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Heat Waves and Broomrape Are the Major Constraints for Lentil Cultivation in Southern Spain

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Abstract: There is potential for expanding lentil cultivation to dry and warm Mediterranean rain-fed environments at low altitudes, where early sowings are recommended to profit from winter rains and escape drought and excessive heat at the grain filling stage. In cooler areas, frost might be a problem in the early sowings, however, in warmer areas such as our low altitude warm southern Spanish environments the most detrimental factor on lentil seed yield appeared to be high temperatures at grain-filling stage, particularly heat waves of more than 5 days with T_{max} > 30 °C. This was followed by broomrape infection, the combination of both being dramatic. We detected variation for stress tolerance, with S17 and R7 accessions outstanding for all stress indexes used, followed by S23, Nsir, S6, and S12. Broomrape infection ranked second risk in the area. No complete resistance to broomrape was identified, but there was a significant variation in the level of infection, with accessions S14 and R17 being the more resistant across environments. This offers prospects for combining heat tolerance and broomrape resistance by breeding.

Keywords: *Lens culinaris*; heath stress; *Orobanche crenata*; genetic resistance; genotype × environment interactions

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1. Introduction

Lentil (*Lens culinaris* Medik.) is an annual temperate grain legume highly valued for food grown worldwide over 5 Mha [1]. Although lentil crop originated in the Middle East, the largest lentil producer today is Canada with *circa* 3 Mton, followed by India with 1 Mton. In spite of the high appreciation of the product in Western Asia and Mediterranean Basin, lentil cultivation is not sufficient to satisfy domestic demand, forcing to imports that in 2019 were of 980 Kton in Western Asia, 230 Kton in Northern Africa, and 104 Kton in Southern Europe [1]. There is therefore, sufficient domestic demand to increase lentil cultivation not only in all Mediterranean Basin and West Asia but in all Europe, which resulted in a true regained interest in the lentil crop.

Average world lentil yields (1200 kg/ha) are small due to biotic and abiotic constraints and to the fact that, generally, lentil is produced on marginal lands with low inputs. Understanding the adaptation constraints of diverse lentil genotypes in differing environments is needed to assist breeders in the expansion of the genetic diversity. Phenology is an important factor influencing adaptation in lentil, by matching the needs of the crop with the available resources and limitations of a particular environment [2]. In temperate environments, lentils are commonly planted in spring and grown during the summer under warm temperatures and long days. By contrast, in Mediterranean Basin and West Asia where winters are mild and summers are too hot and too dry, winter sowings are recommended to profit from winter rains and to avoid high temperatures [3]. However, early sown lentils could suffer from cold in some areas particularly in continental areas or even in Mediterranean ones at high altitudes, which made winter hardiness and frost tolerance the major objectives in lentil breeding [4–6].

In Spain, lentil cultivation is concentrated in central plains (Castilla-la-Mancha and Castilla-León), being low in Southern regions like Andalusia and Extremadura, characterized by higher temperatures and high incidence of the parasitic weed broomrape (*Orobanche crenata* Forsk.). Early sowings are known to favor broomrape infection, which is regarded a major limiting factor for lentil in Mediterranean basin [7]. In fact, broomrape has been suggested as the reason for abandonment of lentil cultivation in southern Spain [8]. In the lack of resistant cultivars and of economically viable chemical control, delaying the sowing date or the use of very early maturing cultivars have been the only recommended methods to escape from infection in broomrape-prone areas. However, in these areas, delayed sowings can expose the lentil to excessive temperatures at flowering, which can be very problematic, as lentil is particularly sensitive to high temperatures during the seed-filling stage [9,10]. Expansion of lentil crop in this area requires therefore a deeper understanding of environmental factors affecting yield and their interactions. The objective of the present experiment was to study the genotypic and environmental effects limiting yield in lentil grown in warm, low altitude, broomrape-prone Andalusian environments.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Performance of 17 lentil accessions (Table 1) was studied at 13 location—year environments (Table 2). Accessions studied included 14 breeding lines derived from local selections made on ICARDA materials, the Spanish landrace Armuña, and the Tunisian cvs. Nefsa, and Nsir, all being Mediterranean types. At each location, a randomized complete block design with three replications was used. The experimental unit consisted of 1-m^2 plots with three replications. Each plot consisting of three 1-m long rows, separated by 0.33 m, with 10 plants per row. Sowing took place by middle December each season, according to local practice. Weeds were controlled by hand weeding. Days to flowering (dtf) was estimated by weekly recording the date in which 50% of the plants of each plot had at least one fully opened flower. Number of emerged broomrape plants per row were recorded and referred as number of broomrapes per lentil plant (Oc/pl). Attention was paid to record presence and to quantify naturally occurring pests and disease. The harvest of the plants took place by late April, May, depending on the environment. Harvested plants were threshed and seed yields recorded.

Table 1. Lentil accessions included in the study.

Accession	Origin/Derived from Accession no.
R4	ILL5755
R5	ILL6002
R7	ILL6258
R14	ILL7517
R17	ILL8707
R19	ILL9903
S6	ILL10074
S7	ILL10079
S8	ILL10170
S12	ILL10174
S14	ILL10273
S17	ILL10278
S23	ILL10648
S24	ILL10653
Armuña	Spanish landrace
Nefza	Tunisian cv.
Nsir	Tunisian cv.

