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RESEARCH ARTICLE

PHENOTYPIC DIVERSITY STUDIES IN CHICKPEA (*CICER ARIETINUM* L.) GERMPLASM OF ETHIOPIAN COLLECTIONS

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ABSTRACT

Eighty chickpea germplasm and four standard checks (i.e Arerti, Harbu, Minjar, and Natoli) were characterized at D/Ziet agriculture Research Center using quantitative traits to determine the level of chickpea germplasm variation and to classify the test genotypes into clusters based on similarity and also to identify promising genotype for further breeding activities. Augmented design was used with five blocks. There was a strong significant differences among germplasm for plant height (PLH), primary branch per plant (PBPP), secondary branch per plant (SBPP), number of pod per plant (NPPP), number of days to mature (DTM) and thousand grain weight (TGW). 14 genotypes gave better thousand grain weights to the best standard check, Harbu (420gm) and 12 genotypes matured earlier than the earliest check, minjar (107days). Wide mean range values were recorded for all the traits. The high values of genotypic and phenotypic coefficient of variation indicated the existence of genetic variation among genotypes for all the studied quantitative traits. Based on cluster analysis the genotypes grouped into four classes irrespective of the origin of germplasm collection. The first two principal components explained 72.9% of the total variation among the genotypes for all quantitative traits. The genetic diversity observed among the genotypes will have a practical value to chickpea breeders to use the identified desirable trait for utilization in breeding program for chickpea genetic improvement.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important food legumes grown in Ethiopia in terms of area coverage and production (CSA, 2013). It is commonly cultivated in vertisol type using residual moisture which gives to the farmers for double cropping. Currently, chickpea is one of the export commodity crops in Ethiopia. And its area coverage and productivity have been increasing over a period of time. However, chickpea production is becoming a risky business because of the current climate change that occur in the country since the amount and pattern of rainfall becoming variable, temperature rise, insect pest and disease pressure increases time to time. This demands chickpea breeder to develop chickpea varieties which can adapt to a rapidly changing environmental conditions and give higher, stable yields and also for enhanced resistance to pests and diseases is one of the immediate objectives of many chickpea variety development program. Ethiopian farmers have been cultivated farmers variety for a long period of time because of the array of genetic diversity they hold, better adaptation and resistance to diseases and insect pests. The diverse agro-climatic conditions in Ethiopia make it very suitable for the development of genetic variation among chickpeas farmers' variety. Because of this, Ethiopia is recognized as a secondary center of diversity for chickpea (Vavilov, 1951). This genetic diversity created in the farmers' fields over millennia provides the raw material for improving crop productivity through plant breeding (Frankham *et al*., 2002; Hari *et al*., 2008; Upadhyaya *et al*., 2008) which could adapt a rapidly changing environmental conditions. For example, Keneni *et al*. (2011a) identified some Ethiopian chickpea germplasm that have resistance to Adzuki Bean Beetle damage. Owing to this, Ethiopian Biodiversity Institute has collected a total of 1224 chickpea accession from chickpea growing areas of Ethiopia (Fig 1) and conserved in the gene bank since 1977. And 811 accessions were characterized so far and also similar activities were undertaken to characterize another 80 chickpea farmer's variety together with standard checks in 2014. The objectives of the present studies were to assess the amount of genetic diversity in a collection of chickpea genotypes using multivariate techniques based on quantitative traits and to identify the potential genotypes for future utilization in chickpea breeding programs.

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MATERIALS AND METHODS

Eighty chickpea farmers' varieties (Table 1) collected from Amhara, Oromia, tigray and SNNP regions and four standard checks (i.e Arerti, Harbu, Minjar, and Natoli) were evaluated at D/Ziet Agricultural Research Center, on station during 2014 cropping season. The experiment was laid out in Augmented Design in five blocks. Each genotype was planted in 5 rows spaced at 10cm between plants and 20cm between rows. DAP and other management practices were applied as per recommendations. 20 individual plants were selected and the following data were recorded as per chickpea descriptor list (i.e days to maturity (DTM), plant height (PLH), primary branch per plant (PBPP), secondary branch per plant (SBPP), number of pod per plant (NPPP), and thousand grain weights (TGW). The collected data were subjected to statistical analysis of variance using IRRISTAT, MINTAB and SAS. Variances and coefficient variation were calculated as per formula suggested by Falconer and Markay (1996).

