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Genetic diversity studies for morphological traits of hot pepper (*Capsicum annuum* L.) genotypes in Central Zone of Tigray Region, Northern Ethiopia

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Hot pepper is the dominant vegetable crop grown in different parts of Ethiopia with long history of cultivation and considerable genetic diversity for most important morphological traits. However, shortage of varieties, the prevalence of fungal and bacterial as well as viral diseases, information is lacking on genetic diversity and genetic information to design genetic resource conservation to improve yield and yield components of hot pepper. The study was undertaken to assess the morphological diversity of 64 hot pepper genotypes at Axum Agricultural Research Centre in Mereb Leke District during the year 2017/2018, using 8×8 simple lattice design. Analysis of variance revealed that there were a significant (P<0.01) differences in genetic variation among genotypes for 19 morphological and fruit characters. The genetic distances measured by D^2 and Ward's clustering method was grouped (the 64 genotypes) into seven distinct clusters. The maximum and minimum distances were observed between Clusters III and VII (189.09) and clusters I and V (29.24). This indicated the existence of a possibility to improve genotypes through hybridization from pair of clusters and subsequent selection can be made from the segregants generations. Principal component analysis showed that the first five principal component analysis explained about 79.45% of the total variation. Generally, the study confirmed presence of adequate genetic diversity between any pair of clusters which could be exploited for future variety improvement program.

Key words: *Capsicum*, cluster analysis, principal component analysis.

INTRODUCTION

The genus *Capsicum* belongs in the family Solanaceae comprises five domesticated species, *Capsicum annuum* L., *Capsicum baccatum* L., *Capsicum chinensis* Jacq., *Capsicum frutescens* L. and *Capsicum pubescens* Ruiz and Pavan (IBPGR, 1983; Padilha and Barbieri, 2016) and 30 wild species. These species are grown in the

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tropical and temperate regions across the continents. In Africa, they are generally considered together as *C. annuum* L. (Grubben and Tahir, 2004). *C. annuum* has grown widely in tropical agro climate conditions of Ethiopia (Berhanu et al., 2011). In the world, it is the most important crop uses as spice and vegetables (Amare, 2013). In Ethiopia, it is a high value crop due to its high pungency which serve as in the preparation of local flour called “Berberie” and it used as food for consumption and source of cash earning for smallholder farmers/or producers in both green and dry form (Amare, 2013). Moreover, in areas where it is suitably grown, hot pepper contributes 40 to 60% to the household income (Shimeles et al., 2016).

According to CSA (2017) the national average yields of hot pepper are 6.3 t ha^{-1} for green pod and 1.8 t ha^{-1} for the dry pod, which is far below the dry pod yield (2.5-3.7 t ha^{-1}) of improved varieties harvested at research fields of Ethiopia (MoANR, 2016) and world average yield of 3 to 4 t ha^{-1} (FAO, 2015). The productivity of the crop is low due to many limiting factors such as shortage of adapted high yielding varieties, using unknown seed sources and poor-quality seeds, poor irrigation system, lack of information on soil fertility, the prevalence of fungal and bacterial as well as viral diseases. However, Ethiopia has less benefited from research activities although some research centers are working on hot pepper variety development which mainly focused on adaptation and release of locally adapted varieties. Hence, generating information on the degree and pattern of genetic diversity of the hot pepper genotypes were less evaluated scientifically either using molecular or morphological studies in Ethiopia.

Diversity studies are an essential step and pre-requisite in plant breeding and could produce valuable knowledge for crop improvement programmes (Pujar et al., 2017). Genetic diversity studies are also useful for conservation, evaluation and utilization of genetic resources and for determining the uniqueness and distinctness of genotypes (Saleh et al., 2016a). The presence of genetic variability in crops is essential for its further improvement by providing options for the breeders to develop new varieties and hybrids (Shimeles, 2018). In Ethiopia, farmers usually save their own seed and transfer it from one generation to the next. However, proper seed production methods including isolation techniques are not in practice within and among farmers, giving chance to out cross and introgression forces to take place. In addition, seed exchange across the border with Eritrea, Kenya and Sudan has been active for a long period of time and numerous exotic varieties have been introduced. This is the reason as to why local pepper sold in the market in mixed pods containing wide range of fruit size, color, pungency, etc., reflecting the rich genetic variation existing in the local genotypes. Although the yield of the Ethiopian landraces is very low as compared with that of other countries, they are highly heterogeneous heterogeneous and could serve as a reservoir of genetic variability for improvement. The evaluation and the documentation of existing diversity are essential to maintain an active basis for the exploration of the genetic variability in pepper breeding programmes (Shimeles et al., 2016). Analysis of genetic diversity using quantitative or predictive methods has been used in the analysis of composition of populations. However, the magnitude of this diversity has not yet evaluated. Therefore, the objectives of this study were to evaluate local hot pepper genotypes for diversity using morphological characteristics, and make the necessary information available for future breeding and crop improvement programs in genotypes in case of central zone of Tigray region, Northern Ethiopia.

MATERIALS AND METHODS

Experimental site

The study was conducted at Axum Agricultural Research Center (AxARC) experimental field, Mereb Leke District, in the central zone of Tigray region, Northern Ethiopia during 2017/2018 cropping season through irrigation. It is located at about 1041 km away from Addis Ababa 257 km from Mekelle and 67 km to the north of Aksum town, at 14° 25’26” and 14° 18’48” N latitude, and 38° 42’15” and 38° 48’30” E longitude with an altitude of 1390 m.a.s.l. The site is found in semi-arid tropical belt of Ethiopia with “kola” agro climatic zone and the rainy season is mono - modal concentrated in one season from late July to early September and receives from 400 to 600 mm of rain fall per annum. The mean minimum and maximum temperatures ranged from 13.33 to 33.71°C, respectively. The soil texture of the specific site of the study area is sandy clay loam textural class with bulk density of 1.72 g cm^{-3}, very low in organic carbon (0.73%) with an alkaline pH of (8.2).

