

Article

Performance of Winter-Sown Chickpea Breeding Lines with Contrasting Levels of Resistance to Ascochyta Blight

Diego Rubiales ^{1,*}, Ana Moral ^{1,†} and Fernando Flores ²¹ Institute for Sustainable Agriculture, CSIC, Avda. Menéndez Pidal s/n, 14004 Córdoba, Spain² Departamento de Ciencias Agroforestales, E.T.S.I., Campus El Carmen, Universidad de Huelva, Avda. Fuerzas Armadas S/N, 21007 Huelva, Spain

* Correspondence: diego.rubiales@ias.csic.es

† The author has passed away.

Abstract: Multi-environment field testing of chickpea accessions winter sown in Southern Spain showed that environmental effects on yield were more important than genotypic effects and GEI. The most detrimental factor on grain yield was ascochyta blight infection. We did not find a significant effect of low temperatures on yield in the environments studied, probably due to the mild winters in the area. On the contrary, we found detrimental effects of high temperatures at the reproductive stage, particularly with numbers of days with Tmax >30 °C. We found that genotypic effects were larger than the environmental on ascochyta infection as we included accessions previously selected for their levels of resistance or susceptibility. Biplots based on the WAASB/productivity ratio highlighted AS19, AS30, AS23, AS26, and AS18 accessions as the best for productivity and stability of yield, matching with those with a lower ascochyta blight infection. The MTSI index also identified these as the best accessions for the region.

Keywords: ascochyta blight; *Cicer arietinum*; genetic resistance; genotype x environment interactions; heat stress; MTSI; WAASB



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1. Introduction

Chickpea (*Cicer arietinum* L.) is a cool season grain legume traditionally important in the human diet of Mediterranean and Asian countries that has been increasingly adopted as food globally. In Mediterranean environments, chickpea is typically grown as a rainfed crop sown in early spring, relying on the residual moisture of the soil. This practice facilitates the escape to cold temperatures and to ascochyta blight (*Ascochyta rabiei* (Pass.) Lab., teleomorph *Didymella rabiei* (Kov.) Arx) damage [1–3]. However, mild winter conditions of this area offer the possibility to sow chickpea in autumn, with increased yield and yield stability, profiting from winter rains and minimizing the effects of terminal heat and drought stress [4,5]. Unfortunately, winter sowing also increases the risk of ascochyta blight devastation. Therefore, control of ascochyta blight is essential to increase chickpea production and yield stability. Host resistance is the most practical and economic way to manage the ascochyta blight problem, but its level has to improve. Concern has also been commonly raised regarding the damage of low temperatures in winter-sown chickpea [2], however less attention was paid to heat stress. Even though high temperatures at the reproductive stage are known, it was assumed that winter sowings would escape from heat stress [2,4,5]. Long term breeding efforts has resulted in a number of breeding lines and cultivars resistant to ascochyta blight [2,3,6] whose performance and agronomic value must now be validated in different environments.

Under multi-environmental trials, yield, abiotic, or biotic stress resistance are influenced by the effects of the genotype (G), the environment (E), and their interactions (GEI). GEI can be studied by a number of methods such as AMMI (additive main effect

and multiplicative interaction) analysis [7] or GGE biplot (genotype plus genotype-by-environment) [8]. However, as long as these methods assume genotypes as random variables, they are not appropriate for analyzing the structure of the linear mixed-effect model (LMM) [9]. WAASB (weighted average of absolute scores) has been proposed [9] to better characterize ideal genotypes and a superiority index, WAASBY, to select genotypes based on both yield performance and the WAASB stability score [9].

The objectives of this research were to evaluate the performance and stability of ascochyta blight resistance and of yield among chickpea breeding lines in Southern Spain under winter sowings.

2. Materials and Methods

2.1. Plant and Experimental Design

Performance of 10 chickpea accessions (Table 1) was studied at three Southern Spanish locations (Córdoba, Escacena, and Tomégil) over five consecutive field seasons (2008–2009, 2009–2010, 2010–2011, 2011–2012, and 2012–2013) (Table 2). Accessions studied were selected out of 80 Kabuli type accessions previously field studied at Córdoba and Escacena during 2007–2008 (data not presented). At each location, a randomized complete block design with three replications was used. The experimental unit consisted of small plots, with three 1 m long rows per accession separated 0.35 m, 10 plants per row. Sowing took place by the middle of December each season, according to local practice. Weeds were controlled by hand weeding. Ascochyta blight (*Ascochyta rabiei*) and any other occurring disease or pest were monitored, recording disease severity (DS), and estimated as a percentage of the canopy covered by lesions. The harvest of the plants took place by late May, depending on the environment. All plants were harvested, threshed, and grain yields recorded. Climatic data were obtained from Red de Información Agroclimática de Andalucía [10] and provided in Table 2.

Table 1. Chickpea accessions included in the study.

