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Genetic diversity for drought tolerance in lentils from Central Asia and the Caucasus: CACLentil

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Abstract:

The drought tolerance of 96 lentil accessions collected from Central Asia and Caucasus (CAC) region was analysed through two sets of field experiment conditions: irrigated and rain-fed. Several yield components of these accessions were evaluated and compared by means of statistical analyses. Analysis of variance revealed significant variation among the genotypes between and within the two experimental conditions. Based on regression analysis, seed and pod number per plant had significant associations with seed yield per plant. Cluster analyses based on drought tolerance index (DTI) grouped accessions into five subgroups with different numbers of accessions in each group. Three groups out of five were characterized by high DTI values and high yields were observed in both, under irrigated and rainfed conditions. This collective group of genotypes demonstrated valuable germplasm traits under stress and may therefore serve as source of useful genes in breeding lentils for drought tolerance.

Keywords: Lentil, drought tolerance, yield components, utilization of germplasm.

1. Introduction

Ladizinsky [14], while studying wild progenitors of lentils, has stated that modern cultivated lentil was domesticated in the 'fertile crescent' around 8800 years before present (BP), although the oldest lentil seed remains were found in a cave in Greece dated to 13000 years BP. Central Asia and the Caucasus (CAC) countries lie within the general region where lentil is though to have been domesticated and first brought in to cultivation [9]. Lentils form an important part in the human diet of CAC for their relatively excellent digestibility when compared to many other legumes. Higher protein content, essential amino acids, various vitamins and micronutrients are some of the other attributes of lentils [4, 5]. Their use with cereal flours in bread-making in some parts of the world greatly enhances the value of the protein in the bread. Lentils' ability to adapt to a limited number of environmental conditions depends in part on the genotype of the plants. Therefore, a thorough assessment of genetic diversity among the genus is needed.

Traditionally, the Asian lentil is grown in rain-fed agricultural systems in the areas with <400 mm annual rainfall in rotation with cereals and exposed to numerous biotic and abiotic stress factors during its life-cycle [16]. All these stresses reduce the biosynthetic capacity of plants and may cause damage that could lead to the destruction of the plants [15]. Among them, drought stress is one of the most widespread abiotic stress, that affects growth and consequently loss in productivity [1, 3, 19, 26]. Worldwide, the major abiotic restrictions on yield of lentil are drought (usually linked with high temperature) and cold [2]. Although lentils are well-adapted for tolerating dry conditions, a considerable decrease in productivity (up to 54 %) has been reported by several investigators [13]. Hamdi et al. [12] were able to report an increase of over 20% in yield, based on 100-seed weight, when lentils were provided with supplementary irrigation. When almost all of the rainfall occurs early in the season, lentils face a drought-like situation near to maturity and yields are depressed. However, when supplementary irrigation was provided after the annual rainfall had ceased completely, Saxena and Wasimi [23] were able to obtain seed yields 60% higher than where no irrigation was provided. These results underscores the importance of supplementary irrigation in cultivating

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lentils, especially in the dry areas. Not all farmers can afford or have access to water when needed and hence the need to breed lentils that can yield well without supplementary irrigation, while at the same time deliver excellent harvests when rainfall is well-distributed.

One of the major objectives of lentil breeding strategy in the world today is to develop cultivars with important agricultural characters, as well as high yielding varieties with drought tolerance. The success of lentil as a crop depends greatly on the ability to produce economically-viable and stable yields when ground moisture is limited [8]. Breeding programs to this end, which were formerly conducted in West Asia, have now extended to the CAC countries in close collaboration with the International Center for Agricultural Research in the Dry Areas (ICARDA) because of that center's world mandate for lentil improvement among the international agricultural research centers (IARCs). Agriculture is now the dominant land-use in the CAC countries and reflects the traditional way of life of the indigenous peoples of this region that was strengthened by the social and economic changes in the 1990s [18].