Mean and range values

The mean and range values of the genotypes indicated that there are genotypes performing better than the best standard checks for most traits. For example 14 genotypes (239923, 239909, 239930, 239922, 239903, 239919, 239924, 239895, 239972, 239917, 239908, 239980, 18207, and 239976) are superior in thousand grain weights to Harbu (420gm). 12 genotypes (239945, 239959, 225743, 232286, 9647, 239922, 9645, 16584, 16592, 229955, 16340 and 228658) matured earlier than the earliest chick Minjar (107days) (Table 3). Generally wide mean range value was observed for all the traits (Fig 2). Mean range of 30.7 to 60.5, 4 to 10, 2 to 21, 28 to 199, 80 to 133 and 92.7 to 1390 were recorded for PLH, PBPP, SBPP, NPPP, DTM and TGW, respectively. Similar finding were also discovered in chickpea (Malik *et al*., 2014; Archak *et al*., 2016). The result clearly indicate that the variability and wide ranges of mean of the studied traits give an opportunity for chickpea breeder for chickpea genetic

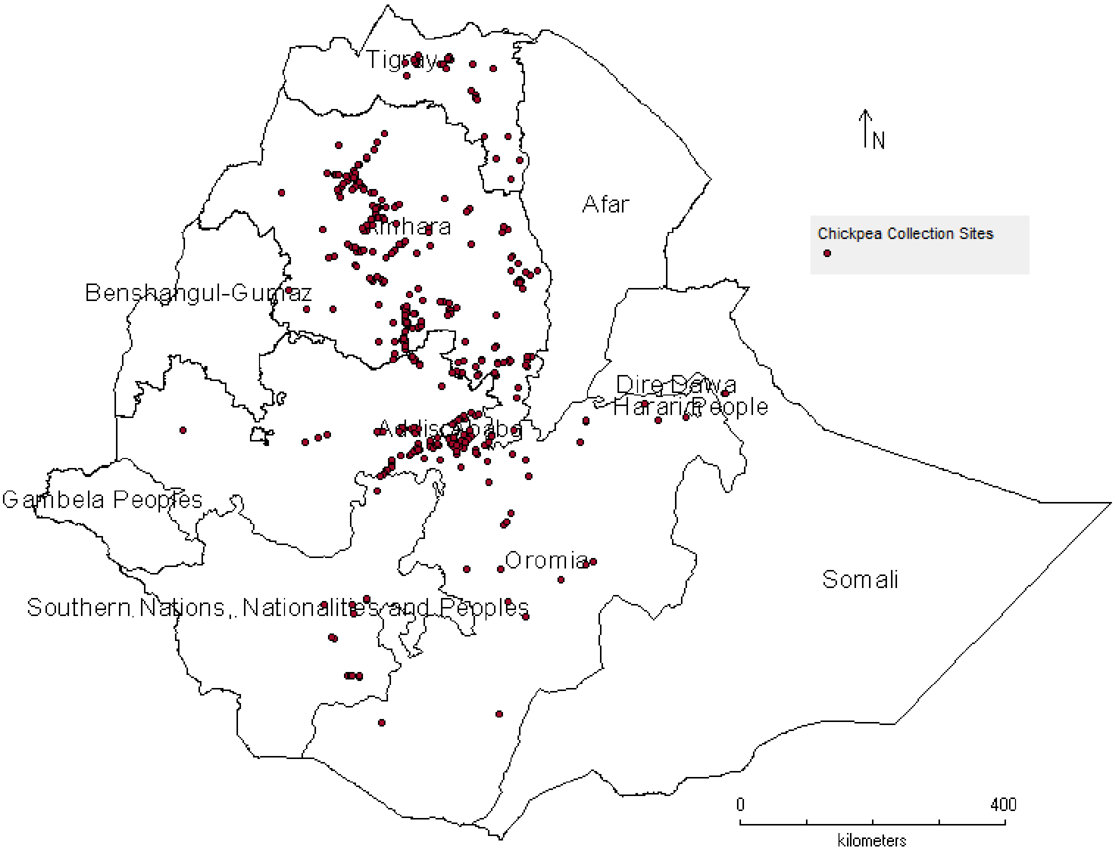

Fig. 1. The geographical distribution of chickpea farmers 'varieties of Ethiopian Biodiversity Institute collections

RESULTS AND DISCUSSION

Many Authors used quantitative traits to classify and estimate chickpea genetic diversity using simple statistical measures such as mean, range, including principal component and cluster analyses (Jayalakshmi and Ronald, 2011; Shiv Sewak *et al*., 2012; Hajibarat *et al*., 2014; Ahmad *et al*., 2012)

