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Genetic divergence and biology of adaptation in *Cicer arietinum* L.*

R. G. Dani** and B. R. Murty
Indian Agricultural Research Institute, New Delhi, India

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Summary. The role of 19 structural, developmental and biochemical traits in relation to specific adaptation was analysed in a set of 17 diverse lines with quantified adaptation, representing contemporary cultivars and land races of chickpea (*Cicer arietinum* L.), using multivariate analysis. Significant varietal variation was observed for most characters, particularly for the activity of the enzyme nitrate reductase (NR) and protein content in the plant. The distance analysis (D^2-statistic) revealed that seed size and pod number and their associated attributes were important forces of divergence. The additional forces of divergence were NR activity at the flower initiation stage, yield components such as number of primary and secondary branches, and other features such as plant habit and duration of flowering. The principal component analysis revealed some similarities and also differences from the distance analysis. Leaf size, days to flower initiation, seed size and, to some extent, NR activity at flower initiation stage, were important in the first vector. Developmental traits such as chlorophyll depth, NR activity at the pod initiation and grain filling stages, and the percent protein content in the plant at flower initiation were important in the second vector. In general, the clustering pattern was not related to the geographical origin, seed colour, size of regression coefficient for yield, or deviation from linearity. The importance of the developmental and biochemical attributes in the divergence of cultivated chickpea, such as days to flower initiation, duration of flowering, NR activity and the rates of protein accumulation in developing seeds, and in adaptation, suggests the critical role of these attributes. NR activity at the flower initiation stage would appear to have a major role in the domestication of this crop and its intra-specific differentiation, as an increased seed size could not have been possible without better nutrient uptake and utilization.

Key words: Distance analysis – *Cicer* – Nitrate reductase (NR) – Principal components – Stability parameters – Protein content

Introduction

Cicer arietinum L., a crop of antiquity, is mainly cultivated in the semiarid regions and is the third most important food legume in the world. It is native of South-West Asia and the Mediterranean regions, while Ethiopia is considered as a secondary center of diversity. The considerable variability found in chickpea material for several morphological characters and the reports of two chromosomal races ($2n = 14$, and $2n = 16$) which are not related to the region of origin, moderate heritability for economic characters such as yield, number of seeds and number of pods, the lack of any relationship between early and late maturity and high yields, the limited experiments on hybridization in this highly autogamous species and the marginal conditions of cultivation and limited human selection, as summarized by van der Maesen (1972), show that natural selection has played a major role in the diversity found in the present-day cultivated forms, which are adapted to specific environmental conditions and therefore have restricted adaptation. In spite of a wide range of variation, an optimum combination of variables for selection has not yet been recommended in this crop, mainly due to a lack of adequate basic information,

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** Present address: Central Institute for Cotton Research, 95 New Ramdaspeth, Nagpur-440010, India

particularly with reference to biochemical-physiological parameters responsible for the adaptation and improved reproductive potential.

The planning of a programme of improvement in a crop such as chickpea displaying low productivity but demonstrating a potential for high yield in specific regions, needs an understanding of the processes of adaptation and their relation to the genetic diversity in the material, since adaptation is a major component of productivity (Frankel and Bennett 1970).

Multivariate analysis has been successfully used to classify biological populations and to identify the factors influencing their divergence in a number of crop plants (Murty and Arunachalam 1965; Vairavan et al. 1973). The available evidence on divergence in chickpea (Murty 1975; Narayan and Macefield 1976; Jain et al. 1981), is based mostly on structural developmental components of yield, and characters heavily weighted in favour of seed, and simply inherited traits such as flower colour (Moreno and Cubero 1978).

In the present study, basic information was collected on the pattern of genetic variation of 19 characters including biochemical, developmental, also agronomical components of fitness and adaptation. The nature of genetic divergence was assessed using the D^2 statistic of Mahalanobis (1936), and the principal component analysis, with a representative set of genotypes with quantified adaptation. A set of land races of chickpea was also included to know if some of the highly selected elite cultivars have a different pattern of divergence. The results are presented in this communication.

Materials and methods

From a world collection of 500 cultivars of diverse geographic origin evaluated in a common International trial conducted in India, Turkey, Lebanon, Morocco, Spain, Jordan and Egypt (Murty 1975), a set of the genotypes including land races with different degrees of adaptation as measured by regression analysis, was selected for the present study. In addition to these, seven prominent contemporary Indian varieties were included. The details of the 17 cultivars, their origin and satability parameters based on yield per plant, have been included in Table 3. This material was grown on the experimental farm of the IARI, New Delhi, during the autumn-winter seasons of 1977–78 and 1978–79, in randomized complete blocks, as single row plots of 6 m with 20 plants in each of three replications, with 30 cm spacing between plants and 50 cm between rows.

The following 19 characters were scored: plant type (spreading –1, medium –5, erect –10), chlorophyll depth (yellow –1, medium green –5, deep green –10), disease score (fully wilted –1, partly wilted –5, immune –10), leaf size (small –1, medium –5, broad –10), days to flower initiation, duration of flowering (period between flower and pod initiation), nitrate reductase (NR) activity in leaves (at stages A, B, and C representing flower initiation, pod initiation and grain filling stage, respectively), percent protein content in the plant at stages A, B, and C, and the percent protein content in young seeds. In addition, the number of primary branches, secondary branches, plant height at maturity, 100 seed weight and grain yield per plant were also noted. Data were recorded on five randomly chosen plants in each line.

