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Lentil Cultivar Evaluation in Diverse Organic Mediterranean Environments

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Abstract: Lentil (*Lens culinaris* Medik.) production faces challenges due to shifting environmental conditions, potentially leading to a transition towards cooler or highland Mediterranean environments. This study assessed the responses of five lentil genotypes across five diverse locations (L1–L5) managed under organic cropping systems over two seasons, focusing on key parameters including seed yield (SY), crude protein (CP), cooking time (CT), seed loss percentage (SL), and yield loss per hectare (YL) caused by bruchid (*Bruchus* sp.). Excessive seasonal rainfall (500 mm), low winter temperatures (−17.9 °C), bruchid SL, and spring sowing were identified as crucial, particularly in challenging environments like highlands. Genotype selection was highlighted as essential for balancing yield and stability, with the small-seeded cultivar 'Dimitra' demonstrating lower YL due to bruchid. Additionally, increased CP was noted in response to heightened bruchid infestations. Specific recommendations were proposed for different environments: In productive lowland areas with low bruchid pressure and high CTs (L1), prioritizing cultivars like 'Samos', 'Dimitra', and 'Thessalia' enhances quality. Locations with high bruchid populations (L4) were not favored organic production but can serve as genetic resistance screening sites. High-elevation environments (L3, L5) proved significantly less productive, underscoring the requirement for earlier and winter-hardy cultivars. These insights guide lentil cultivation, emphasizing the need for tailored breeding strategies adaptable to changing environments.

Keywords: *Bruchus*; cooking time; cool season legumes; crude protein; *Lens culinaris* Medik; pulses; seed yield; organic farming; varieties

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1. Introduction

Lentil (*Lens culinaris* Medik.) is a globally cultivated ancient pulse that is significant in organic agriculture due to its alignment with sustainability and regenerative farming principles. Rich in protein, minerals, dietary fibers, folate, and vitamins, lentils are vital for human and animal nutrition, promoting food security and health [1–3]. They are a key component of the Mediterranean diet, known for its health benefits and association with reduced risks for chronic diseases and cancer [4,5].

Organic lentil farmers face a significant challenge posed by seed bruchids, small beetles of the subfamily *Bruchinae* whose larvae develop within a single seed. Major species such as *Bruchus lentis* Frölich, *Bruchus signaticornis* Gyllenhal, and *Bruchus ervi* Frölich are widely recognized as field pests in lentil cultivation across Europe, Africa, and Asia [6]. The infestation and development process of bruchids in the field has been detailed by Stevenson et al. [7]. Upon hatching, young larvae enter developing seeds, consuming part of the cotyledons over six weeks before exiting through an exit hole in the pod. The economic damage caused by emerging bruchid adults can be significant regarding seed weight loss or quality degradation [8]. Organic farmers employ integrated pest management strategies such as crop rotation and interplanting with less attractive crops to disrupt the pests' life cycle naturally, aligning with organic farming principles [6].

Untreated fungus disease damage in lentil crops can result in substantial yield losses, reaching as high as 70% [9–11]. Natural pesticides show promise in mitigating the chemical control's adverse effects [12]. Additionally, introducing resistant or nearly resistant lentil cultivars, a form of host-plant resistance, is a cost-effective strategy [10,13,14]. However, developing lentil varieties specifically tailored for organic cultivation is still pending [15]. Consequently, organic farmers often rely on conventional breeding varieties, which may lack traits optimized for organic farming but still demonstrate broad adaptability [15,16].

The cooking time of lentils is crucial, affecting nutrient digestibility and consumer satisfaction. Longer cooking times are inconvenient and costly in terms of electricity or fuel for processors and consumers alike [17]. Different lentil cultivars can have varying cooking times due to differences in seed size, seed coat anatomy, and cotyledon color; green-seeded lentils tend to boil faster than red-seeded varieties [18,19].

Lentil production in Greece fails to meet consumption demands, leading to reliance on imports due to factors like drought-induced yield declines. In 2020, lentil cultivation expanded to 11,500 hectares, with a total production of 13,538 tons. However, drought caused the average yield of lentils to decline to 1.17 t h^{-1} [20]. Lentil is a versatile crop, thriving in various climates and soils without requiring nitrogen fertilizers due to its ability to fix atmospheric nitrogen (N_2) [21,22]. However, the success of lentil cultivation in Greece is influenced by a combination of edaphic (soil-related) and climatic conditions [23]. Given the projections of more frequent drought events and higher temperatures in the Mediterranean region due to climate change, there is a pressing need to explore strategies to adapt lentil cultivation to these changing conditions [24]. One such approach could be relocating winter legume production, including lentils, to higher-elevation plains where cooler temperatures and potentially higher water availability during spring may mitigate some of the impacts of climate change [25]. However, the transition to high-elevation plains poses its challenges, such as the harsh winter conditions that can significantly affect yields. Research indicates that yields of spring-sown lentils in winter frost-prone regions may be significantly lower (50–100%) than traditional autumn-sown lentils [26]. Additionally, the winter survival of lentils in such environments is influenced by various factors beyond frost tolerance, including waterlogging, root diseases, and ascochyta blight (*Ascochyta lentis*) [27]. The longstanding cultivation of lentils in the traditional lentil regions makes these regions possibly unsuitable for organic lentils due to high bruchid populations [28]. Overall, bridging the gap between environmental factors, lentil genetics, and organic farming practices is crucial for optimizing lentil cultivation in Mediterranean regions.

This study tested five lentil cultivars across five locations in Greece with diverse pedoclimatic conditions. The objectives of the study included: (i) Enhancing sustainable lentil cultivation in organic farming by studying genotype responses. (ii) Recommending ideal production sites to optimize yields and quality while reducing environmental risks. (iii) Offering insights for future breeding to enhance lentil resilience in regions facing similar climate challenges.

2. Materials and Methods

2.1. Locations and Experimental Management

The lentil cultivar trials were evaluated during the growing seasons 2018/2019 (referred to as 2019) and 2019/2020 (referred to as 2020). These assessments were conducted at five different locations distributed across Greece, identified by codes L1 to L5, as depicted in Figure 1. The locations are Orestiada (L1) in the region of Thrace (41°30′14″ N, 26°32′99″ E), Thessaloniki (L2) in the region of Central Macedonia (40°32′69″ N, 22°59′83″ E), Anatoliko (L3) in the region of Western Macedonia (40°33′09″ N, 21°44′46″ E), Larissa (L4) in Thessaly Central Greece (39°36′81″ N, 22°25′94″ E) and Domokos (L5) in the region of Sterea (39°1′13″ N, 22°19′74″ E).