Analysis of variances (ANOVA)

Analysis of variance revealed highly significant differences ($P < 0.01$) among the genotypes for all the traits considered (Table 2).

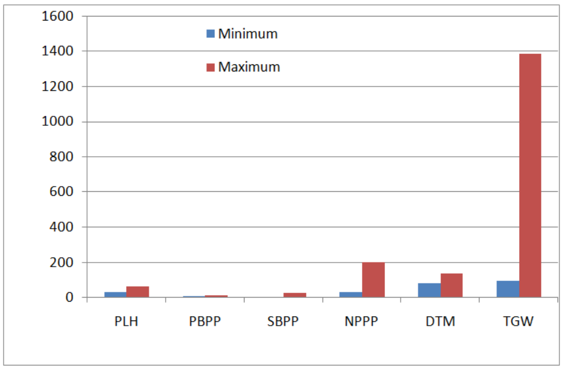

Fig. 2. Minimum and maximum values of six traits of 84 chickpea genotypes

improvement either through direct selection for the target traits or by using as a crossing material.

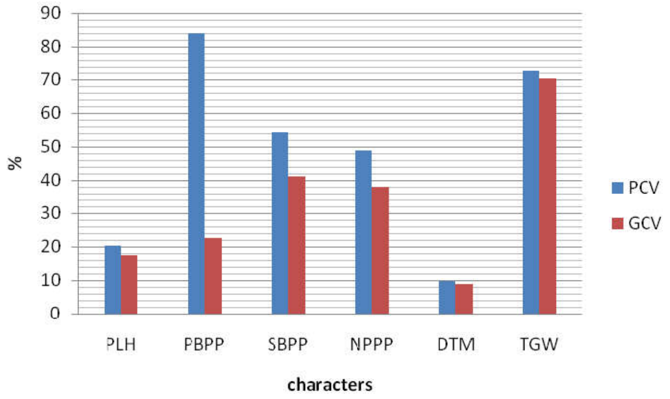

Fig.3. Phenotypic and genotypic coefficient of Variation of all character considered

Phenotypic and genotypic coefficients of variation

The variability in a population arises as a result of the differences in the genetic makeup of individual in the population, the environment and the interaction between genes with the environments. These three sources of variation can be measured and expressed in terms of variance. The variation in the population that is due to the genotypic composition of individuals is called genotypic variation (Allard, 1960; Mather and Jinks, 1971). In the present study, results from the estimation of GCV and PCV of the quantitative traits considered have shown the existence of genetic variation among chickpea germplasm. Low to high PCV and GCV values were observed for the traits considered (Fig 3). The result agreed with the report from Keneni *et al.*, 2012; Malik, 2014). There is no wide variation between GCV and PCV for all traits except PBPP which indicates that the variation observed in the genotypes have strong genetic bases.

