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Short communication. Genotype × environment interaction analysis in two chickpea RIL populations

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Abstract

In order to introduce new chickpea germplasm in Argentina, two recombinant inbred line (RIL) populations $F_{6:7}$ of twenty lines, each one derived from crosses between kabuli and desi types, were evaluated for yield components in different sites and years. Additive main effects and multiplicative interaction (AMMI) analysis was applied to study the performance of different genotypes in different environments. Genotype (G), environment (E) and GE interaction effects were highly significant in both populations for seeds/plant, yield/plant and seed size (100-seed weight). Large differences were observed between the two populations for seeds/plant and seed size. We recommend that some genotypes from these two populations with good performance in a range of environments could be used to introduce new germplasm to the Argentine chickpea breeding programme. The significant GE interactions seem to be related to differences between two geographical areas (Salta and Córdoba/San Luis), at different latitudes and altitudes. These results suggest that these regions should be considered as different macro-environments from the point of view of the chickpea breeding programme.

Additional key words: yield component; desi × kabuli cross; macro-environments.

Chickpea (*Cicer arietinum* L..) is a self-pollinating diploid annual grain legume. Together with cowpea (*Vigna ungiculata* L.), it is the second most cultivated grain legume in the world after common bean (*Phaseolus vulgaris* L.) (http://faostat.fao.org). The crop is widely grown in arid and semi-arid areas across the world though it is mostly cultivated in the Mediterranean basin, the Middle East, central and south Asia, east Africa, America and Australia. Breeders often divide chickpea into two types, desi and kabuli, based mainly on seed morphology and colour, desi having small pigmented seeds and kabuli larger white ones. These types are considered to have different genetic backgrounds (Maynez *et al.,* 1993; Gil *et al.*, 1996).

In Argentina, chickpea was introduced in the 17th century, during the Spanish colonial period in the province of Córdoba by Jesuits missionaries and from this area spread north to Salta province. Local cultivars were developed from Spanish landraces and these were the basis for the first cultivar released in Argentina, 'Chañaritos S-156' (PI636327) (Biderbost & Carreras, 2005). Later, using a Mexican accession in a crossing programme, another cultivar, 'Norteño', was released. The cultivated area is increasing, from 3,000 ha in 2000 year to about 40,000 ha in 2010 (Bolsa de Cereales de Córdoba, 2011). Currently chickpea is being grown at a wide range of sites from latitudes of 20° to 33° South. In order to develop new cultivars better adapted to these different environments it will be necessary to broa-

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This work has four Supplementary Tables that do not appear in the printed article but that accompany the paper online.

Abbreviations used: AMMI (additive main effects and multiplicative interaction); GE (genotype-environment interaction); PCA (principal component analysis); RIL (recombinant inbred line).

den the genetic base of chickpea Argentine germplasm. The low temperature at the beginning of flowering may be an important limiting factor for yield in this country and, further, it is an autumn-winter crop and winter (21st June-21st September) is the dry season in the chickpea area.

The behaviour and adaptation ability of genotypes (G) to different environments (E) leads to sizeable GE interactions. The additive main effects and multiplicative interaction (AMMI) model is a powerful tool for analysing the performance of genotypes in multi-environment trials (Zobel *et al.*, 1988), and analysis of GE interactions is an important aspect of plant breeding programmes. In particular, may help us classify genotypes according to their stability, which can be defined from an agronomic point of view by the cultivar's capacity to perform according to the productive potential of each environment (Becker & Leon, 1988), *i.e.* without departing from the expected behaviour estimated from its average genotypic value. Given this, our aim was to study the performance of new chickpea germplasm across a range of environmental conditions corresponding to the chickpea-growing areas in Argentina.

Two recombinant inbred line (RIL) populations derived from the crosses CA2990 × WR315 and JG62 × ILC72, with twenty $F_{6:7}$ randomly selected lines each, were employed in this study. The code used to identify the 40 selected lines was the letter M or J for the first or second population respectively followed by the RIL number. The parental line CA2990 is a kabuli type from Mexico with white flowers, unifoliate leaves and large seeds, while WR315 is a desi landrace from Central India with pink flowers and resistance to all races of Fusarium. JG62, on the other hand, is a desi cultivar from India with pink flowers, double pods and resistance to race 0 of Fusarium. Finally, the parent ILC72 is a kabuli line from the former Soviet Union [maintained by the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria], with white flowers, that is late flowering and resistant to Ascochyta blight. The populations were sown between 2005 and 2008 in four sites (three regions) in Northwest Argentina, which represents 80% of the growing area in the country [Suppl. Table 1 (pdf on line)]. The field trials at each site and year were performed following a randomized block design with three replications, except in 2005 in which we were only able to sow two replicates. Plot units were 3 rows 4 m long, 0.70 m apart and with 15 seeds m^{-1}. Seeds were inoculated with *Rizhobium* sp. The characters evaluated were: 100-seed weight (g), seeds per plant (mean of 10 plants/plot) and yield per plant (mean of 10 plants/plot in g). All measures were taken from the central row in each plot unit. The Argentinean cultivar 'Chañaritos S-156' was included as control in the trails.