The in vivo assay of NR was done by a method similar to that of Hageman and Hucklesby (1971); NR activity was expressed as micromoles of nitrate reduced per gram fresh weight per hour. Nitrate was estimated colorimetrically by Evans and Nason's (1953) method. The top six leaves of five randomly chosen plants were used for NR estimation. For protein estimation, five plants were destructively sampled. Developing seeds at the 25–30 day stage were also analysed for protein content. Nitrogen was determined on a Technicon Auto Analyser; the N value was then converted into percent protein.

In the calculation of D^2 values, firstly the uncorrelated linear combinations (Y's) were obtained by pivotal condensation of the common dispersion matrix of the correlated variables (X's), following Rao (1952). The mean values of the 19 characters of different populations were then transformed into the mean of uncorrelated linear combinations (Y's).

$D^2_{ij}=\sum_{t=1}^{k}(Yij-Yjt)^2$ gives the D^2 between ith and jth populations for k characters. Following the method suggested by Rao (1952), the genotypes were grouped into clusters. Inter and intracluster distances were determined and their relationship was diagrammatically represented. In canonical analysis, the between product sum matrix (A matrix) was computed using the mean values of the uncorrelated linear combinations of all the characters for all the varieties. From the fourth power of the A-matrix, canonical vector 1 was retrieved by repeated iterations on a trial vector. The first canonical root is extracted after standardizing the first canonical vector.

Results

The character means of the 17 varieties are given in Table 1. The uncorrelated transformed means are not presented. The variance-covariance matrix is given in Table 2. Varieties were found to differ significantly, based on Wilk's Λ criterion, when all the characters were considered simultaneously. The analysis of varietal variation and genotype × environmental interactions observed in this material has been reported earlier (Dani and Murty 1982). After computing all pair-wise D^2 values, the cultivars were grouped into eight clusters by Tocher's method (Rao 1952). The composition of clusters, inter and intra-cluster distances, and the cluster means are given in Tables 3, 4 and 5, respectively.

The eight exotic types, including land races from Morocco, Iran and Uganda, were distributed in three clusters, I, II and III. Cultivars of similar country of origin were not grouped together. Clusters II and III were heterogenous. Three elite varieties from the Punjab were grouped together in cluster V, with comparable regression coefficients for stability for grain yield per plant. The maximum intercluster divergence ($D=20.49$) was observed between clusters I and VIII (Fig. 1) which include types from Morocco and Iran, and the popular variety 'L550' from the Punjab. Clusters I, II and V had substantial intracluster genetic diversity. 'ICP 60' formed a distinct cluster. This variety is characterised by moderate yield but a low regression

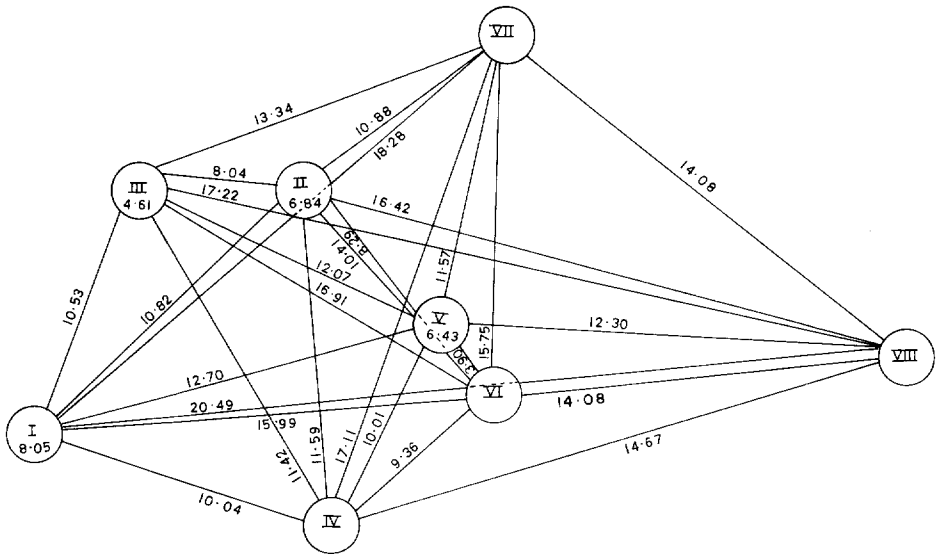

Fig. 1. Clusters and their interrelationships in 17 chickpea varieties (1977–78/78–79) (D^2 analysis based on 19 characters)

