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Water Use Efficiency, Spectral Phenotyping and Protein Composition of Two Chickpea Genotypes Grown in Mediterranean Environments under Different Water and Nitrogen Supply

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Abstract: Chickpea is a drought-tolerant crop and an important source of protein, relevant to its beneficial effects. The aim of this study was to assess the response to agronomic management, including water and nitrogen supply, of crop physiological and agronomic traits in relation to water use efficiency and grain protein composition. Two varieties, Pascià and Sultano, were grown at two different sites in South Italy under rainfed and irrigated conditions, with and without starter nitrogen fertilization. Crop physiological assessment was carried out by hyperspectral phenotyping at flowering and during grain filling. Increases in grain yield and grain size in relation to water supply were observed for water use up to about 400 mm. Water use efficiency increased under starter nitrogen fertilization, and Pascià showed the highest values (4.8 kg mm^{-1}). The highest correlations of the vegetation indexes with the agronomic traits were observed in the later growth stage, especially for the optimized soil-adjusted vegetation index (OSAVI); furthermore, grain filling rate showed a strong relationship with photochemical reflectance index (PRI). Experimental factors mainly influenced protein composition rather than protein content. In particular, the 7s vicilin protein fraction showed a negative correlation with grain yield and water use, while lectin showed an opposite response. Both fractions are of interest for consumer's health because of their allergenic and antinutritional properties, respectively. Data from spectral phenotyping will be useful for digital farming applications, in order to assess crop physiological status in modern agricultural systems.

Keywords: *Cicer arietinum*; vegetation index; legume proteins; vicilin; legumin; lectin

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1. Introduction

The introduction of legumes in the cropping systems plays a key role in promoting environmental sustainability, especially within cereal-based systems, because of their symbiosis with nitrogen-fixing soil bacteria. Among legumes, in chickpea (*Cicer arietinum* L.) residual soil nitrogen appears to not affect nitrogen fixation, and a starter fertilization supply is frequently adopted [1]. Chickpea is a valuable source of proteins, dietary fiber, phytochemicals, and minerals, and as grains to cook or new flours used in addition or as an alternative to wheat [2,3]. Globulins represent the major fractions within storage proteins, including the cupin proteins convicilin, 7s vicilin, and 11s legumin, characterized by lower cysteine content. Lectin, lipoxygenase, 2s albumin, and other protease inhibitors represent the other minor fractions [4,5]. While the existence of a genetic variability is well known [6,7], less information is available on the effect of environment and management on legume storage protein composition and technological performance [2,8,9].

Abiotic and biotic stresses can strongly affect seed production, and agronomic management can play an important role in improving yield productivity and stability [10–13];

indeed, chickpea is considered a crop adapted to semi-arid environments, with a good level of drought tolerance, and one which can be cultivated also under rainfed conditions [14]. Water availability and drought can affect chickpea crop growth rate and yield [15]; flowering and podding are, in particular, very sensitive to abiotic stress conditions and genetic improvement for heat and drought tolerance could be promising for chickpea [16,17]. On the other hand, the environment can affect grain quality in chickpea [18] and this is particularly relevant under high rainfall variability, such as in the Mediterranean basin [19].

The need to improve resource-use efficiency is leading to increasing adoption of precision agriculture, with the target of using the right input at the right time [20]. Indeed, the use of vegetation indexes (VIs) on the canopy, by remote or proximal sensing, allows the assessment of crop water and nutritional status [21]. The normalized difference vegetation index (NDVI) is commonly the most adopted VI, and is based on the higher reflectance of the crops in near-infrared (NIR) with respect to the visible (VIS) spectra; many other spectral indices are available in the literature [22]. Major applications are generally carried out on cereals, while less information is available on the relationships of VIs on pulses. The requirement of more efficient crop systems is mandatory in a scenario of climate changes where resources, such as water, might be more and more limited. For this reason, the optimization of management, also relative to irrigation and water use efficiency, is valuable for chickpea, also considering its good ability to use a water supply, especially during flowering [23]. Most of the studies available on chickpea are conducted in Asia, North America, and Australia [15,24,25], while fewer observations are available in the Mediterranean basin, with also little information on the effect on protein quality.

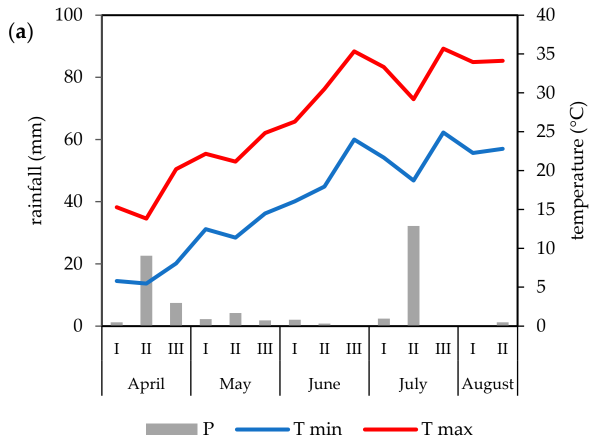
In a previous study conducted in Mediterranean environment, chickpea protein composition was found to be more affected by agronomic conditions than protein content [9]; thus, this study was carried out in order to test the hypothesis that water and nitrogen supply could affect protein composition as well as other crop physiological and agronomic traits. To this aim, the relationship between the productive response of chickpea genotypes and hyperspectral crop reflectance, including VIs, also in relation to changes in grain protein composition, was evaluated. To this purpose, two chickpea varieties were grown with and without irrigation and a starter nitrogen supply, in two locations selected for different climatic conditions; crop physiological assessments by spectral phenotyping and the changes of the main grain protein fractions were carried out.

2. Materials and Methods

2.1. Field Experiments

Two experimental field studies were conducted, in 2021, at two different sites (environments, E) in South Italy. The first field trial was conducted at Troia, in the province of Foggia, Apulia Region (41°21′39″ N and 15°25′18″ E, 128 m above sea level) and the second was conducted at Experimental Agricultural Farm “Pantano of Pignola” (40°33′31″ N and 15°45′31″ E, altitude 400 m above sea level) of ALSIA (Agency for the Agricultural Development and Innovation of Lucania), Basilicata Region; these two sites were, respectively, named as S1 and S2 (Figure 1).

The two environments were selected after being characterized by different meteorological conditions. Long term (1984–2020) climatic information of the two environments was acquired from the NASA POWER database [26], as reported in Table 1, with S2 characterized by lower mean temperatures and higher precipitation. Seasonal meteorological data were obtained from proximal regional weather stations. Crop seasonal minimum and maximum temperature are reported in Figure 1. As for soil texture, field capacity (−0.03 MPa), wilting point (−1.5 MPa), available phosphorous (Olsen), and organic carbon (Walkley–Black) were determined, as reported in Table 2. In particular, the soil at S1 was classified as loam, while at S2 it was classified as silt loam, according to USDA classification.



