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Genotype-by-Environment Interaction Influenced the Crude Protein and Total Amino Acid Content in Lentil Varieties

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ABSTRACT

Lentil (*Lens culinaris* Medik.) is a globally important grain legume valued for its high-protein content and nutritional quality. However, the genetic and environmental factors influencing protein and amino acid composition, particularly the role of genotype × environment interaction (GEI), remain partially uncovered. This study evaluated 15 diverse lentil genotypes across seven agronomic trials spanning multiple years, sowing seasons, and locations to assess the effects of genotype, environment, and GEI on crude protein (CP), crude protein yield (CPY), total amino acids (TAA), and total amino acid yield (TAAY). Advanced statistical models such as Additive Main Effects and Multiplicative Interaction (AMMI), Genotype plus Genotype-by-Environment (GGE), and Weighted Average of Absolute Scores (WAASB) were employed to dissect the contributions of genetic and environmental components and to identify stable, high-performing genotypes. Results revealed significant phenotypic variation for CP, amino acids (AA), and TAA among genotypes. Seasonal variation, especially between autumn and spring sowings, was the primary environmental driver of GEI for both CP and CPY. Notably, some landraces (PI_431710_LSP, PI_431739_LSP, PI_431753_LSP, and IG_1959) demonstrated both high productivity and stability across environments, while others excelled in specific mega-environments identified through GGE analysis. Our findings emphasize the importance of integrating into lentil breeding programs, stability and adaptability, and a more comprehensive approach to measure the yield (e.g., CPY, MegaJoule, amino acid composition, and TAAY), which takes into account the quality and the effective energetic production of a crop. From this perspective, we highlight landraces as valuable sources of genetic diversity for improving yield. This work provides a foundation for targeted breeding strategies aimed at developing lentil varieties with enhanced protein content, balanced amino acid profiles, and resilience to environmental variability.

1 | Introduction

Lentil (*Lens culinaris* Medik.) is an important grain legume cultivated globally due to its high nutritional value and adaptability to various agroclimatic conditions (Khazaei et al. 2019; Kumar et al. 2016). It plays an important role in global food security, especially in regions where a plant-based diet is a staple (Stagnari et al. 2017). It is a diploid (2n = 14), self-pollinating annual crop, and originated in the Middle East (Cokkizgin and Shtaya 2013). Lentil is known to be one of the earliest domesticated plants

cultivated, tracing back to about 10,000 years (Guerra-Garcia et al. 2022; Liber et al. 2021). Currently, lentils are cultivated in more than 52 countries, covering approximately 5.58 million hectares, with an annual production of 6.65 million metric tons (FAOSTAT 2022). Canada and India are the leading producers, together contributing over 60% of the total global production (Khazaei et al. 2019). The crop's ability to fix atmospheric nitrogen through symbiosis with *Rhizobium* spp. can contribute to enhancing soil fertility, decreasing the reliance on synthetic fertilizers, and promoting sustainable agricultural practices (Siddique et al. 2023; Stagnari et al. 2017). Furthermore, lentils exhibit moderate drought tolerance, making them suitable for cultivation in arid and semiarid regions (Kumar et al. 2016; Subedi et al. 2021). Nutritionally, cultivated lentils are highly valued for their rich protein content, with an average of 26% of dry biomass (Dhull et al. 2023; Iqbal et al. 2006; Joshi et al. 2017). Earlier studies performed in various regions of the world have recorded wide variabilities in the crude protein (CP) content, ranging between 20% to 36% of dry biomass (Karaköy et al. 2012; Tahir et al. 2011; Wang and Daun 2006). In addition to protein, lentils provide essential amino acids, fiber, micronutrients (iron, zinc, and folate), and bioactive compounds with health-promoting properties (Carbonaro and Nucara 2022; Tessari et al. 2016; Wu 2013). However, like most legumes, lentils are low in sulfur-containing amino acids, such as methionine and cysteine, which can be resolved by dietary complementation with cereals (Margier et al. 2018; van Vliet et al. 2015).

Despite their nutritional and agricultural benefits, lentil production encounters challenges related to genotype-by-environment (G×E) interactions, which significantly impact yield, protein content, and amino acid composition (Acuña and Wade 2013; Annicchiarico 2002; Aruna et al. 2016; Subedi et al. 2021). Environmental factors such as temperature, precipitation, and soil fertility influence seed composition, often resulting in significant variation across environments (Bansal et al. 2023; Chen et al. 2022; M. S. Erskine 1985; Zeroual et al. 2023). The identification of genotypes with superior adaptability and nutritional traits relies on robust agronomic characterization of genetic resources. The INCREASE project (Bellucci et al. 2021) exemplifies this effort by integrating genomic, phenotypic, and phenomic data across major legumes such as common bean and lentil (Cortinovis et al. 2021; Guerra-Garcia et al. 2022). Moreover, understanding the genetic basis of protein content and other nutritional profiles is vital for developing improved cultivars with enhanced nutritional traits (Johnson et al. 2024; Margier et al. 2018; Salaria et al. 2022). Since expression of nutritional traits is polygenic and strongly influenced by environmental variability, conventional breeding approaches are often insufficient for identifying stable, high-protein genotypes (Jin et al. 2013). Consequently, advanced statistical frameworks that explicitly model genotype-by-environment (G×E) interactions have become essential for evaluating performance stability and guiding selection in lentil improvement programs (Sabaghnia et al. 2008; ElBradei 2015; Gebremedhin et al. 2024). Statistical models have been established to assess G×E interaction and genotype performance based on multienvironment trials. The Additive Main Effects and Multiplicative Interaction (AMMI) model partitions genetic and environmental effects and provides insights into both the stability and adaptability of genotypes (Gauch 2006; Gauch et al. 2008; Yan et al. 2007). Similarly, the Genotype plus Genotype-by-Environment (GGE) Interaction biplot method evaluates both genetic and environmental effects on trait performance (Crossa et al. 1999; Yan et al. 2000). More recently, the Weighted Average of Absolute Scores (WAASB) index has been employed to quantify genotype stability across diverse environments (Olivoto et al. 2019; Piepho 1994). Integrating these statistical approaches enables plant breeders to select genotypes with superior nutritional attributes while ensuring adaptability to different environmental conditions. In lentils and other pulses, protein content and yield tend to be negatively correlated; that is, a higher yield can be associated with a lower protein content. Thus, simultaneously selecting for high yield and protein content can be challenging for breeders (Erskine et al. 2003; Tziouvalekas et al. 2022; Vlachostergios et al. 2021).

Furthermore, while there is been an appreciable number of studies on lentil protein and yield stability, limited studies have integrated amino acid profiling with stability analysis across environments. This leaves a gap in understanding the consistency of nutritional quality in lentil breeding. Given the increasing demand for plant-based protein sources and the importance of global nutritional security, it is crucial to identify lentil genotypes that display high and stable nutritional content across multiple environments (Hang et al. 2022; Pugliese et al. 2024). Therefore, this study aims to investigate the variation of nutritional profiles in lentil, while assessing the effects of genotype, environment, and G×E interactions on CP content, crude protein yield (CPY), total amino acid composition, and amino acid yield in lentils. By employing AMMI, GGE, and WAASB models, we seek to identify high-performing and stable genotypes that can serve as potential candidates for future lentil breeding programs.

2 | Materials and Methods

2.1 | Plant Material

A total of 324 Single Seed Descent (SSD) lentil genotypes from the AGILE project (*Application of Genomic Innovation in the Lentil Economy, KnowPulse*, led by the University of Saskatchewan, Saskatoon, SK) were previously tested in a field trial carried out in the 2016–2017 season in Metaponto (Italy), in collaboration with the University of Basilicata (Wright et al. 2021). The lentil panel mostly includes landraces from 45 different countries. Out of the entire panel, we initially selected 30 lentil lines that showed the highest yield in the Metaponto field trial (Rocchetti et al. 2025; Wright et al. 2021). We added 11 Italian landraces previously collected from local farmers and seed companies, three Italian cultivars provided by AgroService ISEA, and two French cultivars provided by Agri-Obtention, reaching a total number of 46 lentil lines (Table S1) (Rocchetti et al. 2025).

The 46 lines were evaluated across seven agronomic trials that have been carried out in 3 years (2019, 2020, and 2021), two sowing seasons (autumn and spring) and two Italian localities, that are the Research Centre for Cereal and Industrial Crops (CREA-CI) experimental station of Osimo (Ancona, latitude 43°45′04″, longitude 13°49′98″) and the experimental station of Metaponto (University of Potenza, Matera, latitude 40°23′24″, longitude 16°46′48″) (Table 1).

TABLE 1 | List of seven field trials (environments) given by the combination of localities, sowing season, and years of cultivation.

Locality	Metaponto			Osimo					
Sowing	Autumn			Autumn			Spring		
Year	2019	2020	2019	2020	2021	2020	2021		
Name of the trial	Metaponto autumn 2019	Metaponto autumn 2020	Osimo autumn 2019	Osimo autumn 2020	Osimo autumn 2021	Osimo spring 2020	Osimo spring 2019		

Those trials allowed further testing of the agronomical value of the selected material.

The following environmental variables were recorded for the Metaponto and Osimo locations for the 3 years (2019, 2021, and 2022) and sowing seasons (Autumn and Spring): average temperature (°C), max temperature (°C), minimum temperature (°C), and rainfall (mm) (Figure S1). Field trials were conducted using a Randomized Complete Block Design (RCBD) with three replicates of $10m^2$ plot size, and six rows with 0.15 m distance between rows per plot. Sowing was conducted during December for the autumn sowing and during March for the spring sowing. For details, see (Rocchetti et al. 2025).

Out of the 45 lines evaluated in seven environments, 15 diverse lines (potential prebreeding candidates) (Table 2) were selected for protein and amino acids quantification based on the availability of seed materials in at least two replicates per trial, ensuring consistent representation across environments. Although the experimental design included three replicates per genotype within each trial (expected $n = 315$), some genotypes were represented by two replicates as some were also represented by three, resulting in an overall sample count of 284 analyzed samples (Table S2) (Table 2).

2.2 | Crude Protein Determination

For crude protein analysis, dry seeds from each sample (284) were ground into a fine powder using liquid nitrogen with a prechilled mortar and pestle to ensure minimal degradation. Approximately 70 mg of the powdered sample was aliquoted into prelabeled tin foil cups (Leco 502–186) and sealed into teardrop-shaped pockets for analysis.

Nitrogen (N), carbon (C), and hydrogen (H) contents were determined using the Dumas combustion method with an elemental analyzer (TruSpec Micro, LECO Corp., St. Joseph, MI, USA). Nitrogen content was used to quantify the total CP for each analyzed sample, based on the Jones factor ($N \times 6.25$), which is a widely accepted conversion factor for protein determination in dry biomass (Mossé and Baudet 1983; Salo-väänänen and Koivistoinen 1996).

The CPY was estimated as follows:

$$\text{CPY}\left(\text{kg ha}^{-1}\right) = \text{Crude Protein Content}\,(\%) \times \text{Seed Yield}\left(\text{kg ha}^{-1}\right)$$

2.3 | Amino Acid (AA) Extraction and Determination

For amino acid quantification, a total of 284 samples were analyzed, using 20 mg of the freeze-dried material previously used for crude protein analysis, which was hydrolyzed with 6 M HCl (500 μL) at 110°C for 24 h. The hydrolysate was then dried under a speed vacuum for 1 h, reconstituted with 0.1 M HCl (500 μL), and centrifuged (6000 rpm, 5 min, 4°C). A tenfold dilution of the supernatant was prepared, and 50 μL of the diluted extract was combined with DL-norvaline (50 μL of 0.05 mg/mL in 0.1 M HCl) as an internal standard. The mixture was dried again and derivatized using the AccQ·Tag Ultra Derivatization Kit (Waters

TABLE 2 | List of genotypes for which at least two replicates (seed material for nutritional analysis) was available at the seven environments evaluated in this study.