Accessions	Donor's Code	Origin/Derived from Accession no.
AS19	FLIP 03—40C	98TH83/[(ILC1306 X S 94321) X ILC4236] X S 96114
AS8	FLIP 98—129C	95TH47/(FLIP 88—6C* ILC3373)XFLIP 89—4C
AS30	FLIP 03—128C	00TH51/FLIP 98—52C X FLIP 98—47C
AS23	FLIP 03—48C	99TH13/FLIP 93—62C X FLIP 93—50C
AS26	FLIP 03—76C	00TH28/FLIP 98—15C X S 98588
M38	LMR 186	X99TH155/ILC5901XILC5309
AS18	FLIP 03—37C	98TH38/S 96114 X FLIP 92—159C
M7	LMR 29	X99TH151/ILC3805XILC5901
AS41	ILC 263	PI 339223
M42	ILC 3397	
M8	LMR 40	X99TH151/ILC3805XILC5901
M35	LMR 164	X99TH154/ILC5901XILC3397
M30	LMR 158	X99TH154/ILC5901XILC3397
Blanco Lechoso	Local check	Extra-large seeds, superior market quality but highly susceptible to ascochyta blight

Table 2. Description of the environments (combination of location and season) of the trials for the multi-environment study. Climatic data.

Environment	Season	Soil Type	Soil pH	Latit.	Longit.	Altit.	Average Tmax (°C)	Average Tmin (°C)	Rain (mm)
Cor-09	2008–2009	Cambisol	6.5–7	37°50' N	4°50' W	90	19.2	4.1	279
Cor-10	2009–2010	Cambisol	6.5–7	37°50' N	4°50' W	90	20.1	8.4	1053
Cor-11	2010–2011	Cambisol	6.5–7	37°50' N	4°50' W	90	22.5	10.3	514
Cor-12	2011–2012	Cambisol	6.5–7	37°50' N	4°50' W	90	20.8	5.6	163

Table 2. Cont.

Environment	Season	Soil Type	Soil pH	Latit.	Longit.	Altit.	Average Tmax (°C)	Average Tmin (°C)	Rain (mm)
Cor-13	2012–2013	Cambisol	6.5–7	37°50' N	4°50' W	90	18.3	7.5	497
Esc-10	2009–2010	Fluvisol	7–7.5	37°25' N	6°15' W	88	19.3	9.4	1206
Esc-11	2010–2011	Fluvisol	7–7.5	37°25' N	6°15' W	88	21.6	11.4	534
Esc-12	2011–2012	Fluvisol	7–7.5	37°25' N	6°15' W	88	20.4	9.1	134
Esc-13	2012–2013	Fluvisol	7–7.5	37°25' N	6°15' W	88	18.2	8.7	473
Tom-09	2008–2009	Vertisol	7–7.5	37°30' N	5°57' W	12	21.3	6.9	219

2.2. Statistical Analysis

2.2.1. Variance Components

Each environment was a combination of the year and location; therefore, 6 environments were included for stability analysis of the 15 genotypes. All analyses were done by executing the 'metan' package on R Studio statistical Software version 4.1.0 [11]. For the percentage data (i.e., *Ascochyta* blight data), arcsine transformations of the square root of proportions were carried out, and statistical analyses were performed on the transform data. A combined analysis of variance (ANOVA) for randomized complete-block designs across environments was performed to deduce the variance components of different sources of variation and to detect the presence of GEIs by assuming all effects as random factors. The data from all the environments were subjected to Shapiro–Wilk test for normality and a Bartlett's test for homogeneity of variances.

2.2.2. GEI Analysis

Means vs. Stability Biplot

Using the weighted average of absolute scores from the singular value decomposition of the matrix of BLUPs (WAASB), we estimated to evaluate the stability of grain yield or of *ascochyta* infection of genotypes across environments [9]. With the purpose of better characterizing ideal genotypes based on both high performance and stability, a biplot was rendered based on the WAASB and mean performance trait. In this biplot, four quadrants are seen [9]: the genotypes or environments placed in quadrant I are unstable or environments with high discrimination ability and low performance below the grand mean. In quadrant II, the performance of the genotype is above the grand mean but unstable and the environments were good discriminating environments with high magnitudes of the response variable. Genotypes in quadrant III have a low but stable performance due to the lower values of WAASB, whereas the environments are considered poor performing and with low discrimination ability. The genotypes in quadrant IV are high performance and broadly adapted due to the high magnitude of the response variable and the high stability [9].

Cluster Analysis

Based on the WAASBY superiority index [9], weights for WAASB/trait ratio (stability/performance trait) were allotted from the 50/50 to the 0/100 scenarios so that the performance of the trait had more weight than stability.

A Euclidean distance-based dendrogram is used for grouping the genotypes based on their ranks in scenario matrix from 50/50 to 0/100 for WAASB/trait ratio, so the groups formed were indicated as ellipses in the mean vs. stability biplots to identify groups of genotypes so that each group with a different color could have similar performance regarding stability and performance [9].