Cluster analysis

The result Hierarchical cluster analysis indicated that 84 genotypes grouped into four clusters (Fig 4) with variable number of accessions per cluster (Table 5). Genotypes having similar characteristics grouped into one cluster, while genotypes with different characteristics grouped at different cluster group. The highest number in Cluster I and the least is in cluster IV (Table 4). The accessions were clustered irrespective of the origins of the landraces and accession from similar source of origin grouped into different cluster. Similar observations were reported by Keneni *et al.*, 2012 and Archak *et al.*, 2016. This happened because of a high germplasm movement from one locality to other. Cluster II and IV have biggest distance (147) while Cluster I and III are closer (10.9) (Table 5).

Principal Component Analysis

Principal component analysis is helpful for breeder to focus on certain traits which can be improved through conventional selection. In the present study, the first two principal components (PCs), with eigenvalues greater than unity, explained about 72.9% of the total variation among accessions for all traits (Table 6). PBPP, SBPP, NPPP and DTM are the most important contributing traits for the relative magnitudes of eigenvectors for the first principal component (44%), while PLH, NPPP and TGW for the first principal component (28.8). These results were in line with reports from Keneni *et al.* (2011b), Hajibarat *et al.* (2014), and Archak *et al.* (2016).

Table 1. List of Chickpea accessions with their respective region, district (Woreda) and altitude

Entry	Accn no	Region	Zone	District /Woreda	Altitude	Remark
1	9645	Amara	Semen Gondar	Dembia	1861	-
2	16590	Amara	Semen Gondar	Dabat	2629	-
3	16592	Amara	Semen Gondar	Maksegnet	1942	-
4	16594	Amara	Debub Gondar	Libo	1955	-
5	16595	Amara	Misrak Gojam	Dejen	2459	-
6	16596	Amara	Misrak Gojam	Bichena	2536	-
7	16597	Amara	Misrak Gojam	Bichena	2562	-
8	18218	Amara	Semen Shewa	Baso na Werena	2792	-
9	18219	Amara	Semen Shewa	Baso na Werena	2791	-
10	18220	Amara	Semen Shewa	Baso na Werena	2743	-
11	18221	Amara	Semen Shewa	Mafudmezezo Mojana	2898	-
12	18223	Amara	Semen Shewa	Mafudmezezo Mojana	2623	-
13	18224	Amara	Semen Shewa	Mafudmezezo Mojana	2629	-
14	18225	Amara	Semen Shewa	Mafudmezezo Mojana	2659	-
15	18226	Amara	Semen Shewa	Mafudmezezo Mojana	2725	-
16	18227	Amara	Semen Shewa	Mafudmezezo Mojana	2435	-
17	18229	Amara	Semen Shewa	Menzkeya	2755	-
18	235036	Amara	Semen Shewa	Mafudmezezo Mojanaq	1540	-
19	235825	Amara	Semen Gonder	Alefa	-	-
20	236193	Amara	Semen Wello	Kobo	-	-
21	236194	Amara	Debub Wello	Kutaber	-	-
22	236197	Amara	Debub Wello	Tehuledere	-	-
23	236455	Amara	Debub Wello	Kombolcha	1470	-
24	9233	Oromiya	Misirak Harerge	Deder	2020	-
25	9647	Oromiya	Semen Shewa	Wara Jarso	2545	-
26	16340	Oromiya	Semen Shewa	Boneya Boshe	1680	-
27	18206	Oromiya	Misirak Shewa	Adama	2227	-
28	18207	Oromiya	Misirak Shewa	Adama	2315	-
29	18208	Oromiya	Misirak Shewa	Adama	2301	-
30	18212	Oromiya	Misirak Shewa	Lome	1953	-
31	18214	Oromiya	Misirak Shewa	Lome	2022	-
32	18215	Oromiya	Misirak Shewa	Lome	1984	-
33	18216	Oromiya	Misirak Shewa	Lome	2263	-
34	18217	Oromiya	Misirak Shewa	Leben Chukala	1657	-
35	18231	Oromiya	Semen Shewa	Wara Jarso	2552	-