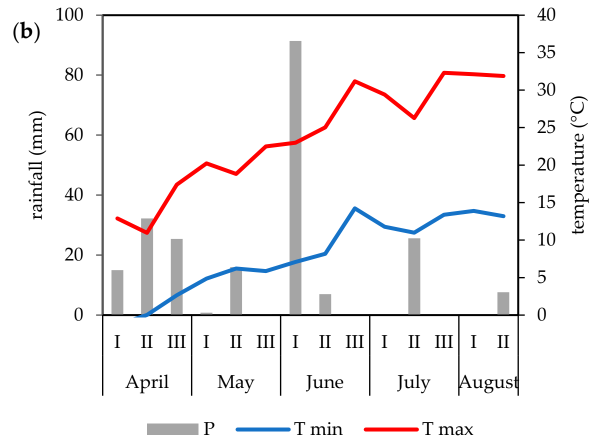


Figure 1. Decadal minimum and maximum temperatures and rainfall distribution during the crop cycle at the two experimental sites, S1 (**a**) and S2 (**b**).

Table 1. Climatic information, expressed as mean and standard deviation, from long-term data (1984–2020) of the field experiments.

E	SRAD	T min	T Max	T Mean	Rainfall	Relative Humidity	Wind Speed
	MJ m^{-2} d^{-1}	°C	°C	°C	mm	%	m s^{-1}
S1	15.2 ± 0.6	11.7 ± 0.5	22.2 ± 0.6	16.9 ± 0.6	511 ± 96	68.2 ± 1.9	2.7 ± 0.1
S2	15.2 ± 0.7	8.0 ± 0.5	18.4 ± 0.7	13.2 ± 0.5	619 ± 144	73.5 ± 2.3	1.9 ± 0.1

Abbreviations are as follows: E = environment; SRAD = solar radiation.

Table 2. Soil physical and chemical characteristics in the investigated fields.

E	Sand	Silt	Clay	FC	WP	AW	BD	pH	EC	tot N	Olsen P	org. C	exch. K
-	%	%	%	%	%	%	g cm^{-3}	-	mS cm^{-1}	%	mg kg^{-1}	%	mg kg^{-1}
S1	47.9	36.1	15.9	34.5	16.4	18.1	1.36	7.3	360	0.214	78.9	1.94	590
S2	25.1	52.3	22.7	36.9	18.4	18.5	1.26	7.4	337	0.208	96.6	0.40	317

Abbreviations are as follows: E = environment; FC = field capacity (%m^3 water/m^3 soil); WP = wilting point (%m^3 water/m^3 soil); AW = available water (%m^3 water/m^3 soil); BD = bulk density; EC = electric conductivity.

The soil preparation consisted of hoeing and harrowing before sowing. Two chickpea (*Cicer arietinum* L.) genotypes were used for field trials, namely Pascià and Sultano (Isea, Macerata, Italy), large-sized and medium-sized seed varieties, respectively, which were adapted to Mediterranean conditions, widely used, and characterized for quality traits in a previous study [9]. For each experimental site, two water regimes (rainfed and irrigated) were adopted. Seeds were sown on 31/03/2021 (S1) and 05/05/2021 (S2) at a final density of 70 seed m^{-2}, with 20 cm of inter-row distance. Irrigation with a micro-flow drip irrigation method, equal to 20 mm corresponding to 200 m^3 ha^{-1}, took place immediately after sowing to assure good seed germination. As for fertilization management, 100 kg ha^{-1} of P_2O_5 was applied before sowing as triple super-phosphate. Two nitrogen (N) fertilization rates were adopted, as follows: with 0 kg ha^{-1} (−N), and with 40 kg ha^{-1} (urea) of N starter (+N). A split-plot design with water irrigation as the main plot, and genotype and N rate as subplots, and four replications was carried out in each environment. Weeds were manually controlled, and fungicide application was provided before flowering (azoxystrobin, 200 g a.i. ha^{-1}).

2.2. Crop Water Demand and Irrigation Supply

Daily potential evapotranspiration (PET) was calculated according to the Penman–Monteith method [27]. The two environments were also characterized in relation to aridity index (AI), calculated as the ratio between P/PET and climatic rainfall deficit as the differences between P and PET. Crop water demand (ET) was calculated according to the following formula: ET = PET × Kc, with Kc as the crop coefficient equal to 0.40, 1.0, and 0.35 at the initial, mid-season and late stage, respectively [27]. Supplementary irrigation (+I) was scheduled weekly, with a replenish of 50% ET demand according to the following formula: I = 50% ET − Peff, with Peff as effective rainfall (Table 3). Water use was calculated as WU = P + I + ΔSWC, with ΔSWC as the difference between soil water content (0–50 cm) at sowing and harvest. Other terms in the water balance, surface runoff, and drainage were negligible.

Table 3. Agrometeorological information on growth stages in terms of thermal units.

Environment	Growth Stage	das	Thermal Units	P	PET	I	P-PET	AI
			GDD	mm	mm	mm	mm	mm mm^{-1}
S1	Sowing	0	14	0.0	2.9	20.0	−2.9	0.00
	Flowering	66	1013	39.4	247.4	68.3	−208.0	0.16
	Grain filling	86	1515	42.2	359.1	136.7	−316.9	0.12
	Maturity rainfed	94	1735	44.6	407.9	-	−363.3	0.11
	Maturity irrigated	100	1906	44.6	444.7	151.9	−400.1	0.10
S2	Sowing	0	12	0.0	4.2	20.0	−4.2	0.00
	Flowering	61	980	114.6	279.3	104.2	−164.7	0.41
	Grain filling	82	1404	140.2	384.9	162.9	−244.7	0.36
	Maturity rainfed	97	1760	140.2	476.2	-	−336.0	0.29
	Maturity irrigated	106	1964	140.2	518.5	209.3	−378.3	0.27

Abbreviations are as follows: das = days after sowing; GDD = growing degree days; P = rainfall; PET = potential evapotranspiration; I = irrigation amount; P-PET = rainfall deficit; AI = aridity index.

2.3. Crop Physiological and Agronomic Measurements

Thermal unit, expressed as growing degree days (GDD), was calculated, with 0 °C as the baseline temperature [28]. Growth stages and grain filling duration (GFD) were recorded in terms of days after sowing (das) and GDD (°C d^{-1}). Grain filling rate (GFR) was calculated as the ratio between grain weight and GFD [29].