Multi-Trait Stability Index (MTSI)

The Multi-Trait Stability Index (MTSI) based on the WAASBY index [12] was used to allow for simultaneous selection of stability and mean performance based on several

traits (grain yield and ascochyta blight) assigning a selection intensity of 25%. We drafted 11 scenarios, assigning different relative weights to performance vs. stability of the trait, starting from 50:50 to 100:0.

2.2.3. Correlations

Correlation analysis was applied to describe the impact on grain yield of ascochyta blight infection and of the various climatic parameters, including temperatures, humidity, and rain during pre-flowering and at the flowering and post-flowering period. This analysis was performed using the genotypic best linear unbiased prediction (BLUP) of each genotype in each environment for each trait. Analysis and visualization of the correlation matrix was made using the ggcorrplot function from the ggcorrplot package in R.

3. Results

3.1. Pooled ANOVA

Pooled ANOVA (Table 3) showed that all the main (E and G) and multiplicative (GEI) effects were highly significant ($p < 0.0001$) for grain yield and ascochyta response. E effects were more important for grain yield explaining 38% of total variation, with 26% explained by G, and only 11% by GEI. However, G effects were more important for ascochyta response explaining 44% of total variation, with 35% explained by E and only 13% by GEI.

Table 3. Pooled analysis of variance and estimated variance components of grain yield (kg/ha) and ascochyta blight (transformed data) of a chickpea performance trial, consisting of 14 genotypes (G) grown in 10 environments (E).

Trait	Random Effects	Estimate	Standard Error	Pr > Chisq	% of Total Variance Explained by
Grain yield (kg/ha)	E	398911	198702	<0.0001	38
	Block(E)	24957	13523	<0.0021	2
	G	274398	115428	<0.0001	26
	GEI	116124	26865	<0.0001	11
	Residual	245612	21538	<0.0001	23
Ascochyta blight	E	0.043	0.02	<0.0001	35
	Block(E)	0.0			0
	G	0.054	0.02	<0.0001	44
	GEI	0.016	0.002	<0.0001	13
	Residual	0.01	0.0009	<0.0001	8

3.2. Grain Yield

Average grain yield over accessions and environments was 1248 kg/ha, with great differences across environments (Table 2), being higher than 1100 kg/ha (Table 4) at Tom09, Cord09, Cord12, Cord13, Esc10, Esc11, Esc12, and Esc13 but only 644 Kg/ha at Cord12 and 113 kg/ha at Cord11, confirming the high effects of the E and of G*E, higher than those of G on grain yield (Table 3). The genotypes performing better across environments were AS19, As8, AS30, AS23, AS26, M38, and AS18 with an average yield over environments higher than 1500 kg/ha. The yield of Blanco Lechoso was the lowest of all the environments, with an average of 256 kg/ha. These yields are in line with the reported yield in the region, with Blanco Lechoso yielding very poorly (in the range of 300 kg/ha) in winter sowings and approximately 1400–1600 kg/ha for ascochyta resistant accessions [13].

Table 4. Grain yield (kg/ha) of 14 chickpea accessions grown at 10 location–year environments.

Accessions	Tom09	Cord09	Cord10	Cord11	Cord12	Cord13	Esc10	Esc11	Esc12	Esc13	Average Over Environments	SE *
AS19	1686	2224	1028	571	2247	1974	4304	1675	1777	2458	1994	193
AS8	1305	2358	853	100	1791	1729	4779	1383	1255	2480	1803	236
AS30	1935	2058	775	282	1509	1664	2896	1771	1897	2824	1761	174
AS23	1735	2910	1035	100	1718	1763	2118	1742	1427	2524	1707	176
AS26	1664	1971	940	42	1476	1869	2467	1787	1738	2920	1687	159
M38	1847	945	900	20	1586	1424	3381	1089	1200	3522	1592	250
AS18	1858	1520	911	467	1229	1589	2697	1260	1116	2571	1522	148
M7	1303	3	323	0	1504	1355	1873	1155	1658	1742	1092	138
AS41	978	1024	547	0	320	1050	1962	1327	810	2264	1028	123
M42	826	402	468	0	686	1202	1666	1080	1055	1649	903	100
M8	861	8	298	0	1031	979	1403	1031	622	1954	819	120
M35	606	179	400	0	940	884	1357	498	973	1957	779	125
M30	510	22	533	0	540	494	1213	150	155	1660	528	97
Blanco Lechoso	281	0	0	0	367	329	0	299	298	987	256	55
Mean	1243	1116	644	113	1210	1308	2294	1160	1142	2251	1248	
SE	109	168	55	32	109	94	231	107	100	107	49	

* SE = standard error.