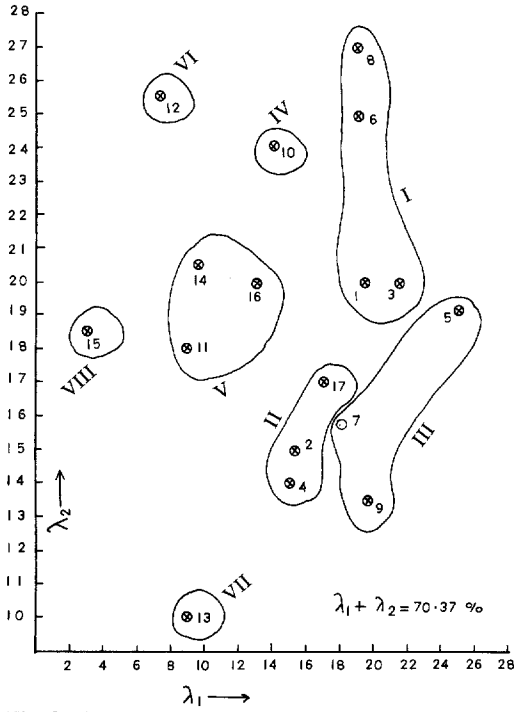

Fig. 2. Group constellations of 17 chickpea varieties of varying adaptation in a λ_1–λ_2 chart (1977–78/78–79). Grouping from D^2 analysis is superimposed over canonical representation (based on 19 characters)

coefficient and moderate deviations from linearity, as compared to the rest. Similarly, the prominent Indian cultivars 'C235' and 'L550' formed distinct individual clusters. The cluster means for the number of pods per plant and seed size and the number of secondary branches were distinctly different. However, NR activity at the flower initiation stage is also responsible for inter-cluster differences. In general, the clustering pattern was not related to the geographical origin, seed colour, size of the regression coefficient for yield, or deviations from linearity (Table 3). This was evident particularly in clusters I, II and III. Similarly, the three Indian types, 'C-235', 'BG203' and 'L550', distributed in clusters VI, VII and VIII, appear to be far diverse from the other lines. Such a diversity could either be due to their introduction into India from different countries or due to different selection forces operating in the regions of their cultivation. Since all three types are yellow seeded and distinctly different from the traditional brown type of India, they could be derived from the Kabuli type introductions from Middle East and Iran. The grouping of Morocco and the Iranian types in the first cluster indicates that they may also be related in their origin and local adaptation. The climatic conditions of Iran, although different from the Mediterranean climate of Morocco, did not disturb the intercorrelations in the 19 variables included in this study. Similarly, the intra-cluster diversity in Cluster V, consisting of only the cultivated types from the Punjab, is similar to that of Cluster II containing two Iranian types and one Indian type. In both cases, the yellow seed coat of the Indian types denotes their exotic origin from Iran or the Middle East.

It may be simpler to represent multivariate analysis in a two-dimensional chart ($\lambda 1$–$\lambda 2$) if the other λs do not contribute much to the variation. It would also help in verifying the grouping based on D^2 analysis. Such a two-dimensional representation (Fig. 2) revealed differences from those observed in D^2 analysis, since $\lambda 1 + \lambda 2$ accounted for only 70 percent of the total variation. The principal component analysis (Table 6)

Table 1. Character means in 17 chickpea varieties (average of two seasons). NR = Nitrate reductase activity

Sl. no character	Range	'ICP117'	'P4311'	'ICP49'	'P5378'	'ICP58'	'P529'
[a]1. Plant type score	(1.44– 1.83)	1.83	1.64	1.66	1.54	1.53	1.60
[a]2. Chlorophyll depth	(1.23– 1.79)	1.24	1.77	1.50	1.66	1.55	1.55
[a]3. Disease score	(0.45– 1.51)	1.37	1.42	1.30	1.51	1.20	1.42
[a]4. Leaf size	(1.44– 1.83)	1.73	1.53	1.53	1.44	1.66	1.53
5. Days to flower initiation	(72.50–103.00)	98.00	71.50	101.75	95.75	95.00	97.50
6. Duration of flowering	(30.00– 39.79)	31.25	30.25	33.25	32.75	39.00	35.75
7. NR-A (flower initiation)	(0.77– 2.64)	2.02	1.68	1.84	1.50	1.14	2.64
8. NR-B (pod initiation)	(0.83– 1.49)	1.29	1.33	0.89	1.45	1.22	1.16
9. NR-C (grain filling)	(0.44– 0.76)	0.75	0.44	0.53	0.61	0.56	0.78
10. % protein in plant-A	(13.11– 20.39)	13.32	16.55	14.13	15.47	14.61	16.17
11. % protein in plant-B	(13.75– 20.00)	17.29	14.85	13.75	16.32	14.98	17.24
12. % protein in plant-C	(13.78– 20.72)	16.73	16.70	15.93	19.51	20.65	15.90
13. % protein in young seed	(20.03– 27.28)	22.15	23.72	22.80	21.89	22.48	23.60
14. No. of primary branches	(5.13– 11.08)	8.00	7.00	6.05	6.68	6.62	5.13
15. No. of secondary branches	(22.03– 49.98)	22.03	29.55	22.73	31.42	31.42	27.95
16. Plant height at maturity (cm)	(44.13– 69.95)	51.13	55.73	44.13	52.30	47.43	46.40
17. Seed size (100 seed wt) (g)	(9.48– 19.15)	10.50	13.20	13.85	10.08	14.65	9.48
18. No. of pods/plant (g)	(36.00–190.50)	40.50	63.00	63.25	120.25	79.25	36.00
19. Grain yield/plant (g)	(4.30– 23.80)	6.78	7.58	8.65	15.48	13.45	8.45