Table 2. Description of the environments (combination of location and season) of the trials for the multi-environment study. Summary climatic data corresponding to each growing season are provided.

Environment	Season	Site, Level of Broomrape (*Oc*) Infestation	Soil type	Soil pH	Latit.	Longit.	Altit.	Average T_{max} (°C)	Average T_{min} (°C)	Rain (mm)
Cam-08	2007–2008	Campillo: low *Oc*	Vertisol	7.5–8	37°20′ N	4°51′ W	461	18.0	6.8	264
Cor-09	2008–2009	Córdoba: high *Oc*	Cambisol	6.5–7	37°50′ N	4°50′ W	90	19.2	6.9	279
Cor-10	2009–2010	Córdoba: high *Oc*	Cambisol	6.5–7	37°50′ N	4°50′ W	90	20.1	8.4	1053
Cor-11	2010–2011	Córdoba: high *Oc*	Cambisol	6.5–7	37°50′ N	4°50′ W	90	22.5	10.3	513
Cor-12	2011–2012	Córdoba: high *Oc*	Cambisol	6.5–7	37°50′ N	4°50′ W	90	20.8	5.9	163
Esc-08	2007–2008	Escacena: high *Oc*	Fluvisol	7–7.5	37°25′ N	6°15′ W	88	20.1	9.3	391
Esc-09	2008–2009	Escacena: high *Oc*	Fluvisol	7–7.5	37°25′ N	6°15′ W	88	20.3	8.8	252
Esc-10	2009–2010	Escacena: high *Oc*	Fluvisol	7–7.5	37°25′ N	6°15′ W	88	19.2	9.4	1206
Esc-11	2010–2011	Escacena: high *Oc*	Fluvisol	7–7.5	37°25′ N	6°15′ W	88	21.6	11.4	534
Esc-12	2011–2012	Escacena: high *Oc*	Fluvisol	7–7.5	37°25′ N	6°15′ W	88	20.5	9.1	134
Esc-13	2012–2013	Escacena: high *Oc*	Fluvisol	7–7.5	37°25′ N	6°15′ W	88	19.4	9.2	411
Tom-08	2007–2008	Tomejil: low *Oc*	Vertisol	7–7.5	37°30′ N	5°57′ W	12	21.7	8.7	399
Tom-09	2008–2009	Tomejil: low *Oc*	Vertisol	7–7.5	37°30′ N	5°57′ W	12	21.3	7.1	219

2.2. Statistical Analysis

2.2.1. Variances Analyses

A combined ANOVA for randomized complete-block designs was carried out using SAS® 9.3 (SAS Institute Inc., Cary, NC, USA) for all traits. A mixed model procedure (PROC MIXED) was then fitted by considering genotype (G) as fixed effects; and environment (E), G × E and block as random effects using REML algorithm. Prior to each ANOVA, tests for normality and homogeneity of variance were conducted for each dependent variable. The normality was tested with the UNIVARIATE procedures which have the NORMAL option to produce four test of normality, whereas, with the statement "REPEATED/GROUP=;" option of PROC MIXED we tested for homogeneity of variance among the specified variables. In all cases there was a good fit to the normal distribution and to provide the evidence of acceptance of equality of variances.

2.2.2. HA-GGE Biplots

HA-GGE biplot takes into consideration any heterogeneity among environments by giving weights to the test environments proportional to their root square heritability and is therefore, appropriate for visual evaluation of the test environments and genotypes [11–13]. Analyses were made with the SAS® 9.3 (SAS Institute Inc., Cary, NC, USA) program developed by Burgueño et al. [14], to graph GGE biplots. The target environment axis is represented by a corresponding straight line drawn through the biplot origin and the Target Environment Axis abscissa (TEAa) defines the mean ordinates of all environments in the biplot. Genotypes located on the polygon vertices reveal the best or the poorest for a particular environment.

2.2.3. Non-Metric Multi-Dimensional Scaling Ordination (NMDS)

In order to assess their influence on grain yield, numbers of broomrapes per plant, days to flowering, and 11 climate variables were subjected to non-metric multi-dimensional scaling ordination (NMDS) [15]. These climate variables were obtained from the Andalusian Network of Agro-climatic Information [16], and included average maximum and minimum temperature, and accumulated rain during pre-flowering, at flowering and post-flowering period plus average maximum and minimum temperature in post-flowering stage and number of days with maximum temperature higher than 30 °C. To reduce the probability that the result of the NMDS analysis would reflect a local stress minimum rather than the overall minimum, we repeated the NMDS analysis 20 times, each time starting from a different random configuration, and selected the two-dimensional solution with the lowest stress. Analysis was made by PAST software (Version 4.07) [17].

2.2.4. Heat Tolerance Indices

Seven heat tolerance indices were calculated by the given formulae:

Geometric mean productivity (GMP) = $\sqrt{(Ypi \times Ysi)}$ [18]
Yield index (YI) = Ysi/Ys [19]
Mean productivity (MP) = (Ypi + Ysi)/2 [20]
Stress susceptibility index (SSI) = (1 − (Ysi/Ypi))/SI [21]
Stress tolerance index (STI) = (Ypi × Ysi)/Y^2 p [18]
Harmonic mean (HARM) = 2 (Ypi × Ysi)/(Ypi + Ysi) [22]
Heat tolerance index (HTI) = (Y^2 si/Ypi) × (Yp/Y^2 s) [23]

Ysi and Ypi are the mean grain yield of individual genotype in heat stress (HS) and non-HS conditions, respectively. SI is stress intensity, where SI = 1 − (Ys/Yp); Ys = total mean grain yield of all genotypes in HS condition; Yp = total mean grain yield of all genotypes in non HS condition. Tom-09 was selected as the harsh environment for heat (Ys), as it was free of broomrape, so yield penalty could be ascribed to high temperature. Esc-08 was selected as non-stress environment (Yp) as there was little broomrape and no heat damage.