Figure 1 shows the WAASB vs. GY biplot where we can see a simultaneous interpretation of productivity and stability. The lines perpendicular to these axes divide the biplot into four parts. Quadrant I contains the Blanco Lechoso and M7 genotypes and the Cord09 and Cord11 environments. These genotypes and environments performed lower than average grain yields and played the largest role in GEI. Quadrant II contains genotypes AS19, AS8, and M38 and environment Esc10, with a good grain yield but little stability, playing also a big role in GEI. So, this environment in quadrant II deserves special attention since it provides above-average production and a high ability to discriminate genotypes. Quadrant III contains M30, M35, M8, M42, and AS41 genotypes, with low performance although high stability, along with the Cord10, Cord12, Esc11, Esc12, and Tom09 environments, with the lowest WAASB values among all environments. The Tom09 environment was exactly on the frontier of the third and fourth quadrant, meaning that the grain yield was equal to the total average and low discriminate power. Finally, quadrant IV includes AS30, AS23, AS26, and AS18 genotypes, with high productivity and stability and Cord13 and Esc13 environments, which had a high production capacity and less WAASB (Figure 1).

Biplots similar to that in Figure 1 are in fact a snapshot allowing for selection of the best genotypes based on productivity and stability. However, a reality might be that the breeders do not always have a clear criterion to pre-assign relative weights to stability *versus* performance for a given trait. In this case, one option [14] might be to rank the genotypes based on the WAASB/GY ratio, giving different weights for stability/productivity. After the cluster analysis (Supplemental Figure S1) based on the Euclidean distance of the ranking matrix of the genotypes based on the WAASB/productivity ratio with different weights for stability/productivity from the 50/50 to the 0/100, the three clusters formed (ellipses of different colors) are shown in Figure 1. Cluster 1 (ellipse marked in green) includes the best genotypes, more productive and more stable (AS19, AS30, AS23, AS26, and AS18). Note that these genotypes remained the firsts-ranked regardless of when the WAASB/GY ratio was low (greater weight for yield). Cluster 2 (marked in blue) includes AS8, M38, and AS41 genotypes, with very different productivity but showing similar profiles in the WAASB/GY ratio ranking. Cluster 3 (marked in red) includes genotypes with poor yields, well below the average. We can highlight the “Blanco lechoso” genotype with both the lowest stability and yield.

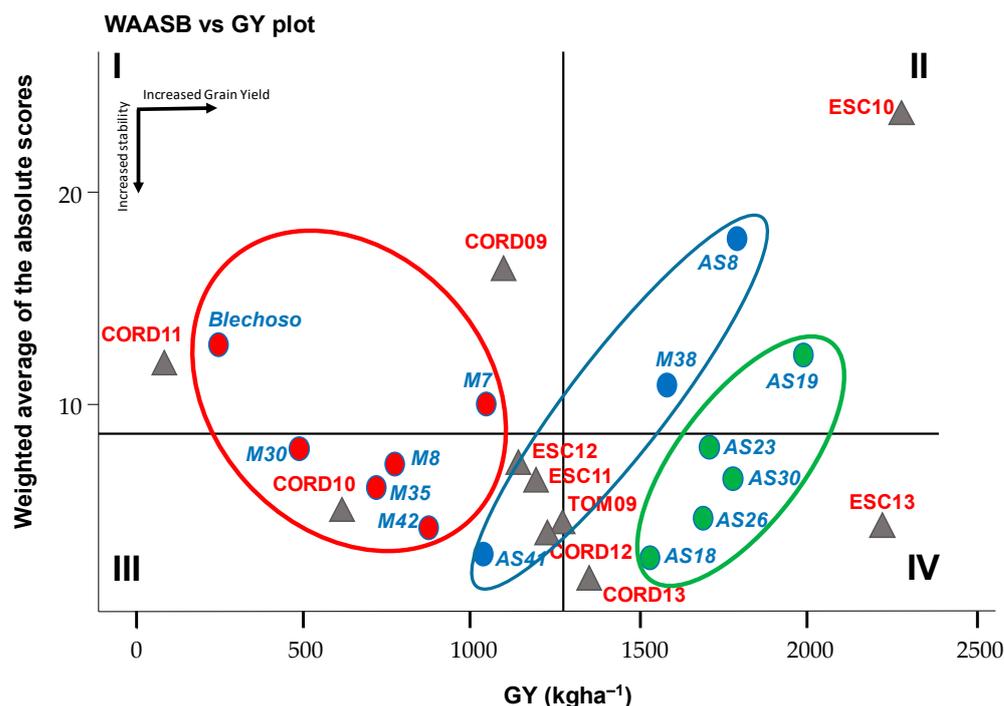


Figure 1. Biplot of grain yield vs. WAASB of 14 chickpea genotypes evaluated in 10 environments. Horizontal black arrows indicate the direction of the increase in grain yield; vertical arrow indicate increase of stability. Triangle for environments and circle with different colors for genotypes. Groups of genotypes with similar performance regarding stability and performance in ellipses according to cluster analysis of the ranking matrix from the 50/50 to the 0/100 scenarios for the WAASB/GY ratio.