For statistical analysis we used an AMMI model (Zobel *et al.*, 1988). As sites and years were unbalanced, we considered each year-site combination as an environment. The AMMI analysis was split into two parts: (1) the additive part where the main effects of the model are analysed by analysis of variance; and (2) the interaction GE, or multiplicative part, which is analysed by principal component analysis (PCA), in order to capture most of the interaction in a few components. The stability of each line was assessed using its PC score expressed as unit vector times the square root of λ_k (genotype PCA score = $\lambda_k^{0.5}\gamma_{ik}$, where λ_k is the eigenvalue of the PCA for axis k, and γ_{ik} is the genotype eigenvector value for axis k). We selected the significant components explaining a high percentage of the GE interaction and a weighted score for each genotype was obtained as a measure of its stability in terms of these components: $\Sigma_1^N|(\lambda_k^{0.5}\gamma_{ik})\lambda_k/t|$ where $t = \min(i-1; j-1)$ (Rubio *et al.*, 2004). A line or environment is expected to be more stable when its weighted score is closer to zero. All statistical analyses were performed using the SAS statistical software (SAS Ins, 1996). Phenotypic correlations were calculated for each assay.

The combined analysis of variance for the three traits studied showed that all main effects (genotypes, environments and GE interactions) were highly significant in both populations (Table 1). When PCA was applied to the interaction terms in all characters, the first three PCs were significant and explained a high percentage (> 77%) of this interaction in both populations (Table 1). A high variability for all three characters was found in both populations across the different environments, the general mean of the JG62 × ILC72 population being higher for seeds/plant and lower for 100-seed weight compared to the figures for CA2990 × WR315 (Table 2). These results indicate that the JG62 × ILC72 and CA2990 × WR315 populations may carry genes for a higher number of seeds per plant and larger seeds respectively. Kabuli × desi crosses have been previously reported to generate high variation showing transgressive segregation for agronomic characters (Hawting & Singh, 1980; Maynez *et al.*, 1993). Positive and moderate to strong correlation coefficients were found between seeds/plant and yield/plant in both

Table 1. AMMI analysis of variance for seeds/plant, yield/plant and 100-seed weight in two populations of chickpea (CA2990 × WR315 and JG62 × ILC72) growing under different conditions (sites and years) in Argentina. Values in parenthesis indicate percentage of variation against G × E sum of squares for principal components (PCs)

Variation sources	Populations $F_{6:7}$					
	CA2990 × WR315			JG62 × ILC72		
	d.f.	Mean square[a]		d.f.	Mean square	
Seeds per plant						
Environment (E)	7	20,400.39***		6	46,438.91***	
Block (Env.)	14	568.60		13	936.36	
Genotype (G)	19	7,300.75***		19	8,355.54***	
GE	133	1,145.25***		114	2,261.12***	
PC1	25	2,071.54***	(34%)	24	5,047.96***	(47%)
PC2	23	1,788.09***	(27%)	22	2,226.18***	(19%)
PC3	21	1,305.59***	(18%)	20	1,804.37***	(14%)
G × Macro-E[a]	19	1,793.01***		19	3,695.88***	
Within Macro-E[a]	114	1,037.30***		95	1,974.17***	
Error	246	207.76		228	474.42	
Yield per plant						
Environment (E)	7	1,329.29***		6	1,700.62***	
Block (Env.)	14	30.55		13	33.86	
Genotype (G)	19	165.27***		19	101.48[ns]	
G × E	133	52.27***		114	69.33***	
PC1	25	133.49***	(48%)	24	174.54***	(53%)
PC2	23	54.41***	(18%)	22	68.26***	(19%)
PC3	21	49.66***	(15%)	20	47.42**	(12%)
G × Macro-E	19	68.57***		19	119.89***	
Within Macro-E	114	49.56***		95	59.23***	
Error	246	16.35		229	16.35	
100-seed weight						
Environment (E)	7	519.88***		6	250.89***	
Block (Env.)	14	9.90		13	18.91	
Genotype (G)	19	695.48***		19	270.70***	
G × E	133	33.01***		114	23.24***	
PC1	25	75.04***	(47%)	24	80.59***	(73%)
PC2	23	29.50***	(17%)	22	14.45*	(12%)
PC3	21	24.71***	(13%)	20	9.27[ns]	(7%)
G × Macro-E	19	27.79***		19	14.13[ns]	
Within Macro-E	114	30.38***		95	25.06***	
Error	254	6.05		242	9.61	

[a] G × Macro-E and Within Macro-E are the split of interaction G × E taken into in account the division of environments in two regions or macro-environments. * Significant at $p < 0.05$. ** Significant at $p < 0.01$. *** Significant at $p < 0.001$. [ns] Non significant.

populations in all eight environments (0.53 to 0.89 in CA2990 × WR315 and 0.57 to 0.95 in JG62 × ILC72). Further, though 100-seed weight was not significantly correlated with yield/plant, it showed a moderately significant negative correlation with seeds/plant in both populations (–0.32 to –0.76 in CA2990 × WR315 and –0.43 to –0.76 in JG62 × ILC72).