Higher values of MP, GMP, HARM, YI, STI, and HTI and lower values of SSI are indicative of higher tolerance to the stress [18–24]. Principal component analysis (PCA) of heat tolerance indices and genotypes were calculated using R stats package.

3. Results

Combined analysis of variance on seed yield, broomrape infection, and date flowering from the 13 environments showed that the fixed effects, genotype (G), was significant ($p < 0.0001$; Table 3). The random effects environment (E), block(environment) (Block(E)) and genotype by environment interaction (G × E) were all also significant.

Table 3. Combined environment variance analysis of seed yield (kg/ha), broomrape infection (Oc/plant), and flowering date (dtf) of a lentil performance trials, consisting of 17 genotypes (G) grown in 13 environments (E), from 2008 to 2013.

	Random Effects	Estimate	Standard Error	Z value	Pr > Z
Grain yield (kg/ha)	E	45084	20177	2.23	0.0127
	Block(E)	4073	1915	2.13	0.0167
	G × E	34344	5238	6.56	<0.0001
	Residual	47537	3296	14.42	<0.0001
	Fixed Effects	Numerator df	Denominator df	F value	Pr > F
	G	16	192	5.3	<0.0001
	Random Effects	Estimate	Standard Error	Z value	Pr > Z
Broomrape infection (Oc/plant)	E	0.0483	0.0247	1.95	0.0255
	Block(E)	0.0080	0.0035	2.28	0.0113
	G×E	0.0078	0.0032	2.39	0.0084
	Residual	0.0524	0.0041	12.65	<0.0001
	Fixed Effects	Numerator df	Denominator df	F value	Pr > F
	G	16	144	5.18	<0.0001
	Random Effects	Estimate	Standard Error	Z value	Pr > Z
Dates to flowering (dtf)	E	28.19	12.22	2.31	0.0105
	Block(E)	0	.	.	.
	G × E	23.57	3.05	7.73	<0.0001
	Residual	18.17	1.22	14.87	<0.0001
	Fixed Effects	Numerator df	Denominator df	F value	Pr > F
	G	16	192	30.63	<0.0001

Average yield over accessions and environments was 445 kg/ha, with great differences across environments. Average yields were higher than 700 kg/ha (Table 4) at Cor-09, Esc-08 and Esc-09, whereas they were lower than 350 kg/ha at Cam-08, Cor-11, Cord-12, Esc-10, Tom-08, and Tom-09, confirming the high effects of the E and of G*E, higher than those of G on seed yield (Table 3). The accession performing better across environments was cv. Nsir with, average 770 kg/ha, ranging between 370 and 1854 kg/ha in different environments. These results are rather in line with the reported yield of this cv. in optimal conditions in Tunisia of *circa* 1500 kg/ha where long-term average seed yield of lentil is 600 Kg/ha [25]. Nsir was followed by lines S23, S17, S12, R7, and S6, with average yields over environments higher than 500 kg/ha, higher than the local check Armuña (average across environments 423 kg/ha). Average yields achieved are in line with the average regional yields of 825 kg/ha in Castilla-la-Mancha and 717 kg/ha in Andalusia [26].

Table 4. Mean seed yield (kg/ha) of 17 lentil accessions grown at 13 location–year environments.

Accession	Cam-08	Cor-09	Cor-10	Cor-11	Cor-12	Esc-08	Esc-09	Esc-10	Esc-11	Esc-12	Esc-13	Tom-08	Tom-09	Mean	SE
Nsir	635	901	1854	370	437	1125	933	921	871	567	387	510	493	770	71
S23	476	1450	358	139	150	966	1413	516	775	938	247	470	461	643	85
S17	424	1215	684	163	170	1254	965	332	342	982	251	630	635	619	73
S12	416	794	145	135	144	974	1174	139	829	1504	487	404	385	579	76
R7	128	970	669	108	105	1204	577	174	847	602	264	625	606	529	66
S6	342	748	227	89	93	892	1127	162	1189	429	429	406	388	502	68
S14	345	800	325	105	84	888	917	269	480	306	596	318	318	442	55
Armuña	268	608	1311	325	225	807	640	261	171	276	119	283	200	423	54
R17	293	395	420	74	85	594	516	166	396	978	836	303	261	409	53
Nefza	216	609	829	53	41	741	643	244	311	276	409	244	204	371	43
S8	234	292	469	76	76	771	536	588	558	233	618	203	164	371	44
S7	183	847	339	117	109	780	546	355	164	239	336	330	272	355	42
S24	313	315	312	62	69	579	1017	202	591	169	429	163	146	336	50
R5	157	549	589	108	72	733	581	257	191	211	220	209	142	309	36
R14	174	557	120	73	66	806	265	166	725	250	342	218	141	300	48
R4	162	454	541	68	98	553	291	108	575	231	689	237	135	319	37
R19	146	559	404	87	95	533	444	240	389	208	356	174	163	292	37
Mean	289	710	564	127	125	835	740	300	553	494	412	337	301	445	
SE	20	50	67	22	23	31	51	47	67	59	44	21	28	15	