3.3. Ascochyta Blight

Ascochyta blight infection was the only significant biotic constraint observed at all sites, with only negligible levels of leaf miner infection at some sites (data not shown). Levels of ascochyta blight infection (Table 5) varied with genotypes (average infection over environments varying from 10% in AS19 to 73% in Blanco Lechoso) and environment (average over genotypes varying from 13,8% at Esc12 to 66.5% at Cord11).

Table 5. Ascochyta blight (%) of 14 chickpea accessions grown at 10 location–year environments.

Accessions	Tom09	Cord09	Cord10	Cord11	Cord12	Cord13	Esc10	Esc11	Esc12	Esc13	Average Over Environments	SE
AS19	8.7	13.7	10	31.7	14.7	6.7	6.7	0	2.3	6	10.0	1.8
AS23	2.3	6.7	15	35	17	9.7	3	1.7	1.3	14.3	10.6	2.1
AS18	4	13.3	13.3	35	15.7	6.3	13.3	2.3	2	10	11.5	1.9
AS8	2	3.7	10	50	17.3	8	7	2.7	5	21	12.7	3.1
AS30	3.7	18.3	9.3	40	17	6.3	16.7	3.3	0.7	14.3	13.0	2.5
AS26	5.3	15	13.3	56.7	21.3	5.3	8.7	5	1.3	6	13.8	3.1
M38	8.3	33.3	5	50	22.3	5.3	10.3	7.3	7.3	5.3	15.5	2.7
AS41	15.7	45	21.3	95	31.7	19.7	24	18.3	16.3	26.3	31.3	4.3
M7	11.7	86.7	23	95	23	15	28.3	14	13	24	33.4	5.5
M35	30	73.3	20	73.3	32.7	16.7	12.7	26	22.3	27.3	33.4	4.1
M30	26.7	70	15	86.7	31.3	22.3	30	21.3	31.7	31.7	36.7	4.2
M8	23.3	90	26	90	40.7	27	27.7	24.7	20	30.3	40.0	4.8
M42	20.7	87	24	93.3	40.3	28.7	28	26.3	22	44	41.4	5.0
Blanco Lechoso	46.7	83.3	100	100	65.3	60.7	100	51.3	48	76	73.1	3.9
Mean	14.9	45.7	21.8	66.5	27.9	17.0	22.6	14.6	13.8	24.0	26.8	
SE	2.2	5.3	3.6	4.4	2.2	2.5	3.7	2.2	2.2	2.9	1.3	

Quadrant I in the ascochyta blight biplot response (Figure 2) includes genotypes AS8 and AS23 with the lowest levels of ascochyta infection but with only moderate stability.

Quadrant III includes genotypes AS18, AS19, AS26, AS30, and M38 genotypes with stably low ascochyta infections, being the most desirable ones. Quadrants II and IV include genotypes with above-average ascochyta infections, highlighting the genotype Blanco Lechoso with the highest infection and instability high infection (>25%). The cluster analysis (Supplemental Figure S2) highlighted three clusters. Cluster 1, in green includes AS8, AS23, AS18, AS19, AS30, and AS26 with the lower level of infection and higher stability based on ranking profiles for the WAASB/ascochyta infection. On the contrary, in red we highlight the genotypes with a high level of infection and medium to high instability.