The most effective method to overcome drought stress is to screen those accessions that are selected for planting in the low-rainfall areas. This, in turn, depends on evaluation and screening of introductory breeding materials. This is sometimes called pre-breeding. In spite of many laboratory screening techniques now available, field screening of materials based on yield comparison under drought conditions and wet conditions still remains one of the more accurate methods delivering accurate results [7, 22]. The first step for the development of drought tolerant cultivars was to find plants that can survive the low rainfall regimes. ICARDA has a diverse collection of >10,500 cultivated and >550 accessions of wild species of lentil germplasm [22]. Previous evaluation for drought tolerance revealed that enormous variability and sufficient level of cold tolerance is present among cultivated species [10]. These accessions are the base materials that ICARDA is using for the development of winter-hardy genotypes. Among wild species, the most winter-hardy accessions were observed in *Lens orientalis.*

Among eight CAC countries, Azerbaijan, Uzbekistan, Georgia and Kazakastan have taken the lead in food legume research after the collapse of the Soviet Union. Lentil had become a neglected crop in the region due to exclusion in collective farming systems during the Soviet era. Lentil is being re-established in the region for both winter and spring cultivation [22].

To increase the effectiveness of this crop improvement work, lentil genetic resources conserved in genebanks should be evaluated in-depth and greater attention must be focussed on those accessions that prove to have abiotic stress tolerance. This study was, therefore, conducted in order to characterise phenotypic differences of lentil accessions in response to drought, determine yield components directly affecting seed yield, and to identify germplasm with greater drought tolerance.

2. Material and Methods

Ninety-six cultivated lentil accessions (*Lens culinaris* subspp. *culinaris* Medik.) of CAC origin, selected from ICARDA's world germplasm collection, were grown under near optimum (irrigated) condition at Absheron experimental station of Genetic Resources Institute of the Azerbaijan National Academy of Sciences, and under dry (rainfed) condition at Jalal-Abad experimental station of Azerbaijan Scientific Agriculture Research Institute in two replications. At maturity stage 3, single plants from each plot were collected and evaluated based on 6 main yield components. The characters studied were: plant height, number of pods per plant, number of seeds per plant, weight of seeds per plant, and 100 seed weight. Weight of seeds per plant was used in this investigation instead of plot yield.

The significance of variation in the yield components for both conditions was estimated based on data over replicates using ANOVA. Phenotypic correlation between traits was calculated. Stepwise linear regression was conducted to derive empirical models using mean seed yield per plant as the dependent variable and other yield components as independent variables. The graph was also constituted to visually illustrate the association between dependent variable and the most important predictor of yield.

Among several stress tolerance attributes (mean productivity, tolerance, stress susceptibility index, geometric mean productivity), drought tolerance index (DTI) was chosen to quantify the response of genotypes to drought stress.

DTI was computed as the fraction of irrigated yield maintained under drought, normalised by the mean yield across all genotypes in the trial [11].

$$DTI = \frac{(y_p)(y_s)}{(y_{\bar{p}})^2}$$

y_p = the yield of a given genotype in a non-stress (irrigated) environment;
y_s = the yield of given genotype in a drought (rainfed) environment;
$y_{\bar{p}}$ = mean yield in non-stress environment.

The higher the value of DTI for a genotype the higher its drought tolerance and yield potential. Hierarchical cluster analyze was used as a tool to classify genotypes according to their drought tolerance levels by the use of Statistical Package for the Social Sciences (SPSS) version 10 computer program [25].

3. Results and Discussion

The effect of dry (rainfed) condition was significant ($P<0.001$) for all six characters observed as that revealed by ANOVA. Water deficits during reproductive development significantly reduced yield. Considerably large differences among genotypes were also observed within both conditions as shown in Table 1.

Genotypes showed nearly same variation range in plant height (~25 cm) for both conditions. In the irrigated field, maximal height was observed in accession ILL 123629 of Tajikistan origin, whereas in rainfed condition ILL 123466 from Uzbekistan was the highest at 45.33 cm. Number of productive boles among genotypes ranged from 6 to 15.29 in irrigated and from 5 to 11.94 in dry conditions. Also the number of pods and seeds per plant varied significantly within the two conditions and decrease of mean indices for these traits under rainfed condition was 26% and 27%, respectively.