In HA-GGE biplots, the further to the right to TEA_0 axis is the accessions, the higher is the value for the trait; and the closer the accession is to TEA_a the more stable is the trait. We can see therefore in Figure 1 that the accessions with higher average grain yield (more to the right: Nsir, S17, S23, Armuña, and S12) are however little stable over environments (far from TEA_a). Nsir and Armuña appear to be more adapted to Córdoba environments, and S17 and S23 to Escacena and Tomejil, although with exceptions in some years, further complicating the interaction. S7, Nefza, and R14 are the accessions with more stable yield, although they are below the average.

There was a large variation in flowering date among the studied accessions, with dtf ranging from an average of 79 in the earliest R14 accession, to 113 in the latest Armuña (Table 5). Biplot analysis (Figure 2) shows how precocity of some accession is more influenced by the environment than others, with R4, S24, and R7 being more stable across environments (closer to TEA_a) and others, either early (R14 and R17) or late (Armuña and Nsir) being more affected by the environment (further to TEA_a).

The most significant biotic constraint recorded in some of the environments was broomrape (Table 6), with little or negligible incidence of any other pest or disease observed (data not shown). There was high variation on average broomrape infection over environments, with no infection at Campillo and Tomejil, but high at Córdoba and Escacena sites, with levels of infection affected by the year due to temperature and rain influence on infection. Like this, average infection over accessions at Córdoba was highest in 2011 (0.87 Oc/pl) and lowest in 2010 (0.11 Oc/pl). At Escacena, average infection over accessions was overall lower and more stable, being highest in 2013 (0.38 Oc/pl) and lowest in 2008 (0.09 Oc/pl). Overall infection of accessions across environments was rather high (0.26 Oc/pl) indicative of the high problem that broomrape represents in the area and of the limited resistance available in breeding lines. Still, accessions S14, R17, R7, R4, and S23 displayed an overall reduced infection (<0.2 Oc/pl), although could still be severely infected in very conducive environments such as Cor-11, with more than 0.4 Oc/pl.

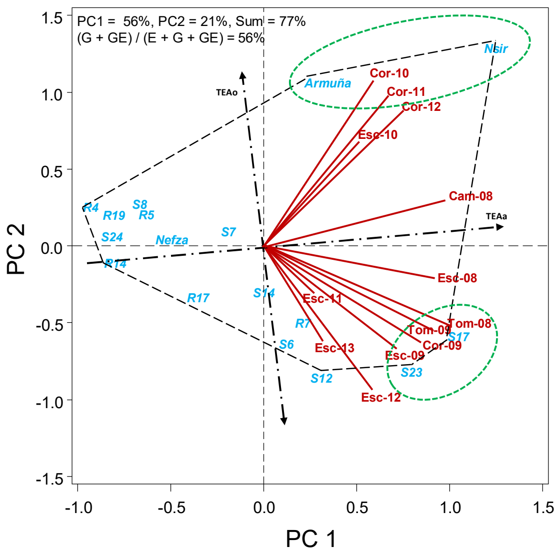

Figure 1. HA-GGE biplot based on the seed yield (kg/ha) of 17 lentil accessions grown at 13 field-year environments, from 2008 to 2013.

Table 5. Days to flowering (dtf) of 17 lentil accessions grown at 13 location–year environments.

Accession	Cam-08	Cor-09	Cor-10	Cor-11	Cor-12	Esc-08	Esc-09	Esc-10	Esc-11	Esc-12	Esc-13	Tom-08	Tom-09	Mean	SE
Armuña	115	121	122	125	99	111	115	102	107	109	108	115	117	113	1.2
Nsir	105	110	106	118	96	102	105	94	103	95	100	107	107	104	1.1
Nefza	100	105	102	113	90	97	99	90	99	87	94	101	102	98	1.2
S8	97	95	106	120	90	94	90	84	93	91	90	99	96	96	1.5
S7	95	95	106	119	93	90	90	78	86	87	87	98	92	94	1.7
R4	95	96	106	113	77	93	95	75	92	96	90	98	96	94	1.7
R19	96	94	106	116	90	90	88	76	98	86	88	97	95	94	1.8
R7	91	91	92	88	79	90	86	80	99	86	88	91	90	89	1.0
S12	91	95	99	98	85	87	84	74	95	85	85	92	88	89	1.4
S17	91	95	101	90	83	88	84	72	101	82	85	94	86	89	1.4
S14	88	91	92	88	79	89	84	87	90	84	89	88	86	87	0.7
S23	87	91	92	88	81	87	84	74	96	84	85	90	86	87	1.0
S24	88	91	98	88	82	87	90	76	87	85	86	87	86	87	0.9
R5	86	91	92	77	67	89	85	88	91	81	87	87	88	85	1.3
S6	87	95	101	88	80	84	84	61	101	85	82	88	86	86	1.8
R17	82	85	76	77	65	84	84	75	89	78	83	82	86	81	1.4
R14	81	89	59	78	74	81	84	75	74	81	80	81	86	79	1.4
Mean	93	96	97	99	83	91	90	80	94	87	89	94	93	91	
SE	1.2	1.2	2.0	2.4	1.3	1.0	1.2	1.8	1.3	1.0	1.0	1.2	1.2	0.4	

