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Genetic Variability and Path Analysis of Chickpea (*Cicer arientinum* L.) Landraces and Lines

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Abstract: Identifying suitable parental materials is an important phase in the development of hybrid seeds. Thus, a study was conducted to determine the genetic variability among 360 chickpea land races and lines. Appropriate parents selected from a pool of genes of 360 chickpeas which was carried out during the year 2000-2001 in DARI, Sararoud of Iran. The experiment included 12 blocks, each contains 20 plots of 2 m rows. The traits studied were growth type, number of leaflet per leaf, leaflet size, plant height, days taken for 50% flowering, flower color, flowering period, days to maturity, pod size, pod per plant, seed numbers per pod, seed color, seed shape and 100 seed weight. Data based on morphological and phonological traits were analyzed using SPSS software and the statistical procedures: correlation coefficient, cluster analysis, principal component analysis and path analysis. Among the morphological characters, numbers of branches, pod numbers with CV: 41.77 and 37.25% had higher variation, respectively while leaflet with CV: 10.49% had minimum variation. Among the phonological traits the flowering period with CV: 22.02% had highest and flowering time had the least variability. The seed yield per plant ranged from 4.27 to 0.41 g and CV: 51.43% reflected highest variation. The highest correlation coefficient (r = 0.78) was between seed yield per plant and pod numbers. Chickpeas genotypes could be classified into four clusters and 63% of the variance were explained by five PCAs. Path analysis revealed that the pod numbers with 0.745, seed numbers with 0.386, 100 seed weight with 0.268 and single seed with 0.267 had highest direct effect on seed yield.

Key words: Chickpea, cluster analysis, path analysis, genetic variability

INTRODUCTION

Plant genetic resources are the basis of global food security. They comprise diversity of genetic material contained in traditional varieties, modern cultivars, crop wild relatives and other wild species. To meet the need for more food, it would be necessary to make better use of a broader range of the world's plant genetic diversity (Karaöz and Zencirci, 2005; Farshadfar and Farshadfar, 2008).

Chickpea (*Cicer arientinum* L.) with 17-24% protein, 41-50.8% carbohydrates and high percentage of other mineral nutrients and unsaturated linloeic and oleic acid is one of the most important crops for human consumption. Chickpea with low production cost, wide climate adaptation, use in crop rotation and atmospheric nitrogen fixation ability is one of the most important legume plants in sustainable agriculture system (Anonymous, 2002; Singh, 1997). Gene pool of chickpea with 39 species included 31 perennial and 8 annual land races, mutants, cultivars and wild types of *Cicer*. Chickpea has high variation for different quality and quantity traits, included ideal plant type (tall type), shape and color grain, flower color, podding, color of seed coat, earliness, resistance to disease and pests, which helps breeders to release improved and advanced lines and varieties (Dasgupta *et al*., 1987; Singh, 1997).

First systematic attempt to establish, gene pool of chickpea was done in 1977 by Indian IKWIST and 11195 lines were registered by 1978. In order to avoid genetic erosion and releasing new varieties, cultivars and recombinant lines the genetic variation for desirable traits should be broadened. To study the genetic diversity among population, varieties and species, the multivariate statistical procedures viz. cluster analysis, principal components, factor analysis, discriminant function and path analysis have been widely applied (Romesburg, 1984; Singh and Bejiga, 1991).

Traditionally, diversity is assessed by measuring variation in phenotypic and morphological characters traits, which are of direct interest to users. In the 1960s, biochemical methods based on seed protein and enzyme. Electrophoresis were introduced, which proved particularly useful in analysis of genetic diversity as they

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reveal differences between seed storage proteins or enzymes encoded by different alleles at one (allozymes) or more gene loci (isozymes). Molecular methods such as RAPDs, AFLPs, SSRs and microsatellites used for detecting DNA sequence variation are being used as complementary strategies to traditional approaches for assessment of genetic diversity (Karp *et al*., 1997; Karp, 2002).