Figure 1. Locations (L1–L5) of field trials conducted in organic environments across Greece.

The locations varied in terms of soil and weather conditions. Details are provided in Table 1. The soils of the tested locations are classified in the orders of *Fluvisols*, *Luvisols*, and *Vertisols* according to FAO soil system classification [29]. Briefly, *Fluvisols* are mainly young soils with little or no pedogenetic horizon development and are mainly found in alluvial deposits. Annual and woody crops are grown on these lands. *Luvisols* are developed on alluvial deposits with clayey horizons. They are productive soils for a wide range of crops. *Vertisols* are considered productive soils; however, due to high content of expanding clay minerals they must be properly managed to avoid constraints in productivity. Soils were fine-textured but varied across the locations: L1 is silty clay loam (SiCL), while L2 and L3 have a clay loam (CL) and L4 and L5 have a clayey (C) texture [30]. The soil pH was neutral or slightly alkaline, ranging from 7.0 to 8.0 [31]. The electrical conductivity (EC) was low (<1.00 mS cm^{-1}, 25 °C) [32]. The soil organic matter (SOM) content was either medium (1.8–2.0%) or low (<1.5%) [33], and the calcium carbonate equivalent ($CaCO_3$) ranged from 1.2% to 4.5% [34]. Soils were either deficient (<10.0 mg P kg^{-1} soil, L1, L2, L4) or moderately sufficient (10–20 mg kg^{-1} soil, L3, L5) in available phosphorus (P_{Olsen}) [35], with medium or mostly high cation exchange capacity (CEC > 25 $cmol_c$ kg^{-1}). The soil-extractable zinc measured by DTPA in locations L2, L3, and L4 was low, while Cu, Fe, and Mn were found to be high in the soil across all sites [36].

The climate in Greece is typically semiarid Mediterranean. According to the *Köppen-Geiger* climate classification [37,38], three types of climates are found in the study locations: L1 (Orestiada) and L5 (Domokos) feature a hot-summer Mediterranean climate (*Csa*), locations L2 (Thessaloniki) and L3 (Anatoliko) a humid subtropical climate (*Cfa*), while there is a combination of *BSk* (arid, steppe, cold)/*Csa* in L4 (Larissa). Regional climate variations are influenced by altitude.

Table 1. Pedoclimatic conditions of the 5 locations (L1–L5) organically cultivated with lentil across Greece over 2019 and 2020.

Location	Orestiada (L1)		Thessaloniki (L2)		Anatoliko (L3)		Larissa (L4)		Domokos (L5)	
Soil Order [1]	*Fluvisols*		*Fluvisols*		*Luvisols*		*Fluvisols*		*Vertisols*	
Soil texture	SiCL		CL		CL		C		C	
pH $_{(1:1)}$	7.3		8.0		7.2		7.8		7.0	
EC [2]	0.72		0.70		0.57		0.52		0.39	
SOM [3] %	2.0		1.3		2.0		1.8		1.4	
$CaCO_3$ %	2.0		4.5		1.3		1.2		1.5	
P_{Olsen} mg kg^{-1}	7.3		6.7		13.0		7.3		18.5	
CEC [4]	28.6		38.3		44.9		13.3		27.7	
Cu [5]	2.5		2.1		2.0		2.2		5.2	
Fe [5]	19		12		21		10		40	
Mn [5]	31		16		32		19		59	
Zn [5]	3.0		0.86		0.60		0.68		1.10	
B mg kg^{-1}	1.40		1.23		0.54		1.40		0.74	
Altitude (m)	26		5		624		77		570	
Year	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
PrS [6] (mm)	367.2	432.6	399.6	524.8	558.3	402.5	479.2	453.2	566.1	684.7
PrA-M [7] (mm)	118.3	182.8	107.8	168.4	144.7	158.4	72.0	99.0	81.8	186.4
Ts (°C) [8]	12.6	12.5	14.0	14.4	10.1	11.1	13.9	14.4	11.3	11.5
TA-M (°C) [9]	15.0	14.5	16.4	15.6	13.2	13.5	16.4	16.2	13.6	13.7
Tmin Jan (°C) [10]	0.7	−3.1	0.5	0.4	−5.9	−3.7	−0.2	−1.0	−2.2	−3.5
TDmin Jan (°C) [11]	−6.2	−9.5	−8.7	−6.1	−17.9	−9.4	−10.7	−6.1	−12.8	−10.5

[1] Soil class [29], [2] EC = Elec. Cond. (mS cm^{-1}) (25 °C), [3] SOM = Soil organic matter, [4] CEC = Cation Exch. Cap., (cmolc kg^{-1}), [5] DTPA-extractable trace elements mg kg^{-1}, [6] PrS = Prec. during the growing season (November to July), [7] PrA-M = Precip. April–May, [8] Season Avg. Ts (°C) = Average temp. in the growing season (November to July), [9] TA-M = Average temp. April–May, [10] Tmin Jan = Average min. temp. in January, [11] TDmin Jan = Daily record min. temp. in January.

In each location and trial, the daily mean air temperature and monthly precipitation during the growing seasons were recorded via a wireless automatic weather station (Pessl iMetos OEM Model-1, Weiz, Austria). In the 2019 growing season (November to July), the mean precipitation (PrS) for the five sites was 474.1 mm, ranging from 367.2 mm (L1) to 566.1 mm (L5), whereas the 2020 growing season, which was in general wetter, the mean precipitation for the five locations was 500.6 mm and ranged from 402.5 mm (L3) to 684.7 mm (L5) (Table 1). Accordingly, the precipitation between April and May (PrA-M) (early reproductive till pod filling growth stages) was much lower in 2019 (PrA-M = 104.9 mm, mean of the five locations) compared to 2020 (PrA-M = 159.0 mm). Among the five locations, the southernmost locations had either the highest season (November to July) precipitation (PrS) (L5) or the lowest precipitation between April and May (PrA-M) (L4). Season (November to July) mean temperature was slightly higher in 2020 compared to 2019 but without substantial differences. The highest season mean temperatures (Ts, °C) were recorded in L2 and L4 and the lowest in the location with the highest altitude (L3). The daily recorded minimum temperatures (TDmin, °C) were recorded in January 2019 at the locations with the highest elevations: TDmin = −17.9 °C in L3 (624 m) and TDmin = −12.8 °C in L5 (570 m) (Table 1).

The experimental arrangement consisted of a randomized complete blocks design (RCBD) with four replications. Each plot comprised seven (7) plant rows, each 4 m long, with a row spacing of 0.25 m. The number of seeds sown was 225 seeds m^{-2}, corresponding to approximately 180 plants m^{-2} uniformly distributed. Sowing occurred during the last week of November in 2018 and 2019 in all locations, except for location L3 (Anatoliko), where sowing occurred at the end of February due to a high incidence of frost.

