedling emergence to crop maturity adopting standard descriptors for *Capsicum* sp.
 102 developed by IPGRI [20] with slight modifications by Asian Vegetable Research and
 103 Development Center (AVRDC). Each qualitative trait was scored by observing four tagged
 104 plants per genotype. For quantitative traits, measurements were recorded on 10 tagged
 105 plants and the mean value used.
 106 Descriptive statistics (mean, standard error, standard deviation, coefficient of variation and
 107 frequency distribution), principal component analyses (PCA) and correlations analysis of the
 108 morphological traits were carried out using GenStat software version 12.1 [21]. Principal
 109 coordinate analysis (PCoA) was carried out to determine the grouping of the genotypes
 110 using DARwin software programme version 5 [22]. Cluster analysis based on Euclidean
 111 similarity coefficient was used to generate a dendrogram using GenStat software version
 112 12.1 [21].

113 Table 1. Pepper accessions used in the study

<i>Genotype</i>	<i>Code</i>	<i>Origin</i>	<i>Region</i>	<i>Taxonomy</i>
VR HOE 11	VR 4	Hohoe	Volta	<i>Capsicum annuum</i> L.
VR KPV 1	VR 7	Kpeve	Volta	<i>Capsicum annuum</i> L.
VR KTS 2	VR 8	Kpogen	Volta	<i>Capsicum annuum</i> L.
BA TAN 3	BA 1	Hianmunchend	Brong Ahafo	<i>Capsicum annuum</i> L.
VR HOE 1	VR 3	Ve-Koloenu	Volta	<i>Capsicum annuum</i> L.
VR KTS 13	VR 2	Nogokpo	Volta	<i>Capsicum annuum</i> L.
BA TAN 12	BA 4	Kyekyewere	Brong Ahafo	<i>Capsicum annuum</i> L.
ER UMK 2	ER 1	Asesewa	Eastern	<i>Capsicum annuum</i> L.

BA JMS 3	BA 2	Babiania	Brong Ahafo	<i>Capsicum annuum</i> L.
UE KNW 7	UE 1	Paganiani	Upper East	<i>Capsicum annuum</i> L.
UE TND 1	UE 6	Talensi	Upper East	<i>Capsicum annuum</i> L.
UE BOM 2	UE 9	Bolgatanga	Upper East	<i>Capsicum annuum</i> L.
NR TAM 4	NR 5	Tamale	Northern	<i>Capsicum annuum</i> L.
UE BAW 2	UE 2	Bawku	Upper East	<i>Capsicum annuum</i> L.
UE BAW 7A	UE 3	Bawku	Upper East	<i>Capsicum annuum</i> L.
UE BAW 7B	UE 5	Bawku	Upper East	<i>Capsicum annuum</i> L.
NR WMP 4	NR 2	Walewale	Northern	<i>Capsicum annuum</i> L.
GA ACC 1	GA 2	Madina	Greater Accra	<i>Capsicum annuum</i> L.
VR KTS 8	VR 9	Kpogen	Volta	<i>Capsicum annuum</i> L.
UE KNW 3	UE 8	Paganiani	Upper East	<i>Capsicum annuum</i> L.
VR KTS 9	VR 5	Kpogen	Volta	<i>Capsicum annuum</i> L.
ER UMK 3	ER 2	Asesewa	Eastern	<i>Capsicum annuum</i> L.
BA SYW 8	BA 7	Ayakomaso	Brong Ahafo	<i>Capsicum annuum</i> L.
VR HOE 8F	VR 10	Gbi, Hohoe	Volta	<i>Capsicum annuum</i> L.

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115

Table 1. Cont'd

Genotype	Code	Origin	Region	Taxonomy
UE KNE 4	UE 11	Goo, Paga	Upper East	<i>Capsicum annuum</i> L.
NR TKB 9	NR 1	Cheschegu	Northern	<i>Capsicum annuum</i> L.
NR WMP 6	NR 3	Diany	Northern	<i>Capsicum annuum</i> L.
UE BNG 1	UE 15	Bongo	Upper East	<i>Capsicum annuum</i> L.
UE KNE 8	UE 14	Zone J	Upper East	<i>Capsicum annuum</i> L.
UE KNW 13	UE 12	Navrongo	Upper East	<i>Capsicum annuum</i> L.
NR TKB 6	NR 7	Nwogu	Northern	<i>Capsicum annuum</i> L.
GA DWW 7	GA 3	Shai hills	Greater Accra	<i>Capsicum annuum</i> L.
GA ACC 5	GA 1	Madina	Greater Accra	<i>Capsicum annuum</i> L.

