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Cluster Analysis of Agronomic Traits in Chickpea Genotypes under Cool Upland Semi-arid Region

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Abstract: This study was conducted to evaluate the genetic diversity among 50 chickpea lines grown in a randomized complete block design with three replications. The days to flower initiation, canopy width, days to maturity, plant height, chlorophyll content, ground coverage, number of subsidiary branches, the first pod height from the ground, number of pods per plant, pod weight per plant, shuck weight per plant, plant fresh weight, number of seeds per pod, number of unfilled pods per plant, protein percent, plant dry weight, hundred seed weight and seed yield were recorded. The coefficient of variation was high for chlorophyll content, ground coverage, number of subsidiary branches, first pod height from ground, number of pods per plant, number of seeds per pods, number unfilled pods , canopy width and seed yield. The chickpea traits were grouped into three groups; Cluster-I is seed yield while Cluster-II consists of chlorophyll content, ground coverage, days to flower initiation, dry matter and hundred seed weight and the other remained traits were grouped as Cluster-III. The cutoff point divided the dendrogram of chickpea genotypes into four clusters, Cluster-A, Cluster-B, Cluster- C and Cluster-D consisted of 16, 4, 11 and 19 genotypes. Four genotypes of Cluster-B namely FLIP09-228C-S00794(30 KR)-2/, TDS-Maragheh90-90, TDS-Maragheh90-373 and TDS-Maragheh90-266 based on high mean yield, high numbers of seeds and pods per plant were identified as good candidates for commercial release and can be advised for cultivation.

Key words: Cluster analysis, chick pea, rainfed, semi-arid area, seed yield, yield components.

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In most developing countries, crops from the Leguminosae family are considered the most important source of protein because they have been an important part of the human diet for a long time and are used as a substitute for meat due to their high protein content. Although some of these crops have adapted well to rainfed conditions, their production capacity is mostly low (Kakaei *et al.*, 2015; Amiri *et al.*, 2020). More than ten mha out of 17 mha of cultivated land in Iran are rainfed and the lower yield of rainfed legumes is attributed to the use of low-yielding cultivars and rising temperature during spring (Kheyruri *et al.*, 2024). Therefore, it is necessary to evaluate the genetic reserves and investigate the genetic diversity to

be able to identify more compatible and high-yielding cultivars to replace the present low yielding cultivars.

Chickpea is a significant cold-season legume known for its high nutritional value, ranking third among the most important legumes after soybeans and beans. Chick pear accounts for approximately 19% of global legume production, primarily cultivated in the rainfed conditions of semi-arid regions. With an annual production of around 18 million tons from roughly 15 million hectares of cultivated land, the average yield is estimated at 1.2 tons per hectare (FAOSTAT, 2022). Chickpea is the most cultivated legume crop in Iran, with approximately 561,000 ha of cultivated land and an annual production of 300,000 tons (Kheiri *et al*., 2021; Singh *et al*., 2023). Regarding its high nutritional value, due to the high amount of protein, vitamins, minerals, and fiber, chickpea is used as human food and animal feed, and it is the most important food legume in most countries of West Asia and North Africa, including Iran. Due to the its higher relative tolerance to drought stress, more than 90% of the area under chickpea cultivation in Iran is rainfed. The total harvested area of chickpeas in Iran is about 561,000 hectares, which ranks fifth in the world after India, Pakistan, Australia, and Turkey while its mean yield is very low, 400 kg ha^{-1} (FAOSTAT, 2022). In rainfed conditions of semi-arid regions, breeding is done for some characteristics of roots like volume or length. Also, considering the ability of nitrogen fixation in the legume roots, using them in crop rotation helps the sustainability of agricultural systems (Kebede, 2021). The chickpea possesses a robust, vertical root system characterized by a dense network of secondary roots. In rainfed conditions, the primary root tends to grow longer and develop more branches. Most of the root volume is concentrated within a soil depth of 60 centimeters (Rao *et al.,* 2021).