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36	18232	Oromiya	Semen Shewa	Wara Jarso	2530	-
37	18233	Oromiya	Semen Shewa	Wara Jarso	2555	-
38	18234	Oromiya	Semen Shewa	Debre Libanos	2633	-
39	228658	Oromiya	Mirab Shewa	Dendi	-	-
40	229955	Oromiya	Bale	Nensebo	1940	-
41	229958	Oromiya	Bale	Mennana Harena Bulu	1430	-
42	236882	Oromiya	Semen Shewa	Hidabu Abote	-	-
43	236886	Oromiya	Mirab Shewa	Jeldu	-	-
44	241128	Oromiya	Misrak Harerge	Fedis	1760	-
45	225743	SNNP	Semen Omo	Gofa Zuria	1550	-
46	9643	Tigray	MIRABAWI	Semema	1952	-
47	16584	Tigray	Mehakelegnaw	Hager Selam	2588	-
48	234050	Tigray	Mehakelegnaw	Laelay Maychew	2120	-
49	235392	Tigray	Debubawi	Endamehoni	-	-
50	235398	Tigray	Debubawi	Mekele	-	-
51	236463	Tigray	Mehakelegnaw	Enticho	1960	-
52	238263	Tigray	Mehakelegnaw	Laelay Maychew	2050	-
53	232286	-	-	-	-	Market Collection
54	232288	-	-	-	-	Market Collection
55	239836	-	-	-	-	Market Collection
56	239852	-	-	-	-	Market Collection
57	239861	-	-	-	-	Market Collection
58	239873	-	-	-	-	Market Collection
59	239895	-	-	-	-	Market Collection
60	239903	-	-	-	-	Market Collection
61	239908	-	-	-	-	Market Collection
62	239909	-	-	-	-	Market Collection
63	239915	-	-	-	-	Market Collection
64	239917	-	-	-	-	Market Collection
65	239919	-	-	-	-	Market Collection
66	239922	-	-	-	-	Market Collection
67	239923	-	-	-	-	Market Collection
68	239924	-	-	-	-	Market Collection
69	239925	-	-	-	-	Market Collection
70	239930	-	-	-	-	Market Collection
71	239935	-	-	-	-	Market Collection
72	239945	-	-	-	-	Market Collection
73	239950	-	-	-	-	Market Collection
74	239957	-	-	-	-	Market Collection
75	239959	-	-	-	-	Market Collection
76	239967	-	-	-	-	Market Collection
77	239972	-	-	-	-	Market Collection
78	239976	-	-	-	-	Market Collection
79	239979	-	-	-	-	Market Collection
80	239980	-	-	-	-	Market Collection
81	Arerti	-	-	-	-	Improved Variety
82	Harbu	-	-	-	-	Improved Variety
83	Minjar	-	-	-	-	Improved Variety
84	Natali	-	-	-	-	Improved Variety

Table 2. Mean square and mean range for the tested traits of 84 chickpea genotypes, 2014

Source of Variation	Degree of Freedom	Mean Squares					
		PLH	PBPP	SBPP	NPPP	DTM	TGW
Blocks	4	53.58**	45.51**	234.41**	4028.21**	417.76**	75849.5**
Treatment	83	325.40**	10.01**	102.28**	5871.51**	523.15**	225277**
Error	12	20.62	1.50	13.02	678.8	20.86	3111.65
CV	99	10.17	21.42	35.27	30.68	4.09	18.59
LSD (5%)		6.26	1.69	4.97	35.9	6.29	76.87

Key: PLH=plant height, PBPP=primary branch per plant, SBPP=secondary branch per plant, NPPP=number of pod per plant, DTM=number of days to mature, TGW=thousand grain weight, **=Significant at 1% probability level