The trials were carried out in organically cultivated environments relying on natural precipitation. No chemical fertilizers, pesticides, or other agrochemicals had been used for at least the last 5 years preceding the experiments. In Larissa (L4), the trials were conducted in a field where lentils and other pulses are frequently grown organically and that is considered highly infested by *Bruchus* sp. In the other locations, the trials were conducted in fields where lentils were occasionally included in rotational schemes with cereals and no agrochemicals had been applied for the past five years. In all locations, the preceding crop was cereal and weed control was achieved through deep plowing in summer and manual weed control. No pesticides were used to control *Bruchus* sp.

2.2. Genetic Materials

Four green-seeded cultivars, namely 'Samos' (G1), 'Dimitra' (G2), 'Thessalia' (G3), and 'Elpida' (G4), property of Institute of Industrial and Forage Crops Larissa Greece (IIFC), along with one red-seeded local population, '03-24L' (G5), which was introduced from ICARDA and subsequently improved by IIFC [39], were evaluated. The cultivars were selected based on their high market share, high yield potential and stability, diversification in flowering date, maturity earliness, and seed size (Table 2).

Table 2. Seed characteristics of the five lentil genotypes.

Name/Code	Cotyledon Color	Seed Size	Flowering [1]	Maturity
Samos (G1)	Yellow	Medium	22	Medium
Dimitra (G2)	Yellow	Small	20	Medium
Thessalia (G3)	Yellow	Medium	21	Medium
Elpida (G4)	Yellow	Large	4	Very early
03-04L (G5)	Red	Medium	12	Early

[1] Days after 1 April in L4 (Larissa).

2.3. Yield, Yield Components, and Seed Bruchid Infection

Plant height (PH) and number of pods per plant (PP) were assessed on 10 randomly selected plants from the three inner plant rows in each plot. The extended BBCH scale was utilized to describe the phenological development of 50% of the plants in each plot [40]. PH was recorded after flowering was completed (BBCH growth stages 69–71), while the PP was determined at physiological maturity (BBCH stage 89). Upon reaching harvest maturity, plots underwent manual harvesting and threshing using a laboratory thresher (Wintersteiger LD350, Wintersteiger Holding AG, Ried im Innkreis, Austria) to evaluate seed yield (SY) and 1000-seed weight (1000 SW). SY was determined on a plot basis (3 m^2 per plot) and was then converted to t ha^{-1} after normalizing the seed weight to 13% seed moisture.

To determine the seed loss percentage (SL) due to bruchid infestation, two random samples of 300 seeds per plot were examined. Seeds were placed in plastic boxes and observed at room temperature until emergence holes appeared, indicating infestation. Infestation levels were assessed by counting emergence holes and examining seeds for adult bruchids [41]. SL per plot was calculated as the mean percentage of infected seeds from the two samples. To further assess the economic implications, the yield loss per hectare (YL) was estimated based on the 1000 SW per plot.

2.4. Cooking Time and Protein

The cooking time (CT) for each cultivar was determined using the tactile method as detailed by Taiwo et al. [42], albeit with several adjustments. In this procedure, five grams of lentil seeds were placed into 100 mL beakers, which were then covered with aluminum foil. Next, 50 mL of boiling distilled water, maintaining a ratio of 1:10 (seed to water), was added to each beaker. Subsequently, the beakers were positioned on a hotplate to initiate cooking. Approximately 20 min later, the softness of the cooked seeds was assessed every 30 s until they achieved a uniform, transparent consistency devoid of any opaque core. This determination was made by sandwiching the cooked seeds between two glass slides and ensuring there was no lateral movement. The optimal cooking time (CT) in minutes was then calculated by averaging the results from triplicate measurements.

Additionally, the seed crude protein percentage (CP) was assessed for each seed sample on a 0% moisture basis. This process included finely grinding a sample from each plot using the Kjeldahl method. The protein percentage was determined by multiplying the total nitrogen (N) content by 6.25 (CP = total N $\times$ 6.25).

2.5. Statistical Analysis

A combined ANOVA for balanced RCBD experiments was conducted, where genotypes (G) and locations (L) were treated as fixed effects and Y was considered a random effect [43]. The treatment sum of squares (SS_{TRMT}) was divided into cultivars (SS_G), locations (SS_L), years (SS_Y), and the two- and three-way components, expressed as a percentage of the sum of squares of the SS_{TRMT}.

Levene's test was employed to assess the equality of variances and residual plots were examined to identify any outliers. Trial means were compared using the LSD test at a significance level of $p \leq 0.05$. Additionally, Pearson correlation coefficients (r) were calculated to identify significant correlations, using the statistical software JMP 11.0.0 [44].

The multivariate GGE biplot model was employed for: (i) grouping the environments based on the best cultivars by the 'which-won-where' pattern and (ii) ranking genotypes for mean performance and stability. The combinations of location and year (e.g., L1 $\times$ 2019) were utilized to reveal GEI crossover interactions or to rank genotypes [45]. The GGE biplot analysis was performed using the R package GGEBiplotGUI version 1.0-9 [46].

3. Results

3.1. Locations, Yields, and Agronomic Traits

Genotype (G), location (L), and the two-way interactions (Y $\times$ L, G $\times$ L) were found to be significant for SY, PP, 1000 SW, and PH (Table 3). Additionally, the G $\times$ Y and the three-way interaction (Y $\times$ L $\times$ G) were significant for SY and 1000 SW but not for PP and PH. The contribution of the L effect was the highest for SY and PP, accounting for over 67% of the variation. Parameters such as PP, 1000 SW, and PH were predominantly genetically controlled, with G accounting for over 9% of the variation, particularly for 1000 SW (73.81%) compared to SY (1.49%). The G $\times$ L contribution to the variation was much higher in comparison to G $\times$ Y for all the above traits. Due to the $SS_{G\times L}/SS_{G\times Y}$ ratio for SY being 3.2-fold higher, the GGE biplot analysis based on the G $\times$ L interaction was utilized to assess the cultivars' adaptation across the growing seasons (Figure 2).