[a] Values transformed into $\log^e$

Table 2. Variance-covariance matrix for 19 characters in 17 chickpea varieties

	Plant type 1	Chloro-phyll depth 2	Disease score 3	Leaf size 4	Days to flower initiation 5	Duration of flowering 6	NR activity 'A' 7	NR activity 'B' 8	NR activity 'C' 9
1.	0.0348	– 0.0014	– 0.0278	0.0210	– 0.1348	0.1677	0.0215	0.0011	0.0055
2.		0.0170	0.0041	0.0018	– 0.0103	– 0.0204	– 0.0122	0.0080	0.0077
3.			0.1631	– 0.0539	0.7916	0.6982	– 0.0438	– 0.0099	0.0126
4.				0.0425	– 0.2020	0.0014	– 0.0368	0.0065	– 0.0159
5.					81.5309	– 10.3600	– 0.0126	– 0.0721	– 0.1602
6.						38.5809	– 0.5829	0.9072	0.9257
6.							0.3341	0.0156	– 0.0063
8.								0.1265	0.0822
9.									0.1616
10.									
11.									
12.									
13.									
14.									
15.									
16.									
17.									
18.									
19.									

indicated that in vector 1, the important characters responsible for genetic divergence in the major axis of differentiation are duration of flowering (+0.7250), followed by leaf size (+0.2722). The roles of NR activity at the flower initiation stage, days to flower initiation, number of pods per plant, and disease score, were also sizeable and positive. In vector 2 (Z_2), which is the second axis of differentiation, chlorophyll depth, percent protein content in the plant at flower initiation, NR activity at pod initiation and at grain filling stage, were important, in that order, for divergence at the secondary level. Therefore, the principal component analysis has to be considered along with D^2 analysis, in such cases when the first two roots are inadequate to represent the total variation between genotypes. However, the clusters IV, V and VI are near each other, both in the distance analysis and principal components. Similarly, clusters II and III are close to each other in

Table 1 (continued)

'ICP61'	'P5418'	'ICP113'	'ICP60'	'F378'	'C235'	'BG203'	'H208'	'L550'	'G543'	'K468'	Mean
1.74	1.74	1.64	1.78	1.60	1.73	1.74	1.64	1.78	1.81	1.44	1.67
1.23	1.30	1.24	1.53	1.60	1.79	1.60	1.70	1.35	1.60	1.70	1.51
1.06	1.24	1.34	1.30	0.75	0.58	0.58	0.86	0.55	0.45	1.17	1.06
1.83	1.52	1.81	1.83	1.48	1.64	1.59	1.57	1.66	1.53	1.53	1.62
95.75	98.25	97.00	96.75	98.25	96.25	96.75	100.75	91.75	103.00	101.25	96.19
33.75	39.75	30.75	31.00	39.50	37.50	39.00	30.00	30.00	35.00	32.25	34.16
1.50	2.12	1.71	1.43	1.28	0.92	0.76	1.29	0.97	1.53	1.56	1.52
1.23	0.83	1.49	0.88	0.94	0.91	1.21	1.11	1.09	1.04	1.00	1.12
0.53	0.62	0.69	0.54	0.56	0.53	0.62	0.70	0.62	0.50	0.74	0.58
15.78	13.11	14.40	13.35	16.47	16.56	20.39	14.14	13.13	16.44	18.28	15.43
17.09	15.56	17.09	18.55	16.94	20.08	19.23	18.39	15.93	15.50	19.63	16.90
14.78	17.56	18.42	17.69	18.74	22.04	20.72	2.072	19.85	13.78	16.01	14.37
23.10	26.00	22.96	23.59	22.28	27.28	24.12	24.47	20.03	24.08	22.81	23.53
6.73	7.35	7.25	8.23	11.08	8.95	7.68	9.85	9.18	10.35	7.03	7.84
25.15	38.32	23.73	27.00	36.80	38.05	46.75	37.98	31.00	48.68	49.98	34.39
53.03	58.68	49.50	55.80	58.53	65.82	55.55	61.19	69.95	52.80	47.63	54.50
16.15	9.65	14.25	17.63	10.10	11.33	10.87	11.10	19.15	9.15	10.70	12.48
54.00	87.00	62.50	51.50	190.50	97.50	170.00	112.25	138.25	161.50	102.25	95.85
4.30	8.50	7.60	10.10	14.65	12.05	23.80	16.28	15.25	15.95	17.17	12.11

Table 2 (continued)