The effect of drought stress on 100-seed weight of genotypes was varying. For example, in some accessions (ILL 123467, ILL123634, ILL 123648 and ILL 134440) there were low 100-seed weights due to the drought, while in some accessions such as ILL 134465, ILL 123613 and ILL 134458 from Azerbaijan the 100-seed weight increased insignificantly. There was no change in 100-seed weight in ILL 123624 and ILL 123576 that came from Tajikistan. Considerable variation was also noted in seed yield per plant. Stress caused up to 1.5 times or 33% decrease in mean seed yield per plant in comparison with the Absheron region. In the present study maximum seed yield per plant (9.3 g) under irrigated condition was obtained in ILL 123613 of Azerbaijan origin and under rain-fed conditions ILL 123466 (5.9 g) of Uzbekistan origin.

Table 1. Comparative analysis of yield components of lentil accessions sown under irrigated (I) and rain-fed (II) conditions at two different locations in Azerbaijan

Characters		Min	Max	Mean ± S.E.	S.D.	Significance
Plant height, cm	*I*	23.6	48.0	36.0 ± 0.47	4.56	***
	II	19.9	45.3	32.7 ± 0.46	4.52	
Number of productive boles	*I*	6.0	15.3	9.7 ± 0.19	1.90	***
	II	5.0	11.9	7.9 ± 0.15	1.50	
Number of pods per plant	*I*	36.8	337.0	122.1 ± 4.09	40.06	***
	II	31.9	283.6	90.2 ± 3.44	33.75	
Number of seeds per plant	*I*	57.0	331.2	159.2 ± 4.83	47.30	***
	II	30.7	265.7	116.4 ± 4.19	41.09	
Weight of seeds per plant, g	*I*	1.9	9.3	4.3 ± 0.13	1.26	***
	II	0.7	5.9	2.9 ± 0.10	1.00	
Weight of 100 seeds, g	*I*	1.6	4.2	2.7 ± 0.05	0.52	***
	II	1.5	3.9	2.5 ± 0.04	0.48	

Based on the data from irrigated field stepwise linear regression offered 3 empiric models where the number of seeds per plant accounted for 66% of the variance in mean seed yield per plant with the equation Y = 0.0215x + 0.8438 and with 0.74 standard error (Table 2). The addition of 100-seed weight in the second model raised the percentage of variance to 95%, whereas the addition of number of pods per plant (the third model) did not contribute significantly to the results.

Only two models were designed for rainfed condition, which were the first two models for irrigated conditions also. Number of seeds explained 66% of variance in mean seed yield per plant as in irrigated condition with the equation Y= 0.0245x. Slight difference was observed in the percentage raised in the second model which was 80%.

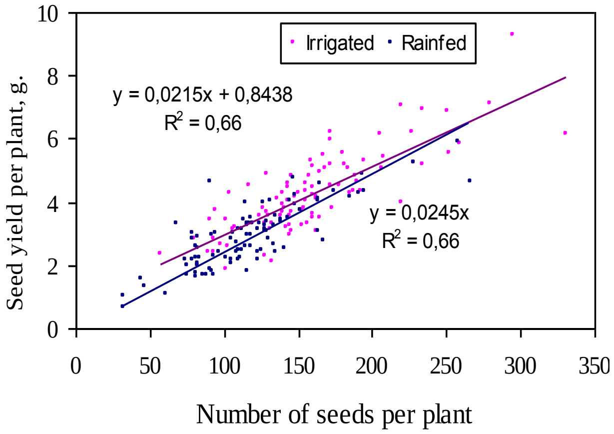
The correlations between dependent variable and variables enumerated above as well as their significance degrees were calculated for both conditions. The seed yield per plant showed a significant positive correlation (r =0.81) with number of seeds per plant, number of pods (r =0.68) per plant and plant height as well. 100 seed weight also showed significant correlation at P<0.05* level. Sinha and Singh [24] observed strongly and positive associations between seed weight per plant and number of seeds and pods per plant in lentil. Seed yield was significantly correlated with plant height and seed per pod was correlated with plant height only [24]. Malhotra et al. [16] were able to reveal that yield per plant exhibited a positive correlation (r = 0.93) with stem length that was significant at the 1% level probability. And, Salehi et al. [21] showed that their ANOVA on different traits in Iranian lentils carried out during an evaluation study at the Zanjan University Research Farm in Iran revealed significant differences for all characters studies except for the number of primary branches. The important positive relationship between plant height and yield per plant was noted in our investigations. The similar results (** = P<0.01) were obtained for rainfed condition, with only difference that the positive correlation with 100-seed weight was nonsignificant in this study.