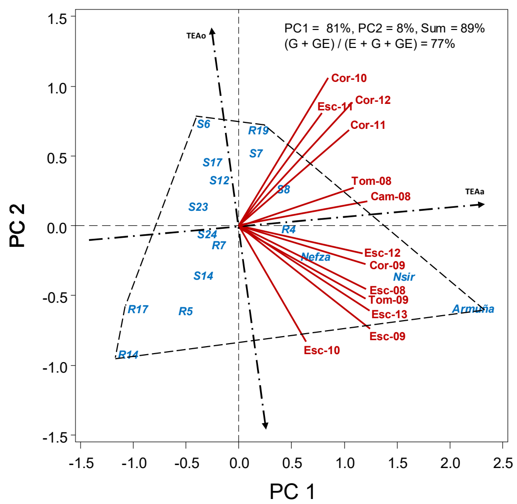

Figure 2. HA-GGE biplot based on days to flowering (dtf) of 17 lentil accessions grown at 10 field–year environments, from 2009 to 2013.

Table 6. Mean broomrape infection (Oc/plant) of 17 lentil accessions grown at 13 location–year environments.

Accession	Cam-08	Cor-09	Cor-10	Cor-11	Cor-12	Esc-08	Esc-09	Esc-10	Esc-11	Esc-12	Esc-13	Tom-08	Tom-09	Mean	SE
Armuña	0.00	0.35	0.33	1.03	0.72	0.41	0.96	0.41	0.67	0.67	0.62	0.00	0.00	0.49	0.10
Nsir	0.00	0.20	0.07	0.99	0.65	0.19	0.81	0.53	0.13	0.41	0.43	0.00	0.00	0.34	0.10
S7	0.00	0.11	0.13	1.07	0.73	0.20	0.08	0.62	0.63	0.47	0.42	0.00	0.00	0.34	0.08
R5	0.00	0.26	0.10	1.01	0.52	0.20	0.87	0.06	0.47	0.47	0.51	0.00	0.00	0.34	0.08
S8	0.00	0.21	0.23	1.02	0.68	0.07	0.11	0.26	0.80	0.43	0.27	0.00	0.00	0.31	0.08
Nefza	0.00	0.06	0.07	0.94	0.28	0.06	0.67	0.11	0.17	0.32	0.43	0.00	0.00	0.25	0.07
S6	0.00	0.18	0.23	1.24	0.37	0.04	0.00	0.33	0.17	0.22	0.44	0.00	0.00	0.25	0.08
S12	0.00	0.11	0.10	0.90	0.46	0.03	0.08	0.37	0.43	0.36	0.46	0.00	0.00	0.25	0.07
S24	0.00	0.15	0.13	0.73	0.77	0.04	0.05	0.41	0.33	0.25	0.18	0.00	0.00	0.24	0.07
S17	0.00	0.08	0.03	1.24	0.33	0.02	0.19	0.11	0.23	0.23	0.52	0.00	0.00	0.23	0.09
R19	0.00	0.09	0.20	0.97	0.39	0.05	0.39	0.13	0.37	0.24	0.22	0.00	0.00	0.23	0.07
R14	0.00	0.10	0.03	0.56	0.26	0.06	0.08	0.11	0.47	0.28	0.56	0.00	0.00	0.21	0.06
S23	0.00	0.09	0.13	0.88	0.33	0.08	0.01	0.22	0.27	0.19	0.35	0.00	0.00	0.20	0.06
R4	0.00	0.03	0.03	0.63	0.53	0.03	0.36	0.09	0.20	0.26	0.37	0.00	0.00	0.19	0.06
R7	0.00	0.00	0.03	0.59	0.24	0.05	0.69	0.20	0.17	0.23	0.10	0.00	0.00	0.18	0.09
R17	0.00	0.12	0.00	0.59	0.48	0.06	0.16	0.11	0.07	0.15	0.28	0.00	0.00	0.15	0.06
S14	0.00	0.06	0.03	0.41	0.30	0.01	0.17	0.06	0.20	0.12	0.28	0.00	0.00	0.13	0.03
Mean	0.00	0.13	0.11	0.87	0.47	0.09	0.33	0.24	0.34	0.31	0.38	0.00	0.00	0.26	
SE	0.00	0.02	0.02	0.04	0.06	0.01	0.06	0.04	0.04	0.02	0.04	0.00	0.00	0.02	

Broomrape response of accessions over environments is further shown by HA-GGE biplot (Figure 3) in which accessions to the left of TEAo axis have lower broomrape infection, this being more stable as the closer they are to TEAa axis. Like this, accessions S14, R17, and R4 are the more resistant and stable, with R7 being also among the more resistant ones, but being more affected by the environment.