Earlier studies had established that evaluation of genetic variability is one the important breeding objectives. Singh (1973) studied the variability of 75 chickpea cultivars (DC and Kabuli type) by D^2 statistic and cluster analysis. Each DC and Kabuli type was grouped in separate cluster. The 1000 grain weight, flowering time and pod size had highest variation. Variability of chickpea has been studied using multivariate statistical methods by different scientists (Bahl *et al*., 1976; Singh *et al*., 1990). Grain yield of chickpea depends on many related traits. Correlation coefficient could show linear relationship between them, however, path analysis would elucidate direct and indirect relationship among these traits, hence on the basis of that the breeder could select the most effective traits to release varieties (Ulukan *et al*., 2003; Yucel *et al*., 2006). Saleem *et al*. (2002) concluded that pod number and 100 grain weight were the most important traits in chickpea breeding programs. Yucel *et al*. (2006) with the path analysis results showed that grain number and pod number were the most desirable traits for chickpea improvement. Similar results were reported in many studies for chickpea and *Vicia* (Yücel, 2004; Ulukan *et al*., 2003; Ciftci *et al*., 2004; Güler *et al*., 2001). Padi (2003) studied relationship between different characters of chickpeas and found that harvest index and pod number had the greatest direct effect on yield. Similar finding was reported by Toker (2004). Noor *et al*. (2003) found that pod number and 100 grain weight had most important traits to improve chickpea.

The purpose of the study is to find out the genetic variability which is bared on different characters.

MATERIALS AND METHODS

To study genetic variability of 360 chickpea line and land races and lines, the experiment was carried out at Dry Land Agriculture Research Institute (DARI), Sararoud, Kermanshah, Iran. The experiment included 12 blocks, each contains 20 plots of 2 m rows. The traits studied were growth type, number of leaflet per leaf, leaflet size, plant height, days taken for 50% flowering, flower color, flowering period, days to maturity, pod size, pod per plant, seed numbers per pod, seed color, seed shape and 100 seed weight. The Bivanij (famous local chickpea) was the control.

Data based on morphological and phonological traits were analyzed using SPSS software and the statistical procedures: correlation coefficient, cluster analysis (UPGMA), principal component analysis and path analysis.

RESULTS

The statistical parameters viz. means, standard deviation, minimum, maximum, Coefficient of variation (CV), skewness and kurtosis for different traits were shown in Table 1. Among the morphological characters, stem numbers, pod numbers with CV = 41.77 and 37.25%, respectively; had higher variation while the leaflet with CV = 10.49% had minimum variation. Among the phonological traits the flowering period with CV = 22.02% had the highest and flowering time had the least variability. The seed yield per plant ranged from 4.27 to 0.41 g and CV = 51.43% revealed the highest variation. The height character also showed high variation; consequently it can be rich gene pool for breeder.

To find out relationships between different traits and yield, correlation coefficient between different quantitative and qualitative characters were shown in

Table 1: The statistical parameters of chickpea genotypes

Traits	Min.	Max.	Mean	SE	CV	Skewness	Kurtosis
Leaflet No.	3.000	7.000	5.9100	1.0100	17.09	0.081	-1.834
Leaflet size	3.000	5.000	3.0500	0.0320	10.49	6.092	35.312
Branches No.	2.000	22.000	7.7800	3.2500	41.77	0.701	0.482
Plant height	13.000	37.000	21.9700	3.8800	17.66	0.781	0.914
Flowering date	66.000	91.000	75.7000	4.7000	6.21	0.965	1.209
Flowering period	5.000	24.000	11.9000	2.6200	22.02	0.486	1.338
Maturity	100.000	134.000	112.4200	5.1400	4.57	0.420	1.051
Flower No.	2.000	9.000	5.5040	1.1600	20.94	-0.225	0.637
Pod size	3.000	7.000	4.3700	0.9700	22.20	-0.575	-1.078
Pod No.	3.000	51.000	16.6700	6.2100	37.25	1.076	2.843
Seed No.	1.000	2.000	1.8500	0.3500	18.92	-2.007	2.042
100 seed weight	8.300	34.350	13.7520	3.3320	24.22	2.181	7.172
Plant density	11.000	31.000	22.3600	4.6900	20.97	-0.160	-0.953
Single seed weight	0.015	1.040	0.1398	0.0059	42.25	10.576	155.960