Experimental materials

Sixty-three local hot pepper Ethiopian landraces along with one released variety Mareko fana as a check were used in this study. The landraces were collected from different agro-ecologies of varying altitude, rainfall, temperature, and soil type by the Ethiopian Biodiversity Institute (EBI), Shire Maitsebri Agricultural Research Center (SMARC) and Melkassa Agricultural Research Center (MARC). Data depicted in Table 1 showed detail description of the accession numbers and source of the genotypes.

Experimental design

The experiment was laid out in 8 × 8 simple lattice design at Axum Agricultural Research Center Crop improvement program research filed during 2017/2018 cropping season under irrigation condition. Seeds of each hot pepper genotypes were sown in seed bed of 0.6 m^2 (3 rows, 0.2 m spacing between rows, 1 m row length) during October 2017 to raise seedlings. Seedlings were transplanted to main field 48 days after seed sowing, that is, when the seedlings attained 15 cm height. Each genotype was planted in the main field in a plot size of 8.4 m^2 (2.8 m × 3 m). Each plot consisted of four rows of 3 m length with inter and intra-row spacing of 0.7 and 0.3 m, respectively, containing a total of 40 plants. Each incomplete block and replication was spaced 1 and 1.5 m, respectively. The

Table 1. Hot pepper accessions, local name, area of collection, origin and sources.

No.	Accession Name	Local Name	Site of Collection	Sources	No.	Accession Name	Local Name	Site of collection	Sources
1	Acc-1	Berebere Hormat	Raya Kobo	Amhara	33	Acc-229701	Hulet Ejenese	Misrak Gojam	Amhara
2	Acc-2	Berbere Aberegelle	Tanqua Abergelle	Tigray	34	Acc-237528	Enticho	Ahferom	Tigray
3	Acc-3	Berebere Birisheleko	Bure	Amhara	35	Acc-9102	Achefer	Mirab Gojam	Amhara
4	Acc-4	Felege Da'ero	Mekelle	Tigray	36	Acc-9094	Gooda	Mirab Gojam	Amhara
5	Acc-5	Berebere Agew	Ofla(Zata)	Tigray	37	Acc-9098	Achefer	Mirab Gojam	Amhara
6	Acc-6	Berebere Dibdibo	Ahferom	Tigray	38	Acc-9104	Merwi	Mirab Gojam	Amhara
7	Acc-7	Shamba berbereAdi	Welkait	Tigray	39	Acc-9099	Amestya	Mirab Gojam	Amhara
8	Acc-8	Berebere korir	Kilte Awulalo	Tigray	40	Acc-9082	Meacha	Mirab Gojam	Amhara
9	Acc-9	Berebere Tsalaiet	Kola Temben	Tigray	41	Acc-9101	Achefer	Mirab Gojam	Amhara
10	Acc-10	Berebere Agbe	Abergelle	Tigray	42	Acc-9086	Kudmie	Mirab Gojam	Amhara
11	Acc-11	Laelay Dayu	Alamata	Tigray	43	Acc-229696	Dibata	Metekel	B/Gumz
12	Acc-12	Berebere Hewane	Walkait	Tigray	44	Acc-9106	Bure Wemberma	Mirab Gojam	Amhara
13	Acc-13	Abat Berebere	Walkait	Tigray	45	Acc-9007	Galioch Buare town	Mirab Gojam	Amhara
14	Acc-14	Bora(Gemelo)	Embalaje	Tigray	46	Acc-9107	Guzamn	Misrak Gojam	Amhara
15	Acc-15	Abat Berebere	Welkait	Tigray	47	Acc-28334	Abdigudina	Illubabor	Oromiya
16	Acc-16	Berbere Rama	Mereb Leke	Tigray	48	Acc-48	Berbere Alaba	Alaba	SNNPRS
17	Acc-28336	Durame	Illubabor	Oromiya	49	Acc-49	Tedele	Guragae	SNNPRS
18	Acc-230800	Bedeno	Misrak Harerge	Oromiya	50	Acc-229694	Mentawuha	Metekel	B/Gumz
19	Acc-28337	Elammo	Illubabor	Oromiya	51	Acc-51	Abeshigie	Guragie	SNNPRS
20	Acc-229699	Adet	Misrak Gojam	Amhara	52	Acc-52	Wegedadi	Mirab Gojam	Amhara
21	Acc-212912	Kedida Gameala	Kembata Alaba	SNNPRS	53	Acc-9093	Solmeda	Mirab Gojam	Amhara
22	Acc-9097	Achefer/Durbate	Mirab Gojam	Amhara	54	Acc-229692	Dinkara	Agew Awi	Amhara
23	Acc-9084	Merawi	Mirab Gojam	Amhara	55	Acc-55	Debremarkos	Misrak Gojam	Amhara
24	Acc-229697	Wonbera	Metekel	B/Gumz	56	Acc-56	Finote Selam	Mirab Gojam	Amhara
25	Acc-212913	Humbo	Semen Omo	SNNPRS	57	Acc-57	Guragie Berebere	Butajira	SNNPRS
26	Acc-229700	Bibugn (Astero M.)	Misrak Gojam	Amhara	58	Acc-58	Berebere Merb	Mereb Leke	Tigray
27	Acc-9085	Merawi	Mirab Gojam	Amhara	59	Acc-59	Adiha Local	Abi Adi	Tigray
28	Acc-230798	Dogo Midi Jara	Misrake Harerge	Oromiya	60	Acc-23880	Meskele Kirstos	Semien Gonder	Amhara
29	Acc-230799	Girawa	Misrake Harerge	Oromiya	61	Acc-61	Myweni	Mereb Leke	Tigray
30	Acc-236436	Bako Tibe	Mirab Shewa	Oromiya	62	Acc-62	Berebere Hesea	Mereb Leke	Tigray
31	Acc-229698	Dibate	Metekel	B/Gumz	63	Acc-63	Yeyeju Bereberie	Woldia	Amhara
32	Acc-229698	Dibate	Metekel	B/Gumz	64	Acc-64	Mareko fana(St. check)	Melkassa	Oromiya