Comparative and visual relation between mean seed yield per plant and number of seeds per plant for irrigated and rainfed conditions with similar coefficient of determination (r^2=0.66) was shown in Figure 1.

Loss of yield is the main concern of plant breeders and they hence emphasize on yield performance under drought-stress conditions. Several stress tolerance attributes which provide a measure of drought have been used for screening drought-tolerant genotypes, among them Mean productivity, Tolerance, Stress susceptibility index, and Stress tolerance index [17].

A larger value of TOL and SSI represent relatively more sensitivity to stress, thus a smaller value of TOL and SSI are favoured. Selection based on these two criteria favours genotypes with low yield potential under non-stress conditions and high yield under stress conditions. Fernandez defined a new advanced index (STI= stress tolerance index), which can be used to identify genotypes that produce high yield under both stress and non-stress conditions. Selection based on STI will be resulted in genotypes with higher stress tolerance and yield potential will be selected [11].

Figure 1. The relationship between weight and number of seeds per plant for lentil accessions in two conditions.



Correlations between Yp, Ys and drought tolerance indicators were calculated to determine more desirable tolerance criteria. Drought and irrigated yields were positively associated with each other. The results showed that DTI with similar coefficient of determination for both conditions (R^2= ~0.9) was better predictors of Yp and Ys than Tol and SSI. The observed relationship is in consistent with those reported by Fernandez [11] in mungbean. In our studies significant and positive correlation observed between yield under stress and drought tolerance index (Table 3), while correlation coefficients with other indicators was negative. Therefore selection for these criteria should decrease yield in rainfed condition and increase in irrigated. Limitations of using the SSI and TOL indices have already been described in wheat [6] and in common bean [20].

Table 3. Correlation coefficients between Yp, Ys and several drought tolerance indices

	Yp	Ys	DTI	TOL	SSI
Yp	1,00	0,63**	0,88**	0,64*	0.25*
Ys	0,63**	1,00	0,90**	-0,2	-0.57**
DTI	0,88**	0,90**	1,00	0,22	-0,18
TOL	0,64**	-0,20	0,22*	1,00	0,87**
SSI	0,25*	-0,57**	-0,18	0,87**	1,00