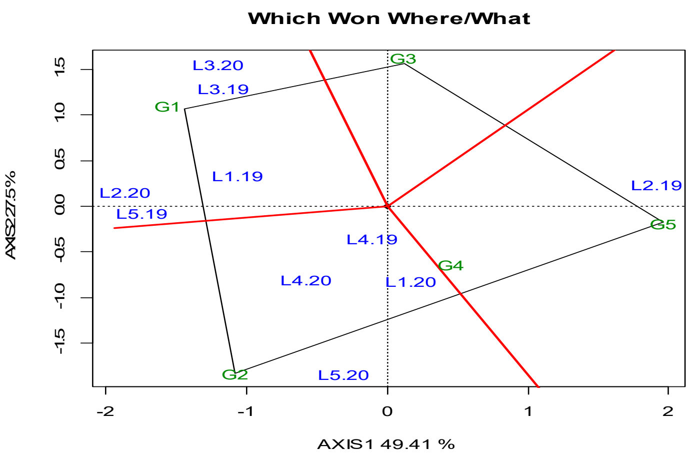

Figure 2. GGE biplot analysis for grouping environments based on the best genotype for seed yield among the five lentil genotypes (abbreviated as G1–G5) across five locations (abbreviated as L1–L5) during 2019 and 2020 (abbreviated 19 and 20). The vertex genotype for each sector, marked by red lines, represents the genotype that yielded the highest for the environments within that sector.

Table 3. Analysis of variance, sum of squares (SS), and percentage (%) contribution to treatment SS (SS_{TRMT}) of the five lentil genotypes tested across five locations for two growing seasons (2019 and 2020) under organic cropping systems.

Source	df	SY	PP	1000 SW	PH	SL	YL	CP	CT
Year (Y)	1	2.07 **	933.12 **	189.54 **	22.78 ^{ns}	999.04 **	0.07 **	8.60 **	3.54 **
Location (L)	4	62.37 **	11,680.93 **	1280.83 **	1390.08 **	35,074.40 **	6.18 **	848.13 **	680.23 **
Genotype (G)	4	1.3 *	1632.83 **	13,652.65 **	1111.58 **	350.20 **	0.10 **	67.51 **	1418.79 **
Y × L	4	8.19 **	802.03 *	1711.044 **	104.4 *	816.44 **	0.06 **	187.84 **	223.07 **
G × Y	4	1.15 *	220.93 ^{ns}	147.787 **	7.95 ^{ns}	90.11 ^{ns}	0.01 ^{ns}	21.55 **	120.00 **
G × L	16	3.74 *	1498.07 *	944.12 **	543.67 **	1329.49 **	0.15 **	77.83 **	75.61 **
Y × L × G	16	8.29 **	482.67 ^{ns}	570.49 **	76.4 ^{ns}	731.83 **	0.09 **	44.65 **	31.45 **
Blocks (LY)	16	3.76 ^{ns}	1044.8 ^{ns}	37.124 ^{ns}	273.83 ^{ns}	506.02 ^{ns}	0.05 ^{ns}	3.48 *	11.74 ^{ns}
Error	120	100.35	3884	94.09	730.1	1516	0.18	8.24	35.58
% of the SS_{TRMT}									
SS_Y		2.38	5.41	1.02	0.70	2.54	1.05	0.69	0.14
SS_L		71.60	67.72	6.92	42.69	89.04	92.79	67.53	26.65
SS_G		1.49	9.47	73.81	34.14	0.89	1.50	5.38	55.58
$SS_{Y\times L}$		9.40	4.65	9.25	3.21	2.07	0.90	14.96	8.74
$SS_{G\times Y}$		1.32	1.28	0.80	0.24	0.23	0.15	1.72	4.70
$SS_{G\times L}$		4.29	8.68	5.10	16.70	3.38	2.25	6.20	2.96
$SS_{Y\times L\times G}$		9.52	2.80	3.08	2.35	1.86	1.35	3.56	1.23

df, degree of freedom; SY, seed yield (t ha^{-1}); PP, number of pods per plant; 1000 SW, thousand seed weight; PH, plant height (cm); SL, seed loss percentage; YL, yield loss (t ha^{-1}); CP, crude protein concentration (%); CT, cooking time (min); * and **: significance at $p < 0.05$ and 0.01, respectively; ^{ns}, not significant.

L1 followed by L2 were the most productive in terms of mean SY, with SYs of 2.00 t ha^{-1} and 1.63 t ha^{-1}, respectively (Table 4). The high-elevation sites L3 (624 m asl) and L5 (500 m) and L4 had low yields (0.53–0.90 t ha^{-1}). Accordingly, site L1 had the highest 1000 SW (47.05 g), followed by L2 (44.67 g). The site L3 had a high PP (40.90 pods/plant) and 1000 SW (44.65 g), despite it being low yielding. The tallest plants were recorded in location L2, with a mean plant height of 39.73 cm, whereas the shortest plants were measured in the late-sowed site L3 (32.18 cm, Table 4).

Table 4. Seed yield t ha^{-1} (SY), number of pods per plant (PP), thousand-seed weight in g (1000 SW), and plant height in cm (PH) of the lentil genotypes tested across five locations for two growing seasons (2019 and 2020) under organic cropping systems.

	SY			PP			1000 SW			PH		
Genotypes	**Mean**	**2019**	**2020**	**Mean**	**2019**	**2020**	**Mean**	**2019**	**2020**	**Mean**	**2019**	**2020**
G1	1.29a	1.25ab	1.34a	35.10a	36.95b	33.25a	39.41d	37.67d	41.16c	37.20a	37.65a	36.75a
G2	1.16bc	1.26ab	1.06b	30.92b	33.05c	28.80b	32.98e	31.75e	34.22d	34.43c	34.45b	34.40b
G3	1.20ab	1.37a	1.02b	35.10a	35.80a	34.40a	45.69b	43.93b	47.46b	37.24a	37.50a	36.98a
G4	1.11bc	1.25ab	0.97b	30.42b	34.40c	26.45b	57.85a	57.17a	58.53a	36.08b	36.70a	35.45ab
G5	1.06c	1.19b	0.92b	27.75c	29.90d	25.60b	41.72c	42.27c	41.17c	30.93d	31.25c	30.60c
Locations												
L1	2.00a	1.78b	2.21a	38.68a	38.30a	39.05a	47.05a	40.59b	53.52a	33.54c	34.15c	32.93bc
L2	1.63b	1.97a	1.30b	28.85c	32.25bc	25.45c	44.67b	46.30a	43.04b	39.73a	41.30a	38.15a
L3	0.53e	0.58d	0.48d	40.90a	43.20ab	38.60a	44.65b	45.81a	43.50b	32.18d	32.10d	32.25c
L4	0.75d	1.06c	0.44d	31.45b	31.80ab	31.10b	40.21d	40.35b	40.08d	36.30b	35.75b	36.85a
L5	0.90c	0.94c	0.87c	19.42d	24.55c	14.30d	41.07c	39.75c	42.40c	34.13c	34.25bc	34.00b
Mean	1.16	1.26	1.06	31.86	34.02	29.70	43.53	42.56	44.51	35.18	35.51	34.84
$LSD_{0.05}$	0.124	0.147	0.207	2.518	3.408	3.774	0.392	0.547	0.641	1.090	1.57	1.67
CV (%)	24.13	18.25	31.03	17.82	11.64	20.16	2.02	2.01	2.28	6.99	7.01	7.63

Different letters within a column indicate significant differences according to LSD test ($p < 0.05$).