Genotypes	Donor	Origin	Biological status
PI_612875_LSP	AGILE/UNIBAS	Syria	Breeding material
W6_27760_LSP	AGILE/UNIBAS	USDA	Breeding material
PI_533693_LSP	AGILE/UNIBAS	Spain	Cultivar
Anicia	Agri-Obtentions	France	Cultivar
Flora	Agri-Obtentions	France	Cultivar
PI_298122_LSP	AGILE/UNIBAS	France	Cultivated material
PI_299351_LSP	AGILE/UNIBAS	Chile	Cultivated material
IG_1959	AGILE/UNIBAS	Ethiopia	Landrace
PI_426778_LSP	AGILE/UNIBAS	Pakistan	Landrace
PI_431663_LSP	AGILE/UNIBAS	Iran	Landrace
PI_431710_LSP	AGILE/UNIBAS	Iran	Landrace
PI_431728_LSP	AGILE/UNIBAS	Iran	Landrace
PI_431739_LSP	AGILE/UNIBAS	Iran	Landrace
PI_431753_LSP	AGILE/UNIBAS	Iran	Landrace
PI_432033_LSP	AGILE/UNIBAS	Iran	Landrace

Corporation, Milford, MA, USA). Subsequently, 1 μL of each derivatized sample was injected into a UPLC (Waters, USA) system equipped with a photodiode array (PDA) detector and an AccQ Tag Ultra Column (2.1 × 100 mm, 1.7 μm). Ultrahigh-Performance Liquid Chromatography (UHPLC) data were normalized to account for run-to-run variability across days, and the resulting normalized matrix was used for amino acid quantification. Standard calibration curves were prepared using a mixture of amino acids (1 pM to 25 pM in 0.1 M HCl) to represent each concentration level. Essential amino acids (EAA) identified included Histidine (His), Threonine (Thr), Lysine (Lys), Tyrosine (Tyr), Methionine (Met), Valine (Val), Leucine (Leu), Isoleucine (Ile), and Phenylalanine (Phe). Nonessential amino acids (NEAA) comprised Alanine (Ala), Arginine (Arg), Aspartic acid (Asp), Cysteine (Cys), Glutamic acid (Glu), Glycine (Gly), Proline (Pro), and Serine (Ser). The total amino acid yield (TAAY) was calculated as follows:

$$\text{TAAY}\left(\text{kg ha}^{-1}\right) = \text{Total Amino Acids}\,(\%) \times \text{Seed Yield}\,(\text{kg ha}^{-1})$$

Data for CP content, total amino acids (TAA), essential amino acids (EAA), CPY, TAA yield, and the 17 detected amino acids are presented in Table S2.

2.4 | Analysis of Variance and Trait Heritability

The phenotypic variability and heritability for nutritional traits in the 15 genotypes grown under different environments have been investigated. A one-way ANOVA followed by post hoc Tukey's Honestly Significant Differences (Tukey's HSD) analysis was performed to identify significant differences among the sample means. For data visualization, boxplots have been generated by using the online software Metaboanalyst 5.0 (Pang et al. 2021). Considering the linear model (1), the phenotypic value of a trait of any individual in a given environment can be obtained based on the overall mean (μ), genetic (G), environmental (E), genotype-by-environment interaction (GEI) effects, and *e*, which is the residual effect within each environment, assuming it is normally distributed:

$$P = \mu + G + E + GEI + e \quad (1)$$

The Residual Maximum Likelihood (RELM) was used to estimate variance components, which are G, E, and GEI effects, and the associated standard errors, as random factors. Broad-sense heritability (H^2) was estimated following the variance partition, using the following formula (2):

$$H^2 = \sigma^2 G / \left(\sigma^2 G + \left(\sigma^2 GEI / n\right) + \left(\sigma^2 e / n^* r\right)\right) \quad (2)$$

where $\sigma^2 G$ represents the genetic variance, $\sigma^2 GEI$ the variance of genotype-environment interaction, *n* the number of environments, *r* is the number of replicates in each environment, and e is the residual error. Based on model (1), we performed ANOVA to test the significance of G, E, and GEI effects, as fixed factors. The environment was dissected into locality (L) and sowing season (S) effects. Statistically significant differences among genotypes, localities, and seasons were determined by using Tukey's test at the probability level of $p < 0.05$. Two multivariate analyses were conducted: (i) a principal component analysis (PCA) was carried out based on the mean value of each trait in each environment to investigate the relationships among the different genotypes by JMP Pro, Version 18.0.2 (JMP 1989); (ii) a heatmap, representing abundances of each trait by false color imaging on the $\log_{10}$-transformed data across the genotypes, combined with two-dimensional

hierarchical clustering, performed with the hclust function in the package stat implemented in Metaboanalyst ver.5.0 (Pang et al. 2021). Similarity was computed through Euclidean distance, employing the Ward D clustering algorithm, which aims to cluster by minimizing the sum of squares of any two clusters. To identify key traits driving variation among the amino acids, we used PLS-DA to generate a Variable Importance in Projection (VIP) plot, highlighting the most influential nutritional compounds based on their relative concentrations (Figure S2). One-way analysis of variance (ANOVA) was computed to compare genotypes grouped by their biological status (landraces versus cultivar and breeding materials) for the nutritional traits considered. Significant differences among groups were tested by applying Tukey's test ($p < 0.05$).

2.5 | Correlation Analysis

Pearson correlation analysis was performed among the mean values of the amino acids, the total amino acid value, and the CP content using JMP Pro version 18.0.2 (JMP 1989).

2.6 | AMMI

Nitrogen content data from all the test environments were subjected to the AMMI (Olivoto et al. 2019). AMMI combines ANOVA and the single value decomposition (SVD) to break down genotype × environment interactions and uses biplots to visually interpret these effects. AMMI analysis was employed to evaluate the GEI effect, identify specific positive genotype-environment interactions, or determine stable genotypes. This is achieved by dissecting GEI into principal components (i.e., PC in a Principal Component Analysis). The first Principal Component Axis (PCA1) and the first two Principal Component Axes (PCA1 and PCA2) were used to generate AMMI biplots (PCA1 vs. PCA2).

2.7 | GGE Interaction

The Genotype plus Genotype-environment interaction (GGE) model (Yan et al. 2000) considers the joint effects of the genotypic main effect, together with GEI. This model was used to investigate trait productivity and to identify similarity among environmental performances, based on the genotype rankings, and the best genotypes performing in each environment. Results are presented using GGE biplots, which approximate overall performance (G + GEI).

2.8 | Stability With WAASB

The stability index WAASB developed by Olivoto et al. (2019) was used to model the genotype performance versus stability across environments. This model applies the SVD (Singular Value Decomposition) of the BLUP matrix for the GEI effect generated by Linear Mixed Model (LMM). The genotype with the lowest WAASB value is considered the most stable: that is, the one that deviates the least from the average performance across environments. Results were reported using scatter plots for each trait, representing the phenotypic values of each genotype (abscissa) and the WAASB index (ordinate).

3 | Results

3.1 | Phenotypic Variation for Nutritional Traits

The total amino acid (TAA) content has been investigated in the seeds from the selected lentil accessions previously grown across different environments and years of trial. The amount of 17 amino acids was quantified by high-throughput UHPLC (Figure 1). CP, CPY, TAA, and TAAY have been quantified for each genotype, considering all replicates in each environment

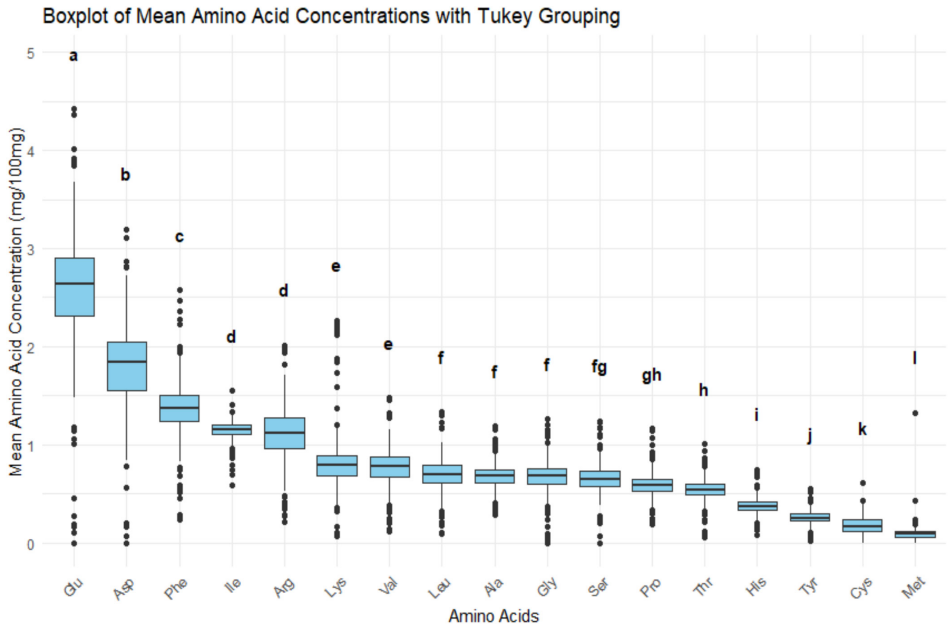

FIGURE 1 | Representation of the individual amino acid profiles (nutritional quality) across 15 lentil genotypes. A Boxplot of the mean and range of all amino acid content for 15 lentil genotypes averaged across localities and years. (Different letters denote statistically significant differences between amino acids based on Tukey's post hoc test ($p < 0.05$)).

(Figure S3). A higher phenotypic variability can be observed among genotypes when considering TAA (CV = 13.77), compared to CP (CV = 7.45). The average values across replicates and environments for each genotype, and summary statistics, are provided for the nutritional traits that are under investigation (Table 3). CP content ranged from 24.31% to 35.90%, with a mean of 29.27%, higher than the typical values reported in the literature for lentil. Similarly, variation was observed in both TAA and total essential amino acid (TEAA) contents, which ranged from 8.57 to 19.80 mg/100 mg (mean: 14.26 mg/100 mg) and from 4.60 to 8.00 mg/100 mg (mean: 6.05 mg/100 mg), respectively (Table 3).

With regards to the single amino acid (AA) content, the sulfur-containing amino acids (Met and Cys) are present in relatively low quantities, that is, 0.09 mg/100 mg and 0.18 mg/100 mg, respectively, as could be expected in lentils and legumes in general. However, variation can be observed among the traits, with TSAA content varying from 0 mg/100 mg (concentrations below the detection threshold of the UHPLC method) to 0.63 mg/100 mg (Table 3). High contents of Glu and Asp and intermediate levels of Phe, Ile, and Arg can be observed in the analyzed samples. (Figure 1, Table 3).

3.2 | ANOVA, Heritability, and Correlation Among the Nutritional Traits

Broad-sense heritability (H^2) was estimated for 22 traits (Table 4). Focusing on each AA, H^2 ranged from 0 (Lys, Met, and Ile) to 0.78 (Cys). The Asp and Glu AAs showed relatively lower heritability (H^2 of 0.21 and 0.12, respectively) when compared to other AAs. Eight AAs (His, Arg, Ala, Pro, Cys, and Tyr) showed high heritability ($H^2 > 0.59$), while four AAs

TABLE 3 | Average values across replicates and environments for crude protein, crude protein yield, and amino acid composition for 15 selected lentil genotypes. Minimum, maximum values, standard deviation, coefficient of variation, and value range are provided.

Trait	Mean	Min	Max	Std dev	CV	Range
Crude protein (%)	29.27	24.31	35.90	2.17	7.45	11.60
Protein yield (kg/ha)	156.95	6.65	514.43	114.14	73.07	507.79
TAA (mg/100 mg)	14.26	8.57	19.85	1.95	13.77	11.27
Amino acid yield (kg/ha)	76.66	2.90	259.42	57.62	75.53	256.51
TEAA (mg/100 mg)	6.05	4.64	8.00	0.60	10.03	3.35
Essential amino acids (mg/100 mg)						
His	0.38	0.26	0.55	0.06	15.31	0.29
Thr	0.54	0.37	0.73	0.07	13.29	0.36
Val	0.77	0.51	1.15	0.12	15.65	0.64
Lys	0.84	0.51	1.60	0.21	25.52	1.09
Ile	1.14	0.91	1.31	0.07	5.97	0.40
Leu	0.69	0.44	1.06	0.11	15.99	0.61
Phe	1.36	0.95	1.94	0.19	14.23	0.98
Met	0.09	0.00	0.73	0.08	85.82	0.73
Nonessential amino acids (mg/100 mg)						
Ser	0.65	0.00	0.96	0.13	19.80	0.96
Arg	1.12	0.70	1.62	0.19	17.00	0.91
Gly	0.68	0.00	1.14	0.13	19.44	1.14
Asp	1.78	0.00	2.83	0.39	21.81	2.83
Glu	2.57	0.00	3.90	0.48	18.89	3.90
Ala	0.68	0.50	0.92	0.08	12.48	0.43
Tyr	0.26	0.12	0.38	0.05	19.30	0.26
Pro	0.59	0.42	0.86	0.08	14.35	0.45
Cys	0.18	0.00	0.43	0.07	40.61	0.43
Total sulfur amino acid (mg/100 mg)						
TSAA (Met + Cys)	0.23	0.00	0.63	0.09	38.94	0.63

(Ser, Gly, Val, and Leu) showed an intermediate estimated H^2 ($0.37 < H^2 < 0.44$). Also, except for TEAA, for which we estimated a lower heritability ($H^2 = 0.13$), CP, TAA, CPY, and TAAY showed high heritability estimates ($H^2 = 0.78$, 0.43, 0.66, and 0.59, respectively).