Table 2: Correlation coefficient between different quantitative traits

Traits	Seed yield per plant	Single seed weight	Plant density	100 seed weight	Seed No.	Pod No.	Pod size	Flower No.	Ripening date	Flowering period	Flowering date	Height	Stem No.	Leaf let size
Single seed weight	0.340*													
Plant density	-0.019	0.043												
100 seed weight	0.434*	0.515	0.020											
Seed No.	0.306*	-0.212	0.009	-0.177										
Pod No.	0.786*	0.000	-0.009	0.110	0.053									
Pod size	0.293*	0.104	0.042	0.283	0.068	0.240								
Flower No.	0.196	-0.132	-0.125	-0.062	0.129	0.325*	0.142							
Ripening date	-0.037	0.115	0.035	0.090	-0.198	0.082	0.251	-0.237						
Flowering period	-0.114	-0.091	-0.084	-0.159	0.034	-0.008	0.154	0.136	0.025					
Flowering date	-0.103	0.106	0.071	-0.006	-0.181	-0.142	0.281	-0.329*	0.669*	-0.304*				
Plant height	0.345*	0.174	-0.023	0.299	-0.043	0.287*	0.371*	0.288*	-0.048	0.046	-0.149			
Stem No.	0.254*	0.127	0.046	0.107	-0.020	0.222	0.010	-0.012	0.198	-0.009	0.116	0.181		
Leaflet size	0.235	0.188	-0.009	0.344*	-0.188	0.207	0.219	-0.013	0.014	-0.008	-0.098	0.146	0.111	
Leaf let No.	0.110	0.112	0.017	0.101	-0.108	0.111	0.069	0.042	0.096	0.008	0.077	0.287*	0.091	0.068

* Significant on 5% probability level

Table 3: Correlation coefficient between different quality traits

Character	Growth type	Plant color	Hairy plant	Flower color	Seed color
Plant color	0.024				
Hairy plant	0.086	-0.083			
Flower color	-0.075	-0.480**	0.094		
Seed color	0.002	0.161**	-0.076	0.156**	
Seed shape	-0.046	-0.048**	0.029	0.386**	0.351**

** Significant on 1% probability level

Table 2 and 3, respectively. The highest correlation coefficient r = 0.78 was between seed yield per plant and pod numbers. Single seed weight, canopy height, seed numbers, pod size, stem numbers and leaflet size were relatively higher than others, respectively. Significant correlation was observed between plant color and flower color, seed color and seed shape with r = 0.48% and 0.35%, respectively. Spearman rank correlation among quality traits is shown in Table 3. There was negative correlation between seed shape and plant color, it means if the stem and leaf contain antocyanine, the seed will be smaller and angled (DC type).

Path and regression analysis: The results of stepwise multiple regression analysis is shown in Table 4. Grain yield per plant was the dependent trait and other traits were independent variables. Pod numbers was entered in the model at first and explained 62% of variation, followed by 100 grain weight and grain numbers entered in the model, respectively.

In order to study the direct and indirect effect of traits entered into the step wise regression analyses on the yield, path analysis was carried out (Fig. 1). According to Table 4, the pod numbers, 100 grain weight, canopy and seed numbers per pod had high correlation coefficient values. Path analysis revealed that the pod numbers with 0.745, seed numbers with 0.386, 100 seed weight with 0.268 and single seed with 0.267 had highest direct effect on seed yield, respectively. Indirect effect via other characters displayed different values.