Acc = Accession, B/Gumz = Benishangul-Gumz Regional State, SNNPRS = Southern Nation, Nationalities and People's Regional State.

middle two rows were used for data collection leaving the two rows as borders. Di-ammonium phosphate (DAP) as a source of Phosphorus was applied at the rate of 200 kg ha^{-1} during planting and nitrogen fertilizer was applied in the form of Urea at the rate of150 kg ha^{-1} in splits, half during transplanting and the rest as side dressing at 45 days

after transplanting. Furrow irrigation method, scheduled at 7 to 10 days interval (AxARC, 2016) was used. Weeding, hoeing and other field management and crop protection activities were done as per the recommendation for hot pepper.

Data collected

Data were collected both from plant and plot basis. The two central rows were used for data collection based on plots, such as flowering and fruiting times, days to maturity, marketable fruit yield (t ha^{-1}), unmarketable fruit yield (t ha^{-1}) and total fruit yield per hectare (t ha^{-1}). Five randomly selected plants from the two central rows of each plot were used for data collection on plant basis and the averages of the five plants in each experimental plot were used for statistical analysis for traits such as plant height (cm), canopy diameter (cm), stem diameter (mm), number of primary branches per plant and number of fruits per plant. Fruit pedicel length (cm), fruit length (cm), width (mm) and pericarp thickness (mm), fruit weight (g) per plant, dry fruit yield per plant (g), 1000 seed weight (g) and seed number per fruit, were measured from 10 fruits from each plot following the methods adapted from IPGRI et al. (1995).

Data analysis

Analysis of variances (ANOVA) was made using SAS Version 9.2 (SAS Institute Inc., 2008) after testing the ANOVA assumptions. Clustering of genotypes into different groups carried out by Ward's method (Ward, 1963) using squared Euclidean distance of the distance metric and standardized variables was performed using Minitab release 17 (Minitab, 1998) to cluster the genotypes based on their agronomic traits.

A measure of a group distance based on multiple characters was given by generalized Mahalanobis D^2 statistics (Mahalanobis, 1936) for 19 quantitative characters and was analyzed using the procedure Procdiscrim of SAS Software. Squared distance (D^2) for each pair of genotype combinations was computed using the following formula:

$$D^2ij = ((Xi - Xj)\ S^{-1}(Xi - Xj)$$

where D^2ij = the square distance between any two groups i and j; Xi and Xj = the vectors for the values for genotype ith and jth genotypes; and S^{-1} = the inverse of pooled variance covariance matrix within groups. Testing the significance of the squared distance values obtained for a pair of clusters was taken as the calculated value of χ^2 (chi-square) and tested against the tabulated χ^2 values at p-1 degree of freedom at 5 and 1% probability level, where p = number of characters used for clustering the genotypes.

The average intra and inter cluster distances were calculated by the formula given by Singh and Chaudhary (1985). Square of intra-cluster distance $=\sum Di^2/n$, Square of inter-cluster distance $= \sum Di^2/ninj$. Where ΣDi^2 = Sum of distance between all possible combinations ni = number of genotypes in cluster i and nj = number of genotypes in cluster j.

Principal components analysis (PCA) was performed using correlation matrix by employing Minitab computer software Released 17 (Minitab, 1998) in order to evaluate the relationships among characters that are correlated among each other by converting into uncorrelated characters called principal components. The contribution of each character in PCA is determined by eigenvector that is greater than half divided by the square root of the standard deviation of the Eigen value of the respective PCA as suggested by Johnson and Wichern (1988). Principal components (PCs) with Eigen value > 1.0 were used as criteria to determine the number of PCs (Kaiser, 1960).

RESULTS AND DISCUSSION

Mean square values of ANOVA of 19 quantitative characters for the sixty-four genotypes showed highly significant difference (P<0.001) for all the characters (Table 2). The significant genetic variation among genotypes might be due to the fact that genotypes were genetically diverse and it could be a good opportunity for breeders to select genotypes for trait of interest for different crop improvement program. Several researchers reported significant differences among hot pepper genotypes studied (Nsabiyera et al., 2013; Birhanu, 2017; Shimeles, 2018).

Efficiency of simple lattice design over randomized complete block design (RCBD) showed that more than half of the traits were efficient. Days to 50% flowering (111.99%), number of fruits per plant (107%), dry fruit yield per plant (107.49%) and number of seeds per fruit (148.93%) are among the traits which indicated high efficiency over RCBD, that is, the experimental plots within replications were heterogeneous; hence, making incomplete block within replication reduces the experimental error. The coefficient of determination (R^2) is used to measure the proportion of variability in a data set that is accounted for by the statistical model. All the traits scored more than 85% estimate of R^2 except plant height (84.7) and stem diameter (84%), showing the adequacy of the model in explaining the variation.

Cluster analysis

Genetic relationships among 64 hot pepper genotypes based on 19 morphological characters in the form of dendrogram using Ward's clustering method are presented in Table 3 and Figure 1 and the mean values for each cluster is presented in Table 4. The genetic divergence analysis has clustered 64 genotypes into seven clusters. Accordingly, Cluster I was the largest cluster which consisted of 20 genotypes (31.25%) followed by Cluster VI comprised of 16 genotypes (25%). Clusters II and V each comprised ten genotypes (15.63%) including one check variety (Mareko fana) at cluster V and Clusters IV and VII each comprised three genotypes (4.69%) while, Cluster III had the lowest number of genotypes that comprises only two genotypes (3.13%). In cluster analysis, if the categorization is successful, individuals within a cluster (homogenous) shall be closer and different clusters (heterogeneous) shall be farther apart (Bijalwan et al., 2018).