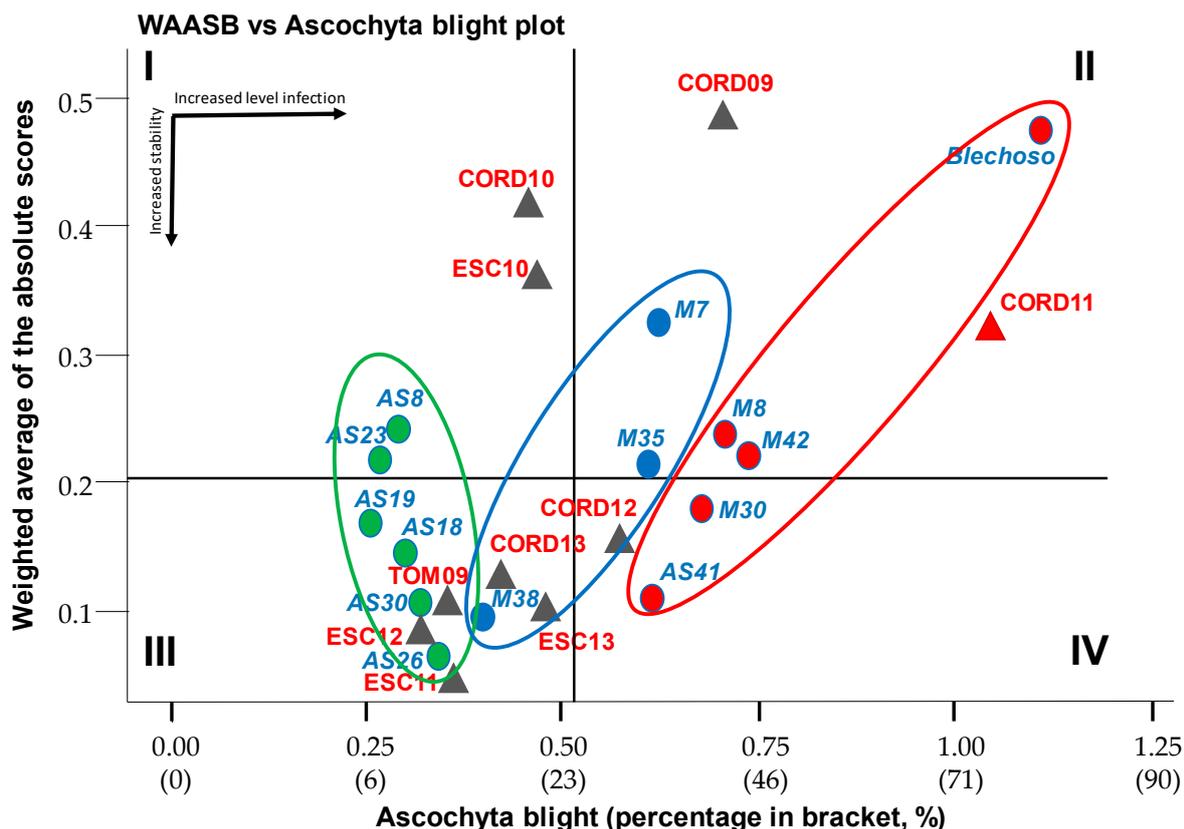


Figure 2. Biplot of ascochyta blight vs. WAASB of 14 chickpea genotypes evaluated in 10 environments. Horizontal black arrows indicate the direction of the increase in level ascochyta infection; vertical arrow indicate increase of stability. Triangle for environments and circle with different colors for genotypes. Groups of genotypes with similar performance regarding stability and performance in ellipses according to cluster analysis of the ranking matrix from the 50/50 to the 0/100 scenarios for the WAASB/GY ratio.

3.4. MTSI Index

Previously described results show the most desirable genotypes in terms of grain yield (AS19, AS30, AS23, AS26, and AS18) or in terms of reduced ascochyta infection (AS8, AS23, AS18, AS19, AS30, and AS26). Currently, there are various indices to select the best genotypes based on several agronomic traits [15]. Thus, we have used the MTSI index [12] that allows for defining the weight to be assigned to the performance and stability. Figure 3 shows the selected genotypes in different scenarios already defined in previous paragraphs. In Figure 3, we can see how from the ratio 25/75 the selected genotypes (AS19, AS30, AS23, and AS26, based on a selection intensity of 25%) are maintained up to the ratio 5/95 where the weight assigned to stability is minimal (where the AS8 genotype was included and the AS26 genotype was excluded). Therefore, we can indicate that genotypes AS19, AS30, AS23, AS26, and AS8 would be selected for the next stage of the breeding program, based on the two agronomic traits studied.