Regarding the differences among the growing seasons, the less humid 2019 was more productive by 15.8% in terms of mean SY compared to 2020 (1.26 and 1.06 t ha^{-1}, respectively). Additionally, the PP was higher by 12.7% in 2019 but the 1000 SW was lower by 4.6% in 2019 compared to 2020. There was a small difference in PH between the two growing seasons (Table 4).

The average environmental temperature T (°C) and precipitation after anthesis (PrA-M mm) were related to SY positively in 2019, but in 2020, these relationships weakened or disappeared. In particular, the SY of each location was related positively to T and PrA-M in 2019 (r = 0.72 and r = 0.90 p < 0.01, respectively), whereas it was not related or only weakly related in 2020 (r = 0.06 and 0.35, respectively.

Among the genotypes, G1 and G3 were the most productive in terms of mean seed yield (SY), with G1 producing 1.29 t ha^{-1} followed by G3 with 1.20 t ha^{-1}. Both G1 and G3 also had the highest PP of 35.10 pods $plant^{-1}$. Genotype 4 had the highest 1000 SW of 57.85 g and G3 and G1 were the tallest, at 37.24 cm and 37.20 cm, respectively (Table 4).

The possible existence of different mega-environments was investigated using a GGE biplot, incorporating SY data from both 2019 and 2020. The biplot revealed crossover G × L interactions, suggesting the potential existence of different mega-environments (Figure 2). Notably, G1 consistently performed well in site L3 and G2 in site L4 across both growing seasons, but the grouping pattern of the other sites varied between years. For instance, in L5, G1 excelled in 2019, while G2 did so in 2020. Similarly, in site L2, G1 dominated in 2020, whereas G5 did so in 2019.

The biplot for ranking genotypes for mean performance highlighted that the mean yield order was G1 > G3 > G2 > G4 > G5. The performance of G2, followed by G5 and G3, was the most variable (least stable), whereas the performance of G4, followed by G1, was highly stable (Figure 3).

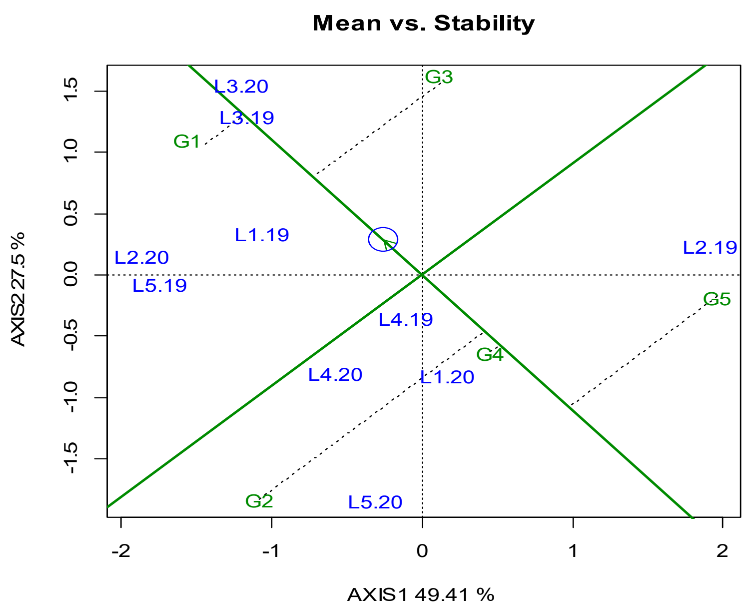

Figure 3. GGE biplot ranking five lentil genotypes (abbreviated G1−G5) for mean seed yield and stability during 2019 and 2020. Entries with vertical projections farther left along the one-arrow horizontal axis represent the highest yielding, while those with vertical projections furthest from the stability line (vertical to the horizontal) are considered less stable.

The comparisons among the L and growing seasons indicated the statistical differences (Table 5). In L4 during both growing seasons, the small-seed genotype G2 exhibited the highest mean SY at 0.92 t ha^{-1}, followed by the red-cotyledon genotype G5 at 0.81 t ha^{-1}. In the short-season site L3, the G1 genotype had a significantly higher yield at 0.73 t ha^{-1}. There were no significant differences in SY observed in L5.

Table 5. Comparison for seed yield (SY t ha^{-1}) in each location (L1–L5) of the lentil genotypes tested across five locations for two growing seasons (2019 and 2020) under organic cropping systems.

	Orestiada (L1)			Thessaloniki (L2)			Anatoliko (L3)			Larissa (L4)			Domokos (L5)		
Cultivar	**Mean**	**2019**	**2020**	**Mean**	**2019**	**2020**	**Mean**	**2019**	**2020**	**Mean**	**2019**	**2020**	**Mean**	**2019**	**2020**
G1	2.12ab	1.72b	2.52	1.97a	1.70c	2.24a	0.73a	0.81	0.65a	0.79c	1.08b	0.45b	0.87	0.91	0.83
G2	2.30a	2.29a	2.32	1.35b	1.69c	1.00bc	0.49b	0.47	0.50a	0.92a	1.13a	0.54a	0.97	0.88	1.07
G3	2.19a	2.41a	1.97	1.67ab	1.83bc	1.50b	0.57b	0.64	0.50a	0.73c	1.05b	0.38c	0.84	0.91	0.77
G4	1.70b	1.37bc	2.04	1.61ab	2.20ab	1.03bc	0.52b	0.55	0.50a	0.73c	0.95c	0.42b	0.97	1.17	0.77
G5	1.68b	1.14c	2.23	1.58b	2.43a	0.72c	0.35c	0.42	0.27b	0.81b	1.09b	0.40b	0.87	0.83	0.90
Mean	2.00	1.78	2.21	1.63	1.97	1.30	0.53	0.58	0.48	0.75	1.06	0.44	0.90	0.94	0.87
$LSD_{0.05}$	0.467	0.461	ns	1.269	0.378	0.644	0.25	ns	0.180	0.101	0.102	0.025	ns	ns	ns
CV%	22.9	16.8	24.4	22.6	12.2	32.3	31.5	31.5	24.2	20.3	15.3	19.9	22.8	18.7	25.7

Different letters within a column indicate significant differences according to LSD test ($p < 0.05$). ns, not significant.