UE KNW 8	UE 4	Banyono	Upper East	<i>Capsicum annuum</i> L.
UE KNW 9	UE 10	Banyono	Upper East	<i>Capsicum annuum</i> L.
UE KNE 2	UE 7	Nangali	Upper East	<i>Capsicum annuum</i> L.
VR HOE 10 B	VR 6	Hohoe	Volta	<i>Capsicum annuum</i> L.
BA SYW 6	BA 8	Ayakomaso	Brong Ahafo	<i>Capsicum annuum</i> L.
NR TAM 6	NR 6	Tampeikukuo	Northern	<i>Capsicum annuum</i> L.
VR HOE 4	VR 1	Ur Avegpo	Volta	<i>Capsicum annuum</i> L.
NR SVN 4	NR 4	Libga	Northern	<i>Capsicum annuum</i> L.
BA JMS 4	BA 5	Babiania	Brong Ahafo	<i>Capsicum annuum</i> L.
UE KNE 6	UE 13	Bonia	Upper East	<i>Capsicum annuum</i> L.
ER UMK 1	ER 3	Asesewa	Eastern	<i>Capsicum annuum</i> L.
Legon 18	S 1	SARI	Northern	<i>Capsicum annuum</i> L.
Shito Adope	S 2	SARI	Northern	<i>Capsicum annuum</i> L.
VR KPD 2	VR 11	Kpando	Volta	<i>Capsicum annuum</i> L.
NR TKB 3	NR 8	Nwogu	Northern	<i>Capsicum annuum</i> L.
BA TAN 11A	BA 3	Nsawkaw	Brong Ahafo	<i>Capsicum annuum</i> L.
BA TAN 11B	BA 6	Nsawkaw	Brong Ahafo	<i>Capsicum annuum</i> L.

116 3. RESULTS AND DISCUSSION

117 3.1 Phenotypic evaluation of qualitative traits

118 Genetic characterization based on standard descriptors such as plant size, plant growth
119 habit, branching habit, stem pubescence, leaf shape, flower position, fruit shape and colour
120 helps to easily describe the morphological features of a genotype and makes diversity
121 assessment easier [23]. The accessions exhibited a wide variation for several morphological
122 characters studied. Most of the accessions had abundant pubescence on the hypocotyl
123 (Table 2). Cotyledon colour ranged from light green to green with the shape varying from
124 ovate to elong-deltoid. Majority of the accessions had sparse stem pubescence with green
125 colour. Two modes of plant growth were observed (62% compact and 38% erect). Leaf

shape varied between deltoid and lanceolate with sparse pubescence characterizing majority of them. Most of the accessions had intermediate plant size with abundant branches and dark green leaves. Yellow-green corolla (Figure 1A) was common among the accessions with filament colour mostly white. Anther colour varied from pale blue to purple (Figure 1B). Fruit shape at pedicel attachment ranged from acute to cordate with or without neck at base of fruit. Fruit shape at blossom end varied from pointed to sunken. Fruit cross-section ranged from smooth to corrugate with two or three locules per fruit (Figure 1C). The study revealed six major classes of fruit shape ranging from elongate to blocky and four fruit colours at mature stage with red as the predominant fruit colour (Figure 1D). The variations observed in the present study are in consonance with observations by Fonseca *et al.* [13] who found four fruit shapes varying from elongate to bell and nine fruit colours when they characterized 38 pepper genotypes from Brazil. Manju and Sreelathakumary [23] also found substantial variations in 32 pepper accessions using 18 morphological markers. The presence of wide genetic variation for a trait within a population indicates the potential for genetic improvement of that trait [24]. The variations among the accessions in the present study could be used in future pepper improvement programmes to breed for specific consumer-preferred traits.

Table 2: Frequency distribution of 20 qualitative traits among pepper accessions

No.	Descriptor		Descriptor state (frequency percentage)			
1	Hypocotyl		Sparse	Intermediate	Abundant	
	pubescence		(6%)	(16%)	(78%)	
2	Cotyledon	leaf	Ovate	Lanceolate	Elong-deltoid	Mixture
	shape		(6%)	(82%)	(10%)	(2%)
3	Cotyledon	leaf	Light green	Green		
	colour		(18%)	(82%)		
4	Stem colour		Green	Green	with	Green with many purple