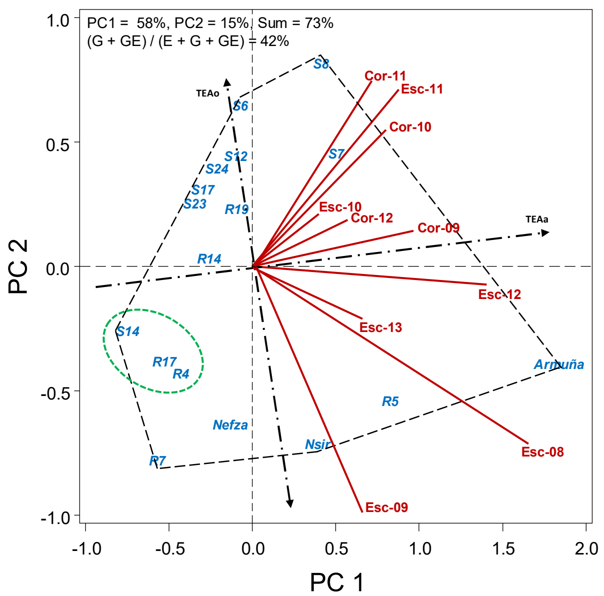

Figure 3. HA-GGE biplot based on the number of broomrape per plant of 14 lentil accessions and 3 elite cultivars grown at 13 field-year environments, from 2008 to 2013.

Correlations between traits and non-metric multi-dimensional scaling ordination (NMDS) (Table 7 and Figure 4) show that grain yield was little affected by precocity and negativity, but not significantly by broomrape infection. Temperature was the most influential parameter on grain yield, whereas rain had little effect. Mild temperatures at pre-flowering (higher T_{min}) favored yield, whereas high temperatures at grain filling were detrimental. The number of days with $T_{max} > 30$ °C during grain filling was the parameter with higher (negative) correlation ($r^2 = -0.72$ **) with grain yield.

Table 7. Pearson correlations among accessed traits.

	Grain Yield	Dtf	Broomrape	Rain	T_{min} Preflowering	T_{Aver} Filling	Numb. Days T_{max} > 30 °C
Grain yield		0.19	−0.36	0.02	0.42	−0.58 *	−0.72 **
Dtf			−0.07	−0.06	0.08	−0.15	−0.25
Broomrape				0.01	−0.15	0.64 *	0.46

*, ** Significant at the 0.05 and 0.001 level of probability, respectively.

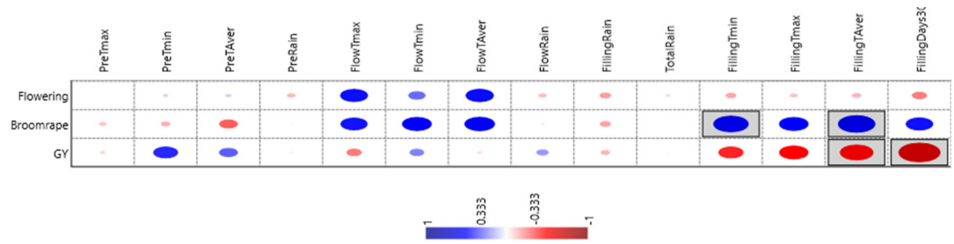

Figure 4. Graphical representation of the effects of climatic parameters on flowering date, broomrape infection, and grain yield. Size of the dot indicates its effect on the trait, blue when positive, red when negative. Only encircled ones are statistically significant.

Table 8 shows the averages at each environment of the parameters more influential on yield. The lowest yields were achieved at the environments with more than 5 days with T_{max} > 30 °C during grain filling (Cor-11, Cor-12, Esc-10, and Tom-09). If this happened in environments with high broomrape infection (Cor-11 and Cor-12), then the consequences were catastrophic.

Table 8. Description by environment of the major drivers on yield: levels of broomrape infection, T_{min} at pre-flowering, T_{aver} at flowering, T_{aver} at grain filling, and number of days with T_{max} > 30 °C at grain filling.

Environments	GY Mean	Broomrape Mean	T_{min} at Pre-Flowering	T_{aver} at Flowering	T_{max} at Grain Filling	Num. Days T_{max} > 30 °C
Cam-08	289	0.00	4.8	9.8	20.6	0
Cor-09	710	0.13	4.1	8.8	25.5	2
Cor-10	564	0.11	5.4	10.1	24.1	2
Cor-11	127	0.87	5.6	10.0	28.9	8
Cor-12	125	0.47	1.2	7.7	29.1	9
Esc-08	835	0.09	8.0	12.6	22.2	0
Esc-09	740	0.33	6.2	10.3	24.8	2
Esc-10	300	0.24	7.3	10.9	27.9	7
Esc-11	553	0.34	7.3	11.1	26.1	3
Esc-12	494	0.31	5.2	10.5	21.7	2
Esc-13	412	0.42	6.7	10.6	24.3	2
Tom-08	337	0.00	6.8	12.8	24.2	3
Tom-09	301	0.00	4.9	10.2	28.1	8

The stress value for NMDS was low (0.051), indicative a good fit for this kind of analysis (i.e., little distortion between the original data and the scaling axes). NMDS Coordinate 1 showed a separation of environments for its yield with lowest at the positive end, and highest ones, at the negative end (Figure 5). NMDS analysis confirmed the results of the correlation table, showing number of days with T_{max} > 30 °C and T_{ave} at grain filling were the climatic factors more detrimental to grain yield (longer green vectors to the right). Broomrape infection also had a detrimental effect on yield. On the contrary, mild temperatures before and during flowering (higher T_{min} and T_{aver}) favored yield although the effect was smaller, as shown by the smaller length of the vectors. Rain had little effect.