Hyperspectral crop phenotyping was carried out on the canopy at flowering (GS 6) and during grain filling (GS 7) by a field spectroradiometer (range 350–820 nm, Apogee SS-110) from 1 m above canopy under clear sky conditions, at around noon. The vegetation indexes (VIs) adopted in the study were calculated from crop spectral reflectance, as reported in Table 4 [30–35].

Table 4. Vegetation indexes (VIs) used in this study.

Vegetation Index	Formula	References
NDVI	$(R_{nir} - R_{red})/(R_{nir} + R_{red})$	[30]
OSAVI	$1.16 \times (R_{nir} - R_{red})/(R_{nir} + R_{red} + 0.16)$	[32]
EVI	$2.5 \times (R_{nir} - R_{red})/(R_{nir} + 6 \times R_{red} - 7.5 \times R_{blue})$	[34]
LAI	$(3.618 \times EVI) - 0.118$	[33]
GCI	$(R_{nir} / R_{green}) - 1$	[35]
PRI	$(R_{531} - R_{570})/(R_{531} + R_{570})$	[31]

Abbreviations are as follows: NDVI = normalized difference vegetation index; OSAVI = optimized soil-adjusted vegetation index; GCI = green chlorophyll index; EVI = enhanced vegetation index; PRI = photochemical reflectance index; R_{nir} = crop reflectance at 800 nm; R_{red} = crop reflectance at 680 nm; R_{blue} = crop reflectance at 450 nm; R_{531} = crop reflectance at 531 nm; R_{570} = crop reflectance at 570 nm.

Plant height (PH) was recorded during grain filling (GS 7). At maturity, each plot was manually harvested, and grain yield (GY), total above ground biomass yield (BY), and single seed weight (GW) were assessed in terms of dry weight. Harvest index (HI) was calculated as the ratio between GY and BY, and seed m^{-2} (GN) was calculated by dividing GY and GW; the number of pods per plant was also determined (pods/plant). Water use efficiency for yield (WUE) was calculated according to the following formula: GY/WU [36]; in addition, agricultural water productivity (AWP) was calculated as the ratio between GY and the sum of I + P [37,38]. Seed protein concentration (PC) was determined by near-infrared spectroscopy (Foss Infratec 1241). Grains were ground in a Cyclotec 1093 mill (Tecator, Hoganas, Sweden) for chemical analysis.

2.4. Grain Water-Holding Capacity

Grain water-holding capacity (WHC_g) was determined on wholemeal flour [9,39]. Briefly, 5 g of flour (dry weight) was suspended in 50 mL of distilled water, mixed thoroughly for 30 min, and then centrifuged at 5000× *g* for 10 min. The free water was removed from the wet flour, which was then weighed. The average of two determinations was reported in grams of water per gram of flour.

2.5. Analysis of Grain Protein Composition

Analysis of chickpea protein composition was assessed by protein separation with SDS-PAGE [9,40]. Briefly, 100 mg of ground flour was suspended with 1 mL of extraction buffer (50 mM Tris–HCl, pH 7.8, 5 mM EDTA, 0.1% 1,4-dithiothreitol) for 1 h at room temperature with constant mixing, and then centrifuged at 10,000× *g* for 30 min. The supernatant, containing the total soluble proteins, was used to prepare samples for electrophoretic separation. The protein concentration in the supernatant was quantified according to the Bradford protocol. For each sample, 10 µL of extracted proteins were separated by SDS-PAGE (at 12%) using an SE 600 apparatus (Hoefer, Inc., Holliston, MA, USA). Gels were stained with Coomassie Brilliant Blue G250 and digitally acquired (Epson Perfection V750pro). Molecular weight markers, from 10 to 250 kDa, were used (Bio-Rad Co, Hercules, CA, USA). Image analysis of gels was performed using ImageQuant TL software (Amersham Biosciences). The relative amount of each protein band abundance was determined by densitometric analysis and expressed as a percentage of the total protein amount in each gel lane. The expression of four groups of proteins was assessed on denatured protein bands, namely lipoxygenase (~90 kDa), 7s convicilin (~68–70 kDa), 7s vicilin (~43, 50 and 53 kDa subunits), 11s legumin (~37 and ~25 kDa as acid subunit α- and basic subunit β-, respectively), lectin (~32 kDa), and 2s albumin (~11 kDa), and the ratio between 7s vicilin and 11s legumin (7s-V/11s-L) was then assessed.

2.6. Statistical Analysis

For each environment, analysis of variance was performed (Figure S1). Means were separated by Tukey's HSD as *post hoc* ($p < 5\%$). The Pearson's multiple regression analysis between Vis, agronomic, and quality traits was also carried out. Statistical analysis was performed using JMP software (SAS Institute Inc., Cary, NC, USA, 2009).

3. Results

3.1. Weather Conditions, Crop Phenology and Water Use

The two investigated environments were characterized by a different meteorological trend, with a higher mean temperature in the final stages at S1 which was also characterized by a lower rainfall amount with respect to S2 (Figure 1). This resulted in a longer crop duration for both chickpea genotypes at S2 (Table 3). A later sowing time was selected according to the local farming practices. In these conditions, an accelerated crop cycle was observed compared to early winter sowing. Crop developmental stages were comparable in the two sites, with the reproductive stage achieved at around 1000 GDD and final maturity at around 2000 GDD, with a slightly shorter cycle in the rainfed conditions (1750 GDD rainfed vs. 1935 GDD irrigated) for both genotypes. However, the higher temperatures and rainfall deficit that occurred, especially in the later stages (Table 3), led to an acceleration of crop senescence, which resulted in a longer grain filling duration (GFD) at S2 (28/34 days at S1, 36/45 days at S2 for rainfed and irrigated, respectively). Crop water use resulted in 287 mm (rainfed) and 346 mm (irrigated) at S1 and in 380 mm (rainfed) and 464 mm (irrigated) at S2.

3.2. Agronomic Response of Yield and Its Components

The shorter grain filling duration observed at S1 was associated with a generally lower grain yield (GY) and grain weight (GW) than at S2 (1209 vs. 1656 and 288 vs. 391, respectively), as shown in Table 5. Indeed, mean grain filling rate (GFR) was also higher at S2, while no differences were observed in terms of water use efficiency (WUE); agricultural water productivity (AWP), on the other hand, was higher at S1. Irrigation supply showed two different productive responses in the two sites, with lower yield in the rainfed treatment (+I vs. –I) at S1, while an opposite behavior was observed at S2 (Table 5). As regards yield components, the lower yield observed for the irrigated treatment at S2 was mainly associated with a lower number of pods/plant (Table 5), since this did not affect grain weight (GW). Crop water consumption (WU), essentially influenced by rainfall and irrigation supply, was higher at S2, and this led to a higher plant height (PH) than at S1. As a consequence of the higher biomass, harvest index (HI) was higher at S1, with a favorable influence of irrigation treatment (Table 5). As for the effect of genotype, Pascià showed higher GY in both sites, associated with a higher GFR, WUE, and AWP. This was mainly due to a higher grain weight (GW) rather than grain number (GN). The same genotype showed a lower PH and a generally higher HI and N uptake (Nupt) in all the environmental conditions. A significant effect of N fertilization was observed in both environments for GY and WUE, with higher values when starter N fertilization supply was carried out (Table 5); this increase was mainly due to an increase in GN; furthermore, the increase in pods/plant with +N treatment was observed only in the S1 field trial.