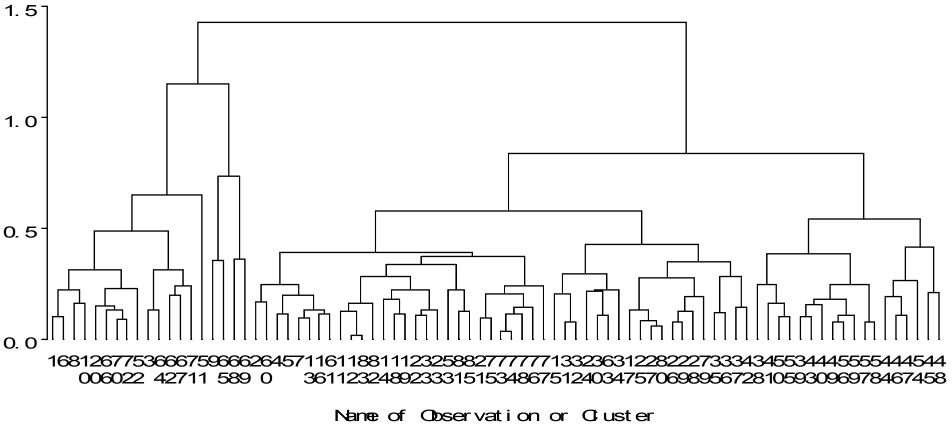

Fig.4. Cluster analysis of 84 chickpea genotypes

Table 3. Mean and Range of plant height in cm (PLH), number of pod per plant (PBPP), secondary branch per plant (SBPP), number of pod per plant (NPPP), days to mature (DTM) and thousand grain weight in gm (TGW) of 84 chickpea genotypes

No	Accn No	PLH	PBPP	SBPP	NPPP	DTM	TGW
1	9233	48	6	13	118	102	189
2	9643	46	6	12	86	102	184
3	9645	52	7	19	129	99	204
4	9647	46	5	15	101	98	197
5	16340	41	4	14	99	100	174
6	16584	45	7	13	121	99	193
7	16590	47	5	9	94	101	190
8	16592	50	6	16	112	99	208
9	16594	52	7	19	182	103	227
10	16595	45	5	12	107	101	93
11	16596	47	5	9	78	102	193
12	16597	44	4	8	73	105	183
13	18206	44	5	9	91	103	180
14	18207	38	4	11	83	104	1099
15	18208	38	4	7	67	103	183
16	18212	40	5	8	94	103	190
17	18214	38	5	5	65	120	214
18	18215	40	5	5	89	108	212
19	18216	36	5	3	90	112	216
20	18217	39	5	5	107	115	214
21	18218	38	5	4	95	114	213
22	18219	37	5	3	84	113	221
23	18220	35	5	3	77	113	259
24	18221	31	4	2	58	112	210
25	18223	32	4	2	63	124	114
26	18224	34	5	3	77	124	206
27	18225	36	4	3	63	124	207
28	18226	32	4	4	70	124	208
29	18227	34	5	4	74	124	198
30	18229	32	5	8	61	104	213
31	18231	36	4	10	66	114	215
32	18232	36	5	7	67	115	216
33	18233	40	5	8	74	119	219
34	18234	32	4	13	44	113	260
35	239852	46	5	13	65	129	333
36	239861	47	6	13	62	133	328
37	239873	52	7	13	60	122	357
38	239895	60	5	11	47	133	581
39	239903	56	4	9	29	131	567
40	239908	50	4	8	28	129	626
41	239909	60	5	9	45	118	523
42	239915	50	6	17	59	116	408
43	239917	56	4	8	34	130	609
44	239919	53	8	15	37	105	567
45	239922	52	7	12	57	99	552
46	239923	59	5	9	39	102	502
47	239924	56	4	11	43	102	567
48	239925	61	6	14	55	105	287
49	239930	57	6	9	54	116	544
50	239935	60	8	13	71	117	350
51	239945	49	8	20	150	80	256
52	239950	48	9	18	117	109	417
53	239957	57	7	15	93	117	487
54	239959	59	7	12	47	98	104
55	239967	58	10	13	67	117	413
56	239972	57	8	11	52	119	606
57	239976	47	6	6	53	113	1390
58	239979	50	6	6	55	113	400
59	239980	54	7	11	49	117	745
60	241128	46	8	18	102	108	195
61	225743	41	7	16	107	98	287
62	228658	41	7	13	148	101	263
63	229955	31	7	13	69	100	157
64	232286	48	10	21	147	98	189
65	234050	43	8	19	199	104	133
66	235036	41	6	10	109	108	235
67	236194	42	7	15	128	108	257
68	236463	40	9	16	162	101	205
69	238263	43	6	10	152	114	220
70	239836	43	5	9	113	110	213
71	229958	41	6	11	139	114	133
72	232288	42	6	10	114	114	131
73	235392	38	5	7	90	118	138
74	235398	38	6	8	89	119	153
75	235825	40	5	7	98	115	118