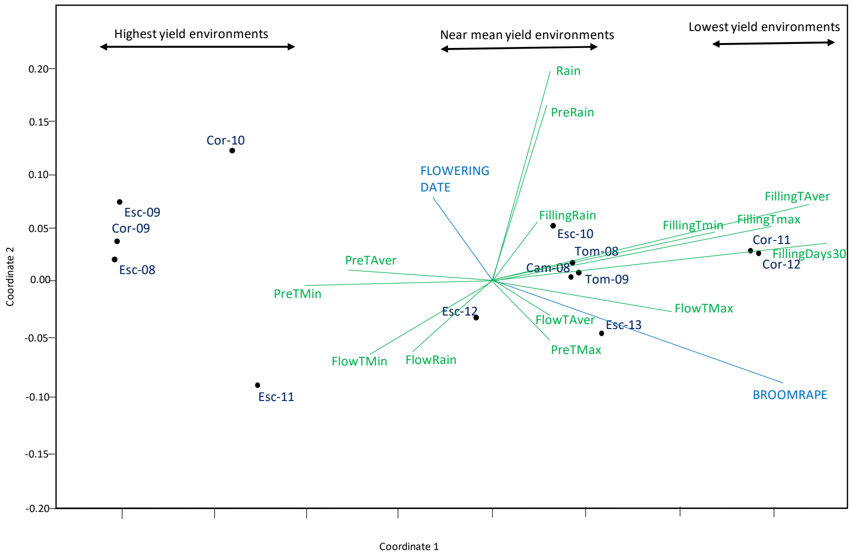

Figure 5. NMDS combined analysis of climate variables including: maximum temperature (T_{max}), minimum temperature (T_{min}) and rain during different growing stages [pre-flowering (Pre), flowering (Flow), grain filling (Filling)], and number of days with $T_{max} > 30$ °C at grain filling and traits assessed on grain yield.

Table 9 shows the grain yield at this no stress (Esc-08) and heat stressed (Tom-09) environments and the calculated heat tolerance indices. According to the authors proposing the various indexes, desirable accessions would be those with higher value for MP, GMP, HARM, YI, and STI indices, and low for SSI, indicative of heat sensitivity.

Table 9. Grain yield under stress and non-stress environments, and heat stress tolerance indices of the studied accessions.

Accession	Ys	Yp	GMP	YI	MP	SSI	STI	HARM	HTI
S17	635	1254	892	2.11	944	0.79	1.25	843	2.86
R7	606	1204	854	2.01	905	0.80	1.14	806	2.71
Nsir	493	1125	745	1.64	809	0.90	0.87	686	1.92
S23	461	966	668	1.53	714	0.84	0.70	625	1.96
S12	385	974	613	1.28	680	0.97	0.59	552	1.36
S6	388	892	589	1.29	640	0.91	0.54	541	1.51
S14	318	888	531	1.06	603	1.03	0.44	468	1.01
S7	272	780	461	0.90	526	1.05	0.33	403	0.84
Armuña	200	807	402	0.66	504	1.20	0.25	321	0.44
R17	261	594	394	0.87	428	0.90	0.24	363	0.89
Nefza	204	741	389	0.68	473	1.16	0.24	320	0.50
S8	164	771	355	0.54	467	1.26	0.20	270	0.31
R14	141	806	337	0.47	474	1.32	0.18	240	0.22
R5	142	733	323	0.47	438	1.29	0.16	238	0.25
R19	163	533	295	0.54	348	1.11	0.14	250	0.44
S24	146	579	290	0.48	362	1.19	0.13	233	0.33
R4	135	553	273	0.44	344	1.21	0.11	217	0.29

Ys = grain yield of individual genotype in the selected stress environment (Tom-09); Yp = grain yield in the non-stress environment (Esc-08); GMP = geometric mean productivity; YI = yield index; MP = mean productivity; SSI = stress sensitivity index; STI = stress tolerance index; HARM = harmonic mean; HTI = heat tolerance index.

In order to determine the desirable selection indices for stress tolerance, correlation coefficient among all indices were calculated (Table 10). There was positive and high significant correlation between Ys and Yp (r = 0.83 ***), suggesting that a high potential yield under optimum condition results in high yield also under stress condition. High and significant positive correlation of grain yield (Yp) under normal condition and Ys under heat stress with all other indices viz., HARM, MP, STI, YI, HTI, and GMP were recorded, which ranged from 0.70 to 0.99 (Table 10), except SSI which exhibited a significant negative correlation with Ys. The significant and positive correlation of Yp and HARM, MP, STI, YI, HTI, and GMP indices showed that these criteria were more effective in identifying high-yielding cultivars under different stress conditions. The low correlation coefficients of Ys and Yp with SSI (−0.49 * and 0.05, respectively) indicates that any of the other indices studied (HARM, MP, STI, YI, HTI, and GMP) were better predictor of Yp and Ys than SSI.

Table 10. Correlation coefficient of Ys, Yp, and heat tolerance indices.