Analysis of variance indicates genotype effects were significant for most nonessential amino acids, while environment and G × E effects were the main determinants of specific amino acids (e.g., Arg, Asp, and Met). The high residual variance observed suggests that unmeasured factors, including management practices or microenvironmental effects, contributed to the unexplained portion (Table 4). Genotype, environment, and GEI effects were significant for CP, CPY, and TAAY, with most variance significantly attributable to the environment (Table 4). For TAA, a significant portion of the variance was due to GEI (13.4%) and genotype (4.5%); however, a high contribution was detected for residual (82.1%), indicating also in

TABLE 4 | Variance partitioning among genotypic effect (Gen), environmental effect (Env), replication within the environment (rep), interaction genotype × environment (GEI), and residual (e). Broad-sense heritability (H^2) is estimated from the regression between BLUP and phenotypic values and their respective ANOVA.

Source of variation	Gen	Env	Rep	GEI	Residual				
Degree of freedom	14	6	2	84			ANOVA		
	% total var	% total var	% total var	% total var	% total var	H^2	Gen	Env	GEI
Traits									
Crude protein (%)	14.71	33.12	1.50	19.39	31.27	0.78	***	***	***
Crude protein yield (kg/ha)	10.42	36.59	3.16	31.22	18.61	0.66	***	***	***
TAA (mg/100 mg)	4.48	0.00	0.00	13.44	82.08	0.43	***	NS	*
TAA yield (kg/ha)	7.92	35.32	2.88	31.60	22.30	0.59	***	***	***
TEAA (mg/100 mg)	0.69	0.22	0.00	0.00	99.10	0.13	NS	NS	ns
Essential amino acids (mg/100 mg)									
Histidine	8.00	1.85	0.00	5.54	84.21	0.62	**	NS	NS
Threonine	6.42	0.85	1.15	0.86	90.71	0.59	*	NS	NS
Valine	3.64	0.00	0.00	1.49	94.87	0.43	NS	NS	NS
Lysine	0.00	10.50	0.00	0.00	89.50	0.00	NS	***	NS
Isoleucine	0.00	16.56	0.00	8.41	75.03	0.00	NS	***	NS
Leucine	3.72	0.00	0.00	1.30	94.98	0.44	NS	NS	NS
Phenylalanine	6.51	0.00	0.00	1.74	91.76	0.59	*	NS	NS
Methionine	0.00	0.00	0.00	31.13	68.86	0.00	NS	NS	*
Nonessential amino acids (mg/100 mg)									
Serine	4.28	0.00	0.84	30.56	64.32	0.37	***	NS	***
Arginine	9.80	7.50	0.00	4.60	78.10	0.69	***	***	NS
Glycine	4.60	0.00	0.00	30.87	64.53	0.38	***	NS	**
Asparagine	2.22	0.56	0.00	39.93	57.29	0.21	***	**	***
Glutamic acid	1.04	0.00	0.00	33.48	65.49	0.12	**	NS	***
Alanine	7.55	0.59	0.00	4.15	87.70	0.61	**	NS	NS
Proline	8.96	0.13	0.00	2.78	88.13	0.66	**	NS	NS
Tyrosine	7.76	0.00	0.00	1.56	90.68	0.63	**	NS	NS
Cysteine	17.85	9.09	0.00	17.55	55.50	0.78	***	***	***

Note: Heritability ranges: low (< 0.30), intermediate (0.3–0.60), and high (> 0.60).
Abbreviation: N.S., not significant.
***= $p < 0.0001$.
**= $p < 0.001$.
*= $p < 0.05$.

these cases that a significant portion of the variance is not explained, and the presence of other factors influences total amino acid level (Table 4).

Pearson correlation coefficients were computed among all traits (Figure 2). A significant and positive correlation was found between CP and TAA ($r^2 = 0.22$, $p = 0.0002$). This correlation was also detected when performing the Simple Linear Regression between the CP and TAA by using single replicate data from all the field trials (Figure S4). CP content shows a significant positive correlation with almost all the amino acids, except for Asp, Met, and Ile, while a negative and significant correlation was detected with Lys. Significant and positive correlations were detected among TAA and almost all the amino acids except for Lys and Ile. Comparing the different amino acid content across samples, we detected strong correlations among almost all of them (Figure 2). Ile and Lys did not correlate with any other amino acid. Met positively and significantly correlates with Asp, Gly, Pro, Ser, and Thr. Cys showed weak but significant and positive correlations with several amino acids, but not with Met, Lys, Tyr, and Ile.

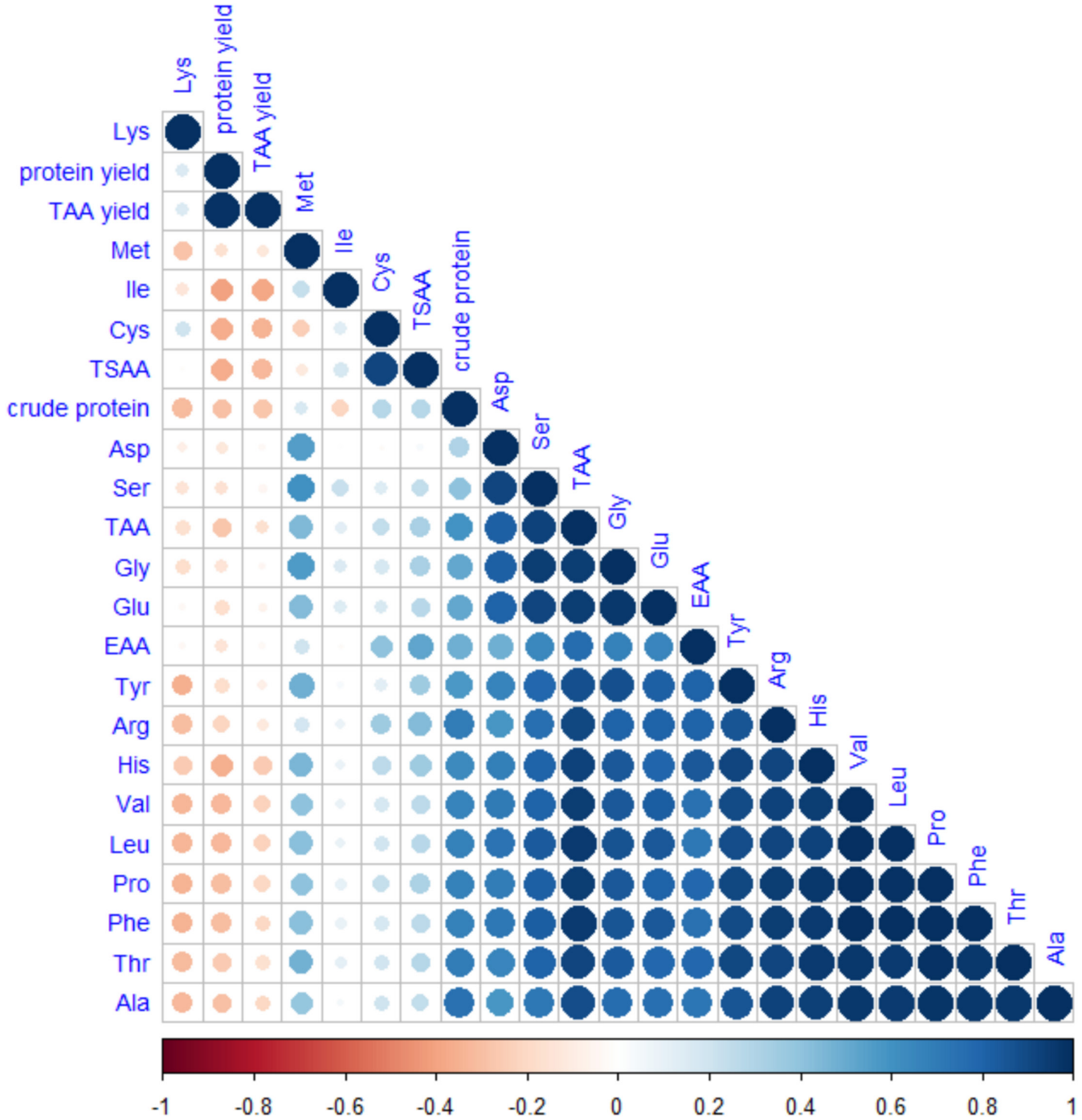

FIGURE 2 | Pearson correlation between amino acids, crude protein, crude protein yield, essential amino acid (EAA), total amino acid (TAA), total sulfur amino acids (TSAA), and total amino acid yield (TAAY). The size and color of each circle represent the strength and direction of Pearson correlation coefficients, with blue indicating positive correlations and red indicating negative correlations.

3.3 | Phenotypic Variability Across Genotypes

A Principal Component Analysis (PCA) representing the phenotypic space among the lentil genotypes for nutritional traits is provided in Figure 3. PC1 and PC2 accounted for 60.9% and 11.7% of the total phenotypic variance, respectively. PC1 allows separate genotypes based on the content of the majority of amino acids (except for Cys, Ile, Lys, and Met) and of CP, TAA, and TEAA (Figure 3 and Table S3). PC2 was significantly and positively correlated with CPY and TAA yield, which are traits influenced by the nutritional composition and the overall yield, while it negatively correlated with Cys and TSAA content (Table S4). The W627760 breeding line showed the highest CPY and TAA yield, while the PI431739 genotype showed the highest content of Cys and TSAA. The cultivars Flora, Anicia, and PI533693 were among the genotypes showing low content of AAs, CP, and TAA. We then compared genotypes, localities, and sowing seasons by performing an ANOVA, considering G, E, and GEI effects as fixed factors for CP, TAA, CPY, and TAAY (Tables 5 and 6; Figures 4 and 5). The analysis revealed that the effects of genotype (G), environment (E), and their interaction (G × E) were statistically significant for CP, CPY, and TAAY content. The influence of genotype (G) and the G × E had a significant impact on TAA composition, while a nonsignificant environmental effect was observed. CP content significantly varied among genotypes, with landraces showing the highest values, and in particular genotypes PI431710 and PI431739 (Figure 4a). Overall, a lower variability among genotypes was observed in TAA content (Figure 5a) across environments; however, two landraces, PI431728 and PI431710, which are also the genotypes showing the highest CP content, exhibit the highest mean TAA content (Figure 5a). Regarding the TAA, landraces still exhibit the highest values, except for a breeding material (line PI_612875) that is among the best lines. Regarding CPY and TAAY, four genotypes (IG1959, PI431663, PI432033, and PI612875) showed the highest values for both traits (Figure 4b and Figure 5b).

No significant differences were detected among the seven environments for TAA content, nor among localities and sowing seasons (Figure 5a). In contrast, significant differences were observed for CP content among the tested environments (Figure 4a). In fact, the highest mean value was found in the OS21 field trial, followed by OA20 and OA19, while the lowest mean values were detected in OS20 and MA19 (Figure 4a). That is, while there was no significant difference in terms of sowing season, we detected an overall higher production at the Osimo location. The OA21 field trial showed the highest nutritional yields, followed by OA19 and MA19; significantly higher mean values were observed in Osimo compared to Metaponto locality, as well as in autumn compared to spring sowing seasons (Figures 4b, 5b).

When we compared nutritional traits by grouping genotypes for biological status (landraces versus commercial and breeding varieties), only for CP, we found that landraces are characterized by a significantly higher content (Figure S5 and Table S5).