3.2. Seed Loss Percentage and Yield Loss

The primary source of variation for seed loss percentage (SL) and yield loss (YL) attributed to bruchid larvae was identified as the location, accounting for more than 89% of the treatment variation. While significant differences were observed among the genotypes, it is noted that genotype alone explained a relatively small portion of the variation (0.89–1.50%). However, the interactions between genotype and location (G × L) were found to be higher than between genotype and year (G × Y) (Table 3).

In both years of experimentation, L4 consistently exhibited the highest level of infestation by bruchid larvae, with SL values of 32.00% and 41.80% in 2019 and 2020, respectively. Correspondingly, the YL of L4 ranged from 0.47 to 0.55 t ha^{-1}. Following L4, L2 exhibited the second-highest level of infestation, with a mean SL of 10.37% (Table 5). Among the genotypes, G3 demonstrated the highest damage in terms of mean SL and YL, with mean values of 13.60% and 0.19 t ha^{-1}, respectively (Table 6). Conversely, the genotype with small seed size, G2 (1000 SW = 32.98 g), exhibited the lowest YL of 0.12 t ha-1, representing a 36% decrease compared to the medium-seeded genotype G3 (1000 SW = 45.69 g) and a 29% decrease compared to the large-seeded genotype G4 (1000 SW = 57.85 g).

Table 6. Seed loss percentage (SL), yield loss in t ha^{-1} (YL), crude protein percentage (CP), and cooking time in min. (CT) of the lentil genotypes tested across five locations for two growing seasons (2019 and 2020) under organic cropping systems.

	SL			YL			CP			CT		
Genotypes	**Mean**	**2019**	**2020**	**Mean**	**2019**	**2020**	**Mean**	**2019**	**2020**	**Mean**	**2019**	**2020**
G1	11.66b	9.70a	13.6b	0.16bc	0.14ab	0.18b	25.14d	25.65d	24.63d	25.12b	25.12b	25.12b
G2	10.50bc	8.47ab	12.6b	0.12d	0.10c	0.14c	25.97b	26.20c	25.73b	24.24a	23.71a	24.77a
G3	13.60a	10.00a	17.2a	0.19a	0.16a	0.22a	25.78c	26.36b	25.19c	26.87c	26.87c	26.87c
G4	10.51bc	8.85ab	12.20b	0.17ab	0.16a	0.18b	24.83e	24.52e	25.15c	27.51d	27.02d	28.01d
G5	9.82c	7.90b	11.80b	0.15c	0.13b	0.17b	26.45a	26.48a	26.43a	27.49d	28.27e	26.72c
Locations												
L1	0.88d	1.40d	0.38d	0.03d	0.04d	0.02d	23.73c	25.01b	22.45d	29.61d	29.61d	30.57d
L2	10.37b	6.50b	14.30b	0.12b	0.08b	0.16b	27.99a	28.61a	27.38a	24.91a	24.91a	25.01b
L3	2.33d	1.77cd	2.90d	0.06c	0.05cd	0.08c	23.17d	23.35d	22.98c	26.14c	26.14c	26.17c
L4	36.90a	32.00a	41.80a	0.51a	0.47a	0.55a	28.04a	28.59a	27.49a	25.54b	25.54b	25.22b
L5	5.65c	3.35c	7.95c	0.07c	0.06bc	0.08c	25.24b	23.65c	26.84b	24.99a	24.99a	24.52a
Mean	11.23	9.00	13.46	0.15	0.14	0.17	25.63	25.84	25.42	26.24	26.24	26.29
$LSD_{0.05}$	1.57	1.592	2.823	0.017	0.021	0.286	0.114	0.095	0.226	0.114	0.158	0.189
CV (%)	31.16	28.01	33.2	24.68	23.74	25.28	1.03	0.58	1.37	1.36	24.22	1.14

Different letters within a column indicate significant differences according to LSD test ($p < 0.05$).

3.3. Protein and Cooking Time

Year (Y), G, and L, as well as their two-way (Y × L, G × Y, G × L) and three-way (Y × L × G) interactions, were highly significant ($p \leq 0.01$) for both CP and CT (Table 3). Crude protein was primarily affected by the L effect (67.53%) and G explained the 5.38% of the variation; the interaction G × L was also more influential than the interaction G × Y. Cooking time was mainly genetically controlled, as genotype explained 55.58% of the variation in CT (Table 3).

The two locations with the highest Infestation with bruchids, L4 and L2, demonstrated the highest CP values (28.04% and 27.99%, respectively). Specifically, the highest CP amount was measured in most infested L4, followed by the moderately infested site L2. The mean CP of the non-infested sites was 24.04%.

Similar mean CTs of 26 min were measured in the growing seasons. The difference in mean cooking times (CTs) among the genotypes was relatively small, 3.3 min. The large-seed-size genotype G4 and the red-cotyledon genotype G5 had the longest cooking times, with durations of 25.51 min and 27.49 min, respectively.

3.4. Correlations among Traits

Based on the small number of mean genotypic values (n = 10), weak and insignificant relationships were found among SY and PP, PH, 1000 SW, and CT (r = 0.2, 0.24, 0.19, and −0.20, respectively). However, the most worth mentioning was the moderate correlation between SL or YL and CP, r = 0.59 and r = 0.53 ($p < 0.01$), respectively (Table S1).

4. Discussion

Analyzing the effects of genotypes, environments, and their interaction (GEI) is crucial for stakeholders such as local organic farmers, seed companies, and breeding programs. Effective interpretation of GEI requires gathering extensive information on critical factors influencing genotype responses, as these interactions are often influenced by environmental, biotic, or abiotic stresses [47–49].

Data analysis has shown a huge environmental effect on SY attributed to the L effect (71.61%), whereas the Y effect was small [19]. The significant impact of the location effect on legumes is often cited as a primary factor contributing to the limited adaptation of legume cultivation [49]. In this study, the impact of L followed by the G × L on SY can primarily be attributed to variations in pedoclimatic conditions, biotic stresses such as bruchid infestations, and sowing time.