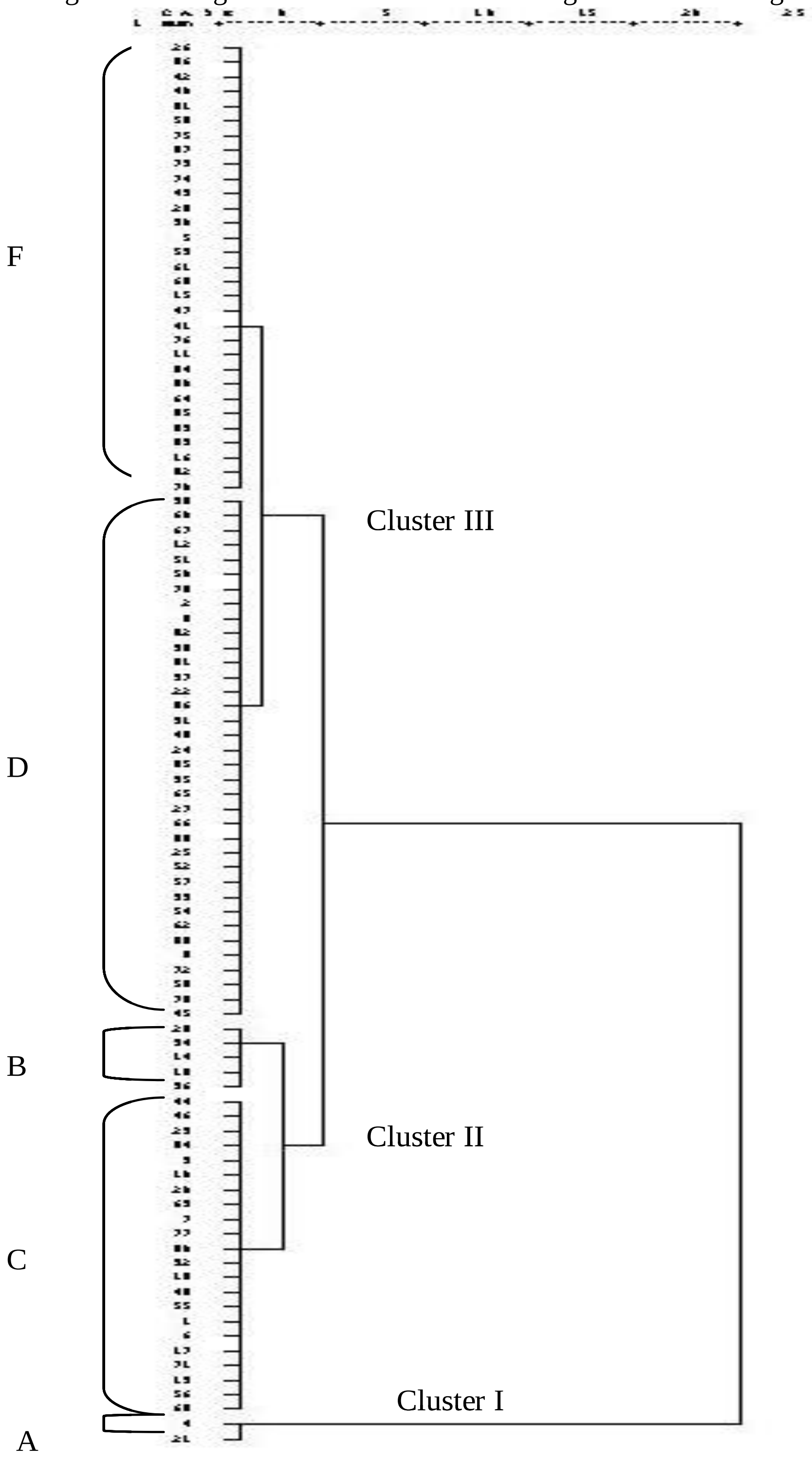
*** and * Significant at the 0.01 and 0.05 levels, respectively.*

So the correlations between yield indices under stress and non-stress conditions as well as several stress tolerance indices would suggest that the most effective approach in breeding for drought resistance in lentil would be based on selection among the high-yielding individuals for high to moderate levels of the drought tolerance index [7].

In this study drought tolerance indices of genotypes calculated based on seed yield potensial per plant in both conditions ranged from 0.11 to 2.42 in accordance with high susceptiblity and high tolerance, mean DTI value was 0.73.

Cluster analysis based on DTI revealed that the studied 96 lentil varieties fell into 3 main clusters, 2 of which in turn also divided into 2 subgroups with different numbers of accessions in each group (Figure 2).

Figure 2. Dendrogram showing the relative tolerance to drought of CAC lentil genotypes.



The most useful character in discriminating between accessions from different countries in a world collection of lentils was 100-seed weight in an evaluation study carried out by Erskine et al. [10]. In that study, the regional grouping indicated the importance of local adaptation through clusters of associated characters with phenological adaptation to the ecological environment as the major evolutionary force in the species. In our study the subgroups were highly unbalanced with respect to number of accessions. Most genotypes were presented in the D and F subgroups (36 and 31 respectively). IG number, origin and DTI values of accessions in each subgroup are shown in Table 4.