With regard to seeds/plant GE interactions, the weighted PC score of the genotypes over the three components ranged from 0.46 to 3.95 in CA2990 × WR315 and from 0.30 to 4.98 in JG62 × ILC72 (Table 2). In both populations the genotype with the highest mean value (M63 and J56 respectively) also had the highest weighted score. This result indicates that these two genotypes

Table 2. Mean for seeds/plant, yield/plant and 100-seed weight of 40 chickpea lines selected from CA2990 × WR315 and JG62 × ILC72 across different environments and their weighted score values on the first three components axes (PC) from AMMI analysis

Seeds per plant						Yield per plant (g)						100-seed weight (g)					
CA2290 × WR315			JG62 × ILC72			CA2290 × WR315			JG62 × ILC72			CA2290 × WR315			JG62 × ILC72		
Genotype	Mean	Weighted score	Genotype	Mean	Weighted score	Genotype	Mean	Weighted score	Genotype	Mean	Weighted score	Genotype	Mean	Weighted score	Genotype	Mean	Weighted score
M63	107	3.95	J56	135	4.98	M30	19	1.54	J56	22	2.13	M01	32	0.51	J32	27	1.08
M14	83	1.57	J99	124	2.17	M60	19	0.89	J99	19	1.04	M25	32	0.95	J22	26	1.05
M75	82	1.64	J86	110	2.63	M18	17	0.54	J71	19	1.86	M02	32	0.52	J75	25	0.68
M46	78	0.86	J84	102	1.27	M79	17	0.38	J84	17	0.72	M85	31	0.36	J71	24	0.82
M79	78	1.02	J12	101	2.35	M04	17	1.04	J22	17	0.59	M30	29	0.84	J90	23	0.69
M59	76	1.69	J96	97	0.57	M63	15	0.98	J86	16	0.65	M18	29	1.05	J95	23	0.96
M19	68	3.35	J19	82	0.90	M27	14	0.83	J19	16	0.78	M27	28	0.28	J77	22	0.13
M60	67	1.63	J55	81	0.38	M85	14	0.36	J12	15	1.07	M60	28	0.37	J60	20	0.39
M04	66	1.47	J64	79	0.86	M75	14	0.65	J60	15	0.59	M51	27	0.44	J19	19	0.85
M18	62	1.18	J71	78	3.28	M46	14	0.24	J32	14	0.56	M04	27	1.17	J04	19	0.51
M30	61	2.49	J61	76	0.59	M51	14	0.34	J75	14	0.77	M11	26	0.35	J98	18	0.87
M64	56	1.91	J04	71	1.64	M19	14	1.01	J77	14	0.54	M66	26	0.62	J64	17	0.25
M51	55	0.88	J60	70	0.30	M14	14	0.45	J96	14	0.36	M64	24	1.03	J61	17	0.38
M27	51	1.06	J77	64	0.70	M25	13	0.82	J64	14	0.42	M19	22	1.21	J84	17	0.83
M85	46	1.06	J98	64	1.48	M64	12	1.17	J55	14	0.10	M79	22	0.50	J56	16	0.27
M11	44	0.46	J22	64	0.56	M01	12	0.44	J04	13	0.64	M46	18	0.24	J55	16	0.39
M25	41	1.58	J95	60	3.08	M59	12	0.66	J61	13	0.32	M75	18	0.15	J99	16	0.71
M01	39	0.85	J75	59	1.60	M11	12	0.49	J95	12	0.89	M14	16	0.73	J96	16	0.50
M66	32	1.41	J90	56	0.44	M02	9	0.40	J90	12	0.33	M59	16	0.12	J12	16	0.98
M02	30	0.87	J32	54	1.64	M66	8	0.67	J98	11	0.67	M63	14	0.31	J86	16	0.70
Mean	61			81			14			15			25			20	
Control	53			46			18			17			34			38	

stand out with respect to the remaining genotypes in particular environments. On the other hand, genotypes such as M30 in CA2990 × WR315 and J71 in JG62 × ILC72 with mean values similar to the general mean and with high weighted scores indicate that they showed highest values for seeds/plant in some of the environments studied but the lowest values in others. The most stable genotypes in each of the populations (M11 and J60 respectively) showed mean values below the general mean. On the other hand, there was high variability in mean values of seeds/plant in the eight environments, Salta region environments (S1, S2, S3 and S4) having the highest values in both populations [Suppl. Table 2 (pdf on line)].