3.4 | Stability Analysis of the Genotypes for CP and CPY

AMMI model-based stability analysis was conducted to partition genotype-by-environment interaction (GEI) effects for both CP content and CPY. For CP, the first and second interaction principal component axes (IPCAs) accounted for the majority of GEI variation, explaining 58.2% and 20.3% of the GEI sum of squares, respectively, capturing 78.5% of the total GEI (Table 7, Figure 6a). In the case of CPY, the first three IPCAs cumulatively explained 93% of the GEI variance (Table 7, Figure 6b).

Two distinct environmental groups, Osimo Spring (2020 and 2021) and Metaponto Autumn (2019 and 2020), were particularly influential in driving GEI for both traits. Notably, lentil genotypes grown in Osimo Spring 2021 exhibited higher CP and CPY levels compared to those cultivated in Osimo Spring 2020. Conversely, the autumn environments were characterized by lower eigenvector values, indicating a reduced contribution to GEI relative to the spring sowing environments.

Most genotypes demonstrated limited interaction with the environment for both CP and CPY, including PI_431753_LSP, PI_431663_LSP, and PI_533693_LSP. In contrast, genotypes PI_612875, PI_431739_LSP, and Anicia exhibited significantly higher GEI effects, suggesting specific adaptation to particular environments (Figure 6a).

To integrate the AMMI analyses in the presence of a significant genotype-by-environment interaction (GEI) effect, genotype main effect (G) and genotype-environment (GGE) biplots for CP and CPY, commonly referred to as "which-won-where" plots, are presented in Figure 7. The combined contribution of the first two principal components (PC1 and PC2) to the variation in CP and CPY was 79.99% and 81.21%, respectively (Figure 7).

The biplot analysis for CP identified a mega-environment consisting of MA_19, MA_20, OA_19, OA_21, and OS_21, which predominantly represent autumn sowing seasons. Within this mega-environment, genotypes PI_299351_LSP, PI_431710, and PI_431739_LSP demonstrated superior performance (Figure 7a), while Flora, Itaca, PI_612875_LSP, and PI_431728_LSP showed the least favorable results. For CPY, the GGE biplot revealed two distinct mega-environments (Figure 7b). The first mega-environment, primarily encompassing the autumn growing seasons (MA_19, MA_20, OA_19, and OA_21), featured PI_612875 and PI_426778_LSP as the highest-performing genotypes. In contrast, the second mega-environment, which included both spring seasons (OS_20 and OS_21) as well as the OA_20 trial, highlighted IG_1959 and PI_431663_LSP as the top-ranking genotypes (Figure 7b).

Genotype stability was further assessed using the Y × WAASB biplot, with mean CP and CPY values plotted on the *x*-axis and WAASB values on the *y*-axis (Figure 8) as described by Olivoto et al. (2019). Genotypes PI_431710, PI_431736_LSP, PI_431753_LSP, IG_1959, PI_431728_LSP, and PI_298122_LSP emerged as the most stable and productive for CP content,

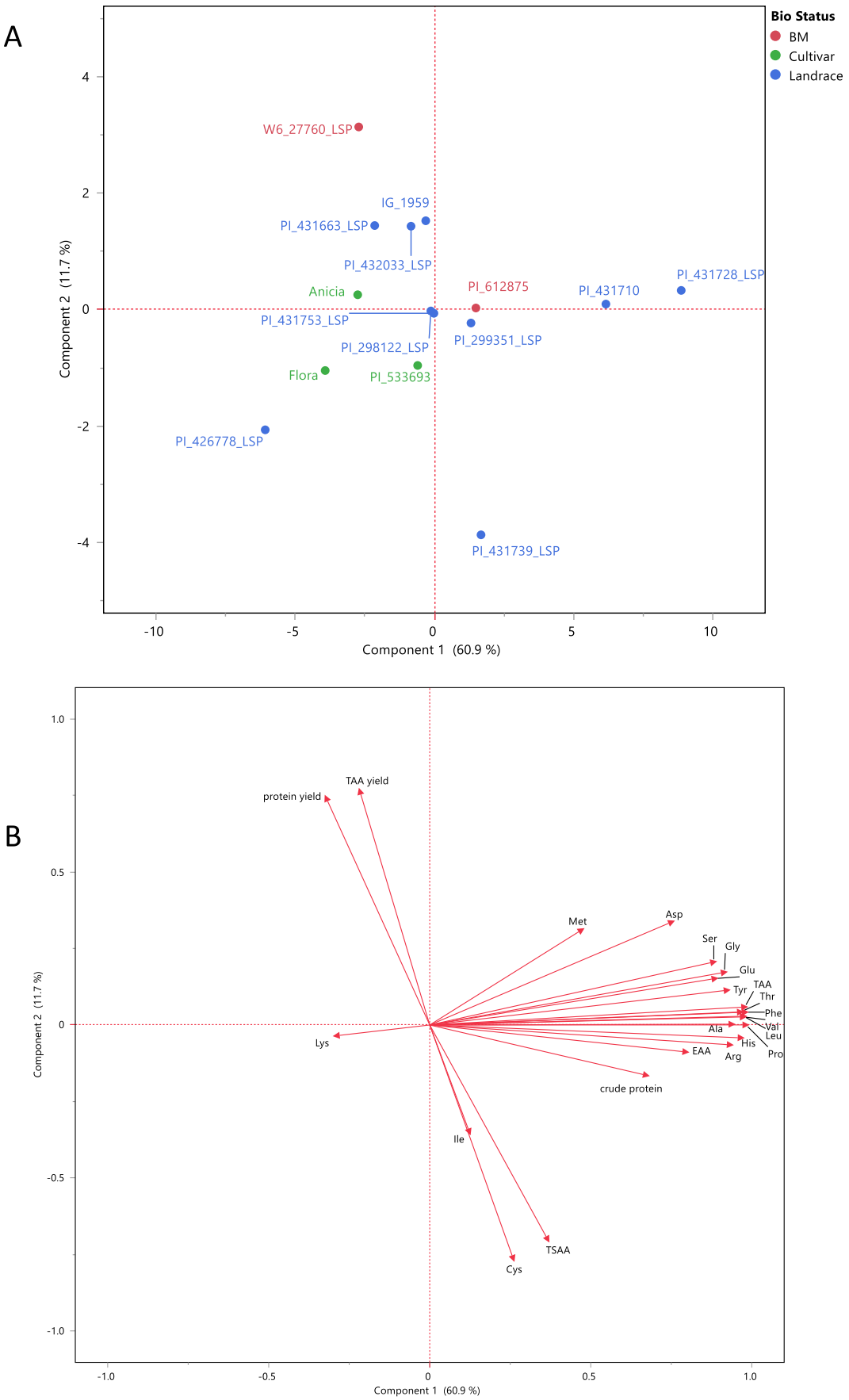

FIGURE 3 | Principal component analysis based on the average values of all the traits for each genotype (a) score plot highlighting the different biological status: landraces (blue), breeding material (red) and cultivar (green); (b) the loading plot for all traits.

TABLE 5 | Analysis of variance for seed crude protein and crude protein yield for 15 lentil genotypes, considering all environments (years/location and sowing season).

ANOVA		Crude protein			Crude protein yield		
Source of variation	**df**	***F*-value**	**Pr (> F)**	**Proportion**	***F*-value**	**Pr (> F)**	**Proportion**
ENV	6	27.65	***	33.12	20.72	***	36.12
GEN	14	11.25	***	14.71	17.97	***	10.42
GEN: ENV	84	2.62	***	19.39	5.98	***	31.22

Abbreviation: N.S., not significant.
***= $p < 0.0001$.
**= $p < 0.001$.
*= $p < 0.05$.

TABLE 6 | Analysis of variance for seed total amino acids and total amino acids yield for 15 lentil genotypes, considering all environments (years/location and sowing season).

ANOVA		Total amino acid			Total amino acid yield		
Source of variation	**df**	***F*-value**	**Pr (> F)**	**Proportion**	***F*-value**	**Pr (> F)**	**Proportion**
ENV	6	0.9	NS	0	20.49	***	35.32
GEN	14	2.5	**	4.48	12.97	***	7.92
GEN: ENV	84	1.36	*	13.44	5.15	***	31.6

Abbreviation: N.S., not significant.
***= $p < 0.0001$.
**= $p < 0.001$.
*= $p < 0.05$.

occupying the fourth quadrant that represents high performance and stability (Figure 8a). For CPY, IG_1959 and PI_431663_LSP were identified as the most stable and productive genotypes (Figure 8b).

3.5 | Stability Analysis of the Genotypes for TAA and Total Amino Acids Yield (TAAY)

An AMMI model-based analysis was conducted to partition the genotype-by-environment interaction (GEI) for TAA. The results revealed that the first principal components significantly accounted for most of the observed variation, contributing cumulatively to 46.9% of the GEI (Table 8; Figure 9a,b). Similarly, for TAAY, the GEI was predominantly explained by the first three components, which together accounted for 94% of the variation (Table 8; Figure 10a,b). The AMMI biplot representation (Figure 10) highlighted two pairs of contrasting environments that primarily exemplified GEI for TAAY: Osimo Spring 2020 and 2021, as well as Metaponto Autumn 2019 and 2020 (Figure 10). Most of the tested genotypes, including PI_431710 and IG_1959, showed limited interaction with the environment, whereas PI_612875 and PI_426778_LSP were most affected by environmental variation (Figure 10).

The GGE biplot analysis for TAA and TAAY (Figure 11), illustrating the "which-won-where" pattern, indicated that the combined contribution of PC1 and PC2 to the variation in TAA and TAAY was 61.82% and 80.01%, respectively. For TAA, the biplot revealed two distinct mega-environments: the first included MA_20, OA_19, OA_21, and OS_20, with PI_431728_LSP and PI_426778_LSP emerging as the top-performing genotypes (Figure 11a). The second mega-environment, comprising MA_19, OS_21, and OA_20, identified PI_299351_LSP as the best performing genotype (Figure 11a). These mega-environments also exhibited a stochastic year effect (Figure 11a). The GGE analysis for TAAY (Figure 11b) produced results consistent with those observed in the CPY analysis (Figure 11b).

According to the WAAB biplot analysis, PI_431710, PI_298122_LSP, and PI_431739_LSP emerged as the most stable and high-performing genotypes for TAA content, as they were located in the fourth quadrant representing high performance and stability (Figure 12a). For TAAY, the genotypes W6_27760_LSP, PI_431663_LSP, and IG_1959 were identified as the most stable and high-performing (Figure 12b).

4 | Discussion

4.1 | CP and Total Amino Acid Characterization of Lentil Genotypes

Lentil is a relevant crop for food in European and Mediterranean regions, which offer significant contributions to feeding the growing world population and agricultural sustainability (Khazaei et al. 2019; Montejano-Ramírez and Valencia-Cantero 2024), being a valid alternative as a protein source (Bellucci et al. 2021). Lentils are recognized for