% protein in plant 'A' 10	% protein in plant 'B' 11	% protein in plant 'C' 12	% protein in young seeds 13	No. of primary branches 14	No. of secondary branches 15	Plant height at maturity 16	Seed size 17	No. of pods/ plant 18	Grain yield/ plant 19
−0.0524	−0.0275	0.0783	−0.0819	−0.0377	−0.2696	−0.3503	−0.0787	1.6841	−0.1081
−0.0035	−0.0024	0.1034	−0.0263	−0.0811	0.2682	−0.1633	−0.0288	1.9367	0.1507
−0.1512	0.0815	0.3972	0.1966	−0.3274	−0.2913	0.3999	0.1756	2.4766	0.5022
0.0315	−0.3840	0.1706	−0.0036	−0.0020	−0.3055	−11819	−0.0564	−0.3508	−0.1679
1.6396	8.5684	−5.0565	0.4459	−6.8718	−9.0175	7.0400	0.0398	39.0634	−0.0763
−0.0523	−2.4663	4.1772	−1.6014	7.7512	6.7578	17.7378	−1.7278	40.0446	1.3852
0.0251	−0.0004	0.5077	−0.1413	−0.0348	−1.0388	−0.9210	−0.0650	6.0049	−0.6359
−0.1482	−0.0728	0.3293	0.0007	0.1686	0.2912	0.7930	−0.1752	2.6148	−0.2064
−0.1280	−0.0958	0.2123	−0.0612	−0.0479	−0.1208	0.0147	−0.1957	0.7157	0.06033
1.6358	−0.0738	−1.1369	−0.0367	0.7472	3.8204	1.3366	0.6908	−4.8988	−1.8676
	3.0320	0.6733	0.2978	−1.7943	−4.2481	−1.0643	0.0814	15.4284	2.0003
		16.2297	0.1782	−0.0310	−6.5408	−2.6472	3.3145	−1.8496	−1.0454
			3.1751	−0.3721	−9.2406	2.7942	−0.4487	−14.3382	0.1098
				7.4154	−6.3651	1.3456	−0.2283	−22.4799	−7.9998
					217.0784	23.0481	1.7766	307.2219	38.6506
						42.6250	1.3723	57.2434	6.9811
							3.2335	−4.1638	2.3170
								2,503.6310	140.6828
									38.6014

both cases. The distinct nature of the varieties 'C-235', cluster (VI), 'BG203' (VII) and 'L550' (VIII) is also confirmed in both analyses.

The coefficients in the vector Z_1 suggest that the duration of flowering, which is important in survival under stress, is the most critical character of divergence in the principal axis of differentiation. Next in importance are the other characters for stability of yield under stress such as days to flower initiation, NR activity at flowering, leaf size and number of pods/plant. Plant type, branching pattern, protein accumulation and seed size played only a minor role in the first axis of differentiation. As in the first vector, plant type, seed size, branching pattern, pods/plant and protein in grain filling stage did not contribute to diversity in the second vector. The role of NR activity at two different

Table 3. Composition of clusters (D^2 analysis based on 19 characters). NA = not available

Cluster no.	Variety name	Origin	Stability parameters for yield[a]			Seed colour
			Mean	Regression coefficient	Squared deviation from linearity	
I	'ICP117'	Morocco	15.34	1.49	23.86	Brown
	'ICP49'	Morocco	14.98	1.44	20.47	Light yellow
	'P5259'	Iran	6.80	0.99	17.31	Black
	'P5418'	Iran	14.26	1.66	27.52	Dark brown
II	'P4311'	Iran	12.56	0.74	4.73	Black
	'P5378'	Iran	12.57	0.57	16.14	Black
	'K468' India	(Kanpur)	7.17	1.05	16.14	Yellow
III	'ICP58'	Uganda	13.93	1.19	4.72	Yellow
	'ICP61'	India	12.88	1.14	19.91	Black
	'ICP113'	Morocco	14.40	1.04	15.71	Black
IV	'ICP60'	India	15.52	0.76	16.12	Black
V	'F378' India	(Punjab)	6.78	0.98	NA	Yellow
	'H208' India	(Punjab)	6.97	1.11	NA	Yellow
	'G543' India	(Punjab)	Not available			
VI	'C235' India	(Punjab)	6.42	1.03	NA	Yellow
VII	'BG203' India	(Delhi)	Not available			
VIII	'L550' India	(Punjab)	892	0.85	NA	Silver white

[a] Based on grain yield per plant

Table 4. Inter- and intra-cluster averages[a] (D^2 analysis based on 19 characters)

	I	II	III	IV	V	VI	VII	VIII
I	64.91 (8.05)	117.11 (10.82)	110.91 (10.53)	121.88 (10.04)	161.32 (12.70)	255.64 (15.99)	334.21 (18.28)	419.88 (20.49)
II		46.79 (6.84)	64.64 (8.04)	134.41 (11.59)	80.29 (8.96)	198.79 (14.01)	118.21 (10.88)	269.56 (16.42)
III			21.52 (4.61)	130.33 (11.42)	145.74 (12.07)	282.56 (16.92)	177.84 (13.34)	296.57 (17.22)
IV				0	100.20 (10.01)	87.55 (9.36)	293.00 (17.11)	215.12 (14.67)
V					41.37 (6.43)	86.60 (3.90)	133.95 (11.57)	151.40 (12.30)
VI						0	248.13 (15.75)	198.34 (14.08)
VII							0	245.75 (15.68)
VIII								0

[a] The D values are given in parentheses

stages in both vectors indicates that mobilization of nutrients is important during the period of flower initiation to pod initiation and is an important component of genetic divergence in this material.