Table 5. Agronomic performance, in terms of yield, water use efficiency, and yield components of Pascià and Sultano chickpea varieties grown under two water regimes and two N rates.

E	Factor	Level	GY	GFR	WUE	AWP	PH	HI	pod/plant	GN	GW	Nupt
			kg ha^{-1}	kg d^{-1}	kg mm^{-1}	kg mm^{-1}	cm	%	no.	no. m^{-2}	mg	kg ha^{-1}
S1	W	−I	1089	9.7	3.8	24.1	30.2	45.1	21.7	4016	271	34.7
		+I	1329	9.0	4.4	6.6	33.3	49.1	24.5	4426	305	42.4
			*	ns	*	*	*	*	ns	*	*	*
	G	Pascià	1447	11.1	4.9	18.6	29.1	50.5	23.2	4258	342	46.1
		Sultano	971	7.6	3.3	11.9	34.4	43.7	22.8	4184	234	31.1
			*	*	*	*	*	*	ns	ns	*	*
	N	−N	1092	9.4	3.7	14.8	30.9	48.8	20.5	3758	291	34.8
		+N	1325	9.2	4.5	15.7	32.7	45.8	25.5	4683	284	42.4
			*	ns	*	ns	ns	ns	*	*	ns	*
S2	W	−I	1810	11.2	4.8	12.9	48.2	42.8	32.3	4486	403	58.0
		+I	1500	8.4	3.7	4.3	50.8	46.8	26.1	3935	378	48.0
			*	*	*	*	*	*	*	*	ns	*
	G	Pascià	1855	10.8	4.7	9.5	44.9	47.7	27.7	4291	432	59.3
		Sultano	1456	8.8	3.7	7.7	54.0	41.2	30.7	4131	350	46.7
			*	*	*	*	*	*	ns	ns	*	*
	N	−N	1462	9.9	3.7	7.8	49.1	43.9	27.5	3678	393	47.3
		+N	1849	9.8	4.7	9.4	49.9	45.1	30.1	4753	389	58.7
			*	ns	*	*	ns	ns	ns	*	ns	*
S1		Mean	1209	9.3	4.1	15.3	31.8	0.47	23.1	4221	288	38.6
S2		Mean	1656	9.8	4.2	8.6	49.5	0.45	29.2	4211	391	53.0
			*	*	ns	*	*	*	*	ns	*	*

Abbreviations are as follows: E = environment; W = water regime; G = genotype; N = nitrogen; S1 = field experiment at Troia; S2 = field experiment at Pignola; −I = rainfed; +I = irrigated; −N = without N fertilization supply; + N = with N fertilization supply; GFR = grain filling rate; WUE = water use efficiency; AWP = agricultural water productivity; GY = seed yield; PH = plant height; HI = harvest index; GW = grain weight; GN = seed/m^2; Nupt = N uptake; * significant difference at $p < 5\%$, according to Tukey's test; ns = non-significant difference.

3.3. Hyperspectral Phenotyping

The hyperspectral phenotyping allowed for the assessment of crop physiological status during reproductive stages, on which agronomic and environmental factors generally play a key role in determining final productivity. Crop reflectance from visible and near-infrared was measured at flowering (GS 6) and grain filling (GS 7), and the main VIs were calculated

(Table 6). Here, NDVI, OSAVI, and EVI-LAI are descriptors of biomass vigor and crop development, while GCI is generally considered an indicator of leaf chlorophyll content and PRI of plant stress.

Table 6. Agronomic performance, in terms of yield, water use efficiency, and yield components of Pascià and Sultano chickpea varieties grown under two water regimes and two N rates.

E	Factor	Level	NDVI		OSAVI		GCI		LAI		PRI	
			GS 6.1	**GS 7.5**	**GS 6.1**	**GS 7.5**	**GS 6.1**	**GS 7.5**	**GS 6.1**	**GS 7.5**	**GS 6.1**	**GS 7.5**
S1	W	−I	0.539	0.437	0.418	0.265	1.336	1.184	1.202	0.523	−0.016	−0.042
		+I	0.595	0.467	0.472	0.279	1.621	1.311	1.347	0.562	−0.011	−0.037
			*	ns	*	ns	*	*	*	ns	ns	*
	G	Pascià	0.574	0.440	0.458	0.262	1.581	1.234	1.322	0.515	−0.016	−0.042
		Sultano	0.560	0.460	0.432	0.283	1.376	1.261	1.230	0.570	−0.010	−0.037
			ns	ns	ns	*	*	ns	ns	*	ns	*
	N	−N	0.551	0.442	0.430	0.264	1.418	1.216	1.227	0.531	−0.014	−0.040
		+N	0.582	0.462	0.460	0.280	1.539	1.279	1.325	0.554	−0.012	−0.040
			ns	ns	ns	*	*	ns	ns	ns	ns	ns
S2	W	−I	0.617	0.450	0.432	0.350	2.001	1.253	1.113	0.853	−0.025	−0.049
		+I	0.462	0.520	0.355	0.387	1.290	1.489	0.986	0.972	−0.044	−0.030
			*	*	*	*	*	*	ns	*	*	*
	G	Pascià	0.532	0.460	0.398	0.354	1.621	1.254	1.054	0.895	−0.037	−0.036
		Sultano	0.547	0.510	0.389	0.384	1.671	1.488	1.045	0.931	−0.032	−0.042
			ns	*	ns	*	ns	*	ns	ns	ns	*
	N	−N	0.543	0.470	0.379	0.360	1.640	1.336	0.979	0.887	−0.031	−0.040
		+N	0.536	0.500	0.407	0.378	1.652	1.406	1.119	0.939	−0.039	−0.038
			ns	ns	ns	ns	ns	*	ns	ns	ns	ns
S1		Mean	0.567	0.452	0.445	0.272	1.336	1.184	1.276	0.543	−0.013	−0.040
S2		Mean	0.539	0.485	0.393	0.369	1.621	1.311	1.049	0.913	−0.035	−0.039
			ns	*	*	*	*	*	*	*	*	ns

Abbreviations are as follows: E = environment; W = water regime; G = genotype; N = nitrogen; S1 = field experiment at Troia; S2 = field experiment at Pignola; −I = rainfed; +I = irrigated; −N = without N fertilization supply; +N = with N fertilization supply; NDVI = normalized difference vegetation index; OSAVI = optimized soil-adjusted vegetation index; GCI = LAI = leaf area index estimated from enhanced vegetation index; PRI = photochemical reflectance index; * significant difference at $p < 5\%$, according to Tukey's test; ns = non-significant difference.