Table 4. IG and serial numbers (given in parentheses) of 96 lentil genotypes (ILL) of CAC origin distributed among clusters (AZE=Azerbaijan; ARM=Armenia; GEO=Georgia; KAZ=Kazakhstan; TJK=Tajikistan; UZB=Uzbekistan)

Origin	**No of access.**	**Cluster I**	**Cluster II**		**Cluster III**	
		A DTI>1.6	**B** DTI (1.6-1.47)	**C** DTI (1.47-0.88)	**D** DTI (0.8-0.6)	**F** DTI< 0.6
AZE	28	123613 (21)	134466 (14), 123684 (19), 123679 (34)	134465 (13), 123682 (17), 123683 (18), 123603 (20), 134447 (44), 134456 (46), 134458 (48), 123599 (69), 134467 (71)	134464 (12), 123618 (22), 134440 (24), 134444 (25), 134450 (45), 134459 (49), 134468 (72), 70167 (82), 123773 (86), 134453 (95)	134461(11), 134446 (43), 134457 (47), 134451 (94), 134454 (96)
ARM	14			3396 (10), 134425 (55), 134426 (56)	134421 (27), 134407 (38), 134409 (39), 134412 (50), 134417 (51), 134423 (54), 134427 (57)	123534 (15), 134418 (26), 70173 (83), 134399 (93)
GEO	9			123619 (23), 134436 (63)	134431 (59), 134432 (60), 134435 (62)	134430 (58), 134433 (61), 134437 (64), 123799 (87)
KAZ	1					123467 (5)
TJK	36		123648 (28), 123629 (36)	123634 (1), 123647 (3), 123624 (7), 123654 (32), 123594 (77), 516 (80), 70181 (84)	123644 (2), 123571 (8), 123651 (31), 123626 (35), 123631 (37), 123584 (52), 123525 (65), 123527(66), 123528 (67), 123595 (78), 85 (79), 123837 (88), 129245 (89)	123568 (16), 123649 (29), 123650 (30), 123576 (40), 123577 (41), 123579 (42) 123585 (53), 123596 (68), 123587 (75), 76281 (85), 129246 (90), 131734 (91)
TKM	2				3395 (9)	123508 (70)
UZB	6	123466 (4)		123500 (6)	123655 (33), 4875 (81)	134470 (73), 134471 (74)
Total	**96**	**2**	**5**	**22**	**36**	**31**

According to dendrogram two accessions originated from Azerbaijan (ILL 123613) and Uzbekistan (ILL 123466) clearly differed from the rest of genotypes for their relative tolerance to drought and formed a separate group with the highest DTI values. Subgroup B contained 5 genotypes (from which 3 were from Azerbaijan and two from Tajikistan) that tended to be relatively high tolerant together with the cluster I, with mean tolerance indices ranging from 1,59 to 1,47. Twenty two accessions from cluster II, with DTI between 1.47-0.9 intervals, assembled in one subgroup (C) also showing relatively high tolerance than subgroups D and F. Lentil genotypes exhibiting the lowest DTI values (<0.6) were grouped together in a subgroup of susceptible to drought. The forth subgroup was comprised of accessions that showed intermediate tolerance for drought tolerance index, which was higher than in subgroup F and lower than in others.

All groups were geographically diverse. The Kazakhstan and two Turkmenistan accessions, that were uniformly low in DTI value, occurred in subgroups D and F.

4. Conclusions

In conclusion we can say that one subgroup contained only two genotypes suggesting phenotypic uniqueness of these accessions with high tolerance and higher yield potential. Three groups out of five were characterized by high DTI values and high yields in both under irrigated and rainfed conditions. This collective group of genotypes probably represents very valuable germplasm and may serve as a source for useful genes in improving drought tolerance in lentil for the CAC.

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