In most of the chickpea producing countries, genetically improved cultivars have been introduced for cultivation. Knowledge of genetic diversity is the first step in breeding any crop because genetic variation and selection are the two main tools of any breeding program (Swarup *et al.,* 2021). Selection of the most favorable genotypes depends on the existence of a desirable diversity in terms of the target traits. In order to benefit from the existing diversity and create new genotypes, it is necessary to evaluate the genetic resources, so considering the large number of genetic resources, grouping individuals into morphological and genotypic groups is very important in the exploitation of genetic resources. Such selected elite genotypes are generated from different hybridization programs after several years of evaluation regarding different physiological and morphological characteristics and are subjected to final investigations to find the most desirable genotype for commercial cultivar release (Soltani and Sinclair, 2011).

Also, the International Center for Agricultural Research in the Dry Areas (ICARDA) tries to provide several treasures of elite lines are prepared and sent to some semi-arid regions of Asia or Africa, so the best lines are identified and studied for compatibility in the target regions. These lines have been evaluated in Iranian rainfed stations and a number of the best superior lines in terms of important traits such as seed yield, plant height and hundred seed weight are selected for propagation and further studies. Toker and Cagirgan (2004) studied 17 genotypes of chickpea in the western Mediterranean region of Turkey to evaluate seed yield performance and found a positively strong association between yield performance with biomass, tall plants, having more branches and pods' frequencies per plant.

Rezaeinia *et al.* (2017) studied several genotypes of chickpea in order to investigate genetic variation and identify yield components and reported relatively a great diversity among genotypes in terms of weight of seeds as well as weight of pods and seeds per plant. Also, they showed that the hundred seed weight, pods' weight and the seeds per plant have the greatest effect on yield performance, so these traits were introduced as main yield components. Nie *et al.* (2015) studied genetic variation among 100 chickpea genotypes and reported high diversity for plant height and hundred seed weight whereas genotypes were clustered into four distinct groups; the genotypes of the first group had high yield performance with moderate plant height, while the genotypes of the fourth group had large seed size and highest plant height. Nabati *et al.* (2023) evaluated many chickpea genotypes for twenty qualitative and quantitative traits and found high genetic variation for plant height,

pods' number per plant, plant dry weight, hundred grain weight, seed yield, and harvest index. while the clustering indicated that the first cluster had many pods and leaflet, with long peduncle length; the second cluster had high plant dry weight and seed yield. The goal of current investigation was to investigate the diversity of morphological traits as well as determining the pattern of genetic diversity and grouping genotypes based on seed yield performance and morphological traits using cluster analysis method.

Materials and Methods

Experiment and plant materials

In order to carry out this research, the number of 50 chickpea genotypes (Table 1). They consist of 49 international lines sent from the ICARDA and a control variety (AZAD) was planted in the form of a randomized complete block scheme with three repetitions. The experimental field is located in the Gavshaleh, Saqqez, Iran; 36°19′54″N and 47°19′07″E with an altitude of 1476 meters, as a semi-arid cool upland region. Each experimental plot included four one-meter lines with a distance of 0.25 m between the lines, the area of each test plot was one square meter, and the harvest area after removing 25 cm from the beginning and end of the lines was 0.25 square meters. Field preparation operations including deep plowing in autumn and surface plowing, disc, and leveling in spring were usually done in chickpea experiments. During the growth period, the usual agricultural cares such as weeding and fighting against pests and diseases were carried out in the field.

Measured traits

Necessary phenological measurements were taken from each plot, such as days to flower initiation (DF), canopy width (CW), and days to maturity (DM). After maturity, 10 random plants were chosen from plots and these traits were recorded: chlorophyll content (CHL), plant height (PH), ground coverage (GC), number of the subsidiary branches (SB), the first pod height from ground (FPH), number of pods per plant (NPP), pod weight (PW), shuck weight per plant (SW), plant fresh weight (PFW), number of seeds per pod (SP), number of unfilled pods (UP) and protein percent (PP). Finally, the harvested plants were exposed to sunlight for several days to dry and then weighed to determine the plant dry weight (PDW g plant^{-1}). After harvesting, the harvested plants were exposed to sunlight for several days to dry and then the separation of seeds from straw and stubble was done manually (beating, sifting, and blowing) and then the seeds were weighed in grams per square meter and converted to kg ha^{-1} as seed yield (SY). Finally, the hundred seed weight (HSW) was computed from the weight of three subsamples from the sed yield of each unit.