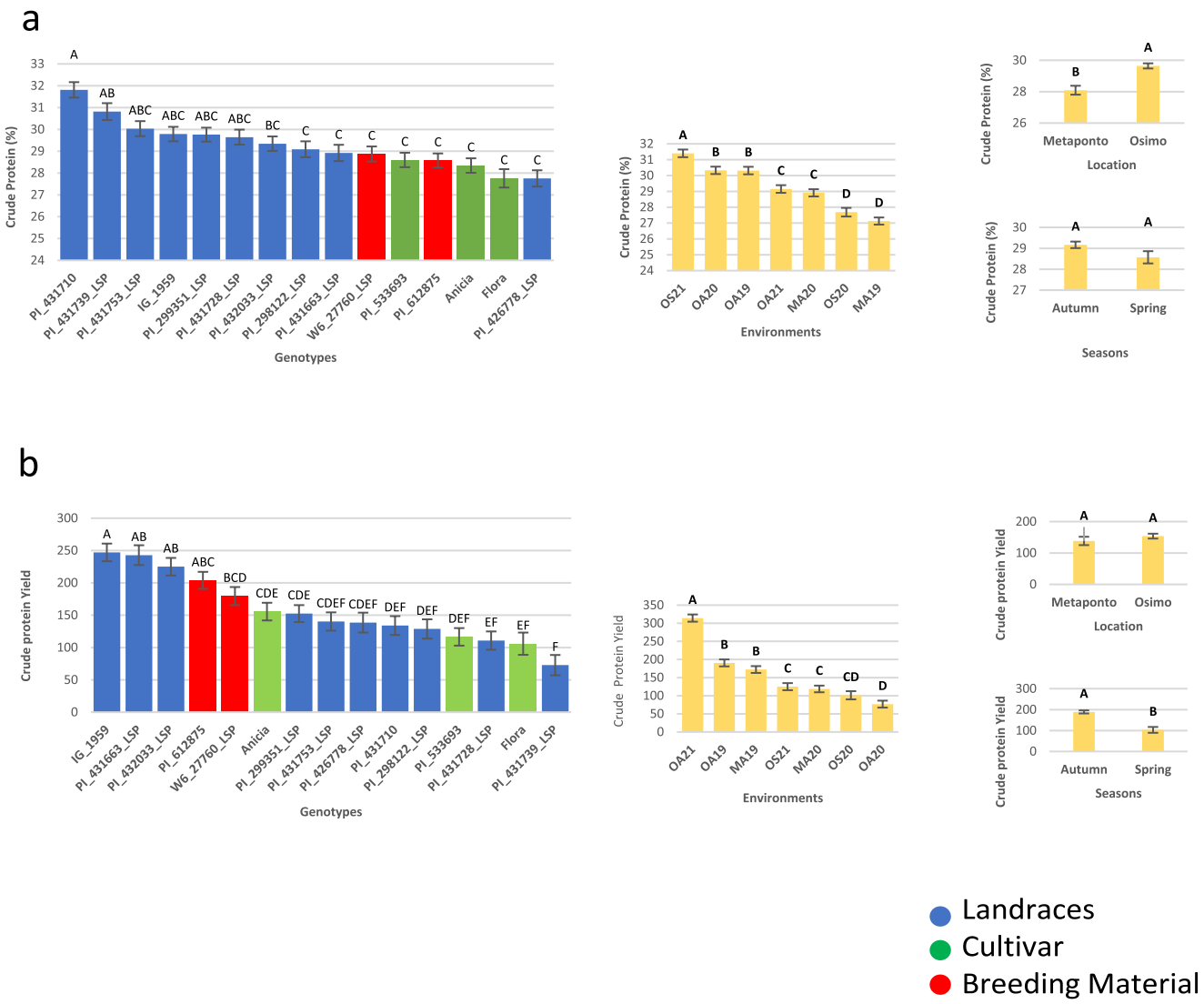

FIGURE 4 | Least square mean plot of seed crude protein (%) (Figure 4a) and crude protein yield (kg ha^{-1}) (Figure 4b) for 15 lentil genotypes averaged across seven environments, a linear model with environments dissected into location and season effect for CP (%) content (Figure 4a) and CPY (kg ha^{-1}). (Different letters denote statistically significant differences between amino acids based on Tukey's post hoc test [$p < 0.05$]).

their high-protein content and balanced amino acid profile, making them an essential protein source in plant-based diets (Joshi et al. 2017). On one hand, it is crucial to identify novel genotypes that are well-adapted to different agroenvironments, ensuring optimal yield and seed quality to cope with the increasing demand for alternative proteins and the parallel climate change challenges. On the other hand, the limited breeding effort that can be recorded so far toward some of the most relevant legume species for food consumption is a major bottleneck for their cultivation and exploitation.

For these reasons, the agronomic characterization of genetic resources is crucial to assess new germplasm with important adaptive and yield traits to be employed in breeding programs. In this study, we investigated the effect of genotype, environment, and their interactions (GEI) on key nutritional traits, such as CP, TAA, and their respective yield components (i.e., CPY and TAAY), that is, the evaluation of seed quality combined with productivity. This work has been carried out considering 15 lentil genotypes of different biological status (i.e., landrace, cultivar, and breeding material), across seven field trials, including different sowing seasons (i.e., autumn and spring), years (2019–2020-2021), and locations (Osimo and Metaponto). Significant differences have been highlighted among genotypes, and environmental and GEI effects were detected. Thus, the study provides an important understanding of how lentil genetic diversity can be exploited for enhanced nutritional quality through targeted breeding programs (Baxevanos et al. 2024; Subedi et al. 2021; Yan et al. 2000).

The amino acid composition of lentil seeds that has been described in this study aligns with previous research, highlighting relatively high levels of glutamic acid, arginine, and aspartic acid among nonessential amino acids (Ghumman et al. 2019; Khazaei et al. 2019; Paucean et al. 2018; Shekib et al. 1986). A deficiency in sulfur-containing amino acids (i.e., methionine and cysteine) was observed, in line with what is known from the literature (Carbonaro and Nucara 2022; Khazaei et al. 2019;

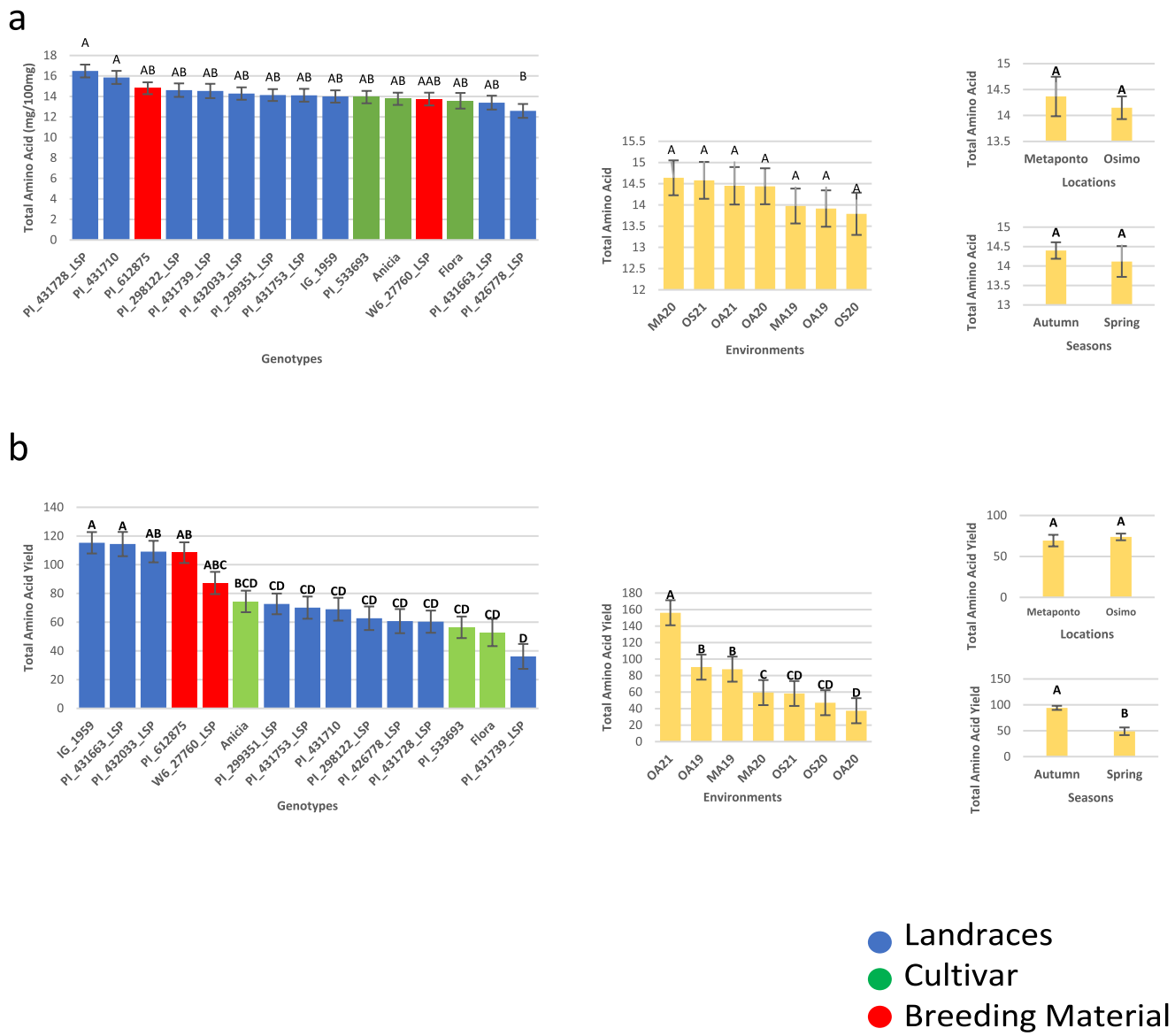

FIGURE 5 | Least square mean plot of seed total amino acid (mg/100 mg) (Figure 4c) and Total amino acid yield (kg ha^{-1}) (Figure 4d) for 15 lentil genotypes averaged across 7 environments, a linear model with environments dissected into location and season effect for TAA(%) content (Figure 4c) and TAAY (kg ha^{-1}). (Different letters denote statistically significant differences between amino acids based on Tukey's post hoc test ($p < 0.05$)).

Salo-väänänen and Koivistoinen 1996; Singh et al. 2023; Sree and Brar 2020; Thavarajah et al. 2009). This emphasizes the need for dietary complementation with cereals, which are a rich source of these limited amino acids (Haug and Lantzsch 1983; Messina 2014; National Research Council 1989; van Vliet et al. 2015). The significant genetic variation observed in non-essential amino acids (NEAAs) is of particular interest, as it supports the identification and selection of high-performing genotypes with stable expression across environments. This suggests strong potential for breeding improvements targeting these traits. In contrast, the environmental effect on essential amino acids such as lysine and isoleucine highlights the role of abiotic factors such as water availability and temperature in influencing nutritional profiles during critical developmental stages, including grain filling (Kumar et al. 2015; Margier et al. 2018). Notably, the heritability estimate of amino acids varies widely, ranging from negligible (0%) in lysine, methionine, and isoleucine to 78% in cysteine. The negligible heritability in these amino acids likely reflects the combined effects of a small number of genotypes evaluated, large residual variance due to experimental or statistical noise, and an appreciable GEI. Traits, such as methionine, that are measured in low concentrations are susceptible to such statistical noise. These emphasize the need for a larger and diverse panel for a multilocation field trial. Furthermore, our correlation analysis revealed a negative relationship between lysine and other nutritional traits, likely due to metabolic trade-offs in biosynthetic pathways (Angelovici et al. 2011; Yang et al. 2020; Zhu and Galili 2004). Such metabolic trade-offs in biosynthetic pathways, where limited resources are allocated between growth and nutrient synthesis, are common in legumes and should be considered in future breeding strategies (Du et al. 2018).

TABLE 7 | Additive main effects and multiplicative interaction (AMMI) results for seed crude protein and crude protein yield of 15 lentil genotypes, considering all environments (years, locations, and sowing seasons). The degree of freedom (Df), *F*-value, and probability are reported in the table.

		Crude protein			Crude protein yield		
Source	**Df**	***F*-value**	**Pr (> F)**	**Proportion (%)**	***F*-value**	**Pr (> F)**	**Proportion (%)**
ENV	6	27.65	***	33.12	20.72	***	36.12
REP (ENV)	14	1.6	NS	1.5	4.36	***	3.16
GEN	14	11.25	***	14.71	17.97	***	10.42
GEN: ENV	84	2.62	***	19.39	5.98	***	31.22
PC1	19	7.47	***	58.2	13.12	***	45.2
PC2	17	2.91	***	20.3	10.42	***	32.1
PC3	15	1.71	NS	10.5	5.76	**	15.7
PC4	13	1.04	NS	5.6	1.52	NS	3.6
PC5	11	0.73	NS	3.3	1.43	NS	2.9
PC6	9	0.58	NS	2.1	0.33	NS	0.5
Residuals	165						
Total	367						

Abbreviation: N.S., not significant.
***$= p < 0.0001$.
**$= p < 0.001$.
*$= p < 0.05$.