This indicates that the tested hot pepper landraces were highly divergent. Saleh et al. (2016a) classified 60 local pepper collections into five clusters. Janaki et al. (2016) classified 63 chilli accessions into eight clusters. Razzaq et al. (2016) classified 25 pepper accessions into five distinct clusters. Pujar et al. (2017) grouped 63 chilli genotypes into five clusters. Birhanu (2017) classified 36 hot pepper genotypes into six clusters. Shimeles (2018)

Table 2. Mean squares of variance for 19 characters of 64 hot pepper genotypes evaluated at Mereb Leke in, 2017/2018.

Character	Mean squares			Error				
	Replication (1)	Treatments Adji (63)	Blocks with in replication (Adj)(14)	Intra Block (49)	RCBD (63)	R^2 (%)	RE to RCBD (%)	CV (%)
DFL	8.508	27.51**	13.936	6.528	8.175	86.95	111.99	3.8
DFR	0.008	30.17**	8.820	6.347	6.897	87.19	102.29	3.3
DM	3.445	44.26**	6.222	5.856	5.937	91.20	100.08	2.0
PH	526.500	62.76**	15.390	17.487	17.021	84.70	97.33	7.8
CD	7.703	14.69**	3.318	3.550	3.499	86.89	98.55	5.0
NPB	0.797	13.45**	1.100	1.117	1.113	94.65	99.66	15.0
SD	1.144	3.37**	1.241	0.963	1.025	84.00	101.37	7.7
FPL	0.054	0.79**	0.119	0.074	0.084	94.29	104.71	7.8
FL	0.002	22.28**	0.426	0.407	0.411	98.66	100.04	7.8
FD	1.533	49.14**	2.355	1.638	1.797	97.70	102.77	7.1
FPT	0.918	0.13**	0.026	0.029	0.028	87.87	97.27	8.3
FW	0.463	4.82**	0.144	0.187	0.177	97.30	94.96	11.9
NFP	2.820	294.20**	8.135	4.555	5.351	98.93	107.00	6.9
NSF	17.331	1453.86**	80.555	18.524	32.309	99.14	148.93	3.1
TSW	0.538	1.50**	0.213	0.189	0.194	91.78	100.32	7.8
DFYP	12.500	1741.51**	37.670	20.676	24.452	99.21	107.49	4.2
MFY	1.304	0.81**	0.069	0.131	0.117	90.52	89.57	14.6
UNMFY	0.008	0.01**	0.001	0.001	0.001	92.09	91.35	19.7
TFY	1.533	0.82**	0.065	0.135	0.120	90.46	88.52	13.8

*and** = significant at 5 and 1% probability level, respectively. Number in parenthesis represented degree of freedom adj = adjusted treatment mean squares, RCBD = Randomized completed block design, RE to RCBD = Relative efficiency to randomized completed block design CV = coefficient of variation, R^2 (%) = coefficient of determination, DFL = days to 50% flowering, DFR = days to 50% fruiting, DM = days to maturity, PH = Plant height, CD = canopy diameter, number of primary branches per plant, SD = stem diameter, FPL = fruit pedicel length, FL = fruit length, FD = fruit diameter, FPT = fruit pericarp thickness, FW = average single fruit weight, NFP = number of fruits per plant, NSF = number of seeds per fruit, TSW = thousand seed weight, DFYP = dry fruit yield per plant, MFY = marketable fruit yield, UNMFY = Unmarketable fruit yield and TFY = total fruit yield.

Table 3. The distribution of 64 hot pepper genotypes in to seven clusters based on D^2 analysis.

Cluster	No. of genotypes	Percentage	Name of genotypes
I	20	31.25	Acc-1, Acc-11, Acc-16, Acc-230798, Acc-229698, Acc-28334, Acc-48, Acc-49, Acc-229694, Acc-51, Acc-52, Acc-9093, Acc-229692, Acc-55, Acc-56, Acc-57, Acc-58, Acc-23880, Acc-61, Acc-62
II	10	15.63	Acc-2, Acc-5, Acc-6, Acc-8, Acc-10, Acc-14, Acc-28336, Acc-229700, Acc-59, Acc-63
III	2	3.13	Acc-3, Acc-212912
IV	3	4.69	Acc-4, Acc-229699, Acc-212913
V	10	15.63	Acc-7, Acc-13, Acc-28337, Acc-236436, Acc-9102, Acc-9094, Acc-9082, Acc-9106, Acc-9107, Acc-64
VI	16	25	Acc-9, Acc-12, Acc-15, Acc-230800, Acc-9084, Acc-229697, Acc-8995, Acc-230799, Acc-237528, Acc-9098, Acc-9104, Acc-9099, Acc-9101, Acc-9086, Acc-229696, Acc-9007
VII	3	4.69	Acc-9097, Acc-9085, Acc-229701