The climate data provided interesting insights into the relationship between seasonal temperature (Ts), precipitation April–May (PrA-M), and SY. In 2019, medium to strong relationships were observed between Ts and SY (r = 0.72) and between PrA-M and SY (r = 0.90). However, in 2020, this pattern was not repeated or was weak. Generally, 80% of the range in lentil SY in the Mediterranean climate may be explained by the difference in seasonal precipitation, as the greatest precipitation falls in winter, whereas in the period from anthesis to pod filling the plants are subjected to water and temperature stresses [27,50]. The significant relationship between rainfall and yield potential is aligned with our study's observations in 2019; however, the response observed in 2020 differed, indicating that factors other than precipitation may have influenced yield potential during that growing season. Possible explanations for the discrepancy in yield response between 2019 and 2020 include variations in water availability, with lentils potentially meeting their water requirements earlier in 2020 due to excessive precipitation, leading to yield reductions. Lentil is well-suited to low-to-medium rainfall regions typically receiving 300–400 mm annually. Yield (SY) can reach greater than 1.0 t ha^{-1} and up to 2.5 t ha^{-1} if sown early [51–53].

In our case, the PrS was more than sufficient in 2020 (500.6 mm) in comparison to 2019 (474.1 mm). During the early reproductive till pod filling growth stages the PrA-M was much higher in 2020 (159.0 mm) in comparison to 2019 (104.9 mm). Lentil plants require ample moisture, particularly during anthesis. However, during the ripening stage, excessive soil moisture can harm plants [54,55]. Excessive rainfall indeed poses a risk to lentil crops, potentially leading to lodging when biomass surpasses a certain threshold, which can result in substantial yield losses [27,56]. Moreover, excessive wet conditions during spring can exacerbate pest problems like aphids [57], numerous aphid-transmitted viruses [11], and foliar disease outbreaks [9]. The observation regarding the low yield achieved in the most humid site (L5) is intriguing and supports the notion that excessive moisture may harm lentil yields. Excessive moisture coupled with the heavy-textured, high-clay-content *Vertisol* in L5 may have led to poor soil drainage conditions (*Vertisols* retain water due to their high clay contents), which can negatively affect crop growth. Well-drained soils (e.g., sandy loam-SL) to medium-textured soils (loamy-L, silty loam-SiL, silty-Si) are considered more favorable for the cultivation of lentils. Heavy soils together with a lack of proper management may cause a reduction in yields. Furthermore, *Vertisols* (high content of expanding clay minerals) are sensitive to changes in rainfall patterns, especially during the critical stages of crop growth, and this limitation can further constrain production.

The low yield observed at the high-altitude (624 m asl) site L3 appears to be primarily attributed to late sowing, which was necessitated by the low temperatures during the typical winter period in the region (daily record minimum in January −17.9 °C and −9.4 °C, in 2019 and 2020, respectively). Despite starting the season with promising indicators such as high PP, the final yield was compromised, likely due to low PH, reduced biomass production, a delay in maturity, and subsequent water stress. Adjusting the sowing time is crucial for maximizing lentil yields, especially in regions with challenging climatic conditions such as high altitudes. Timely sowing ensures proper crop development and minimizes the risk of yield losses due to factors like late water stress [58,59]. Understanding the mechanisms through which lentils combat drought stress is crucial for devising effective cultivation strategies, especially in challenging environments like the Mediterranean. Ludlow [60] outlined various strategies including drought escape, avoidance, and tolerance. In Mediterranean regions, the predominant strategy often involves drought escape, characterized by vigorous growth in winter followed by rapid senescence induced by high temperatures and drought stress in late spring or early summer [51]. Furthermore, utilizing earlier flowering cultivars with improved agronomic traits can enhance productivity in short-season environments [52]. Supplemental irrigation during critical stages such as flowering and early pod filling can help alleviate drought stress in spring-sown lentil crops [51]. However, for Mediterranean conditions, this solution may not be sustainable.

Early sowing in or before winter has the potential to increase water use efficiency, total biological nitrogen fixation, biomass, seed yield, and production by more than 50% [26,61]. However, transitioning lentil cultivation to high-elevation plains, particularly when planted in autumn, presents challenges, primarily due to frost damage. Studies have shown that exposure to temperatures as low as −15 °C for 3 h can distinguish frost-hardy lentil genotypes, but even the hardiest lines may suffer 75% kill at lower temperatures like −18 °C and −20 °C [61]. The observed low yields at the high-elevation site L5, which has low temperatures (−12.8 °C to −10.5 °C), highlight the importance of addressing frost tolerance. Our results show non-significant SY differentiation among cultivars in this site. Moreover, factors beyond frost tolerance, such as waterlogging, root diseases, and ascochyta blight, could also influence winter survival and overall yield [27].

High levels of bruchid infestation at site L4 (Larissa) emphasize the significant impact of this biotic stressor on legume cultivation in the region. The longstanding lentil cultivation in the Larissa region since 1933 has led to prolonged exposure to bruchid infestations, increasing the area's vulnerability to this pest over time [28].

The previous discussion has outlined the most critical abiotic–biotic factors that could explain the differential response of genotypes. The GGE biplots revealed an inconsistent grouping pattern across locations and variability in genotype performance. This suggests that the region cannot be divided into distinct mega-environments, indicating the importance of selecting cultivars that perform well across a range of conditions [62]. The comparison of biplots for mean yield and stability identifies G1 as the top-performing genotype, excelling in both yield and stability. Following closely is G3, which demonstrates high yield but lower stability. These findings suggest that while G3 may offer high yield potential, it may be more susceptible to environmental variability compared to G1. This is in agreement with another study under conventional production systems, confirming genotype G1 as the most productive [19]. The absence of varieties specifically developed for organic cultivation, as highlighted by Vlachostergios et al. [15], presents a challenge for organic farmers who seek cultivars optimized for their production practices. Selecting conventionally bred cultivars with broad adaptability is essential as it allows them to perform reasonably well under organic farming conditions [15,16].

The key agronomic traits PP, PH, and 1000 SW were primarily determined by genetics rather than environmental factors, which is consistent with previous research [19,28]. While previous studies in conventional cropping systems within the region have demonstrated strong positive correlations between SY and PP and PH and a negative correlation between SY and 1000 SW [19], this study revealed a weak correlation between these traits and SY. This inconsistency may be attributed to the higher dependency of yields in organic systems to infestations such as bruchids or foliar diseases and low soil fertility, leading to varied responses across locations.