The area-specific nature of adaptation as measured by yield and its stability reported in this crop was confirmed in this study and the reasons can now be explained more logically. The performance of the exotic types in terms of grain yield per plant was lower than the promising types of Indian origin. The Iranian cultivar 'P4311', which is known to be very early, did not prove to be so. Similarly, the seed protein content in the Morocco line, 'ICP113', was not the highest. In those groups where the flowering duration was lengthier,

Table 5. Cluster means in 17 chickpea varieties (19 characters)

Sr. no. character	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
[a]1. Plant type	1.71 ± 0.09	1.54 ± 0.11	1.64 ± 0.11	1.78 ± 0.19	1.68 ± 0.11	1.73 ± 0.19	1.74 ± 0.19	1.78 ± 0.19
[a]2. Chlorophyll depth	1.35 ± 0.07	1.71 ± 0.08	1.34 ± 0.08	1.53 ± 0.13	1.63 ± 0.08	1.79 ± 0.13	1.60 ± 0.13	1.30 ± 0.13
[a]3. Disease score	1.33 ± 0.20	1.37 ± 0.23	1.20 ± 0.23	1.29 ± 0.40	0.68 ± 0.23	0.58 ± 0.50	0.59 ± 0.40	0.55 ± 0.40
[a]4. Leaf size	1.58 ± 0.10	1.53 ± 0.11	1.70 ± 0.12	1.83 ± 0.21	1.53 ± 0.12	1.64 ± 0.21	1.60 ± 0.21	1.66 ± 0.21
5. Days to flower initiation	98.44 ± 4.51	89.83 ± 5.21	95.58 ± 5.21	96.75 ± 9.03	100.66 ± 5.21	96.25 ± 9.03	96.75 ± 0.03	91.75 ± 9.03
6. Duration of flowering	35.00 ± 3.57	34.50 ± 3.59	31.00 ± 3.59	31.00 ± 6.21	34.83 ± 3.59	37.50 ± 6.21	39.00 ± 6.21	30.00 ± 6.21
7. NR-A (flower initiation)	2.15 ± 0.12	1.58 ± 0.13	1.45 ± 0.13	1.48 ± 0.28	1.37 ± 0.13	0.92 ± 0.28	0.77 ± 0.28	0.97 ± 0.28
8. NR-B (pod initiation	1.04 ± 0.07	1.27 ± 0.08	1.32 ± 0.08	0.88 ± 0.14	1.07 ± 0.08	0.91 ± 1.14	1.12 ± 0.14	1.09 ± 0.09
9. NR-C (grain filling)	0.61 ± 0.08	0.59 ± 0.59	0.59 ± 0.09	0.54 ± 0.16	0.58 ± 0.09	0.53 ± 0.16	0.62 ± 0.16	0.62 ± 0.16
10. % protein in plant-A	14.18 ± 0.64	16.77 ± 0.74	14.93 ± 0.74	13.35 ± 1.28	15.68 ± 0.74	16.56 ± 1.28	20.39 ± 1.28	13.13 ± 1.28
11. % protein in plant-B	15.96 ± 0.87	16.93 ± 1.01	16.38 ± 1.03	18.55 ± 1.74	18.27 ± 1.01	20.08 ± 1.78	19.23 ± 1.74	15.93 ± 1.74
12. % protein in plant-C	16.53 ± 2.01	16.86 ± 2.33	17.95 ± 2.33	17.69 ± 4.03	18.20 ± 2.33	22.04 ± 4.02	20.72 ± 4.03	13.78 ± 4.03
13. % protein in young seeds	23.64 ± 0.89	22.81 ± 1.03	22.84 ± 1.03	23.59 ± 1.78	243.52 ± 1.03	27.28 ± 1.78	24.12 ± 1.78	20.02 ± 1.78
14. No. of primary branches	6.63 ± 1.36	6.90 ± 1.57	6.86 ± 1.57	8.23 ± 2.73	10.43 ± 1.57	8.95 ± 2.73	7.68 ± 2.73	9.18 ± 2.73
15. No. of secondary branches	27.75 ± 7.37	38.39 ± 8.51	30.10 ± 8.51	27.00 ± 14.73	40.49 ± 8.51	38.05 ± 14.73	46.75 ± 14.73	31.00 ± 14.73
16. Plant height at maturity	50.01 ± 3.38	50.35 ± 3.90	49.99 ± 3.90	55.80 ± 6.74	57.74 ± 3.90	65.82 ± 6.65	55.55 ± 6.65	69.95 ± 6.65
17. Seed size (100 seed wt)	10.87 ± 0.99	11.32 ± 1.04	15.02 ± 1.04	17.63 ± 1.80	10.25 ± 1.09	11.33 ± 1.80	10.85 ± 1.79	19.15 ± 1.80
18. No. of pods/plant	56.69 ± 25.01	95.16 ± 28.89	65.25 ± 28.89	51.50 ± 50.04	15.62 ± 3.59	12.05 ± 6.21	23.80 ± 6.21	15.25 ± 6.21
19. Grain yield/plant	8.10 ± 3.11	13.40 ± 3.59	8.45 ± 3.59	10.10 ± 6.21	154.75 ± 28.89	97.50 ± 50.04	170.00 ± 50.04	134.25 ± 50.04

[a] Values transformed into $\log^e$

Table 6. Values of coefficients in Z_1 and Z_2 in 17 varieties (19 characters)

Sr. no. character	Z_1	Z_2
1. Plant type	−0.1446	0.0923
2. Chlorophyll depth	−0.1826	0.5201
3. Disease score	0.2278	−0.0920
4. Leaf size	0.2722	−0.2187
5. Days to flower initiation	0.2610	0.2480
6. Duration of flowering	0.7250	0.2145
7. NR-'A' (flower initiation)	0.2689	0.1383
8. NR-'B' (pod initiation)	0.0075	0.3749
9. NR-'C' (grain filling)	−0.0351	0.2539
10. % protein in plant 'A'	−0.0001	−0.4736
11. % protein in plant 'B'	−0.1071	0.2178
12. % protein in plant 'C'	−0.1611	0.0609
13. % protein in young seeds	0.0781	0.2167
14. No. of primary branches	−0.0770	0.0322
15. No. of secondary branches	−0.0487	−0.0068
16. Plant height at maturity	−0.1399	0.0749
17. Seed size (100 seed wt)	−0.0554	−0.0269
18. No. of pods/plant	0.2512	−0.0305
19. Grain yield/plant	−0.0738	−0.0305