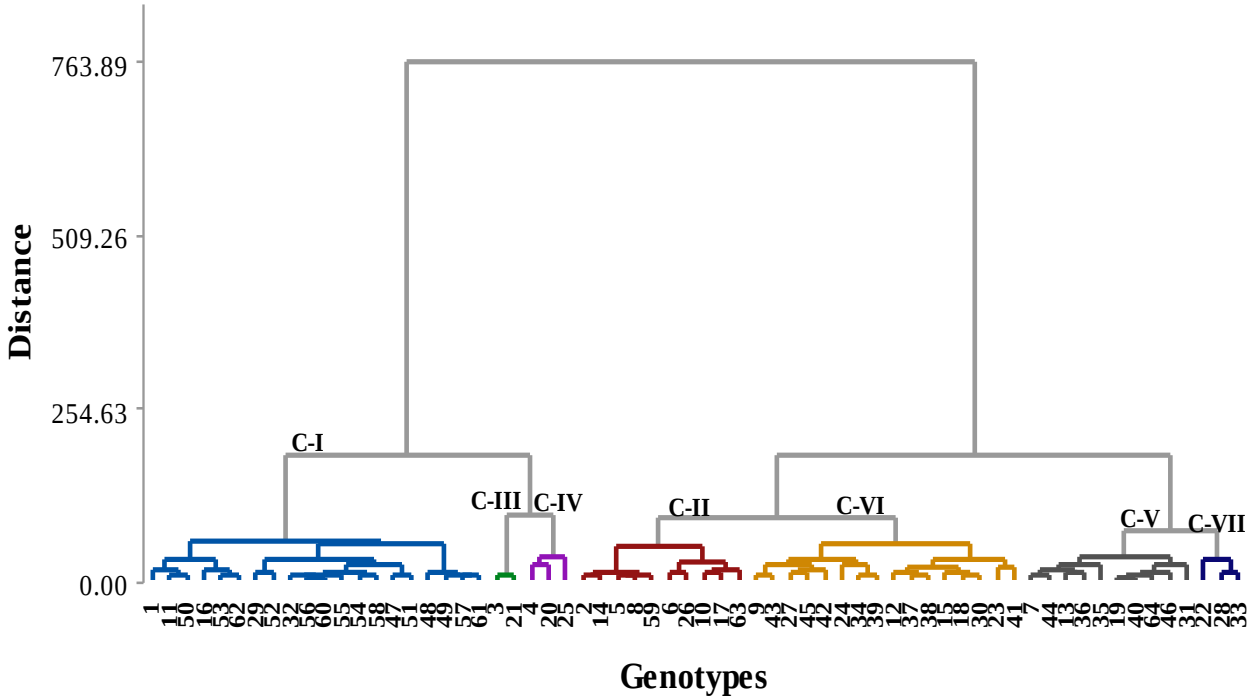

Figure 1. Dendrogram generated by Wards cluster analysis method for 64 hot pepper genotypes for 19 characters evaluated at Mereb Leke in, 2017/2018.

Table 4. Mean values of seven clusters for 19 characters of 64 hot pepper genotypes.

Character	Clusters						
	C-I	C-II	C-III	C-IV	V	C-VI	C-VII
DFL	65.15	71.33	70.97	63.86	66.79	68.47	64.09
DFR	72.10	80.25	79.00	73.67	76.25	77.47	72.67
DM	117.88	127.65	121.25	120.00	122.55	126.50	120.17
PH	51.83	57.01	63.30	52.23	54.32	53.52	40.30
CD	35.89	39.28	40.75	39.53	35.40	38.51	37.20
NPB	4.29	9.92	7.35	8.90	5.78	9.05	7.53
SD	12.06	14.17	14.90	13.10	12.62	12.64	10.72
FPL	3.96	3.14	4.18	3.02	3.60	2.95	3.58
FL	12.21	6.16	10.28	7.06	8.04	5.11	4.98
FD	22.77	13.07	30.20	20.40	18.60	13.73	13.57
FPT	2.30	1.81	2.50	2.34	2.06	1.86	1.83
FW	5.37	2.60	6.56	4.17	2.88	2.34	2.08
NFP	19.55	47.98	19.67	42.85	25.19	36.04	38.30
NSF	150.80	124.58	229.35	153.27	134.10	127.45	114.19
TSW	6.29	5.51	6.63	5.70	5.22	4.83	5.17
DFYP	119.97	118.31	165.80	167.33	79.31	84.23	104.40
MFY	2.69	2.57	4.01	3.86	2.04	2.03	2.17
UNMFY	0.15	0.21	0.37	0.16	0.15	0.28	0.15
TFY	2.84	2.78	4.38	4.02	2.19	2.31	2.32

DFL = Days to 50% flowering, DFR = days to 50% fruiting, DM = days to maturity, PH = plant height, CD = canopy diameter, NPB = number of primary branches per plant, SD = stem diameter, FPL = fruit pedicel length, FL = fruit length, FD = fruit diameter, FPT = fruit pericarp thickness, average single fruit weight, DFYP = dry fruit yield per plant, NFP = number of fruit per plant, NSF = number of seeds per fruit, TSW = thousand seed weight, MFY = marketable fruit yield, UNMFY = unmarketable fruit yield, TFY = total fruit yield.

had grouped 49 hot pepper genotypes in to six distinct clusters.

Cluster mean analysis

The mean values of genotypes were computed in each cluster and registered as mean of the respective cluster and results are presented in Table 3. The cluster mean values revealed that there were considerable differences among the clusters for different morphological and fruit characters.

Cluster I had the largest number of genotypes which were early in flowering, fruiting and maturity period (65.15, 72.10 and 117.88 days, respectively). It showed high fruit length (12 cm). The majority of the genotypes in this cluster showed moderate performance in most of the fruit yield and yield related traits as compared to clusters III, IV and V. It had relatively moderate average single fruit weight (5.37 g), moderate dry fruit yield per plant (119.97 g), moderate fruit pedicel length (3.96 cm), moderate fruit diameter (22.77 mm), thousand seed weight (6.29 g) next to cluster III, moderate fruit pericarp thickness (2.30 mm), moderate total fruit yield per hectare (2.84 t ha^{-1}) as next to clusters III and IV. It also showed relatively low value of number of primary branches per plant (4.29) and number of fruits per plant (19.55).