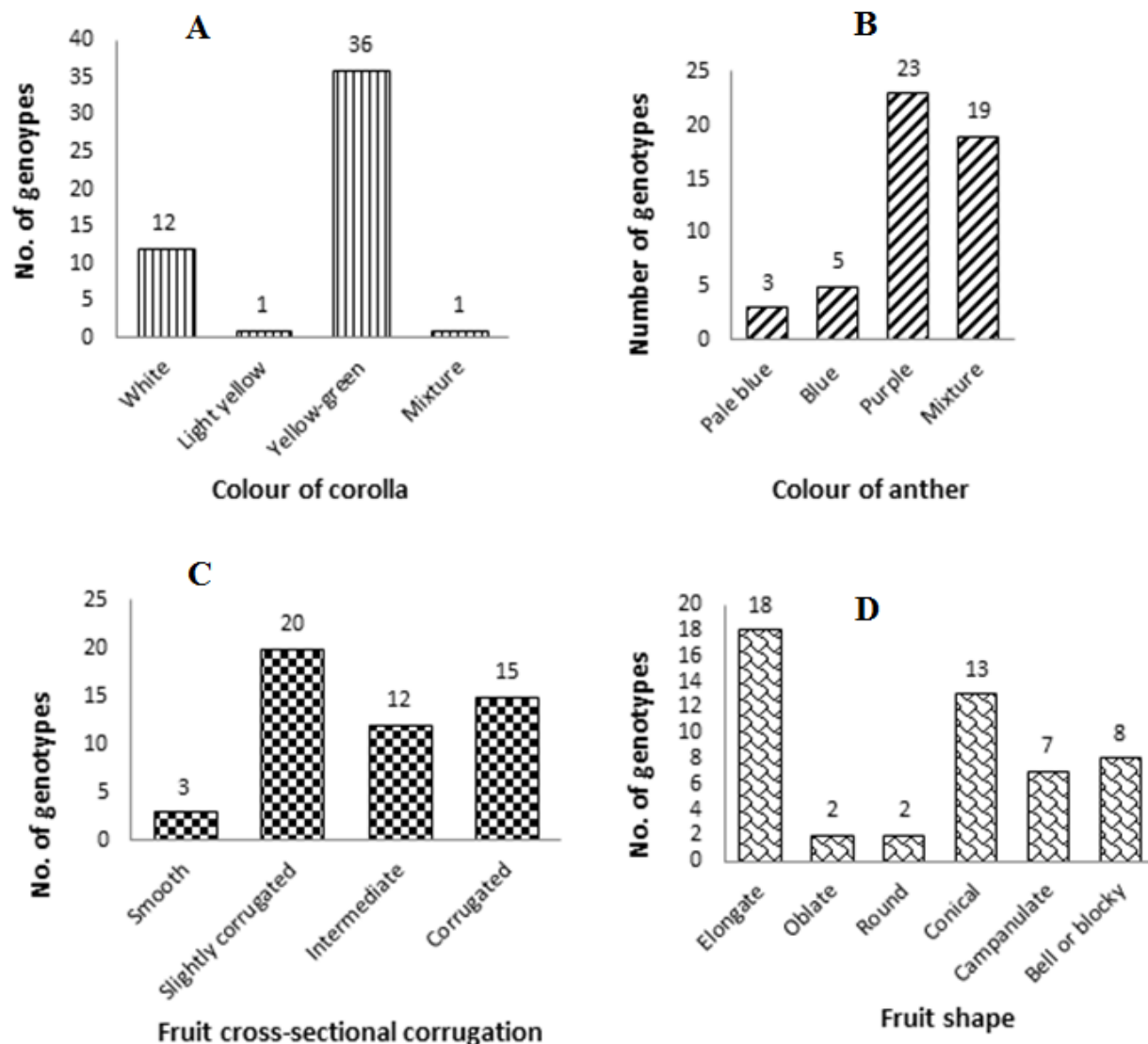
			(72%)	few purple strips		
				strips	(20%)	
				(8%)		
5	Plant growth habit	Compact	Erect			
		(62%)	(38%)			
6	Stem pubescence	Glabrous	Sparse	Intermediate	Abundant	
		(4%)	(66%)	(26%)	(4%)	
7	Leaf pubescence	Glabrous	Sparse	Intermediate	Mixture	
		(16%)	(58%)	(16%)	(10%)	
8	Leaf shape	Deltoid	Ovate	Lanceolate	Mixture	
		(20%)	(54%)	(22%)	(4%)	
9	Leaf colour	Light green	Green	Dark green	Mixture	
		(8%)	(36%)	(50%)	(6%)	

144

145 Table 2. Cont'd

No	Descriptor		Descriptor state (frequency percentage)			
10	Branching habit		Sparse	Intermediate	Abundant	Mixture
			(2%)	(28%)	(62%)	(8%)
11	Plant size		Small	Intermediate	Large	
			(14%)	(70%)	(16%)	
12	Filament colour		White	Light purple	Purple	Mixture
			(76%)	(12%)	(2%)	(10%)
13	Calyx margin shape		Intermediate	Dentate		
			(74%)	(26%)		
14	Calyx annular constriction		Absent	Not clear	Clear	Distinct and Mixture
			(2%)	(16%)	(22%)	uniform in whole (2%)

						plant (58%)	
15	Fruit position		Declining (44%)	Erect (14%)	Mixture (42%)		
16	Fruit colour at mature stage		Green (4%)	Yellow (2%)	Orange (2%)	Red (86%)	Mixture (6%)
17	Fruit shape at pedicel attachment		Acute (6%)	Obtuse (44%)	Truncate (44%)	Cordate (6%)	
18	Neck at base of fruit		Absent (74%)	Present (26%)			
19	Fruit shape at blossom end		Pointed (60%)	Blunt (22%)	Sunken (16%)	Mixture (2%)	
20	Fruit cross- sectional corrugation		Smooth (6%)	Slightly corrugated (40%)	Intermediate (24%)	Corrugated (30%)	



147

148 Fig. 1 Frequency distribution of four traits among 50 pepper accessions from Ghana

149 3.2 Phenotypic analysis of quantitative traits

150 Cotyledon leaf length ranged from 1.24 cm in BA JMS 4 to 2.36 cm in VR HOE 11 with a
 151 mean of 1.76 cm (Table 3). Cotyledon leaf had widths ranging from 0.45-0.87 cm. The
 152 heights of the pepper genotypes ranged from 31.00-91.70 cm with an average height of
 153 49.61 cm. Pepper accessions with intermediate heights would be good for intercropping
 154 purposes. The diameters of the stems were from 0.60-1.73 cm. The average genotype used

about 41 days for 50% of its population to flower. The days to 50% flowering ranged from 22-58 days after transplanting was done. Days to 50% fruiting ranged from 26-29 days after days to 50% flowering were attained. Fruit pedicel length and width ranged from 1.49-3.21 cm and 0.09-0.39 cm, respectively. The mean fruit length was 4.44 cm with BA TAN 3 and VR KTS 2 having the shortest and longest fruit lengths of 0.70 cm and 10.46 cm, respectively. Fruit width also ranged from 0.63-3.90 cm with an average of 1.58 cm. Fruit weight which is the most important economic trait ranged from 4.50-131.50 g with genotypes UE TND 1 and GA ACC 5 having the smallest and greatest fruit weights respectively. In general, accessions belonging to scotch bonnet big group had the widest and heaviest fruits while those from the long cayenne group had longer fruits. Although quantitative traits are highly influenced by the environmental conditions, they are of major agronomic importance. Pepper accessions with diverse quantitative traits could be identified and selected as parental genotypes for hybridization and improvement purposes. For instance in breeding for pepper varieties with intermediate fruit length, BA TAN 3 which had the shortest fruit length could be hybridized with VR KTS 2 to develop progenies with intermediate fruit length to meet the demand for the export market.