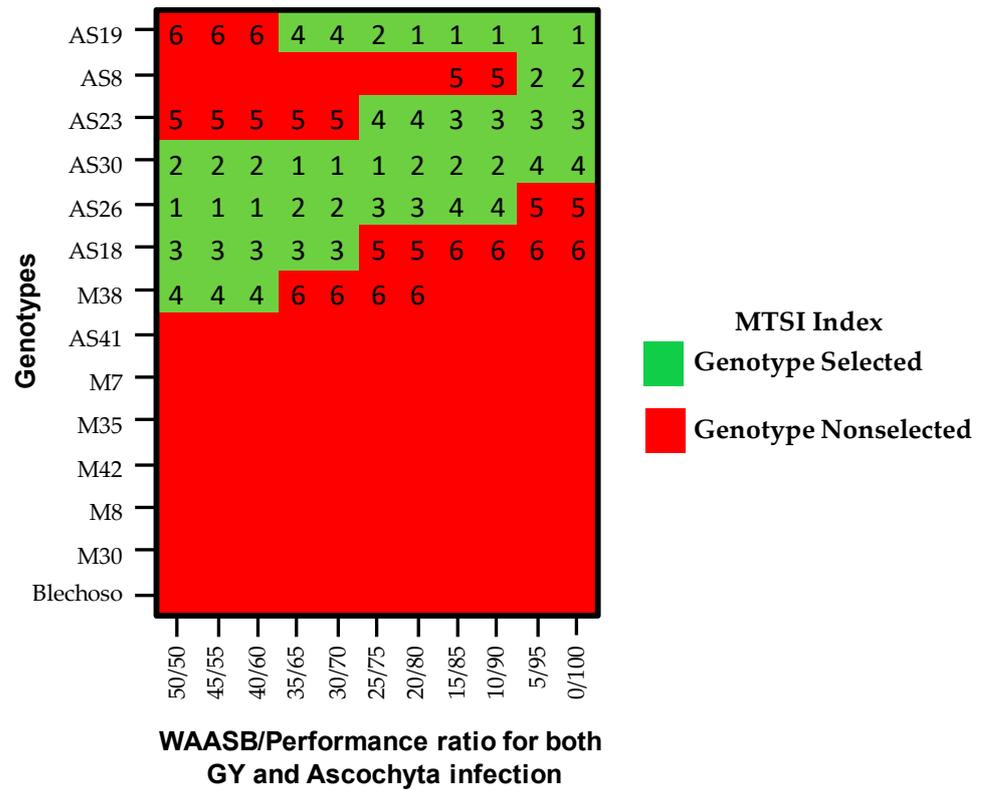


Figure 3. Heatmap showing the MTSI index rank of 14 chickpea genotypes considering different weights for stability and both, GY and ascochyta infection.

An example of the rankings obtained of the MTSI index assigning a 25/75 ratio (25% stability, 75% performance) for grain yield and for ascochyta infection is shown in Figure 4, with AS30, AS19, AS26, and AS23 (red dots) marked as the most desirable to be selected.

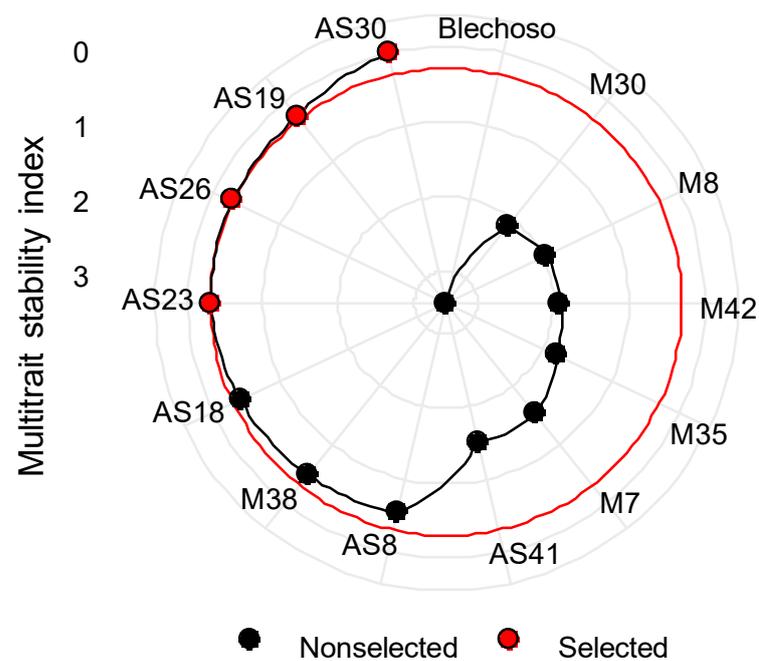


Figure 4. Ranking of accessions based on MTSI index for GY and ascochyta infection assigning a selection intensity of 25/75 (25% stability, 75% performance).

The grain yield of the four selected genotypes (XS, 1787kg/ha) was higher than the original average of all genotypes (XO, 1248 kg/ha) (Table 6). Conversely, the average infection on the four selected genotypes (XO, 11.68%) was lower than the average on the whole set (XS, 26.8%). The magnitude of this difference is given by SD. The broad heritability (96%) and genetic gain (53.7%) were slightly higher for ascochyta infection than for grain yield (93% and 40.3%), revealing the feasibility of improving both traits but with better chances for ascochyta infection than for yield.

Table 6. Estimates of genetic parameters for GY and Ascochyta infection based on MTSI for the 14 chickpea accessions tested in 10 environments.

Trait	XO	XS	SD	h ²	SG(%)
Grain yield (kg/ha)	1248	1787	539	0.93	40.3
Ascochyta infection (%)	26.8	11.8	−15	0.96	53.7

Note: XO: original mean; XS: mean of the selected accessions; SD: selection differential; h²: the broad heritability and SG%: selection genetic gains.

3.5. Correlations among Traits and Environmental Factors

Pearson’s correlation between traits and climatic parameters (Figure 5) shows that grain yield was favored by a higher humidity at pre-flowering and flowering stages ($r = 0.26$ ** and $r = 0.34$ ***, respectively) and by rain at flowering ($r = 0.29$ ***), whereas rain after flowering was detrimental for yield ($r = -0.36$ ***). Similarly, high temperatures at all growing periods were detrimental but particularly at and after flowering ($r = -0.25$ **, $r = -0.46$ *** and $r = -0.45$ ***, for PreTmax, FlowTmax, and PostTmax, respectively). The most detrimental factor on grain yield was ascochyta blight infection ($r = -0.64$ ***) paired with the number of days with Tmax >30 °C (T30, $r = -0.61$ ***). Ascochyta blight infection was favored by warm temperatures at flowering ($r = 0.26$ **) and by rain at post-flowering ($r = 0.23$ **) (Figure 5).