In the case of yield/plant, the weighted PC scores ranged from 0.24 to 1.54 in CA2990 × WR315 and from 0.10 to 2.13 in JG62 × ILC72 genotypes (Table 2). In the CA2990 × WR315 population, the M30 genotype had the highest values for both yield/plant and interaction. In JG62 × ILC72 the three most productive genotypes (J56, J99 and J71) also had high weighted scores. It is notable that genotypes with intermediate values for seeds/plant, such as M30 and J71, were found to have high yield/plant; this may be due to their relatively large seeds. In general, as occurred for seeds/plants, Salta region environments showed the highest yields/plant [Suppl. Table 2 (pdf on line)].

Weighted PC scores for 100-seed weight were lower than for both of the previously mentioned traits, seeds/plant and yield/plant (Table 2).

Due to the differences between regions observed for seeds/plant and yield/plant, we split the total GE interactions from ANOVA into genotype × region and genotype × (within region) considering two geographical areas: on the one hand, the Salta region and, on the other, Cordoba and San Luis. Both interactions where highly significant for yield/plant and seeds/plant in the

two populations (Table 1). Spearman rank correlation between the average genotypic performance in both regions showed a null correlation for yield ($r = –0.04$) in JG62 × ILC72 population and moderate ($r = 0.54$) in the other one. This result suggests a qualitative genotype-region interaction. For seeds/plant correlations were from moderate ($r = 0.54$; JG62 × ILC72) to high ($r = 0.73$; CA2009 × WR315). AMMI analyses for these traits considering separately the two geographical areas were applied. The results showed that PC1 and PC2 explained a high percentage (> 81%) of the interaction in both populations. We observed that the genotypes performed differently in these two geographical areas in both the populations [Suppl. Tables 3 and 4 (pdfs on line)]. For example, in the CA2990 × WR315 population, M63 stands out from the rest of the genotypes for a high number of seeds/plant in the Salta region, while in Cordoba/San Luis combined region it was ranked third for seeds/plant but showed more stability (weighted score = 0.56). For yield/plant, M30 was the most productive in Salta but only moderately so in Cordoba/San Luis. In the JG62 × ILC72 population, a high contrast was observed in the J56 genotype, with it giving the highest yield/plant in Salta, but the lowest value for this trait in Cordoba/San Luis. These results indicate that these two geographical areas could be considered to be different macro-environments from the point of view of chickpea breeding programmes.

Our results suggest the presence of favourable genes for a higher number of seeds per plant in the JG62 × ILC72 population and for larger seeds in CA2990 × WR315. In general, genotypes in JG62 × ILC72 with the highest number of seeds/plant were also those with the best yield/plant (J56 and J99). On the other hand, in CA2990 × WR315 seed size plays an important role in determining overall yield/plant because genotypes such as M60, M30 and M18, with moderate seeds/plant values but large seeds, gave a high yield/plant. These genotypes showed also higher mean values than the control (Chañaritos-S156') mainly for seeds/plant (Table 2). On the basis of our findings we recommend that these genotypes (J56, J99, M60, M30 and M18) could be used to introduce new germplasm to the Argentine chickpea breeding programme.

In Argentina, the growing season is characterised by low temperatures and short-day length, and both factors could affect flowering time and pod setting (Kumar & Abbo, 2001). Indeed, the significant GE interactions detected in this study could be related to the adaptability of the genotypes to the different environments. The two populations studied are segregating for important adaptive traits like resistance to diseases, in particular to Ascochyta blight and Fusarium wilt, and flowering time. In our trials, neither of the aforementioned pathogens were observed; flowering time may, therefore, be playing an important role in the adaptability of the genotypes. Chickpea is considered to have high day-length sensitivity in its centre of origin, while in tropical zones it has evolved towards short photoperiods (Kumar & Abbo, 2001). Given this, it could be interesting to assess the importance of flowering time in adaptability of chickpea under environmental conditions in Argentina. Taking into account the low temperature during the dry growing season in this country, chilling tolerance at flowering could be another interesting trait to consider. Abortion of flowers at temperatures of 15°C and below has been reported in several different countries and chilling tolerant germplasm has been obtained (Millán *et al.*, 2006).

In conclusion, the two populations studied in this work have shown a high genetic variability susceptible to be used in the Argentine chickpea breeding programmes. Significant differences in genotypic performance were observed between the two macro-environments identified in this study and this suggests cultivars should be selected for each geographical area. More research is needed to elucidate the importance of different traits related to adaptability under contrasting environmental conditions in Argentina.

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