The pepper accessions were also analysed based on the area of collection (region) using four quantitative traits to determine the extent of diversity among the accessions based on their origin (Table 4). For mean cotyledon leaf width, accessions from the Volta region had the least width of 0.67 cm while accessions from Greater Accra had the highest width of 0.78 cm. The overall mean plant height among the accessions was 49.61 cm. The average plant height of accessions from the Brong Ahafo (59.50 cm) was higher than the average plant heights of the accessions from the other regions with Volta region having the least height of 45.00 cm. Greater Accra had accessions with bigger fruits (5.56 cm long and 3.96 cm wide) than the other regions. Although variations existed among the accessions from the different regions, it can be observed that variations among accessions within a region were higher than variations among accessions between regions. This means that pepper accessions with

182 diverse characteristics could be identified within regions of choice to minimize the cost
 183 associated with germplasm collection in the entire country.

184 Table 3. Analysis of 11 quantitative traits among pepper accessions

Parameter	Mean $\pm$ SE	Range	SD	CV (%)
Cotyledon leaf length (cm)	1.76 $\pm$ 0.03	1.24-2.36	0.24	13.37
Cotyledon leaf width (cm)	0.69 $\pm$ 0.01	0.45-0.87	0.09	13.30
Plant height (cm)	49.61 $\pm$ 1.66	31.00-91.70	11.71	23.60
Stem diameter (cm)	1.07 $\pm$ 0.03	0.60-1.73	0.22	20.52
Days to 50% flowering	40.78 $\pm$ 1.34	22.00-58.00	9.46	23.19
Days to 50% fruiting	28.46 $\pm$ 0.17	26.00-29.00	1.16	4.09
Fruit pedicel length (cm)	2.30 $\pm$ 0.06	1.49-3.21	0.41	17.70
Fruit pedicel width (cm)	0.17 $\pm$ 0.01	0.09-0.39	0.06	32.11
Fruit length (cm)	4.44 $\pm$ 0.34	0.70-10.46	2.39	53.78
Fruit width (cm)	1.58 $\pm$ 0.10	0.63-3.90	0.68	42.60
Fruit weight (g)	35.55 $\pm$ 3.72	4.50-131.50	26.33	74.08

185 Table 4. Analysis of four quantitative traits among the pepper accessions based on origin (region)

Origin of accessions	Cotyledon leaf width (cm)				Plant height (cm)				Fruit length (cm)				Fruit width (cm)			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
Overall	0.69	0.09	0.45	0.87	49.61	11.71	31.00	91.70	4.44	2.39	0.70	10.46	1.58	0.68	0.63	3.90
Brong Ahafo	0.68	0.12	0.45	0.86	59.50	15.86	42.30	91.70	4.26	2.34	0.70	7.47	1.37	0.48	0.63	2.18
Eastern	0.69	0.10	0.60	0.80	56.57	19.16	39.00	77.00	4.24	3.44	1.15	7.95	1.40	0.37	1.03	1.77
Greater Accra	0.78	0.09	0.68	0.87	46.43	12.61	32.00	55.30	5.56	2.25	3.55	7.99	2.53	1.22	1.58	3.90
Northern	0.71	0.07	0.60	0.84	48.70	10.78	31.00	67.00	3.96	1.38	1.87	6.55	1.40	0.69	0.85	2.71
Upper East	0.68	0.09	0.47	0.84	47.57	9.76	32.30	62.30	4.04	2.02	1.83	9.39	1.60	0.74	0.62	2.94
Volta	0.67	0.07	0.54	0.77	45.00	5.09	38.70	56.00	5.32	3.40	1.52	10.46	1.67	0.44	0.86	2.34

186 **3.3 Influence of agro-morphological traits on total genetic variance as revealed by** 187 **principal component analysis (PCA)**

188 Principal component analyses were carried to determine the most important agro-
189 morphological traits which contributed significantly to total genetic diversity among the
190 pepper accessions. The first three principal components (PC) accounted for 51.65% of the
191 total genetic variance using the 24 qualitative traits (Table 5). The first PC accounted for
192 23.22% of the variance with the larger part of the variance explained by leaf pubescence
193 density, calyx annular constriction, filament colour and fruit position. PC 2 explained 17.82%
194 of variation with anther colour as the predominant trait that accounted for the variation. For
195 PC 3, fruit cross-sectional corrugation, fruit position at blossom end and fruit shape were the
196 most important traits that explained 10.61% the variation observed.