At flowering, all the investigated VIs, except for NDVI and GCI, showed higher values at S1, while an opposite response was observed at grain filling with generally higher VIs at S2. The PRI was significantly different between rainfed and irrigated treatments in both environments at grain filling, when differences with crop water status were marked. No significant differences were generally observed for the effect of genotype and N fertilization, except for GCI at S1 (Table 6).

3.4. Protein Quality

Few variations in protein content (PC) were observed in chickpea samples from the experimental field trials (Table 7), with a significant effect observed at S2 with a higher PC under rainfed conditions (-I). As for water-holding capacity, generally higher values were observed at S1, while irrigation showed no significant effect. As for genotype, higher values were observed in Pascià only at S1; furthermore, higher WHC with N fertilization supply (+N) was observed only at S2.

Table 7. Protein content, grain water-holding capacity, and protein composition of Pascià and Sultano chickpea varieties grown under two water regimes and two N rates.

E	Factor	Level	PC	WHC_g	lipox	conv	7s-V	11s-L	lect	2s-Alb	7s-V/11s-L
			%	g/g	%	%	%	%	%	%	-
S1	W	−I	19.9	1.30	7.5	5.8	15.0	31.5	8.5	9.6	0.48
		+I	20.0	1.28	7.1	5.1	16.0	30.5	7.9	9.5	0.54
			ns	ns	*	*	*	ns	ns	ns	ns
	G	Pascià	19.9	1.30	7.5	5.6	14.4	31.6	9.3	9.0	0.46
		Sultano	20.0	1.27	7.2	5.4	16.6	30.4	7.0	10.0	0.56
			ns	*	ns	ns	*	ns	*	ns	*
	N	−N	19.9	1.29	7.6	5.7	16.3	31.6	7.8	10.1	0.51
		+N	20.1	1.29	7.0	5.3	14.4	30.4	8.6	9.0	0.50
			ns	ns	ns	*	*	ns	ns	ns	ns
S2	W	−I	20.2	1.26	6.9	4.8	14.6	31.9	10.1	7.5	0.46
		+I	19.8	1.27	7.7	5.0	13.7	31.6	9.9	7.0	0.43
			*	ns	*	ns	*	ns	ns	ns	*
	G	Pascià	20.0	1.27	6.9	4.8	14.0	32.2	10.5	7.0	0.43
		Sultano	20.1	1.26	7.7	5.0	14.3	31.3	9.5	7.5	0.46
			ns	ns	*	ns	ns	ns	*	ns	*
	N	−N	20.2	1.25	7.1	4.7	13.9	32.0	10.3	7.1	0.44
		+N	19.8	1.28	7.4	5.1	14.4	31.5	9.8	7.4	0.46
			ns	*	ns	*	ns	ns	ns	ns	*
S1		Mean	20.0	1.29	7.3	5.5	15.5	31.0	8.2	9.5	0.51
S2		Mean	20.0	1.27	7.3	4.9	14.2	31.7	10.0	7.3	0.45
			ns	*	ns	*	*	ns	*	ns	*

Abbreviations are as follows: E = environment; W = water regime; G = genotype; N = nitrogen; S1 = field experiment at Troia; S2 = field experiment at Pignola; −I = rainfed; +I = irrigated; −N = without N fertilization supply; +N = with N fertilization supply; PC = protein content; WHC_g = water-holding capacity; lipox = lipoxygenase; conv = convicilin; 7s-V = 7s vicilin; 11s-L = 11s legumin; 2s-Alb = 2s albumin; 7s-V/11s-L = ratio between 7s vicilin and 11s legumin; * significant difference at $p < 5\%$, according to Tukey's test; ns = non-significant difference.

The few changes observed for protein content were associated with higher variations in terms of protein composition (Table 7). No mean differences between the two sites were observed for lipoxygenase, which followed an opposite trend of grain yield in relation to irrigation treatment, with a higher amount in rainfed crops (−I) at S1 and a lower at S2. Furthermore, the effect of genotype was significant only at S2 (Sultano > Pascià). Contrasting results were observed in relation to convicilin and 7s vicilin, which were generally higher at S2, while no significant differences were observed for 11s legumin and 2s albumin. Furthermore, the ratio between 7s vicilin and 11s legumin was markedly higher at S1 than S2, with a significantly higher content in Sultano than Pascià; on the other hand, lectin showed an opposite behavior. As for yield, contrasting results were observed in relation to the expression of 7s vicilin and lectin that showed, respectively, a negative and a positive association with water use.

3.5. Multiple Regression Analysis

Multiple regression analysis was performed to individuate significant correlations between investigated crop physiological, agronomic, and quality traits (Figure 2). Within the yield components, GW more than pod/plant correlated with GY and, GFR more than GFD. Crop WU showed a good relationship with GY in interaction with the N fertilization rate for both Pascià (Figure 3a) and Sultano (Figure 3b). In particular, this mainly influenced GW (Figure 3c), as well as plant height (Figure 3d). As for the VIs, PRI showed a good relationship with water supply as the sum of rainfall and irrigation (Figure 4a), and then a negative relationship with GFR (Figure 4b) that, instead, showed a positive relationship with the measure of GCI at flowering (Figure 4c). Further, the higher correlation with yield was shown when the measure occurred in grain filling (GS7), with a higher r observed for OSAVI (Figure 4d) and LAI (EVI).