Data analysis

The Normal distribution shape of measured traits in chickpea genotypes was assessed via the Shapiro-Wilk procedure with the Normality statement in Minitab software version 14.0 (Minitab Inc., USA). Agglomerative hierarchical cluster analysis was used to group genotypes as well as traits according to standardized squared Euclidean distances using the obtained dataset. The computed standardized squared Euclidean distances were merged by the minimum variance of Wards method and dendrogram diagrams were generated to represent the patterns and relations among genotypes and traits with the Mult/Exploratory statement in Statitica software version 14.0 (TIBCO Inc., USA).

To verify the number of clusters showing the significant partition in the dendrogram diagrams, the multivariate analysis of variance was used via Wilks' lambda and Hotelling statistic as well as Pillai's trace and Roys' maximum root which were applied on the original dataset for all measured traits of chickpea with the Multivariate statement in SPSS application version 17.0 (SPSS Inc., USA), so the significant borders were selected as the final cutoff point. The above-mentioned statistics were computed as:

$$\text{Wilks' Lambda } \Lambda = |W| / |T|$$

where, $|W|$ is the determinant of the within-group sum of squares and cross-products matrix and $|T|$ is the determinant of the total sum of squares and cross-products matrix, whereas Wilks' Lambda ranges $0 \leq \Lambda \leq 1$, and smaller values indicate greater differences between groups. Also, Hotelling's Trace or T^2 was calculated as:

$$T^2 = trace(W^{(-1)}B)$$

Table 1. Pedigree and origin of 50 chickpea genotypes.

Genotype No.	Pedigree	Origin	Genotype No.	Pedigree	Origin
1	FLIP09-320C-X05TH19/X04TH-138XFLIP01-28	ICARDA	26	TDS-Maragheh90-373	Turkey
2	TDS-Maragheh90-229	Turkey	27	TDS-Maragheh90-266	Turkey
3	FLIP09-228C-S00794(30 KR)-2/	ICARDA	28	FLIP09-441C-X04TH61/X03TH-129XFLIP96-154	ICARDA
4	TDS-Maragheh90-155	Turkey	29	TDS-Maragheh90-112	Turkey
5	TDS-Maragheh90-213	Turkey	30	FLIP09-53C-X04TH175/FLIP95-51XFLIP97-165	ICARDA
6	TDS-Maragheh90-216	Turkey	31	TDS-Maragheh90-205	Turkey
7	TDS-Maragheh90-4	Turkey	32	FLIP09-267C-X04TH72/X03TH-140XFLIP99-48	ICARDA
8	AZAD	Iran	33	TDS-Maragheh90-423	Turkey
9	TDS-Maragheh90-352	Turkey	34	TDS-Maragheh90-221	Turkey
10	TDS-Maragheh90-145	Turkey	35	TDS-Maragheh90-162	Turkey
11	FLIP09-318C-X05TH8/X04TH-127XFLIP97-131	ICARDA	36	TDS-Maragheh90-92	Turkey
12	FLIP09-423C-X04TH127/FLIP98-230XFLIP97-116	ICARDA	37	TDS-Maragheh90-137	Turkey
13	TDS-Maragheh90-357	Turkey	38	TDS-Maragheh90-143	Turkey
14	TDS-Maragheh90-204	Turkey	39	FLIP09-279C-X04TH141/FLIP99-46XFLIP97-91	ICARDA
15	TDS-Maragheh90-325	Turkey	40	TDS-Maragheh90-250	Turkey
16	TDS-Maragheh90-333	Turkey	41	TDS-Maragheh90-434	Turkey
17	TDS-Maragheh90-46	Turkey	42	FLIP09-242C-F2 X01TH187 (45KR)-2/	ICARDA
18	TDS-Maragheh90-210	Turkey	43	TDS-Maragheh90-61	Turkey
19	FLIP09-239C-F2 X01TH183 (45KR)-26/	ICARDA	44	TDS-Maragheh90-87	Turkey
20	TDS-Maragheh90-400	Turkey	45	FLIP09-249C-S00794(30 KR)-6/	ICARDA
21	TDS-Maragheh90-305	Turkey	46	TDS-Maragheh90-235	Turkey
22	TDS-Maragheh90-90	Turkey	47	FLIP09-397C-X04TH65/X03TH-133XFLIP96-154	ICARDA
23	TDS-Maragheh90-150	Turkey	48	FLIP88-85C-X85 TH143/ILC 629 x FLIP 82-144C	ICARDA
24	TDS-Maragheh90-30	Turkey	49	TDS-Maragheh90-152	Turkey
25	TDS-Maragheh90-445	Turkey	50	TDS-Maragheh90-208	Turkey