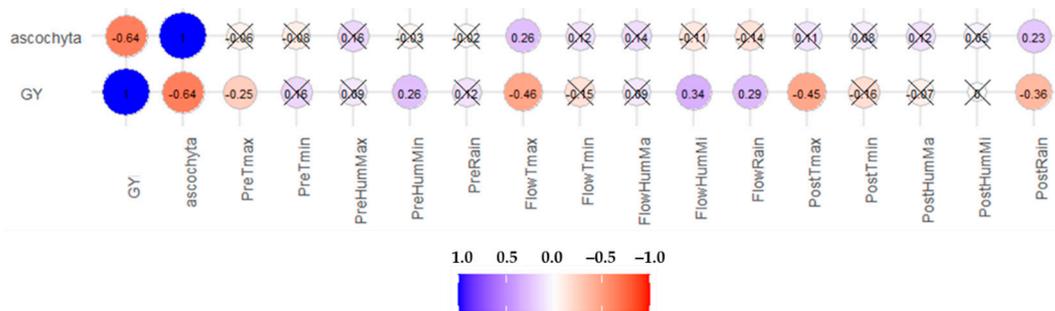


Figure 5. Graphical representation of the effects of climatic parameters and of disease incidence on grain yield and among them. Size of the dot indicates its effect on the trait, blue when positive, red when negative. The not statistically significant crossed.

4. Discussions

Environmental effects were more important than genotypic effects and GEI for grain yield, which agrees with previous reports [16]. Indeed, climatic factors affected yield, with high temperatures at all growing periods being detrimental for yield but particularly at and after flowering. The most detrimental factor on grain yield was ascochyta blight infection, as widely acknowledged in winter-sown chickpea [3]. Ascochyta infection also varied with the environment, being favored by warm temperatures and rain after flowering. As a consequence, even when rain at flowering was beneficial for yield, rains after flowering were detrimental as they favored ascochyta infection. The devastating effect of ascochyta blight is in fact regarded as the reason driving delayed sowing from an autumn- to a spring-sown crop to escape infection, and this is suggested to have already occurred, specifically before the Early Bronze Age [17]. We found that genotypic effects were

larger than the environmental on ascochyta infection as we included accessions previously selected for their levels of resistance or susceptibility. This is the result of long-term breeding efforts with ascochyta resistance as a major target [2,3,6]. As for Chandirasekaran et al. (2009) [18], the relative rank of resistant accessions was maintained across environments. As a result, the set of resistant accessions (AS8, AS18, AS19, AS23, AS30, AS26, and M38, with overall DS < 16%) yielded overall >1500 kg/ha, whereas the non-resistant yielded less than 1000 kg/ha.

We did not find any other significant biotic stress in any of the environments. This is not surprising for fusarium wilt or rust [19,20] as they both can be devastating in spring-sown chickpea in the area, but winter-sown chickpea escapes infection. There has been some concern regarding the risk of broomrape infection in winter chickpea [21]. However, we did not observe any infection even when the experimental sites are heavily infested and nearby fava bean, lentil, pea, or crops suffered from high damage [14,22,23]. This supports earlier reports, suggesting high resistance to broomrape in chickpea [24,25].

In addition to biotic stresses, chickpea production might face drought and low and high temperatures, which are becoming increasingly important with unpredictable climate change and the associated increased frequency of drought and extreme temperatures, which considerably reduces grain yield [26]. Winter-sown chickpea can be damaged by low temperatures at the vegetative and reproductive phase, when mean temperature of the day falls below 15 °C [27–29]. However, we did not find a significant effect of low temperatures on yield in the environments studied, probably due to the mild winters in the area. On the contrary, we found detrimental effects of high temperatures at all growing periods but particularly at and after flowering. Contrary to the long term concern regarding cold temperatures, awareness of heat stress is rather recent but widely acknowledged as a major constraint for chickpea productivity as temperatures >30 °C reduce grain weight and number due to reduced pollen viability and flower and pod abortion [30–33]. Chickpea genotypes tolerant to heat stress have been recently identified [34–36] and QTLs reported [37], which will facilitate chickpea heat tolerance breeding [38,39].

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy12092194/s1>, Figure S1. Dendrogram of the cluster analysis applied to the Euclidean distance of the ranks matrix in scenario from 50/50 to 0/100 for the WAASB/GY ratio, grouped by the Complete-linkage fusion technique. Figure S2. Dendrogram of the cluster analysis applied to the Euclidean distance of the ranks matrix in scenario from 50/50 to 0/100 for the WAASB/ascochyta blight (data transformed) ratio, grouped by the Complete-linkage fusion technique.

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