		GY	AI	GFD	GFR	WU	WUE	AWP	PH	HI	pod/plant	GW	GN	Nupt	PC	WHC_g	lipox	conv	7s-V	11s-L	lect	2s-Alb	7s-V/11s-L
GY		1.00	0.51	0.33	0.57	0.46	0.88	0.00	0.32	0.26	0.63	0.75	0.66	1.00	-0.04	0.06	-0.11	-0.23	-0.35	0.04	0.37	-0.21	-0.23
AI		0.51	1.00	0.72	0.17	0.95	0.06	-0.32	0.86	-0.26	0.47	0.67	0.01	0.52	0.10	-0.28	-0.05	-0.38	-0.41	0.14	0.41	-0.23	-0.37
GFD		0.33	0.72	1.00	-0.21	0.89	-0.07	-0.74	0.77	0.02	0.23	0.52	-0.06	0.32	-0.10	-0.18	0.10	-0.38	-0.36	0.03	0.26	-0.21	-0.26
GFR		0.57	0.17	-0.21	1.00	0.01	0.64	0.46	-0.16	0.37	0.15	0.72	0.05	0.58	0.11	0.03	-0.23	-0.08	-0.33	0.19	0.32	0.01	-0.35
P + I		0.20	0.43	0.94	-0.34	0.67	-0.09	-0.84	0.57	0.17	0.09	0.35	-0.06	0.18	-0.16	-0.12	0.13	-0.34	-0.24	-0.04	0.13	-0.15	-0.12
WU		0.46	0.95	0.89	0.01	1.00	0.00	-0.51	0.88	-0.16	0.39	0.65	-0.03	0.46	0.01	-0.25	0.02	-0.40	-0.44	0.11	0.38	-0.24	-0.37
WUE		0.88	0.06	-0.07	0.64	0.00	1.00	0.25	-0.09	0.40	0.52	0.52	0.75	0.87	-0.06	0.24	-0.12	-0.07	-0.21	-0.03	0.23	-0.11	-0.08
AWP		0.00	-0.32	-0.74	0.46	-0.51	0.25	1.00	-0.51	0.01	-0.04	-0.15	0.13	0.00	0.00	0.28	0.07	0.39	-0.01	0.04	0.04	0.05	-0.05
PH		0.32	0.86	0.77	-0.16	0.88	-0.09	-0.51	1.00	-0.32	0.51	0.40	0.03	0.33	0.03	-0.30	0.03	-0.44	-0.28	0.03	0.22	-0.15	-0.19
HI		0.26	-0.26	0.02	0.37	-0.16	0.40	0.01	-0.32	1.00	-0.19	0.33	0.04	0.24	-0.29	0.22	-0.07	0.03	-0.11	0.00	-0.03	0.16	-0.07
pod/plant		0.63	0.47	0.23	0.15	0.39	0.52	-0.04	0.51	-0.19	1.00	0.30	0.64	0.62	-0.02	0.08	-0.11	-0.27	-0.11	-0.12	0.19	-0.23	0.05
GW		0.75	0.67	0.52	0.72	0.65	0.52	-0.15	0.40	0.33	0.30	1.00	0.02	0.76	0.05	-0.13	-0.17	-0.34	-0.52	0.19	0.46	-0.15	-0.48
GN		0.66	0.01	-0.06	0.05	-0.03	0.75	0.13	0.03	0.04	0.64	0.02	1.00	0.65	-0.10	0.22	0.01	-0.03	0.05	-0.23	0.01	-0.13	0.24
Nupt		1.00	0.52	0.32	0.58	0.46	0.87	0.00	0.33	0.24	0.62	0.76	0.65	1.00	0.04	0.06	-0.12	-0.24	-0.34	0.04	0.38	-0.21	-0.22
NDVI	GS6	0.17	-0.12	-0.40	0.38	-0.27	0.33	0.13	-0.12	0.02	0.23	0.07	0.20	0.19	0.26	-0.16	-0.30	-0.12	0.18	0.00	-0.11	0.19	0.13
	GS7	0.11	0.24	0.53	-0.31	0.39	-0.04	-0.42	0.53	-0.12	0.26	0.08	0.11	0.10	-0.09	-0.03	0.21	-0.05	-0.07	0.01	0.03	-0.09	-0.05
OSAVI	GS6	0.06	-0.30	-0.34	0.28	-0.35	0.27	0.12	-0.25	0.17	0.07	-0.01	0.11	0.07	0.08	0.04	-0.18	-0.08	0.06	-0.10	-0.28	0.32	0.11
	GS7	0.39	0.76	0.76	-0.09	0.82	0.01	-0.45	0.86	-0.26	0.44	0.45	0.09	0.39	0.03	-0.21	0.09	-0.28	-0.28	0.07	0.25	-0.18	-0.24
LAI	GS6	0.02	-0.40	-0.34	0.18	-0.42	0.26	0.15	-0.32	0.23	-0.02	-0.09	0.13	0.02	0.00	0.10	0.00	0.08	0.03	-0.12	-0.29	0.29	0.09
	GS7	0.45	0.86	0.82	-0.03	0.91	0.03	-0.46	0.88	-0.21	0.44	0.56	0.06	0.45	0.02	-0.24	0.06	-0.33	-0.33	0.09	0.30	-0.21	-0.28
GCI	GS6	0.41	0.27	-0.08	0.52	0.11	0.40	0.00	0.20	0.01	0.37	0.42	0.16	0.43	0.26	-0.23	-0.30	-0.28	-0.09	0.04	0.08	0.12	-0.09
	GS7	0.09	0.25	0.52	-0.29	0.39	-0.07	-0.44	0.54	-0.13	0.22	0.09	0.06	0.08	-0.09	-0.06	0.17	-0.11	-0.12	0.01	-0.01	0.00	-0.08
PRI	GS6	-0.22	-0.54	-0.59	-0.01	-0.61	0.08	0.25	-0.46	0.00	-0.04	-0.42	0.19	-0.21	0.13	-0.03	-0.15	0.08	0.47	-0.03	-0.19	0.13	0.35
	GS7	-0.06	-0.03	0.46	-0.43	0.19	-0.14	-0.41	0.12	0.17	-0.14	-0.06	0.01	-0.08	-0.27	0.05	0.17	-0.03	0.04	0.01	-0.04	-0.11	0.04

Figure 2. Pearson's correlation matrix between (heatmap) investigated crop physiological, agronomic, and quality traits on chickpea genotypes under a range of environmental and agronomic conditions. Abbreviations are as follows: GFR = grain filling rate; WUE = water use efficiency; AWP = agricultural water productivity; GY = seed yield; PH = plant height; HI = harvest index; GW = grain weight; GN = seed/m^2; Nupt = N uptake; NDVI = normalized difference vegetation index; OSAVI = optimized soil-adjusted vegetation index; LAI = leaf area index estimated from enhanced vegetation index; PRI = photochemical reflectance index; PC = protein content; WHC_g = grain water-holding capacity; lipox = lipoxygenase; conv = convicilin; 7s-V = 7s vicilin; 11s-L = 11s legumin; 2s-Alb = 2s albumin; 7s-V/11s-L = ratio between 7s vicilin and 11s legumin. Red and green represent low and high R values; values are statistically significant, at $p < 5\%$, with $R > \pm 0.26$.