where, is the inverse of the within-group matrix and B is the between-group matrix. The values of Hotelling's Trace ranges from 0 to infinite (positive values only), and the largest values indicate greater differences between groups. Additionally, the Pillai's Trace (V) is obtained as:

$$V=trace(B(B+W)^{-1})$$

where B is the between-group matrix and W is the within-group matrix and its range is $0 \leq V \leq s$, while s is the minimum of p (number of variables) and degree of freedom. As V approaches 's' there is a strong separation between the groups. Finally, the Roy's Largest Root (θ) is computed as:

$$\theta = \lambda max$$

where, λ maximum is the largest eigenvalue of $W^{-1}B$, and the larger values indicate stronger group separation.

Results and Discussion

Coefficient of variation (CV) of different traits is given in Table 2. The magnitudes of CV were >20% for chlorophyll content (CHL),

Table 2. Descriptive statistics of measured traits of chickpea

Traits	Unit	Mean	Minimum	Maximum	Coefficient of variation CV
Plant height (PH)	cm	25.12	18.33	38.33	16.75
Days to flower initiation (DF)	Day	53.53	50.67	55.67	2.16
Days to maturity (DM)	Day	87.67	85.33	`90.00	1.34
Chlorophyll content (CHL)	SPAD	32.02	17.00	58.67	35.48
Ground coverage (GC)	%	47.03	13.33	73.33	25.26
Subsidiary branches (SB)	Number	4.873	3.000	9.333	25.21
First pod height from ground (FPH)	cm	13.13	9.33	22.67	22.11
Number of pods per plant (NPP)	Number	15.57	4.33	32.00	37.21
Pod weight (PW)	g	0.462	0.175	0.596	17.03
Shuck weight per plant (SW)	g	0.111	0.075	0.175	19.89
Number of seeds per pod (SP)	Number	17.54	6.67	33.00	31.52
Number of unfilled pods (UP)	Number	1.633	1.000	2.667	24.82
Hundred seed weight (HSW).	g	35.72	25.13	45.40	15.02
Plant dry weight (PDW)	g	13.05	10.33	16.00	10.06
Plant fresh weight (PFW)	g	19.81	16.67	23.67	8.09
Canopy width (CW)	cm	23.59	13.33	33.33	20.59
Protein percent (PP)	%	18.68	15.18	22.62	9.52
Seed yield (SY)	kg ha^{-1}	1252.5	636.20	2048.30	25.59

ground coverage (GC), number of subsidiary branches (SB), the first pod height from the ground (FPH), number of pods and seeds (NPP and SP), unfilled pods per plant (UP), canopy width (CW) and seed yield (SY). Its value was moderate (10 to 20%) for plant height (PH), pod weight per plant (PW), shuck weight per plant (SW), and hundred seed weight (HSW) while it was low (<10%) for days to flower initiation (DF), days to maturity (DM), protein content (PP), plant dry weight (PDW) and plant fresh weight (PFW). Thus, the genetic variation of chickpea genotypes was adequate to achieve a favorable combination of traits. Kanouni *et al.* (2012) studied 60 chickpea lines from ICARDA, and reported high CV values for early plant vigor, seed yield and number of pods per plant and low CV values for days to flower initiation and days to maturity.