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76	236193	41	5	7	94	121	126
77	236197	36	5	7	101	124	117
78	236455	37	5	7	85	122	117
79	236882	40	4	5	79	127	124
80	236886	37	5	6	60	123	111
81	ARERTI	46	6	11	83	117	318
82	HABRU	48	7	13	77	111	420
83	MINJAR	44	5	9	76	107	267
84	NATALI	47	6	12	88	121	353
	MEANS	45	6	10	85	112	300
	RANGE	31-61	4-10	2-21	28-199	80-133	93-1390

Table 4. Clustering of 84 chickpea genotypes into four clusters using mean of six quantitative traits

Cluster	Accession						
Cluster I (47 accessions)	9643	16597	18214	18226	239861	236455	236197
	9647	18206	18215	18227	239873	236882	**ARERTI**
	16340	18207	18216	18229	239915	236886	**HABRU**
	16590	18208	18218	18231	239957	235392	**MINJAR**
	16596	18212	18219	18232	241128	235398	**NATALI**
	18220	18223	18225	18233	225743	235825	18221
	236193	18224	239852	18234	229955		
Cluster II (18 accessions)	239895	239919	239930	239976	239909	239924	239967
	239903	239922	239935	239979	239917	239925	239972
	239908	239923	239959	239980			
Cluster III (15)	9233	18217	235036	16584	239950	239836	232288
	9645	239945	236194	16592	228658	229958	232286
	16595						
Cluster IV (4 accessions)	16594	234050	236463	238263			

Table 5. Pair-wise generalized squared distances among four clusters constituting 84 chickpea genotypes

Clusters	1	2	3	4
1	0	28.31601	10.92404	61.68688
2	28.31601	0	58.98305	147.2988
3	10.92404	58.98305	0	22.61266
4	61.68688	147.2988	22.61266	0

Table 6. Eigenvectors and eigenvalues of the six PCs of the traits of 84 chickpea accessions

Traits	Eigenvectors					
	PC1	PC2	PC3	PC4	PC5	PC6
PLH	-0.14	0.658	0.181	0.41	0.563	0.174
PBPP	-0.496	0.263	0.33	0.009	-0.66	0.374
SBPP	-0.564	0.103	0.086	-0.124	0.051	-0.804
NPPP	-0.454	-0.357	0.152	-0.493	0.493	0.396
DTM	0.428	0.051	0.844	-0.275	0.026	-0.16
TGW	0.162	0.598	-0.34	-0.705	-0.045	0.033
Eigenvalue	2.6464	1.7258	0.658	0.4761	0.2963	0.1974
% of total variance explained	44.1	28.8	11	7.9	4.9	3.3
% cumulative variance explained	44.1	72.9	83.8	91.8	96.7	100

Conclusion

The study revealed that there is a vast range of genetic variability among the tested genotypes for days to maturity (DTM), plant height (PLH), primary branch per plant (PBPP), secondary branch per plant (SBPP), number of pod per plant (NPPP), and thousand grain weights (TGW). The variations observed among the genotypes are very essential for chickpea breeding program for specific traits. Genotypes 239923, 239909, 239930, 239922, 239903, 239919, 239924, 239895, 239972, 239917, 239908, 239980, 18207, and 239976 are recommended for further evaluation for yield. Genotypes, 239945, 239959, 225743, 232286, 9647, 239922, 9645, 16584, 16592, 229955, 16340 and 228658 are early maturing type.

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