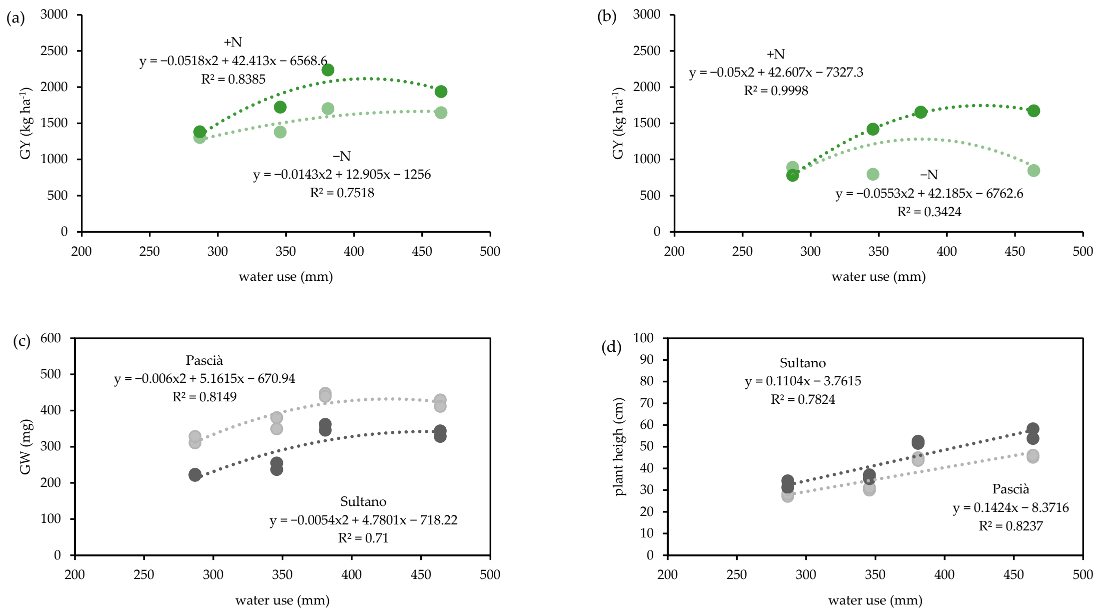


Figure 3. Relationship between water use with grain yield (GY) for at 0 kg ha^{-1} of N (light green) and 40 kg ha^{-1} of N (deep green) in Pascià (**a**) and Sultano (**b**) chickpea genotypes and with grain weight (**c**) and plant height (**d**). Chickpea genotypes Pascià and Sultano are reported in deep grey and light grey, respectively.

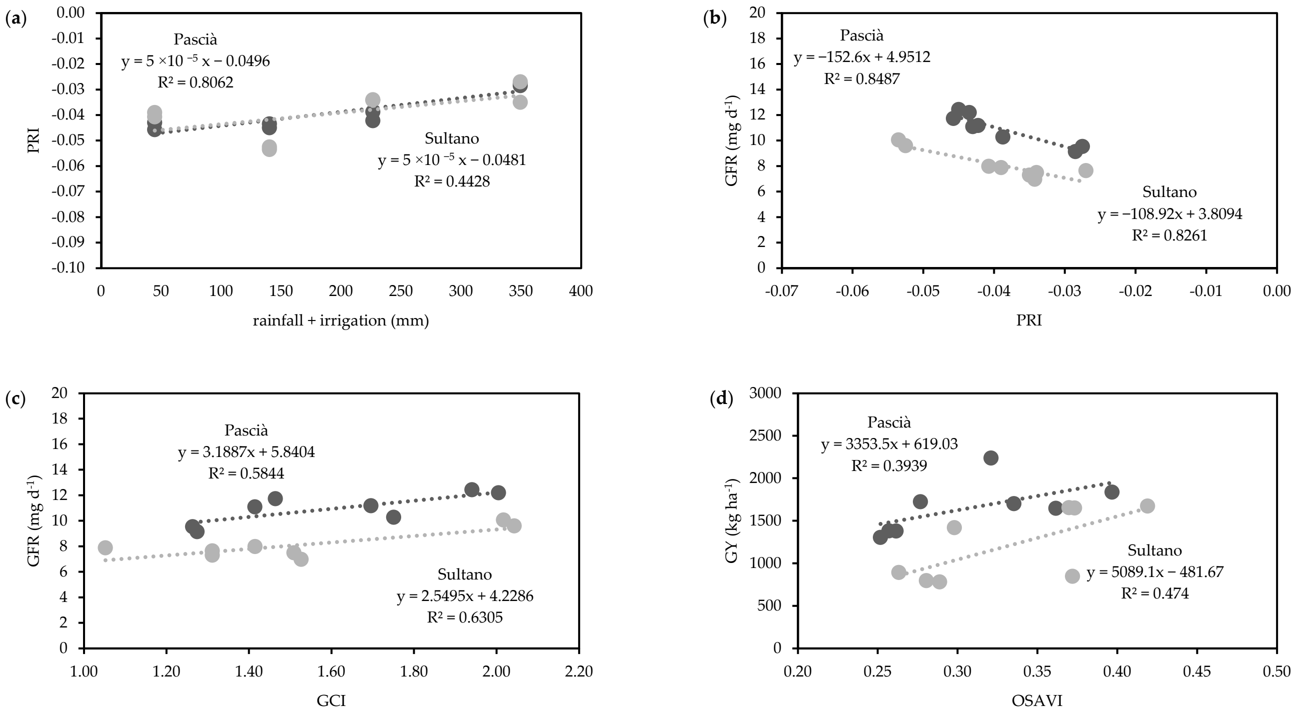


Figure 4. (**a**) Relationship between PRI, measured at grain filling, and water supply (rainfall and irrigation), and (**b**) with grain filling rate; (**c**) relationship between GCI, measured at flowering, with grain filling rate (GFR); (**d**) relationship between OSAVI, measured at grain filling, with grain yield. Chickpea genotypes Pascià and Sultano are reported in deep grey and light grey, respectively.

In regard to quality traits, PC showed a significant positive correlation with GCI (flowering). Furthermore, the ratio between 7s vicilin and 11s legumin (7s-V/11s-L) showed a significant negative correlation with AI (−0.37) and GW (−0.48). On the contrary, lectin showed a significant positive correlation with AI, WU, and GW (Figure 2).

4. Discussion

Yield production obtained by spring sowing in the two environments, characterized by different mean temperatures and climatic rainfall deficits, was in a range comparable with previous observations in the literature [41]. In a previous study [10] conducted in a Mediterranean environment, the same varieties, Pascià and Sultano, showed a higher yield under winter sowing with respect to spring sowing. However, this advantage is critically influenced by unfavorable climatic conditions, especially during flowering, which can lead to a higher risk of biotically stressful conditions [25].