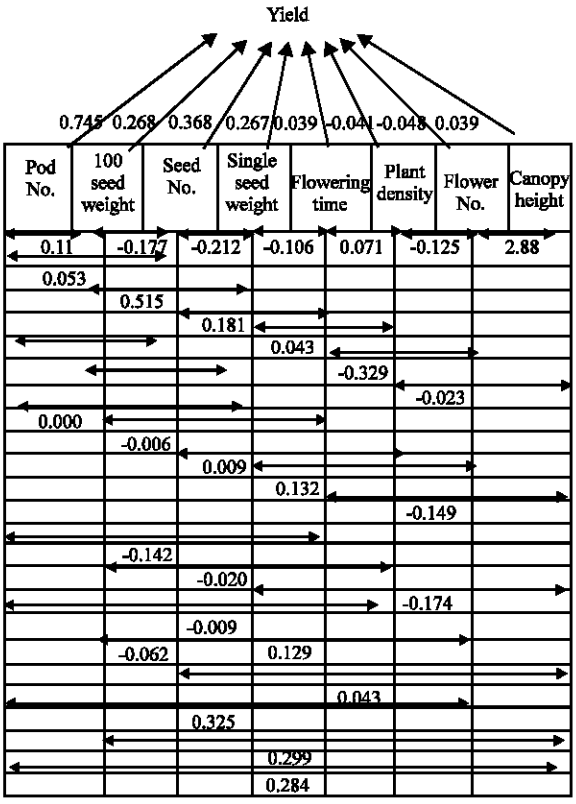

Fig. 1: Diagram of path analysis on yield

Cluster analysis: Cluster analysis using UPGMA method was carried out in two steps. First, for all genotypes regardless of geographical regions and second, for quality and quantity traits separately.

All genotypes were classified into four clusters. Cluster 1 contains 338 genotypes, cluster 2 and 3 had one and cluster 4 includes eight genotypes. Genotypes 41-3550 in cluster No. 2 due to highest plant height, the highest pod size, grain numbers per pod with higher

Table 4: Stepwise regression analysis on different characters

Steps	Entered variable	R^2-value	Adj. R^2	Regression coefficient	SE	t-value	p-value
1st	Pod No.	0.616	0.615	0.745	0.006	42.514**	0.000
2nd	100 seed weight	0.739	0.737	0.268	0.013	13.666**	0.000
3rd	Seed No.	0.851	0.850	0.387	0.104	0.22936	0.000
4th	Single seed weight	0.907	0.905	0.269	0.714	13.977**	0.000
5th	Flowering date	0.908	0.907	0.039	0.008	2.240*	0.026
6th	Plant density	0.910	0.0908	-0.041	0.008	-2.492*	0.013
7th	Flower No.	0.911	0.909	-0.048	0.035	-2.541*	0.011
8th	Canopy height	0.912	0.910	0.039	0.010	2.162*	0.031

*, ** are significant on 5 and 1% probability level, respectively

Table 5: Five principal components on quantity traits

Principal components	1st PCA	2nd PCA	3rd PCA	4th PCA	5th PCA
Eigen values	3.302	2.313	1.522	1.320	1.070
Cumulative variance	0.220	0.374	0.476	0.564	0.635
CV%	0.220	0.154	0.102	0.087	0.071

Table 6: Principal component analysis on different traits

Characters	1st PCA	2nd PCA	3rd PCA	4th PCA	5th PCA
Leaf let No.	0.1347	0.0943	0.1485	0.3527	0.5474
Leaflet size	0.2627	0.1006	-0.1767	0.1504	-0.2106
Stem No.	0.1549	0.1308	0.4081	0.0673	-0.0898
Canopy height	0.3357	-0.0678	0.0911	0.2843	0.3377
Flowering date	-0.1313	0.4916	0.3109	-0.1194	0.0603
Flowering period	-0.0685	-0.1924	0.0367	0.5575	-0.2817
Ripening date	-0.0611	0.4699	0.3401	0.1261	-0.1060
Flower No.	0.1481	-0.3728	0.1644	0.2116	0.0171
Pod size	0.3120	-0.1415	-0.2087	-0.1256	0.3241
Pod No.	0.3378	-0.1740	0.4123	-0.0597	-0.1347
Seed No.	0.0015	-0.3003	0.1953	-0.4827	-0.0461
100 seed weight	0.4036	0.2876	-0.2899	-0.0339	-0.1341
Plant density	-0.0042	0.0968	0.0122	-0.2532	0.4908
Single seed weight	0.4074	0.2879	-0.2908	-0.0311	-0.1306
Seed yield per plant	0.4636	-0.0711	0.3051	-0.2504	-0.1946

average greater than grand mean of genotypes were located in separate cluster. Accession 41-3747 with high 100 grain weight and good performance of other yield components was in separate cluster as well.