Genotypes in Cluster II were late in flowering (71.33 days), fruiting (80.25 days) and maturity (127.65 days) than the genotypes in the remaining clusters. The genotypes had tall plant height (57.01 cm) next to III (63.30 cm), relatively moderate canopy diameter (39.28 cm), stem diameter (14.17 mm) and the highest number of primary branches per plant (9.92). On the contrary, genotypes in cluster II had the least fruit diameter (13.07 mm) and fruit pericarp thickness (1.81 mm) as compared to the rest of clusters.

Cluster III, which comprised the highest yield bearing genotypes, characterized by the relatively late genotypes in days to 50% flowering, 50% fruiting and maturity. Moreover, they had the highest plant height (63.30 cm), canopy diameter (40.75 cm), stem diameter (14.90 mm), fruit pedicel length (4.18 cm), fruit diameter (30.20 mm), fruit pericarp thickness (2.50 mm), average single fruit weight (6.56 g), number of seeds per fruit (229.35) and total fruit yield (4.38 t ha^{-1}). On the contrary, it had the least number of fruits per plant (19.67), number of primary branches per plant (7.35) next to cluster I. It had also high fruit length (10.28) next to cluster I (12.21 cm) and dry fruit yield per plant (165.80 g) next to cluster IV (167.33 g). Hence, genotypes from this cluster could be used in pepper breeding program for fruit yield improvement.

Genotypes in Cluster IV had medium maturity period (120 days) as compared to cluster I (117.88 days). The genotypes in this cluster had the moderate stem diameter (13.10 mm), fruit length (7.06 cm), fruit diameter (20.40 mm) as compared to cluster III, moderate fruit pericarp thickness (2.34 mm) next to cluster III, average single fruit weight (4.17 g) with the high number of fruits per plant (42.85). It also had least fruit pedicel length (3.02 cm) among other clusters and next to cluster VI.

Genotypes in Cluster V had relatively medium maturity (122.55 days) as compared to clusters I, IV and VII. The genotypes also had moderate fruit length (8.04 cm). However, this cluster had the lowest canopy diameter (35.40 cm), total yield per hectare (2.19 t ha^{-1}) and moderate fruit diameter (18.60 mm). It also had the least unmarketable fruit yield per hectare as compared to the rest of clusters.

Genotypes under Cluster VI were matured relatively late (126.50 days) as compared to clusters I, IV, VII, III and V. Those genotypes also had the highest number of primary branches per plant (9.05) next to cluster II, least fruit pedicel length (2.95 cm), least fruit length (5.11 cm) and least average fruit weight (2.34 g) next to cluster VII, least thousand seed weight (4.83 g), least dry red fruit yield per plant (84.23 g) next to V, least marketable fruit yield (2.03 t ha^{-1}) and less total yield (2.31 t ha^{-1}) next to cluster V.

Genotypes in Cluster VII are relatively medium matured (120.17 days) as compared to clusters I (117.88 days). Those genotypes also had the least plant height (40.30), least canopy diameter (37.20 cm) next to I and V, least stem diameter (10.72 mm), medium fruit pedicel length (3.58 cm), least fruit length (4.98 cm), least fruit diameter (13.57 mm) next to clusters II and VI, least fruit pericarp thickness (1.83 mm) next to cluster II (1.81 mm), least average fruit weight (2.08 g) next to VI (2.34 g), least number seeds per fruit (114.19), least thousand seed weight (5.17 g) next to VI (4.83 g), less dry fruit yield per plant (104.40 g) next to V (79.31) and VI (84.23), and less total fruit yield (2.32 t ha^{-1}) next to clusters V (2.19 t ha^{-1}) and V (2.31 t ha^{-1}).

The results of mean and inter cluster distance analysis suggested that parental lines selected from these clusters could be used in hybridization programs, since crossing between divergent parents is likely to produce wide variability and transgressive segregations with high heterotic effects. To get genotypes/varieties with high fruit yield and early maturing genotypes, it is possible to cross genotypes from clusters I and III, III and IV. Janaki et al. (2016), Birhanu (2017), Kumari (2017), Pujar et al. (2017), Bijalwan et al.(2018) and Shimeles (2018) had also reported that selection of parents for hybridization should be done from two clusters having wider inter-cluster distances to get maximum variability in segregating generations.

Estimation of inter cluster square distances (D^2)

Estimation of inter cluster square distances (D^2)

Table 5. Average Intra (Bold) and inter cluster squared distance (D^2) between clusters based on 19 characters of 64 hot pepper genotypes tested in, 2017/2018.

Cluster	I	II	III	IV	V	VI	VII
I	**2.33**	73.87**	85.49**	50.67**	29.24*	64.81**	65.35**
II		**3.71**	143.58**	48.70**	34.72*	21.63^{ns}	38.99**
III			**6.93**	89.77**	110.29**	131.19**	189.09**
IV				**6.12**	46.59**	64.17**	67.13**
V					**3.71**	22.62^{ns}	36.34**
VI						**2.77**	37.25**
VII							**6.12**

* and **, significant (χ^2 = 28.869) and highly significant (χ^2 = 34.805) at 5 and 1% probability levels, respectively. ns= Non-significant.

calculated between pairs of clusters were considered as chi-square values and tested for significance using p-1 degrees of freedom, where "p" indicates the number of characters (Singh and Chaudhary, 1985). Intra cluster and inter-cluster divergence value among 7 clusters and their statistical distance were computed (Table 5). Cluster number I showed the least intra cluster D^2 value (2.33) while the maximum intra cluster D^2 value (6.93) was from cluster number III. This indicates that genotypes under cluster I were the least divergence. While, genotypes under cluster III were the maximum divergence. Accordingly, the χ^2-test for the seven clusters was highly significant difference among the inter clusters, except between clusters II and VI and, V and VI.