As for the role of soil water availability, the main effect was a positive relationship with grain filling duration and rate. The higher water use resulted in higher grain weight and then yield. In the absence of marked biotic stressful conditions, such as anthracnose, a positive relationship between rainfall distribution and biomass and yield traits is reported in the literature for chickpea under Mediterranean environments [42], thus, influencing, in particular, leaf area index duration. In the current study, the significant correlation between estimated LAI and yield traits was markedly relevant for the number of pods per plant and grain weight. The influence of N supply led to a moderate advantage for chickpea growth, with increasing water use efficiency; by increasing the number of pods per plant, this had more of an effective for the large seed variety Pascià. On the other hand, grain weight, which was the most determinant yield component, was more affected by genotype and water supply than starter N fertilization. This result is in accordance with a study conducted on the chickpea genotype Sultano [2], in which the authors observed that supplementary mineral N fertilization led to a non-significant increase in 100 seed weights.

The use of data from spectral phenotyping could be strategically useful for precision agriculture to achieve the sustainable goals of food quality and food safety promoted by climate-smart agriculture [43]. This has a great relevance for pulse crops, such as chickpea, that are strongly influenced by abiotic and biotic conditions [44]. The outcomes of this study, in particular data from OSAVI and EVI-LAI, allowed us to describe crop water status during the key stages to determine final yields, such as flowering and grain filling. The former stage is highly determinant of seed numbers, while the latter is determinant for seed weight. This might be explained by the strong influence of water availability on crop growth rate and duration, especially for late sowing [15]. Recently, a phenomic investigation on pulse crops, including chickpea, was carried out in the Pacific Northwest of the United States, to provide useful tools for plant breeders to accelerate breeding programs [25]. The assessed VIs, including NDVI, correlated with agronomic traits, i.e., seed yield, flowering date, and days to physiological maturity (grain filling duration). The timing of VI measurement is fundamental in order to better predict final yield [45]; in a study in which remote sensing was applied to chickpea subjected to biotic stress conditions, early NDVI observations produced weaker results than late ones for predicting final grain yield [46]. The higher correlation observed with the use of the OSAVI with respect to NDVI is explained by the ability of this VI to correct the influence of soil background, and it is particularly suggested in a semi-arid area, especially for chickpea characterized by a lower soil coverage than other grasses [47]. Furthermore, in a study conducted in India [24], the authors reported observation data using satellite remote sensing (Landsat TM) to describe LAI variations by different VIs on wheat and chickpea; the regressions obtained were better for wheat than chickpea, and the best performances were observed in growing LAI phases; the authors also observed a better regression with the use of SAVI and RVI rather than NDVI for chickpea.

As for water use efficiency (WUE), the results of our observations are in a range reported on chickpea under a Mediterranean environment [42]. The WUE parameters are reported to be related to crop growth length and days to maturity in chickpea [48], and improved with irrigation only in the environment with the higher rainfall deficit; indeed, the values observed in terms of AWP were higher than that observed on common bean grown under comparable conditions and in one of the investigated environment [38]. This result confirms the greater drought tolerance of chickpea [14] with respect to other pulses, particularly valuable in the Mediterranean and semi-arid basins where water limitations are frequent. Furthermore, the relationship between water supply and crop production indicates the presence of a plateau value; further water supply seems to not further increase yield. Bellido et al. [1] reported a quadratic relationship between seed yield and rainfall amount with a plateau around 400 mm, with excessive rainfall having a negative effect on chickpea. On the other hand, the higher water supply may be of interest for merceological quality, since larger seeds are generally appreciated by the market and consumers [49]. In these terms, the significant relationship observed between VIs with grain filling rate may be useful for precision water management to achieve quality requirements. In fact, the amount of leaf chlorophyll (chl-a) estimated by GCI at flowering can influence photosynthetic rate and final dry matter accumulation in grain [50].

Protein content in seeds is generally in the 19–24% range in chickpea [7]; in the current study, a reduced variability was observed, in a range comparable to that observed in a set of genotypes that included Pascià and Sultano grown in South Italy [51]. The limited variation observed confirms that protein content is a quite stable trait in chickpea, despite protein composition, which can be also influenced by environment and management [9]. Most of the studies available on legume storage proteins are focused more on genetic diversity [6,7] than on agronomical factors [52]. In a previous study conducted in a set of chickpea genotypes, including Pascià and Sultano, a strong impact of cropping systems was observed, especially for the ratio of 7s to 11s globulins [9]. This ratio, in particular 7s vicilin, negatively correlated with grain yield; the same result was observed in the current study, confirming the association between agronomic traits and grain protein composition. The outcomes of this study underline the higher contribution of water supply rather than nitrogen on regulating the expression of the vicilin fraction. This result is of interest since the 50-kDa subunit of 7s vicilin has a putative role as an allergen [53]. In these terms, a good agronomic management could promote an increase in yield and grain size together with a reduction in allergenic potential. Furthermore, the changes in lectin amount, negatively associated with rainfall and water use, are of particular interest because of its anti-nutritional properties [54]. In addition, the higher lectin expression observed in the large-seed genotype Pascià with respect to Sultano is consistent with previous investigations on the same genetic material [9]. Finally, since most of the legume storage globulins have enzymatic properties [4], metabolic implications could be expected in relation to chickpea adaptability under contrasting environmental conditions.

5. Conclusions

The two investigated chickpea genotypes showed a differential response in terms of water use efficiency; in particular, irrigation treatment was efficient only in the low rainfall environment. Starter N fertilization, on the other hand, contributed to improving the response of different agronomic traits by improving water use efficiency, especially in combination with the irrigation supply. The hyperspectral phenotyping carried out gave interesting results, in particular in terms of individuating the optimal timing, grain filling, and vegetation indexes that best describe the physiological status of the two investigated chickpea genotypes. In particular, while GCI and PRI showed a good relationship with grain filling rate, OSAVI and EVI-LAI were better with grain yield, and also better than NDVI. These outcomes can be useful for the scientific community, field technicians, and farmers, since little information is actually available on chickpea grown in the Mediterranean basin. As for quality, reduced changes were observed in terms of protein content and grain water-holding capacity; instead, more variations were observed in terms of grain protein composition. In particular, a negative correlation with water supply and yield was observed for 7s vicilin, and a positive correlation was found with grain weight and lectin. Further proteomic investigations, also under different environments and on more genotypes, will be carried out to provide a deep insight into these effects on metabolic protein regulations. Furthermore, data from spectral phenotyping will be useful for digital farming applications, in order to assess crop physiological status in modern agricultural systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12122026/s1, Figure S1: Percentage of the sum of squares (SS) explained by the factors included in the ANOVA model for agronomic and quality traits; Table S1: Mean data (+standard error) of agronomic performance, vegetation indexes and protein quality of two chickpea genotypes (G1, Pascia; G2, Sultano) grown under rainfed (−I) and with supplementary irrigation (+I) and two N rates (−N, +N) at Troia (S1) and Pignola (S2).

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