Babbar and Tiwari (2018) investigated the genetic variation of 40 chickpea genotypes and found high CV values for biomass, seed yield, number of fertile pods per plant, number of total pods per plant, and plant height while the other traits had low CV values including hundred seed weight, days to maturity, days to flower initiation and number of seeds per pod.

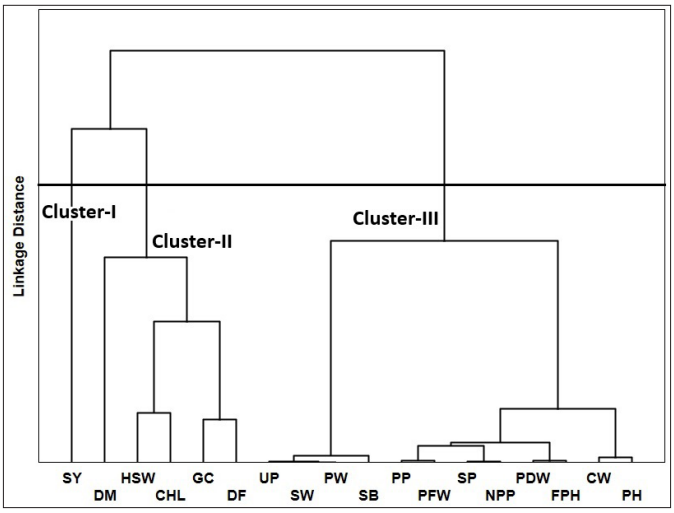
The dendrogram diagram was drawn to explore the structure among traits of chickpea (Fig. 1), the cutoff point is verified via four indices of the multivariate analysis of variance (Table 3). The Wilks' lambda, Hotelling, Pillai's trace and Roys' maximum root was significant and verified the position of cutoff point in the dendrogram.

The studied chickpea traits were grouped into three groups; Cluster-I is seed yield (SY) while Cluster-II is consisting on chlorophyll content (CHL), ground coverage (GC), days to flower initiation (DF), days to maturity (DM) and hundred seed weight (HSW). The other remained traits were grouped as Cluster-III (Fig. 1). According to Pushpavalli *et al.* (2014),

Table 3. Multivariate statistics for determining the cutoff pint of dendrogram for categorizing traits

Statistics	Value	F†	Hypothesis df‡	Error df	Sig.
Pillai's Trace	1.99	33.79	30	4	0.002
Wilks' Lambda	0.023	137.27	30	2	0.007
Hotelling's Trace	3357.35	154.72	30	1	0.000
Roy's Largest Root	3230.65	443.75	15	2	0.000

†F, statistic of F ratio; ‡df, degrees of freedom.



Traits are: days to flower initiation (DF), canopy width (CW), days to maturity (DM), chlorophyll content (CHL), plant height (PH), ground coverage (GC), number of the subsidiary branches (SB), the first pod height from ground (FPH), number of pods per plant (NPP), pod weight (PW), shuck weight per plant (SW), plant fresh weight (PFW), number of seeds per pod (SP), number of unfilled pods (UP), protein percent (PP), plant dry weight (PDW), seed yield (SY) and hundred seed weight (HSW).

Fig. 1. Dendrogram of clustering for eighteen traits of chickpea.

the two main components of a chickpea's performance are the number of pods and seeds, so Cluster-II had one component and Cluster-III had two components while the own seed yield was grouped as another separate cluster which indicating the importance of nonlinear components in the seed yield. Similar findings were also reported by Salgotra (2016). In most seed-yielding crops, yield performance is analyzed as the linear product of the yield components whereas it is related to sink size, which is specified in the vegetative step and the photosynthesis potential in the reproductive step (González-Paleo *et al.*, 2016).

In the chickpea, the active seed-filling step starts when the pod wall has reached its maximum size (Soltani and Sinclair, 2011), and genetic diversity for this process is reported among genotypes, but it is rarely related to seed size (Rao *et al.*, 2021). For an ideal genotype, variation in seed weight is due to changes in seed growth rates, even if the time of filling is variable across environments. This investigation has demonstrated that two components, the number of pods and seeds, were associated but the other component, hundred seed weight, was not completely associated to them. Also, the final seed yield was not related linearly to these components and was clustered separately. Richards *et al.* (2022) pointed out that the number and weight of chickpea seeds are associated with the amount assimilated to the flowers, and dividing biomass to these organs will increase such yield components. A deep physiologic grasp of the interaction between vegetative and reproductive steps is needed for the next breeding programs of chickpea yield. The findings of this investigation showed that selecting both the number and weight of seeds or both pods' number and seed weight would improve the seed yield of chickpeas in various ways.