197 For the 11 quantitative traits, the first three PCs accounted for 59.61% of the total genetic
198 variance (Table 6). PC 1 accounted for 23.32% of the observed variation which was
199 explained by fruit length, fruit pedicel width and fruit weight. PC 2 accounted for 18.79% of
200 the variance with plant height, stem diameter and fruit pedicel length being the most
201 important traits. Fruit width, cotyledon leaf length and days to 50% fruiting were the most
202 significant traits explaining 17.50% of the variance as observed in PC 3. Genetic diversity
203 studies using principal components have been carried out by several authors on pepper.
204 Bozokalfa *et al.* [25] recorded 54.29% variation in 48 pepper accessions using the first six
205 principal components. Similarly Nsabiyea *et al.* [26] working on 37 pepper genotypes
206 indicated that 55.40% of the total genetic variance was explained by the first 2 PCs. Distinct
207 pepper genotypes can be selected as parental lines using the most important traits
208 contributing more to total variability. Se-Jong *et al.* [16] reported the use of morphological
209 traits such as flower and fruit characteristics in pepper as the simplest approach for the
210 detection of genotypes and the assessment of genetic diversity.

211

212

213 Table 5. PCA of qualitative traits to total genetic variance using the first 3 PCs

Traits	PC 1	PC 2	PC 3
Hypocotyl pubescence	0.1113	-0.0657	-0.1092
Leaf pubescence density	0.4453	0.1293	0.3932
Leaf colour	0.0145	-0.1101	0.1212
Leaf shape	-0.1724	0.0688	0.1518
Neck at base of fruit	0.0172	-0.0105	-0.0019
Number of locules	-0.0016	0.0177	-0.0126
Plant size	0.0895	-0.0187	0.0480
Plant growth habit	-0.1301	0.0428	0.1385
Stem pubescence density	0.0596	0.1088	0.1158
Stem colour	-0.1432	0.0383	0.1239
Fruit shape at peduncle attachment	0.1289	0.0659	-0.1774
Fruit shape at blossom end	0.1483	0.0750	0.3978
Fruit shape	0.2949	0.1365	0.3623
Fruit position	0.4577	0.6967	0.1390
Fruit cross sectional corrugation	0.1929	0.1617	0.4541
Fruit colour at mature stage	-0.0154	-0.0752	0.1669
Filament colour	0.3483	0.4628	0.3600
Cotyledon leaf shape	-0.0433	-0.0057	0.0160
Cotyledon leaf colour	-0.0502	-0.0593	0.0905
Corolla colour	0.2779	-0.1143	-0.0664
Calyx margin shape	-0.0715	0.0219	0.0572
Calyx annular constriction	0.3025	-0.0125	-0.0654
Branching habit	0.0714	-0.0658	0.1192
Anther colour	0.1817	0.4057	0.0825
Eigen value	8.042	6.171	3.675
% variance	23.22	17.82	10.61
Cumulative % variance	23.22	41.04	51.65

214 Table 6. PCA of quantitative traits to total genetic variance using the first 3 PCs

Traits	PC 1	PC 2	PC 3
Cotyledon leaf length	0.2635	0.0397	0.2337
Cotyledon leaf width	-0.0876	0.0153	0.0541
Days to 50% flowering	-0.1107	0.3458	-0.1291
Days to 50% fruiting	-0.4557	0.1315	0.2939
Fruit weight	0.3584	0.2948	0.4621
Plant height	-0.1371	0.5840	-0.2065
Stem diameter	-0.3345	0.3657	0.0189
Fruit length	0.4960	0.1437	-0.1143
Fruit pedicel length	0.1905	0.4765	-0.3078
Fruit pedicel width	0.4019	0.0614	0.2895
Fruit width	-0.0042	0.2194	0.6256
Eigen value	2.565	2.067	1.925
% variance	23.32	18.79	17.50
Cumulative % variance	23.32	42.11	59.61

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216 3.4 Correlation analysis of 11 morphological traits

217 Correlation coefficients of 11 quantitative traits measured among the 50 pepper accessions
218 are presented in table 7. Cotyledon leaf length possessed significant negative correlation ($r =$
219 -0.375) with cotyledon leaf width but showed significant positive correlation ($r = 0.305$) with
220 days to 50% fruiting. Cotyledon leaf width had non-significant association with all the traits
221 with the exception of cotyledon leaf length. Days to 50% flowering showed significant
222 positive association ($r = 0.419$) with plant height. This result was in consonance with report
223 by Ghosh *et al.* [27]. Days to 50% fruiting had significant positive and negative correlations
224 ($r = 0.315$, $r = 0.513$, $r = -0.473$) with fruit width, stem diameter and fruit length respectively.

225 Fruit weight disclosed highly significant positive associations ($r = 0.436$, $r = 0.527$, $r = 0.710$)
226 with fruit length, fruit pedicel width and fruit width. This result was in agreement with
227 Muhammad *et al.* [28]. However, they reported significant negative correlation between fruit
228 weight and fruit length in tomato. Fruit weight also shared non-significant association with
229 plant height indicating that fruit weight which is the most important economic trait was not
230 influenced by the height of the pepper plants. Plant height showed significant positive
231 correlations ($r = 0.508$, $r = 0.529$) with stem diameter and fruit pedicel width respectively. Fruit
232 length possessed significant positive associations ($r = 0.419$, $r = 0.408$) with fruit pedicel
233 length and fruit pedicel width.