Research conducted in Greece during 2006–2007 for comparing bruchid infestation levels between organic and conventional lentils revealed that the mean YL was 8.4-fold higher under organic farming. Early flowering and small seed size were traits associated with low YL [31]. However, the early flowering scenario was not realized, as the earliest genotype, G4, demonstrated a high YL, possibly attributed to its large seed size. Conversely, the genotype with a small seed size, G2, exhibited the lowest YL, representing a 29% decrease compared to the large-seeded G4 and a 36% decrease compared to the medium-seeded genotype G3. The lower YL of G2 is primarily attributed to its low 1000 SW, as the SL was approximately the same as the medium- and large-seeded genotypes, indicating similar infestation levels. This aligns with the understanding that smaller seed sizes are often associated with reduced bruchid damage. Additionally, insights from research on vetch species (*Vicia sativa*) and faba beans (*Vicia faba* L.) shed further light on the relationship between plant traits and bruchid infestations. Genotypes with large seeds, many seeds per pod, fewer pods, and fewer branches per plant were found to be more susceptible to bruchid damage. This susceptibility may be attributed to factors such as easier access for female bruchids to lay eggs on a limited number of pods and the availability of more protein content in large seeds [41,63]. Certainly, the ability of certain cultivars, like G3, to maintain high yields despite facing significant yield loss (YL) from bruchids underscores the intricate interplay of multiple factors, including genotypic yield potential and resilience to pests [14,28,64,65], Therefore, while small seed size may play a role in reducing susceptibility to bruchid damage, it is just one aspect of a broader spectrum of factors influencing overall crop performance.

The previous discussion highlighted the importance of considering the cultivation history of specific locations, such as L4, when making decisions about agricultural practices like organic lentil farming. While locations with a history of lentil cultivation may not be ideal for organic lentil farming due to potential pest pressures, they can serve as valuable sites for screening and testing tolerant cultivars to pests such as bruchids [28]. Conversely, high-yielding locations such as L1, characterized by a low YL, are considered ideal for organic farming. Choosing such locations for organic lentil farming can help mitigate the risk of pest-related losses and maximize the success of organic cultivation practices.

Most of the researchers agree that lentil seed CP varies little among locations and there is low G $\times$ L [66–68]. This supports the hypothesis that the nitrogen-fixing ability of lentil and other legumes by *Rhizobium* bacteria makes their CP relatively stable across environments [69]. Recent comparisons between organic and conventional environments have shown higher CP in organic farming (organic: 32.0%, conventional: 27.5%), arguing that the organic environments potentially lead to higher CP due to lower yields [21]. Most researchers have also reported a high negative relationship between CP and SY [68,70]. However, our data show variations in CP levels across sites with different levels of bruchid infestation. Specifically, locations with higher levels of bruchid infestation tend to have higher CP levels. This trend is particularly evident in site L4, which had the highest bruchid infestation levels and consequently the highest CP levels (28.04%). Site L2 also showed a similar pattern with moderately high bruchid infestation. Although there are no specific reports on the relationship between bruchid infestation and CP in lentils, studies on other leguminous crops have demonstrated a positive correlation between infected seeds and CP.

The study by Nikolova et al. [71] demonstrated that spring pea seeds (*Pisum sativum*) damaged by *Bruchus pisorum* L. had increased CP compared to healthy seeds on the same site. Similarly, Zubareva et al. [72] advocated that *B. pisorum* L. damage led to increased CP in spring peas. This could be attributed to the plant's defense mechanisms, wherein plants produce defense-related proteins at high concentrations in response to stressors such as insect infestation [73].

Cooking time is a crucial factor for evaluating pulse cooking quality and is considered highly heritable [74,75]. The current study found that the genotype was the primary factor contributing to CT. Despite variations in growing seasons, the average CT remained consistent at around 26 min. This suggests that environmental factors related to growing seasons did not significantly affect CT in the trials. Similar values were also measured under a conventional cropping system where the ranking of the genotypes in the organic system was pretty much like the one measured in the conventional system [19]. A CT of 30 min is considered acceptable for commercial purposes and cultivars below this threshold are classified as having a short CT [23]. While there were significant differences in CTs among varieties, the range of mean CTs was relatively narrow, at 3.3 min. This variation is considered acceptable by market standards, likely due to the pre-selection of varieties with short CTs before commercial release. The shortest CTs were measured in the small-seeded G2 and in the medium-seeded G1 and G2. These findings support previous results by Iliadis [23], who characterized G1 and G2 as fast cooking, and Theologidou et al. [18], who characterized G1 and G3 as fast cooking. The large-seeded G4 and the red-lentil G5 had the longest in CTs. Cultivar G5 was also evaluated by Theologidou et al. [18], who also found longer CTs for this cultivar. Red cultivars usually require longer CTs and, for this reason, are consumed dehulled to reduce their CTs to as short as 15 min [75,76]. The longer CT of the large-seeded G4 agreed with previous findings that large-seeded lentils have longer CTs than small-seeded ones [74].

Regarding the effect of location on CT, the longest CT was recorded for L1 (29.61 min), whereas the other locations had similar CTs ranging from 24.91 to 26.14 min. However, locations like L1 were expected to have long CTs because of their low precipitation [23]. Finally, a specific pattern linking the environmental conditions to CT was not identified. This lack of a clear pattern may be due to the particular set of lentil cultivars studied. Drawing from the research by Iliadis [23], it suggests that the unfavorable effects of certain soils on CT can be mitigated by using short CT varieties. This finding aligns with the situation discussed, where certain cultivars exhibited shorter CTs despite potentially adverse environmental conditions.

5. Conclusions

Based on the comprehensive analysis of organic lentil cultivation, several specific scenarios for selecting suitable cultivars tailored to different environments were identified. These scenarios likely consider various factors such as the pedoclimatic conditions, pest pressures, and CTs. Here are some potential scenarios:

(i) High-yielding organic environments with long CTs (L1): In such environments, cultivars with short CTs (such as G1, G2, or G3) are recommended for maximizing quality.
(ii) Regions with a high bruchid populations (L4): In areas where there is a significant presence of bruchids, organic production might not be economically viable due to pest pressure. Conventional production methods might be more suitable in such cases.

These areas can serve as screening sites for identifying genetic resistance to bruchids, with attention given to cultivars like G2, which are known for their lower YL.

(iii) High-elevation environments for spring planting (L3): In such areas, where productivity is significantly lower, there is a need for earlier-maturing cultivars and supplemental irrigation during critical stages like flowering and early pod filling. However, it is noted that spring crops may not be sustainable for Mediterranean conditions, indicating the need for alternative approaches.

(iv) High elevation for autumn planting (L5): Similar to spring planting in high-elevation environments, low productivity is observed here as well. To address this, planting winter-hardy cultivars at optimal dates with a good agronomic package is advised.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14040790/s1, Table S1. Correlation coefficients among seed yield in kg ha^{-1} (SY), seed loss percentage (SL), yield loss in t ha^{-1} (YL), number of pods per plant (PP), plant height in cm (PH), thousand seed weight in g (1000 SW), cooking time in min. (CT) and crude protein percentage (CP).

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