The dendrogram was drawn to find the pattern among genotypes (Fig. 2), the cutoff point is confirmed via the Wilks' lambda, Hotelling, Pillai's trace, and Roys' maximum root which were significant and confirmed the cutoff point (Table 4). The cutoff point divided

Table 4. Multivariate statistics for determining the cutoff pint of dendrogram for categorizing genotypes

Statistics	Value	F†	Hypothesis df‡	Error df	Sig.
Pillai's Trace	2.09	3.99	54	93	0.000
Wilks' Lambda	0.043	8.48	54	87.2	0.000
Hotelling's Trace	33.83	17.33	54	83	0.000
Roy's Largest Root	29.00	49.94	18	31	0.000

†F, statistic of F ratio; ‡df, degrees of freedom

Table 5. Means of measured traits for four identified clusters of genotypes

Traits	Unit	Cluster-A	Cluster-B	Cluster-C	Cluster-D
Plant height (PH)	cm	23.73	26.00	25.73	25.75
Days to flower initiation (DF)	Day	53.90	52.58	53.39	53.49
Days to maturity (DM)	Day	87.92	86.50	87.27	87.95
Chlorophyll content (CHL)	SPAD	27.94	33.83	38.45	31.35
Ground coverage (GC)	%	38.23	52.92	50.76	51.05
Subsidiary branches (SB)	Number	4.375	5.917	5.000	5.000
First pod height from ground (FPH)	cm	12.46	13.67	13.12	13.60
Number of pods per plant (NPP)	Number	10.27	29.17	19.64	14.81
Pod weight (PW)	g	0.465	0.341	0.474	0.479
Shuck weight per plant (SW)	g	0.115	0.101	0.118	0.106
Number of seeds per pod (SP)	Number	12.50	31.00	21.42	16.70
Number of unfilled pods (UP)	Number	1.687	1.333	1.576	1.684
Hundred seed weight (HSW).	g	35.00	32.50	35.70	37.02
Plant dry weight (PDW)	g	12.62	13.17	13.67	13.04
Plant fresh weight (PFW)	g	19.15	19.50	20.42	20.09
Canopy width (CW)	cm	23.02	26.67	21.97	24.37
Protein percent (PP)	%	18.40	18.55	19.61	18.41
Seed yield (SY)	kg ha^{-1}	925.3	1995.8	1514.7	1219.7

the dendrogram of chickpea genotypes into four clusters, and average values of the traits in each cluster are presented in Table 5 which explains the features of the identified clusters. Cluster-A contains 16 genotypes (G8, G9, G11, G13, G19, G21, G24, G25, G36, G38, G40, G41, G43, G44, G47, and G48) with the lowest seed yield performance and variety control (AZAD) is grouped in this cluster, so these genotypes have the same properties as local cultivars and did not show any superiority to routinely cultivated cultivars of chickpea in Iran. Cluster-B consists of four genotypes (G3, G22, G26, and G27) and indicated a high mean yield performance as well as high numbers of seeds and pods per plant but its hundred seed weight (HSW) is lower than Cluster-C and Cluster-D. Also, the genotypes of Cluster-B were early flowering and maturity with tall plants which can be suitable for combined harvesting. Cluster-C contains 11 genotypes (G, G5, G6, G14, G16, G17, G20, G23, G28, G30, and G50) with moderate seed yield which showed moderate values for most of the measured traits while Cluster-D contains 19 genotypes (G1, G4, G7, G10, G12, G15, G18, G29, G31, G32, G33,