The χ^2-test for the seven clusters indicated that there was a very highly significant difference among the clusters. The highest inter-cluster distance was exhibited by cluster III and VII (D^2 =189.09), followed by cluster II and III (D^2 = 143.58), cluster III and VI (D^2 = 131.19), cluster III and V (D^2 = 110.29), cluster III and IV (D^2 = 89.77) and cluster I and III (D^2 = 85.49) which implies that these clusters were genetically more divergent from each other than any other pairs of cluster. Cluster I and V showed the least inter cluster distance (29.24) compared to other pair of clusters.

The smallest inter-cluster distance was observed between Clusters I and V (D^2 = 29.24) followed by clusters II and V (D^2 =34.72). Genotypes belonging to these clusters were relatively close to each other. According to Rama (1992) crossing of genotypes from those clusters might not give higher heterotic value in F_1 and may result in narrow range of variability in the segregating F_2 population. Such analysis was meant to avoid selection of parents from genetically homogeneous clusters and to maintain a relatively broad genetic base. Accordingly, it is well recognized that the greater the distance between clusters, the wider the genetic diversity would be between the genotypes. Therefore, highly divergent genotypes would produce a broad spectrum of segregation in the subsequent generations enabling further selection and improvement and it is important for pepper breeding program. Such recommendations were also made by Nsabiyera et al. (2013), Hasan et al. (2014), Hassan et al. (2015), Janaki et al. (2016), Razzaq et al. (2016) and Pujar et al. (2017), in which the greater the distance between clusters, the wider the genetic diversity would be between the genotypes.

Generally, divergence analysis showed presence of high genetic divergence among the tested hot pepper genotypes evaluated at Mereb Leke. Hence, wide genetic distance (inter-cluster) between the genotypes of clusters III and VII is important to do crossing between genotypes of these two clusters for the development of hybrids in hot pepper breeding programs. The clusters I, II, V, VI, and VII were found superior for one or more characters. Therefore, a multiple crossing program can be proposed involving genotypes from these clusters for the development of superior segregants in advanced generations with high yield potential in hot pepper.

Principal component analysis

Principal component analysis (PCA) was used to examine the variability among 64 hot pepper genotypes. To validate the clustering (grouping) observed by the cluster analysis (Table 3 and Figure 1), an ordination analysis (Principal Components Analysis, PCA) was executed using the 19 quantitative characters. Correlation matrix generated using the genotypic mean values of the 19 traits used as an input and were subjected to the principal components analysis (PCA). From the 19 principal components (equal number to the original variables) extracted, the first five PC's with an Eigen value >1 were significant. The first five principal components (PC's) accounted for 79.45% and the first and the second PC's accounted for 40.05 and 19.4% (total 59.45%) of the variance, respectively (Table 6). Component loading of the first three principal components is shown in Table 6. To aid visualization of the overall variability in the tested genotypes, the first two components scores (PC's) are plotted (Figure 2). Out of the total variation, PC1 and PC5 explained the largest and smallest variation, respectively, while PC2, PC3 and

Table 6. Eigen vectors, proportion and cumulative percentage of variation explained by the first five principal components (PC) for morphological and fruit characters of64 hot pepper genotypes evaluated at Mereb Leke in, 2017/2018.

Principal components	PC 1	PC 2	PC 3	PC 4	PC 5
Eigen values	7.61	3.69	1.86	1.15	0.78
Proportion of variance (%)	40.05	19.4	9.8	6.5	4.1
Cumulative variance (%)	40.05	59.45	69.25	75.35	79.45
Characters	**Eigenvectors**				
Days to 50% flowering	-0.180	0.233	0.369	0.035	-0.200
Days to 50% fruiting	-0.246	0.193	0.282	0.034	-0.295
Days maturity	-0.304	0.110	0.144	0.095	0.016
Plant height (cm)	-0.041	0.317	0.423	-0.175	-0.031
Canopy diameter (cm)	-0.112	0.271	-0.141	-0.104	0.772
Number primary branches	-0.282	0.159	-0.193	0.106	-0.051
Stem Diameter (mm)	-0.097	0.372	0.273	-0.250	0.138
Fruit pedicel length (cm)	0.234	-0.017	0.240	0.044	0.114
Fruit length	0.306	-0.009	0.210	-0.231	0.025
Fruit diameter (mm)	0.310	0.060	0.173	0.235	0.036
Fruit pericarp thickness (mm)	0.303	0.065	0.070	0.135	-0.047
Fruit weight (g)	0.321	0.128	0.065	0.008	-0.043
Number fruit per plant	-0.255	0.170	-0.271	-0.213	-0.196
Number seed per fruit	0.189	0.198	0.066	0.545	0.070
Thousand seed weight (g)	0.231	0.144	-0.016	-0.411	0.065
Dry fruit yield per plant (g)	0.172	0.307	-0.289	0.024	-0.390
Marketable yield (tha^{-1})	0.183	0.375	-0.274	-0.055	-0.064
Unmarketable yield (tha^{-1})	-0.170	0.229	-0.019	0.480	0.170
Total fruit yield (tha^{-1})	0.162	0.398	-0.273	0.001	-0.044

PC4 accounted for 19.4, 9.8 and 6.5% of the total variation, respectively.

Most yield and fruit characteristics contribute to PC1 such as fruit length (FL), fruit diameter (FD), fruit pericarp thickness (FPT), total fruit yield per hectare (TFY), marketable yield per hectare (MFY), average single fruit weight (FW), number of seed per fruit (NSF) and dry fruit yield per plant (DFYP) had more contribution to the total diversity and they were responsible for the differentiation of the seven clusters. The long vectors indicate that, they have a large contribution to the total variation (Saleh et al., 2016b).