	Ys	Yp	HARM	MP	SSI	STI	YI	HTI
Yp	0.83 ***							
HARM	0.99 ***	0.88 ***						
MP	0.93 ***	0.98 ***	0.96 ***					
SSI	−0.49 *	0.05	−0.42	−0.15				
STI	0.98 ***	0.87 ***	0.99 ***	0.95 ***	−0.41			
YI	0.98 ***	0.83 ***	0.99 ***	0.93 ***	−0.50 *	0.99 ***		
HTI	0.97 ***	0.70 **	0.95 ***	0.84 ***	−0.65 **	0.95 ***	0.98 ***	
GMP	0.98 ***	0.93 ***	0.99 ***	0.99 ***	−0.31	0.98 ***	0.98 ***	0.91 ***

*, **, *** Significant at the 0.05, 0.001 and 0.0001 level of probability, respectively.

Different indices gave different values, but the general picture did not change, with a number of accessions (S17, R7, Nsir, S23, S12, and S6) at the top of the rankings for any index, and others at the bottom (R5, R19, S24, and R4). As no single index is perfect we performed a biplot of principal component analysis (PCA). The relationship among the genotypes and heat tolerance indices, are graphically depicted by PCA analysis. The PCA reduced all the indices into two components. Principal component analysis biplot of Ys, Yp and heat indices (Figure 6) revealed the correlation coefficient among them [27]. The first component (PC1) explained 95.2% of the total variation, being positively correlated with Ys, Yp, MP, GMP, HARM, STI, YI, and HTI (Figure 6). The second component (PC2) explained 4.4% of the total variation and correlated negatively with SSI (stress susceptibility index). In summary, biplot graph (Figure 6) confirmed correlation analysis (Table 10). Accessions with high positive PC1 (S17, R7, Nsir, S23, S12, S6, and S14) are the more productive both under stress and non-stress conditions (Figure 6).

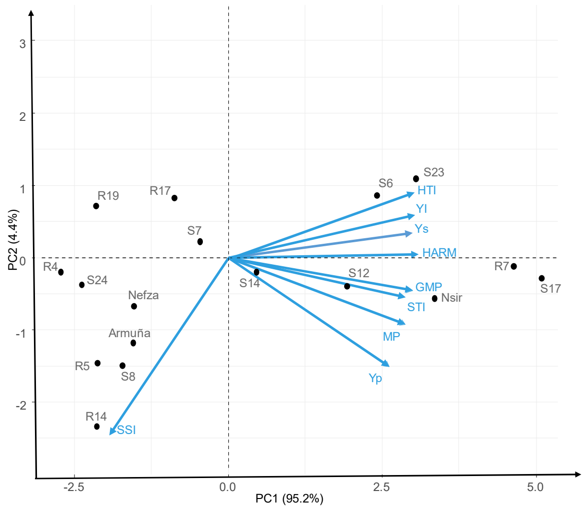

Figure 6. Biplot of principal component analysis of 17 lentil lines for productivity and stress indices.

4. Discussions

Winter sowings are expected to provide greater yield potential than spring sowings in dry and warm areas by taking advantage of winter rains and escaping drought and excessive heat at late spring. Lentil is known to be highly sensitive to high temperatures at the grain-filling stage when exposure to heat shock for several days affects many physiological processes leading to substantial yield losses [9,28–31] which can be avoided by early sowings. Although early sown lentils could suffer from cold in some areas [3–6], this is not the case in the area of study where winters are very mild and we did not observe a significant effect of low temperatures at any plant stage on seed yield. On the contrary, our results show that even at December sowings, high temperature at grain-filling stage was the factor most detrimental on grain yield. This was followed by broomrape infection, with precocity and rain having little effect. Tolerance to heat stress appears therefore as a top lentil breeding priority for the region, as it seems to be in other areas for spring-sown lentils [3,9,28–32]. The most critical period for lentil yield has been established between 50 and 126 degree days after flowering, at the time of pod formation [33]. Site-specific combinations of sowing date and phenology are necessary to reduce the likelihood of excessive heat to coincide with this critical period.

Some variation in response to heat stress appears to be available as assessed by a number of indexes [29]. These indexes have been mainly used to identify tolerance against abiotic stresses in a number of crops [34]. Our results confirm variation for stress tolerance index with S17 and R7 accessions outstanding for all indices used, followed by S23, Nsir, S6, and S12. High correlation was observed among indices, in agreement with previous reports on tolerance to high temperature [34,35] or to drought [36,37]. Although heat stress tolerance is a complex trait, significant progress is being achieved in deciphering the genetics and pathways underlying the heat stress tolerance in lentil [38–40].

Early sowings are known to increase the risk of broomrape infection [41–44] that is acknowledged as a major constraint for most legume crops in the Mediterranean basin [45]. Our results confirm this risk, but ranking second after the one of scenarios of excessive temperatures at grain filling. The use of very early maturing cultivars has been suggested to escape *O. crenata* in several legumes as precocity use to be correlated with reduced infection [46,47]. This could also help to escape from high temperatures. However, we did not find such correlation in the lentil germplasm studied, which is in agreement with a previous field study [48]. No complete resistance to broomrape was identified, but there was a significant variation in the level of infection, with accessions S14 and R17 being the more resistant across environments, which might be further exploited in breeding.

We conclude that the lentil has potential for reintroduction into rain-fed farming systems in Mediterranean Basin, but expanding cultivation to warmer areas requires specific breeding. Contrary to traditional lentil cultivation areas where frost can be a problem in early sowings, high temperatures during grain filling and broomrape infection appear as the two most serious constraints in Southern Spain, the combination of both being dramatic. There is variation for tolerance to both stresses, offering potential for breeding.

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