234 In general, there were significant positive correlations between fruit weight (yield) and yield
235 components such as fruit length and fruit width. Mohammed *et al.* [29] indicated that
236 correlation analysis is helpful in determining main traits influencing variation in traits of
237 economic importance as well as their interrelatedness to facilitate their simultaneous
238 improvement. Based on the correlations between the agro-morphological traits at the
239 phenotypic level, pepper accessions with long fruit length, large fruit pedicel width and large
240 fruit width should be selected as desirable parental lines in efforts towards fruit yield
241 improvement.

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Table 7. Pearson correlation coefficient for 11 morphological traits evaluated among 50 pepper genotypes

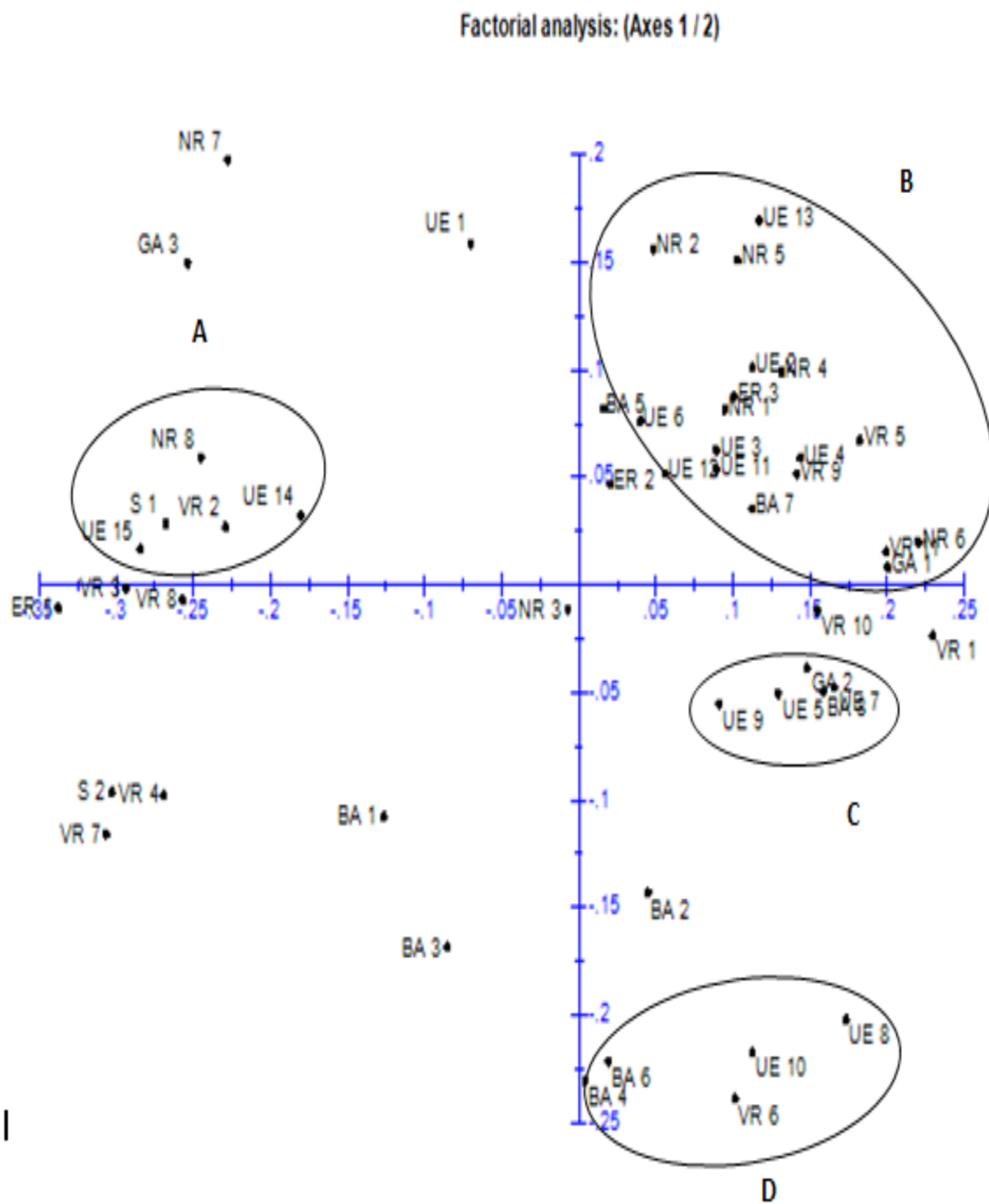
Traits												
CotLL (cm)	1	-										
CotLW (cm)	2	0.305*	-									
D50%Flo	3	-0.026NS	-0.123NS	-								
D50%Fr	4	-0.375**	0.181NS	-0.022NS	-							
F_Wt(g)	5	0.039NS	-0.094NS	0.024NS	-0.122NS	-						
Plt_Ht(cm)	6	0.086NS	0.108NS	0.419**	0.206NS	0.036NS	-					
St_Diam (cm)	7	-0.104NS	0.130NS	0.029NS	0.513***	-0.099NS	0.5081**	-				
Fr_Len (cm)	8	0.232NS	-0.160NS	-0.174NS	-0.473***	0.436**	0.0459	-0.207NS	-			
FrPd_Len (cm)	9	0.142NS	-0.113NS	0.256NS	-0.212NS	0.179NS	0.5298**	0.142NS	0.419**	-		
FrPd_Width (cm)	10	0.168NS	0.115NS	-0.221NS	-0.198NS	0.527***	-0.1339	-0.162NS	0.408**	0.073NS	-	
Fr_Width (cm)	11	-0.174NS	0.045NS	0.134NS	0.315*	0.710***	-0.006	0.089NS	-0.163NS	-0.159NS	0.215NS	-
		1	2	3	4	5	6	7	8	9	10	11