NR activity and percent protein content in plant and seed, were not correspondingly highest or vice versa. Among the contemporary Indian cultivars, those from the Punjab and Delhi were the highest yielders. Taken as a distinct group, the relationship between adaptability and the various characters related to it was more or less well established in the case of the two prominent varieties 'C235' and 'L550', which formed two separate clusters. High mean values of developmental traits such as plant type, chlorophyll depth, days to flower initiation and duration of flowering could be associated with high adaptability of the local variety 'C235' (regression coefficient = 1.03), which also possessed higher protein content in the plant and the seed. 'L550' (regression coefficient = 0.85) is a popular white seeded Kabuli type variety, which is characterised by extensive vegetative growth, as reflected in the high number of primary and secondary branches, high number of pods, as also higher values for NR activity, traits associated with better adaptation under environmental constraints like stress or lower fertility.

Discussion

With the extensive geographic distribution of chickpea, from the Mediterranean to the semi-arid regions of South Asia, from the Indo-Gangetic plains to the highlands in Ethiopia, this crop should have accumulated considerable variability. However, the marginal fertility of the areas of its cultivation and very limited inputs coupled with the predominant role of natural selection under domestication, might have fixed adap-

tive gene blocks in specific regions, as observed in other crops by Frankel and Hawkes (1975).

The range of variation in this material is considerable, including seed size, pod number, and the number of branches (Singh and Auckland 1975). The present conditions of cultivation of chickpea are not very different from its original habitat or the area of distribution of its wild relatives, with no selection for response to optimal ecological conditions. Therefore, in spite of variation, the elite varieties are extensively location-specific in their adaptation. A constellation of characters would appear to have been fixed under predominant natural selection, both in primitive and elite cultivars. The breeding strategy should be oriented in breaking such adverse associations and in incorporating useful adaptive gene blocks into superior yielding cultivars. Since growth and adaptation are dependent upon genetically controlled biochemical and physiological processes, a study of such biochemical attributes related to adaptation, in addition to developmental and agronomical attributes, would help understand the processes involved in adaptation. Adaptation is a complex response affected by many interacting individual and population functions, including morphological, physiological and biochemical, in such a way that taken as a whole, render a population more fit than other populations differently endowed (Bennett 1970). However, this fitness may vary with place to place, and at each stage of growth. The genetic basis of the response to climatic conditions, which is important in planning for crop improvement, can be subjected to biometrical analysis.

The present investigation has thrown new light on the nature of genetic differentiation in diverse chickpea material, and on the nature of the relationship between adaptation and forces of intra-specific differentiation. A number of characters important under natural selection and in intra-specific differentiation, such as days to flower initiation, chlorophyll depth, plant habit, secondary branches and the biochemical attributes, were included in this study. The common features of distance analysis, as also found in other reports, (Murty 1975; Ramanujam 1975; Narayan and Macefield 1976; Katiyar 1978; Jain et al. 1981), are that seed size and pod number and their associated growth attributes are important common forces of differentiation, whether the material is primitive land races or elite cultivars of chickpea. Past studies indicate the course of evolution in this crop towards a large seed size under domestication, and the associated changes in the plant frame, pointing back to the general importance of selection for increased seed size in adaptation. Moreno and Cubero (1978) concluded from their principal component analysis of some 150 selected chickpea lines, that the material could be separated into two complexes differing in a cluster of characters associated with seed and pod size and leaf morphology. However, in a similar analysis using generalized distance with nearly 500 cultivars representing the world collection, Murty (1975) had earlier concluded that among the high yielders, number of pods per plant, number of secondary branches and compact plant type, are major components of divergence, in addition to seed colour and seed size. Leaflet size, lateral spread, and to some extent, days to flower initiation, were also important in divergence in chickpea. Similarly, Narayan and Macefield (1976) observed from the distance analysis in a world collection of nearly 5,000 types of chickpea, that seed weight, seed number, plant type and seed colour contributed maximally to the intercluster divergence. The present analysis nevertheless suggests that nitrate reductase activity at flower initiation stage is specially important among other additional forces of divergence such as number of primary and secondary branches and indirect forces such as plant habit and duration of flowering.

Principal component analysis further confirmed that out of the seven biochemical characters studied, four were important in divergence – NR activity at all three stages, and percent protein content in the plant at flower initiation stage, in addition to common developmental traits such as plant type, chlorophyll depth, disease score, leaf size and days to flower initiation. Thus, some light has been thrown on the mechanism of adaptation, such as an increased seed size through increased initial nutrient uptake. NR activity at the flower initiation stage was found to be important in the first vector, which represents the major axis of differentiation.