Additionally, our study further highlights the importance of environmental factors such as precipitation on CP and CPY (Erskine 1985; Gerrano et al. 2022; Subedi et al. 2021). Whereas genotypic effects are more important for determining CP content, CPY is more influenced by environment, which aligns with observations in other multienvironment studies (Chen et al. 2022; Pugliese et al. 2024). Significant genotype × environment interaction (GEI) for CPY further highlights the complex interplay between genetics and environment (biotic and abiotic factors) in lentil productivity. Interestingly, landrace genotypes were always among the best performing genotypes when considering TAA, TAAY, CP and CPY; in particular, for CPY and TAAY, landraces IG_1959, PI_431663_LSP, and PI_432033_LSP, but also PI_431710 (for both CP and TAA), PI_431739_LSP (CP), and PI_431728 (TAA), are the most interesting lines, making them candidates for future breeding programs. The observed performance among the tested landraces is not surprising, as several studies have demonstrated the importance of genetic diversity within landrace populations. This makes them widely adaptable compared to commercial cultivars under variable temperature and precipitation conditions, supporting their resilience to the environmental stress and suitability for low-input and marginal environments (Tziouvalekas et al. 2022). The reduced protein content in modern cultivars suggests that past breeding efforts may have prioritized yield stability over nutritional quality, emphasizing the need for a balanced approach in future breeding programs (Sá et al. 2024). Although several studies (Sehgal et al. 2018; Behboudian et al. 2001; Singh 2007) have reported reduced nutritional profiles in pulses, our study observed increased CP levels in Osimo Spring 2021 under pronounced drought conditions. This genotype-dependent response suggests that, in some cases, protein synthesis or nitrogen allocation may be redirected toward seed reserves as part of stress adaptation, a phenomenon reported in selected lentil genotypes by Hummel et al. (2018). However, this came at the expense of seed yield and CPY, reinforcing the need for breeding strategies that balance protein content and environmental resilience (Bratković et al. 2024). Total amino acid (TAA) content and yield were primarily influenced by genotype and genotype-by-environment (G × E) interaction (Flores et al. 1998; Sellami et al. 2021; Shi et al. 2023). Environment explained a significant portion of the variation in amino acid yield, underscoring the importance of environmental management in optimizing such nutritional traits (Gerrano et al. 2022). Despite this, amino acid profiles remained relatively stable under moderate drought conditions, suggesting a degree of inherent resilience in lentil nutritional composition (Hang et al. 2022; Wu 2013). TAA overall stability compared to CP may indicate greater genetic control as well as metabolic stability. While nitrogen fixation, assimilation, and translocation processes strongly influence the seed nitrogen and consequently the CP content in legumes, amino acid composition is tightly regulated by intrinsic biosynthetic and enzymatic activities that maintain metabolic balance (Choukri et al. 2020; Bansal et al. 2023). Consequently, during nonuniform nitrogen availability, the amino acid distribution remains the same, which makes it highly heritable (Khazaei et al. 2019).

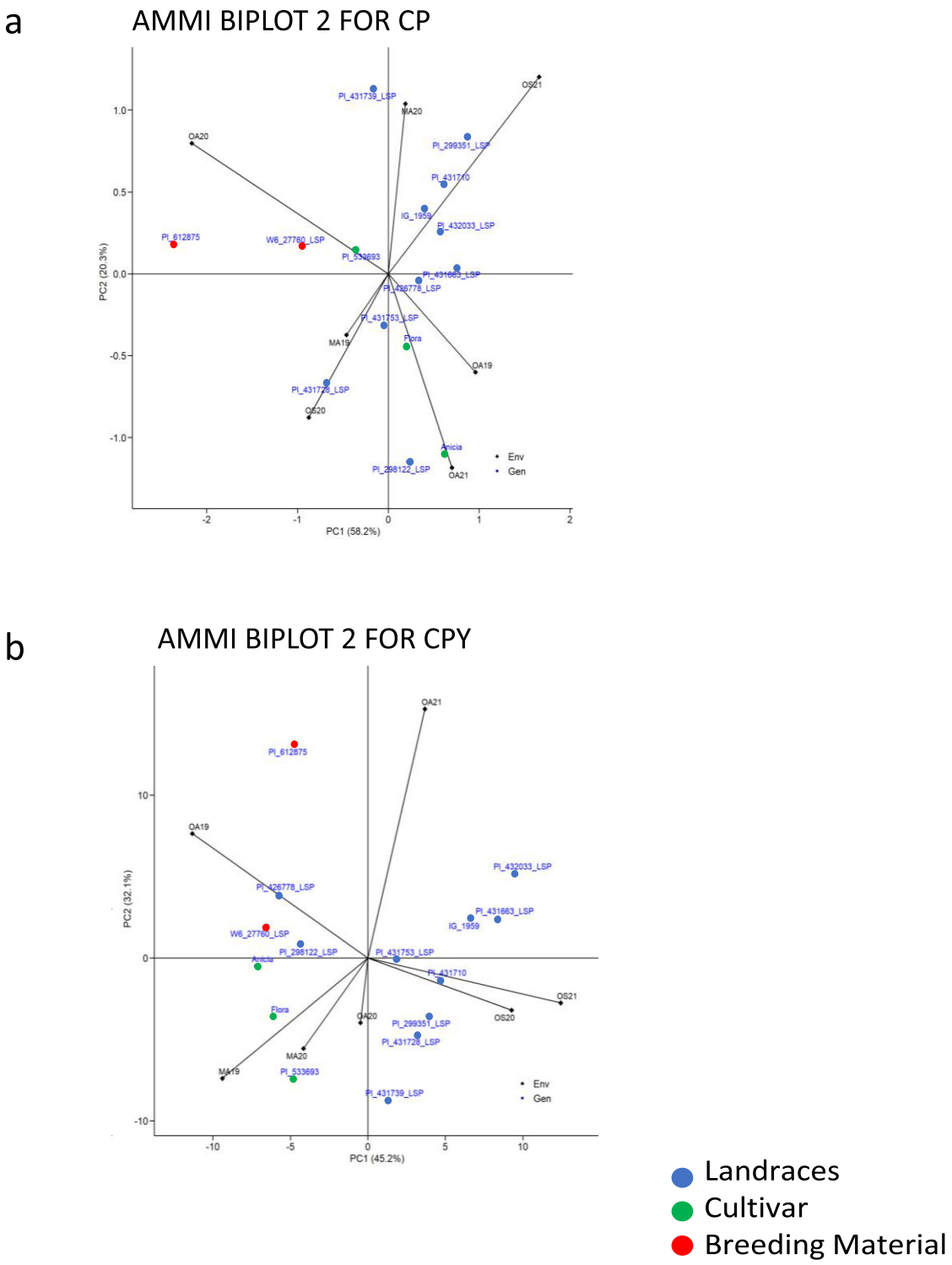

FIGURE 6 | Additive main effects and multiplicative interaction (AMMI) biplots 2 for (a) Crude protein (CP), and (b) Crude protein yield (CPY) showing Principal Component 1 (PC1) (IPCA1) and PC2 (IPCA2) of lentil genotypes based on mean performance and stability across environments and years (Red, breeding material; blue, landraces; green, cultivars), while arrows and diamonds represent the seven environments, which are Osimo (OS) and Metaponto (MA) from 2019–2021.

4.2 | Genotype-by-Environment Interaction (GEI) and Stability

In the study, we analyzed in more detail the GEI effect in order to investigate relevant parameters such as genotype stability or adaptability to specific environments, to support plant germplasm selection. With respect to CP content, the AMMI biplot 2 (GGE) model and the WAASB biplot show that lentil genotypes such as PI_431710_LSP, PI_431739_LSP, and PI_431753 are consistently productive and most stable in most of the tested environments. IG1959 and PI_431663_LSP were the best performing and most stable with regard to the CPY. Particularly,

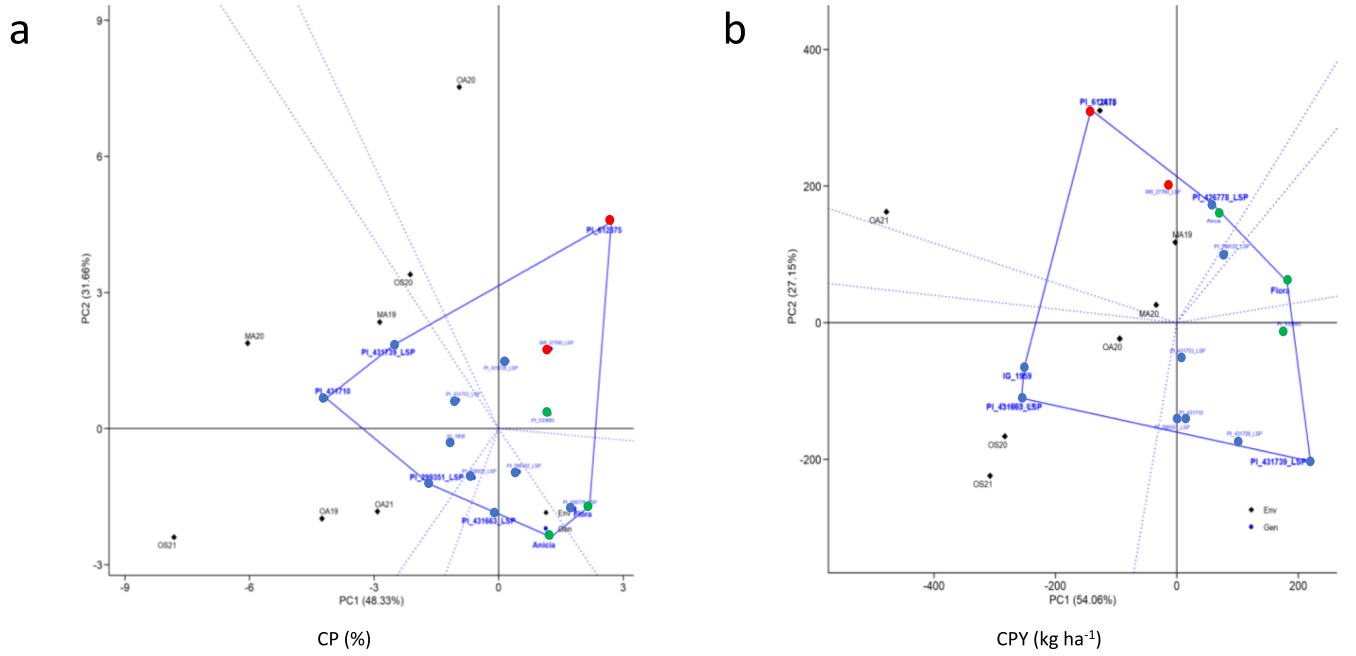

FIGURE 7 | Genotype plus genotype × environment interaction (GGE) biplots generated using site regression (SREG) as an indication of (a) Crude protein content, and (b) Crude protein yield stability for the 15 lentil genotypes evaluated across seven environments in Osimo and Metaponto from 2019–2021.

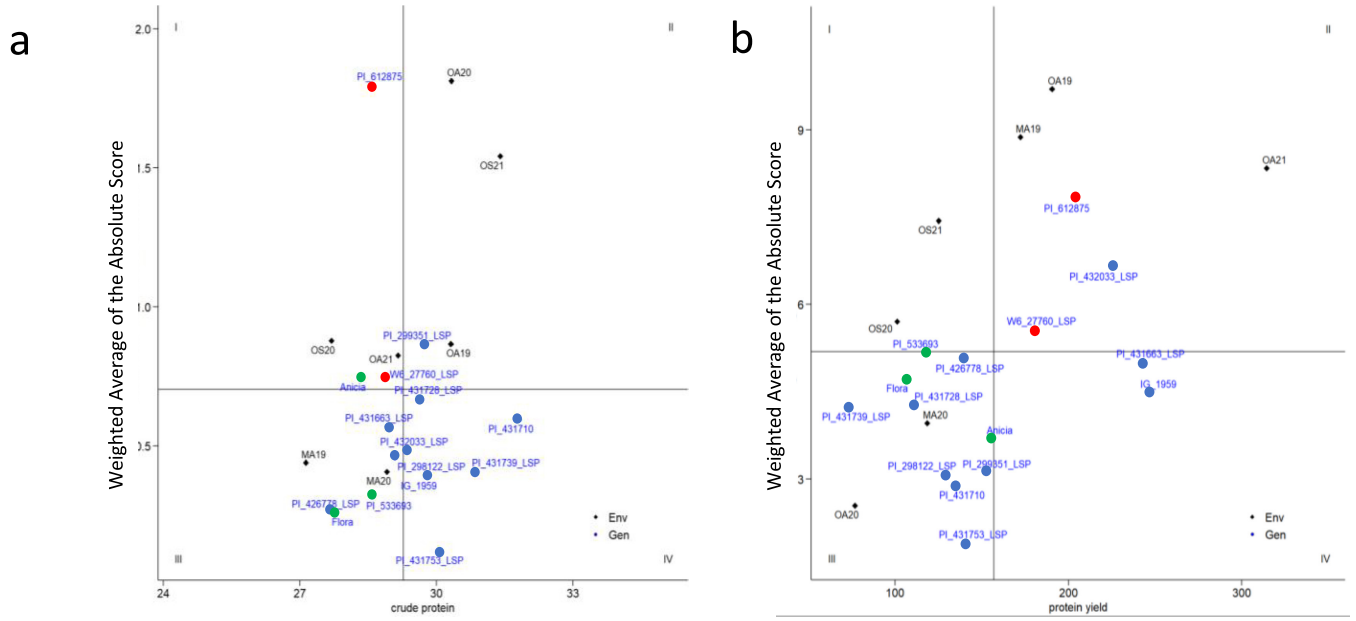

FIGURE 8 | Crude Protein × WAASB (a) and Crude Protein Yield × WAASB (b) biplot based on joint interpretation of productivity and stability (WAASB) for 15 lentil genotypes evaluated under seven environments in Osimo and Metaponto from 2019–2021. Quadrant 1: strong GEI component (high WAASBY values) and a below-average response variable. Quadrant 2: strong GEI component (high WAASBY values) and an above-average response variable. Quadrant 3: weak GEI component (stable) and a below-average response variable Quadrant 4: weak GEI component (stable) and above average of the response variable (recommended for breeding purposes).

the GGE analysis revealed that seasonal effects, particularly between autumn and spring sowing, were the primary drivers of GEI for both CP and CPY, consistent with previous observations (Gauch 2006; Yan and Hunt 1998). Additionally, breeding materials such as PI_612875 and W6_27760_LSP, despite their good performance, showed marked environmental sensitivity. Such genotypes exhibit strong genetic potential under favorable conditions but respond disproportionately to environmental variation, indicating a high level of specific adaptation. From a breeding perspective, these lines represent valuable candidates for targeted breeding in environments with similar conditions to those where they performed best.