Members of cluster 4 had desirable situation for pod per plant, leaflet numbers, days to 50% flowering and maturity. The results of principal components analysis for 360 chickpea genotypes are shown in Table 5. About 63% of variance was demonstrated by five PCAs. The 1st PCA covered 22% of variance, 2nd PCA; 15%, 3rd PCA; 10%, 4th PCA; 8% and 5th PCA with 7% of variance. Contribution of each character in PCAs is given in Table 6. According to these results the seed yield per plant had highest value and 100 grain weight was high, respectively. There was positive significant correlation among these traits with grain yield per plant (Table 2). Stepwise regression analysis was significant and some of the traits were entered into the model for grain yield per plant. Therefore in breeding program grain yield could be focused on. The first PCA is named yield component PCA (with 15.42% variance) and the second one is called phonological PCA (15.42% of variance), the third would be called as morphometrical PCA (10/18% of variance) and fourth PCA is generation phase and fifth PCA is called canopy PCA.

DISCUSSION

Determination of genetic variation is the main step in breeding programs. Different statistical parameters of accessions were estimated and according to the results applied breeding programs would designed. Such procedures have been done by many authors and similar results have been reported. Singh and Bejiga (1991) studied the 38 pea cultivars and concluded that ripening date with CV = 3% was minimum and flowering period with CV = 26% had largest coefficient of variation. These values in this experiment for same traits ranged between 4.57 and 22.02%. They showed that Phenotypic Coefficient of Variation (PCV) for all traits were higher than Genotypic Coefficient of Variation (GCV) indicating the highly environmental effects. Therefore breeders should be careful to select the best parent based on such traits.

Correlation coefficient and regression analysis help the breeders to select an efficient trait, that's why this parameter was estimated. Sandhu and Singh (1972) reported high correlation between seed yield and pod per plant, seed per plant; seed weight per plant and stem number per plant. Also the positive significant correlation among yield and pod number per plant, branch number, plant height were reported by Singh *et al.* (1990). Joshi (1972) reported that if the seed will be circle and angle less, the leaf color will be whiter. Path analysis identified the character having direct and indirect effects on the yield. Breeders would try to find out relationships between different characteristics obtaining new varieties and lines. Pod numbers had highest direct effect on yield in this experiment. Therefore, promising lines could be selected on the basis this trait. Phadnis *et al.* (1970) studied 45 chickpea lines and revealed that seed weight had largest direct effect on seed yield. Bahl *et al.* (1976) also studied yield components by path analysis in 21 chickpeas and similar results were reported. Paliwal (1987), Chaudhary *et al.* (1988) and Dasgupta *et al.* (1992) reported the higher effect of pod numbers and seed numbers on economical yield of chick pea. These results help breeders to select new parents and lines based on pod numbers, seed numbers and 100 seed weight.

Multivariate statistical procedures such as cluster analysis, principal component analysis etc. were used to determine genetic variability based on different characters. These methods have done by many scientists and a lot of results have been reported. Singh (1973) analyzed 75 lines of DC and Kabuli peas by D^2 statistics and classified into 9 clusters and each DC and Kabuli type were located in separate cluster. The 100 grain weight and flowering time had the highest effect on variation.Ansawa (1981) reported such results as well. Govil *et al.* (1980) classified Iranian and Indian pea samples into 13 clusters. In the most studies pod numbers per plant had the most variation (Singh, 1997; Toker, 2004; Noor *et al.*, 2003).

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