The key enzyme and rate limiting step in soil nitrate assimilation in plants is considered by some to be nitrate reductase. This enzyme has been studied in considerable detail and attempts have been made to relate the activity of NR to reduced N, dry matter production, grain protein production, grain yield, and to calculate the contribution of various plant parts towards total nitrate assimilated. On the basis of the correlation of NR activity with grain yield and grain protein production, some investigators have suggested NR as biochemical criteria for selection in some cereals (Beevers and Hageman 1969). In a preliminary experiment, Pokhriyal and Abrol (1980) have shown that in 20-day old *Cicer* seedlings, soil derived nitrogen accounted for 15.1, 8.3 and 7.2% of the total reduced nitrogen at pre-flowering, profuse-flowering and seed-filling stages, respectively. Significant varietal differences noted for the first time in the present study, in world collections of chickpea for NR and the percent protein content in plant at three major developmental stages of flower and pod initiation and grain filling, as also for the percent protein content in maturing seeds, are of considerable interest. The role of nitrate reductase activity in nitrogen uptake in the early seedling stages, and its effect on protein accumulation in grain, would be important in chickpea, which is traditionally grown under semiarid conditions. Thus, the capacity of nutrient uptake and its utilization, important in survival under stress conditions, would appear to have a significant role in the genetic diversity in chickpea which is mostly subjected to natural selection and very limited human selection. Studies on nitrate assimilation in crop plants are now considered also to be important in understanding the general principles for genetic control of mineral nutrition. Genetic variation for characters of mineral nutrition can given rise to nutritional ecotypes, as a result of selection during natural adaptation of diverging plant species to soil conditions (Shumny and Tokarev 1983) and more work of this nature is needed in chickpea.

Smartt (1978) envisages that several biochemical changes could have occurred under domestication during the evolution of pulse crops. A positive selection for such characters as seed size and pod size could have accelerated a complex of selection pressures with correlated responses resulting in a modified shoot architecture in all pulse crops, since a larger seed size and pod size would require a more massive stem with upright plants and a change in branching habit and leaf size for efficient light interception. The large differences in seed size in the very high yielding cultivars of the Mediterranean and the low productive types in the arid regions of India and South Iran, would support the hypothesis of Smartt (1978). Therefore, it is to be expected that the most workable classification of large world collections of cultivated chickpea, would not be on geographical distribution, but mostly on seed characters, as envisaged by van der Maesen (1972). The seed characters are in turn influenced by early growth and nutrient uptake. Thus, the findings of the present investigation are of interest in the evolution of pulse crops, where domestication has resulted in increased seed size and its associated growth attributes.

It is clear that without an efficient nutrient uptake and its utilization, an increased seed size could not have been achieved. Thus, nitrate reductase activity at flower initiation, as observed in this study, could have played a major role under domestication.

In cereals the NR activity is generally high at the flower initiation stage, implying maximum N intake by the plant at that stage, and hence selection for NR at flower-initiation stage would be useful. However, in pulse crops such as chickpea, a considerable amount of flower drop is observed soon after the onset of flowering. In pigeonpea, the percentage of flower-sites developed into pods has been reported to be as low as 2.65 to 16.4% (Dani 1979). According to Sinha (1974), the competition for photosynthesis from leaves is initially confined to roots, nodules, and other vegetative organs. However, when flowering begins, and fruit setting commences, the latter provide apparently a stronger site for the utilization of the photosynthates. At this stage, the nodules are possibly deprived of photosynthates and consequently start degenerating. Thus, at this critical stage, uptake of nonsymbiotic nitrogen through NR directed assimilation process may be useful, and therefore selection at a stage after flower initiation, possibly at pod-initiation, should prove to be beneficial. More recently, the existence of genetic differences has been observed (Schilling 1983) between species of *Lupinus* and in some other legumes, for transformation of such additional mineral nitrogen applied during the period of flowering, into additional proteins, especially seed proteins. The additional nitrogen could be related to the qualitative changes in the reserve proteins of seeds rich in lysine, which may be associated with adaptation to withstand nutritional stress.

In the present investigation, two developmental traits – chlorophyll depth and leaf size – were also found to be important in secondary differentiation, whereas they were found to have no role in divergence of chickpea (Murty 1975). This would be due to the fact that a majority of the lines included in the present study were of Indian origin, which are specifically adapted to their native environment and cultivation during winter/spring, under which a high photosynthetic activity is effected through greater chlorophyll content in leaves, and greater leaf area. On the contrary, the performance of the exotic lines was lower, and they showed greater susceptibility to *Fusarium* wilt, small leaf size and less intensity of chlorophyll depth. Regression analysis of grain yield per plant of the exotic types (Murty 1975) was based on experiments conducted over a range of environments of the major chickpea producing countries of the world. Some of these varieties, grown in two environments, showed no close correspondence to their stability as measured by regression analysis, or the actual means of the varieties in terms of various developmental, agronomical and biochemical characters. Taken as a group, this relationship between stability and various characters was closer in the Indian material. Thus, adaptability is shown to be far more important than yield, under differential conditions of fluctuating environments and high competition. The role of biochemical characters in the genetic and physiological mechanisms which determine adaptation, is important. The present trend of varietal improvement would seem to have more or less neglected the inclusion of such traits and the accent is more often placed on purely structural components such as plant type. As suggested by Bennett (1970) the analysis of adaptation of primitive populations and their comparison with the modern cultivars, needs to be linked with the analysis of differences at the biochemical level.

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