Furthermore, the WAASB biplot for TAA, genotypes were clearly differentiated in terms of mean performance and stability. The landrace PI_431728_LSP, which was identified in

TABLE 8 | Additive main effects and multiplicative interaction (AMMI) results for seed total amino acids and total amino acids yield for 15 lentil genotypes across environments and years.

		Total amino acids			Total amino acid yield		
Source	**Df**	***F*-value**	**Pr (> F)**	**Proportion (%)**	***F*-value**	**Pr (> F)**	**Proportion (%)**
ENV	6	0.90	NS	0	20.49	***	35.32
REP (ENV)	14	0.83	NS	0	3.49	***	2.88
GEN	14	2.50	**	4.48	12.97	***	7.92
GEN: ENV	84	1.36	*	13.44	5.15	***	31.6
PC1	19	3.09	**	46.9	10.56	***	42.7
PC2	17	1.39	NS	18.8	9.21	***	33.3
PC3	15	0.96	NS	11.5	5.65	***	18
PC4	13	0.92	NS	9.6	1.01	NS	2.8
PC5	11	0.84	NS	7.4	1.02	NS	2.4
PC6	9	0.81	NS	5.8	0.42	NS	0.8
Residuals	165						
Total	367						

Abbreviation: N.S., not significant.
***$= p < 0.0001$.
**$= p < 0.001$.
*$= p < 0.05$.

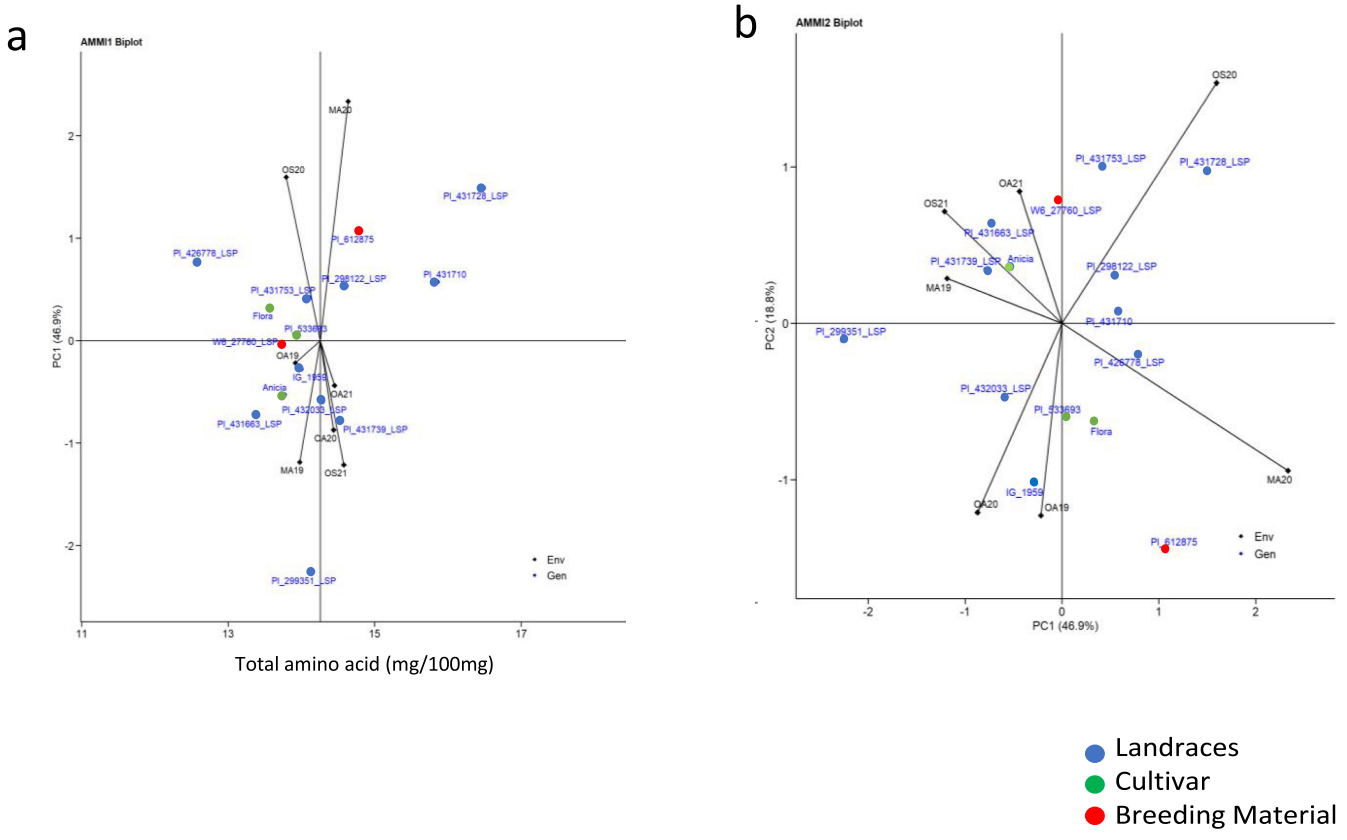

FIGURE 9 | (a) AMMI1 biplot of genotypes by environment interaction of Total amino acid (TAA) (*x*-axis) and the PC1 scores (*y*-axis). (b) AMMI2 biplot (PC1 vs. PC2) for plotting to TAA data. (Red, breeding material; blue, landraces; green, cultivars), while arrows and diamonds represent the seven environments, which are Osimo (OS) and Metaponto (MA) from 2019 to 2021.

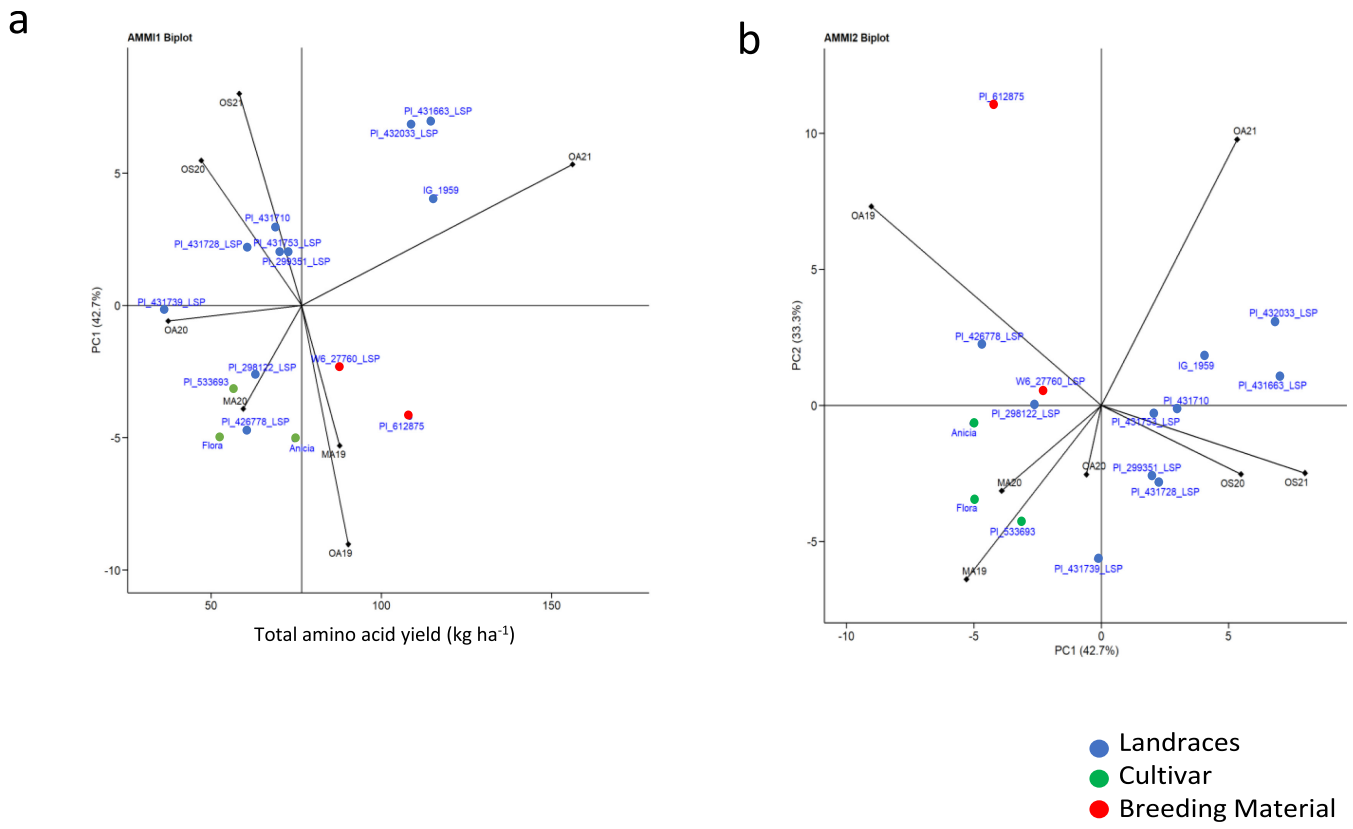

FIGURE 10 | (a) AMMI1 biplot of genotypes by environment interaction of total amino acid yield (TAAY) (*x*-axis) and the PC1 scores (*y*-axis). (b) AMMI2 biplot (PC1 vs. PC2) for plotting to TAAY data. (Red, breeding material; blue, landraces; green, cultivars), while arrows and diamonds represent the seven environments, which are Osimo (OS) and Metaponto (MA) from 2019 to 2021.