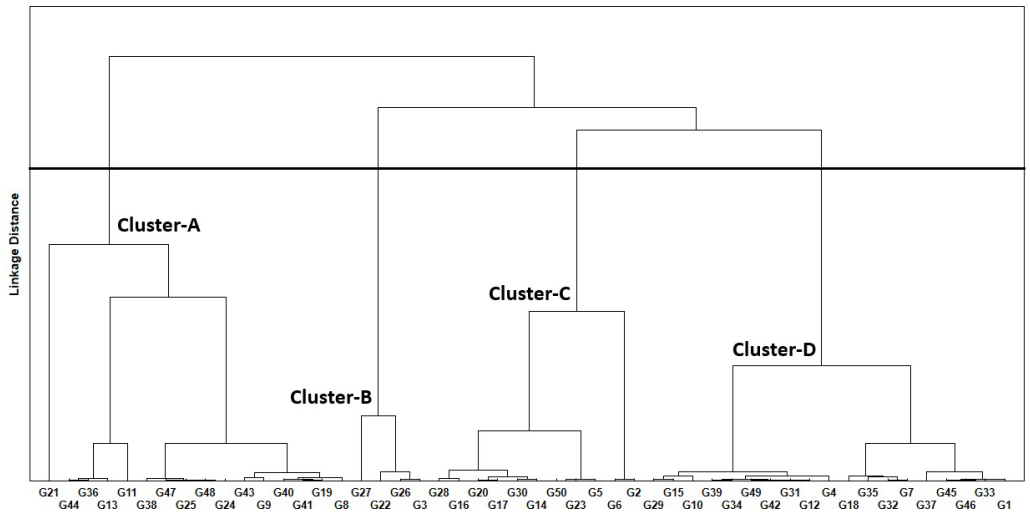


Fig. 2. Dendrogram of clustering for 50 genotypes of chickpea.

G34, G35, G37, G39, G42, G45, G46, and G49) with a relatively low yield performance which indicated the highest hundred seed weight (HSW), so they have very marketable seeds.

The CV values prepared an index of variation for measured traits and demonstrated that an obvious diversity exists in our chickpea genotypes, so such great genetic variation for most morphologic traits is useful for a breeding program. Grasp the pattern of genetic variation in the crop resources would be beneficial to design germplasm perseveration, and to explore the practical variation in gene banks (Zhang *et al.,* 2017). Our results show that there is a remarkable diversity among genotypes and lines of chickpea, that can be used to obtain more seed yield and its components as well as other target traits like tall plants or earliness. The clustering of similar genotypes depends on the distances among them, which can be found by a morphologic variation (Aswathi *et al.,* 2023). Our clustering results show a variation among genotypes, associated to the selection pressures in the breeding programs. The research permits a better grasp of the chickpea genotypes via agronomically morphologic traits and demonstrates the beneficial aspect of statistical tools like cluster analysis was useful in identifying the most variable traits within chickpea and can be useful in the future to succeed in genetic improvement projects. We found each genotype has a similar magnitude of genetic variation which may be found and that the distances among genotypes may be restricted. Also, remarkable genetic variation may exist within chickpea genotypes, so hybridization is possible for obtaining new sources for breeding. The association among traits supports the idea that only a few heritable traits are needed to explain the genetic variation within the chickpea germplasm. These traits may engage plant breeders for effective germplasm management and evaluation. The four desirable genotypes; G3, FLIP09-228C-S00794(30 KR)-2/ from ICARDA; G22, TDS-Maragheh90-90 from Turkey; G26, TDS-Maragheh90-373 from Turkey; and G27, TDS-Maragheh90-266 from Turkey are good candidates for commercial release in Iran. They performed very well and showed high amounts of most target traits, so they can be advised for cultivation in cool upland rainfed conditions of semi-arid environments.

Conclusions

The high genetic diversity found in most of the measured traits showed the genetic factor's impact, so chickpea breeding regarding these traits is possible. The genotypes used in the present investigation indicated various properties that can be used in different breeding projects. The four genotypes (FLIP09-228C-S00794 (30 KR)-2/, TDS-Maragheh90-90, TDS-Maragheh90-373 and TDS-Maragheh90-266) are recommended for farmers due to their better performance and high seed yield.

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