252

253 *, **, *** = significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively, NS = not significant ($P > 0.05$), Cot LL= Cotyledon leaf length (cm), Cot LW =
 254 Cotyledon leaf width (cm), D50%Flo = Days to 50% flowering, D50%Fr = Days to 50% fruiting, F_Wt = Fruit weight (g), Plt_Ht = Plant height
 255 (cm), St_Diam = Stem diameter (cm), Fr_Len = Fruit length (cm), FrPd_Len = Fruit pedicel length (cm), FrPd_Width = Fruit pedicel width (cm),
 256 Fr_Width = Fruit width (cm).

257 **3.5 Principal coordinate analysis (PCoA)**

258 Principal coordinate analysis (PCoA) grouped the pepper accessions into four major clusters
 259 with the accessions fairly distributed on the axes (Figure 2). Cluster A consisted of five
 260 accessions (NR 8, UE 14, VR 2, S 1 and UE 15). These accessions had glabrous leaf
 261 pubescence, elongate fruit shape and slightly corrugated fruit cross section. Cluster B had
 262 the highest number (20) of accessions. These include NR 2, UE 13, NR 5, BA 5, ER 2, UE
 263 6, UE 12, UE 11, UE 3, NR 1, ER 3, UE 2, NR 4, BA 7, VR 9, UE 4, VR 5, VR 11, NR 6 and
 264 GA 1. The accessions in this cluster had sparse leaf pubescence, conical fruit shape and
 265 corrugated fruit cross section. Cluster C was made up of five accessions. This cluster was
 266 made up of accessions UE 9, UE 5, GA 2, BA 8 and UE 7. They clustered together mainly
 267 due to their sparse leaf pubescence, white filament and campanulate fruit shape. The
 268 accessions that clustered in group D comprised, BA 4, BA 6, UE 8, UE 10 and VR 6. These
 269 accessions had intermediate leaf pubescence, distinct and uniform calyx annular constriction
 270 and pointed fruit shape at blossom end. The other pepper accessions were however, fairly
 271 distributed along the two axes signifying substantial variation among them. The factorial
 272 analysis showed the clustering of some of the accessions overlapping or closely placed on
 273 the scatter plot indicating possible duplicates. Most of the accessions clustered together
 274 irrespective of the origin from where they were obtained from. The analysis has provided
 275 additional information by clearly revealing the relationship and the most important traits
 276 which brought about variation among the accessions which could be used as the basis for
 277 selecting desirable parental lines for hybridization purposes.



278

279 Fig. 2: Principal coordinate analysis (PCoA) diagram of the 50 pepper accessions.

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281

282 **3.6 Genetic relationship among pepper accessions using morpho-agronomical traits**

283 Cluster analysis is very useful in revealing complex relationships and genetic relatedness
 284 among populations of diverse origins in a more simplified manner [16]. It is also effective in
 285 indicating accessions with useful traits belonging to different clusters for hybridization.
 286 Genetic similarity based on the 35 morph-agronomical traits ranged from 0.88-0.99 (Figure
 287 3). The 50 pepper accessions were grouped into 2 main clusters at a similarity coefficient of
 288 about 88. Cluster A had a very unique accession, BA TAN 3 (late flowering and tiny fruit
 289 size) while cluster B had 49 accessions. Although majority of the accessions were grouped
 290 into cluster B, there were variations among them resulting in further sub-clustering. Cluster B
 291 was further subdivided into six sub-clusters (Table 8). Sub-cluster B I consisted of accession
 292 UE KNW 7. Sub-cluster B II was made up of accessions VR HOE 10B, BA TAN 11A and BA
 293 TAN 11B. Accession GA DWW 7 alone was found in sub-cluster B III. Sub-cluster B IV was
 294 made up of accession VR KPV 1. Sub-cluster B V had only one accession, BA JMS 4. Sub-
 295 cluster B VI consisted of 42 accessions. Even though majority of the accessions clustered
 296 into their various groups, clustering did not strictly follow the geographic location from where
 297 the accessions were collected but was based on their morphological features. At a genetic
 298 similarity above 98%, NR WMP 4 and UE TND1 as well as UE BAW 7A and UE BAW 2 may
 299 be considered as possible duplicates, respectively. Similar results of possible duplicates
 300 have been observed by other researchers working on different crops [30, 31]. The cluster
 301 analysis revealed VR HOE 1 and BA TAN 3 as the more diverse genotypes. Although the
 302 dendrogram revealed high genetic relatedness, the diversity within the genotypes could be
 303 used for improving desirable traits through selection and hybridization.