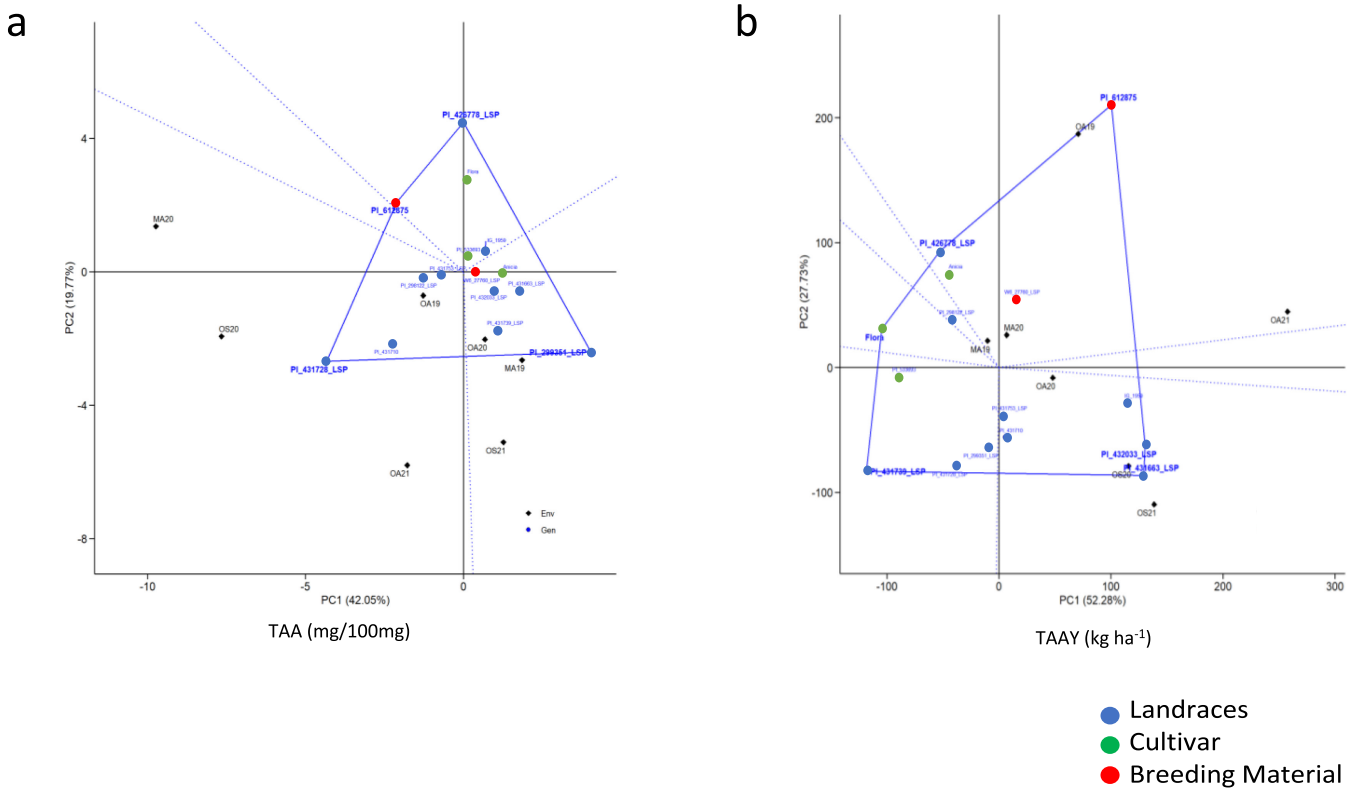

FIGURE 11 | Genotype plus genotype × environment interaction (GGE) biplots generated using site regression (SREG) as an indication of (a) total amino acid (TAA), and (b) total amino acid yield (TAAY) stability for the 15 lentil genotypes evaluated across seven environments in Osimo and Metaponto from 2019–2021.

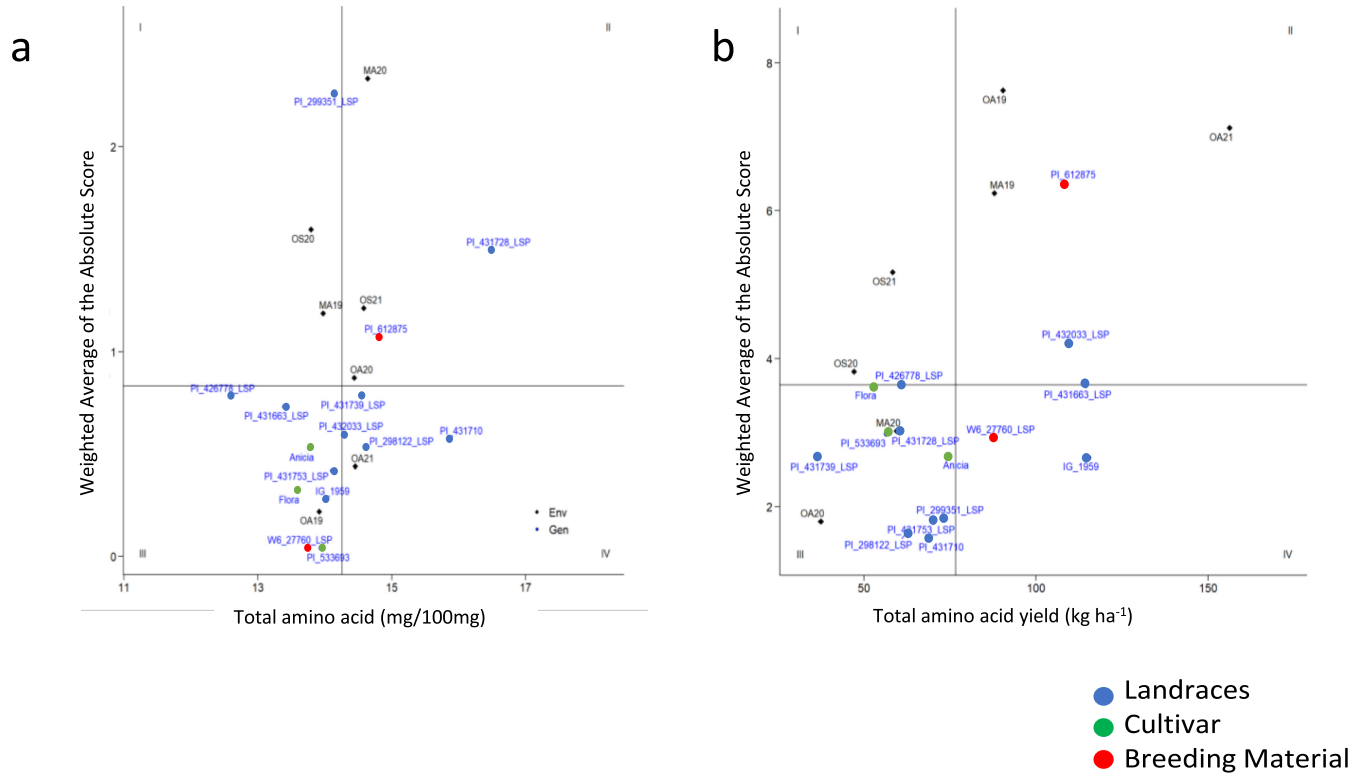

FIGURE 12 | Total amino acid (TAA)×WAASB (a) and total amino acid yield (TAAY)×WAASB (b) biplot based on joint interpretation of productivity and stability (WAASB) for 15 lentil genotypes evaluated under seven environments in Osimo and Metaponto from 2019–2021. Quadrant 1: strong GEI component (high WAASBY values) and a below-average response variable. Quadrant 2: strong GEI component (high WAASBY values) and an above-average response variable. Quadrant 3: weak GEI component (stable) and a below-average response variable. Quadrant 4: weak GEI component (stable) and an above-average response variable (recommended for breeding purposes).

the GGE biplot as the top performer, again recorded the highest TAA mean but showed higher WAASB scores, indicating greater environmental sensitivity. This confirms that its superior amino acid content is strongly influenced by environmental conditions, particularly those prevailing in the autumn sowing trials (MA20, OA21, OA19, and OS20), where it consistently excelled. In contrast, the landrace PI_431710 and breeding material PI_612875 combined moderate to high TAA levels with lower WAASB scores, reflecting greater stability across environments. From a breeding perspective, PI_431728_LSP represents a promising genotype for specific adaptation in favorable autumn environments, while PI_431710 and PI_612875 are more suitable for broad adaptation and could serve as parental lines for improving both performance and stability in lentil breeding programs. Landrace IG1959 can also be considered as a promising material regarding the TAAY, based on the high performance and stability.

Overall, our work highlighted a set of promising materials, considering stability, seed quality, and yield, but also including lines that outperformed in specific mega-environments, suggesting their potential exploitation as cultivated material or as being included in breeding programs for target environments. The identification of "mega-environments" in the GGE analysis further suggests that specific genotypes perform optimally under particular environmental conditions, providing valuable insights for region-specific breeding initiatives (Annicchiarico 2002; Gauch et al. 2008). Of particular interest, landraces have been identified as among the most promising germplasm, making them a potential source of diversity and beneficial traits for seed quality, yield, and adaptability in modern lentil breeding and for direct cultivation (Ates 2019; Babayeva et al. 2025; Lorenzetti et al. 2024). In our work, however, we adopted the CPY as a measure to evaluate the best performing crop, highlighting the importance of considering what is usually classified as "quality" traits, such as nitrogen content and total protein in seeds, as crucial contributors to the overall yield. This is particularly relevant when considering the negative correlation that some crops and legumes, such as lentil, can display between the classical agronomic yield and the protein content, which does not allow a comprehensive evaluation of the overall quality of the energetic production. From the perspective of lentil breeding, we recommend integrating the overall protein yield with a more detailed analysis of the amino acid composition, for which we reported significant differences among genotypes. Despite achieving the aim of this study, the use of a small panel size (15 genotypes) intended for prepreeding programs, however, serves as a limitation that may restrict the extrapolation to the broader lentil gene pool. Future studies should include a larger and diverse panel to integrate genomic markers to validate the observed trait–environment associations.

5 | Conclusion

This study investigated and provided a significant understanding of the intricate dynamics between genotype, environment, and their interactions (G×E) in determining the nutritional

quality of lentil (*L. culinaris* Medik.) seeds, particularly focusing on CP content and TAA. This research identified stable and high-performing genotypes and germplasm to be further utilized for breeding under target environments. The findings reveal that environmental factors play a significant role in determining protein yields, emphasizing the need for careful environmental management, alongside genetic selection. The use of advanced statistical models (AMMI, GGE, and WAASB) provided a comprehensive understanding of these interactions, highlighting the complexity of breeding for stable nutritional traits in lentils. The identified stable genotypes offer promising avenues for breeding programs aimed at producing lentil varieties with enhanced protein content and amino acid profiles, which are crucial for meeting the dietary needs of growing populations. Future research should focus on integrating molecular and genomic tools to identify key genetic markers associated with protein content and amino acid profiles.

Author Contributions

V.D.V. conceived and managed the project. A.K.F. wrote the article. A.K.F., A.K., R.P., E. Bi, and V.D. contributed to the writing and drafting. A.K.F. and A.K. performed data analysis. A.P., G.F., E.Be., L.N., L.R., C.S., and E.Bi. contributed to the analyses. A.K.F., A.K., A.C., F.F., G.C., D.P., and B.F. conducted the nutritional profiling for crude protein and total amino acids. A.P., G.F., E.Be., T.G., L.N., L.R., C.S., and D.P. contributed to the editing of the article. All authors read and approved the article.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** The list of 46 lentil genotypes selected for this study. Genotypes code, donor, country of origin, and the biological status are provided for each genotype. **Table S2:** Crude protein (CP), protein yield (CPY), total amino acid yield (TAAY), total amino acids (TAA), total sulfur amino acid (TSAA), total essential amino acids (TEAA), and amino acid composition are provided for the analyzed samples. **Table S3:** Principal component eigenvectors and probabilities. **Table S4:** Mean value for all traits across the seven environments used for computing PCA. **Tables S5:** Analysis of variance (ANOVA) for crude protein (CP), crude protein yield (CPY), total amino acids (TAA), and total amino acid yield (TAAY) by biological status, across genotypes ($p < 0.05$). **Figure S1:** Thermo-pluviometric graph for Osimo and Metaponto from 2019 to 2021. **Figure S2:** Variable Importance in projection (VIP) scores for amino acids and protein traits in lentil genotypes based on multivariate analysis. The dot plot on the left ranks traits by their VIP scores. The heatmap on the right shows concentration patterns of these traits across 15 genotypes, with colors ranging from blue (low) to red (high) representing relative levels. **Figure S3:** Representation of crude protein (CP), crude protein yield (CPY), total amino acid (TAA), and total amino acid yield (TAAY) profiles (nutritional quality) across 15 lentil genotypes. (a) Boxplots of seed CP content (%), mean CPY (kg ha-1), TAA (mg/100 mg), and TAAY (kg ha-1), content for 15 lentil genotypes averaged across sites and years. Figure S4: Linear regression between the crude protein and TAA using single replicate data from all the field trials. Key: ** = $p < 0.001$. **Figure S5:** Boxplot of crude protein (CP), crude protein yield (CPY), total amino acids (TAA), and total amino acid yield (TAAY) for three biological status groups (BM, cultivar, and landrace), illustrating groupwise differences and distribution patterns among genotypes.