In the second axis (PC 2), traits such as plant height (PH), canopy diameter (CD), stem diameter (SD), days to 50% flowering (DFL), days to 50% fruiting (DFR), days to maturity (DM), dry fruit yield per plant (DFYP) and total fruit yield per hectare (TFY) had a long vector and associated positively with PC 2. Characters like days to 50% flowering (DFL), days to 50% fruiting (DFR) and days to maturity (DM), plant height (PH) and stem diameter (SD), fruit pedicel length (FPL), and fruit diameter (FD) were the characters which contributed to the third principal component (PC 3). Similarly, number of seeds per fruit (NSF) and fruit diameter (FD) were the characters contributed to the fourth cluster (PC4). Fruit diameter is the most traits in PC4. Fifth principal component (PC5) contributed to characters such as canopy diameter (CD), fruit pedicel length (FPL) and stem diameter (SD). Canopy diameter is the most important trait in PC5.

Conclusion

The genetic distances measured by D^2 and Ward's clustering method grouped the 64 genotypes in to seven distinct clusters of which Cluster I comprised 20 (31.25%) genotypes. Cluster VI comprised 16 (25%) genotypes. Clusters II and V consisted of ten genotypes. Clusters III consisted of two genotypes and Cluster IV contained three genotypes. The maximum and minimum distances were observed between Clustesr III and VII (180.53) and cluster VII (30.18). Hence, crossing of genotypes from the divergent clusters may produce a broad spectrum of segregants in the subsequent generations. PCA analysis showed the first five principal components (PC's) accounted for 79.45% and the first and the second PC's accounted for 40.05 and 19.4% of the variance,

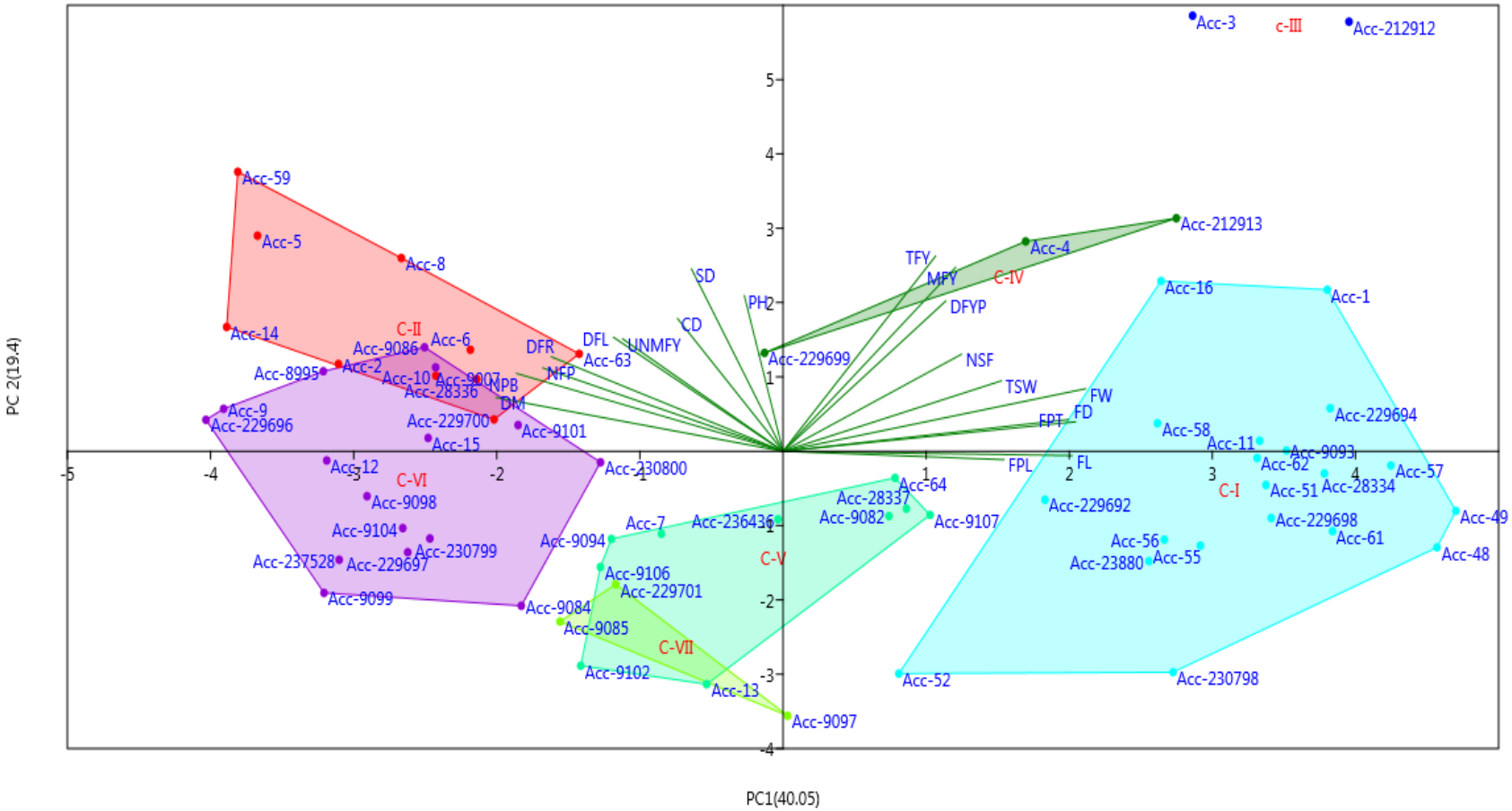

Figure 2. Principal component biplot of 19 quantitative characters of hot pepper genotypes.

respectively, indicating that the investigated traits are useful to consider variation in the hot pepper genotypes. This study generally indicated that there was significant genetic variability or diversity among the test genotypes. Thus, there is enormous opportunity in the improvement program of hot pepper through direct selection or hybridization involving crossing of the genotypes from different clusters would produce viable and potential segregant populations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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