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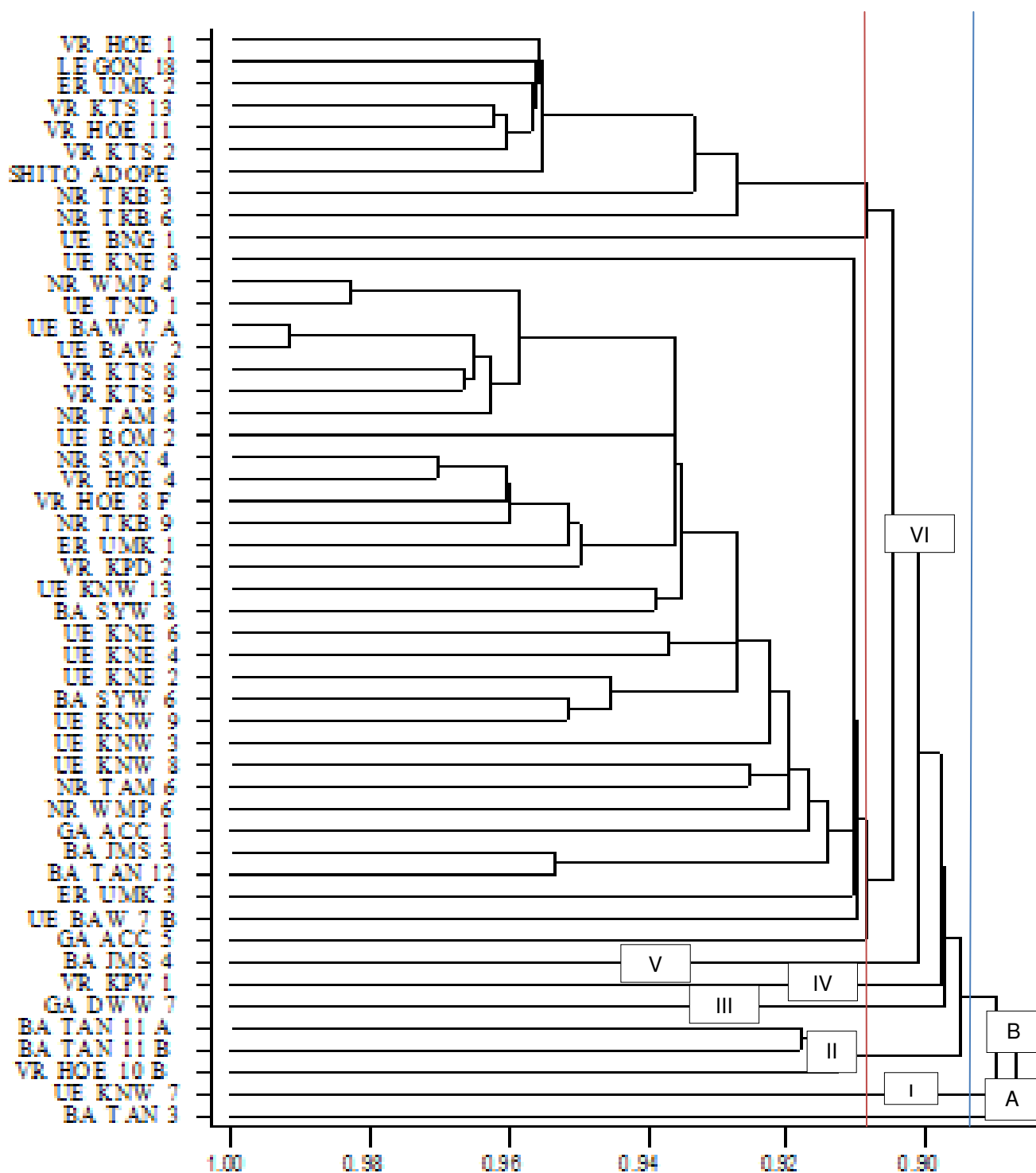
306

307 Table 8. Clustering of accessions based on morphological traits

Cluster	Similarity coefficient	Accessions
A	0.88	BA TAN 3
B I	0.905	UE KNW 7
B II	0.905	VR HOE 10B, BA TAN 11A and BA TAN 11B
B III	0.905	GA DWW 7
B IV	0.905	VR KPV 1
B V	0.905	BA JMS 4
B VI	0.905	GA ACC 5, UE BAW 7B, ER UMK 3, BA TAN 12, BA JMS 3, GA ACC 1, NR WMP 6, NR TAM 6, UE KNW 8, UE KNW 3, UE KNW 9, BA SYW 6, UE KNE 2, UE KNE 4, UE KNE 6, BA SYW 8, UE KNW 13, VR KPD 2, ER UMK 1, NR TKB 9, VR HOE 8F, VR HOE 4, NR SVN 4, UE BOM 2, NR TAM 4, VR KTS 9, VR KTS 8, UE BAW 2, UE BAW 7A, UE TND 1, NR WMP 4, UE KNE 8, UE BNG 1, NR TKB 6, NR TKB 3, Shito Adope, VR KTS 2, VR HOE 11, VR KTS 13, ER UMK 2, Legon 18 and VR HOE 1

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311

312 Fig 3. Genetic relationships among 50 pepper accessions based on morphological traits

313 using coefficient of euclidean, single-linked similarity matrix

314 **4. CONCLUSION**

315 The study revealed the existence of considerable variation among the germplasm
316 accessions. Principal component analysis of both qualitative and quantitative traits revealed
317 the most important agro-morphological traits which contributed more to the total genetic
318 variance. Relevant traits accounting for this variation were plant height, stem diameter and
319 fruit traits such as shape, colour, weight, length and width. Significant and positive
320 associations were found among majority of the traits with fruit width and fruit weight as the
321 highly correlated. The cluster analysis revealed VR HOE 1 and BA TAN 3 as the more
322 diverse genotypes. The observed diversity can be used for improving desirable traits through
323 selection and hybridization.

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