Received: 1 August 2018 | Revised: 3 January 2019 | Accepted: 21 January 2019

DOI: 10.1111/pbr.12691

ORIGINAL ARTICLE

WILEY Plant Breeding

Polymorphisms in cyanogenic glucoside and cyano-amino acid content in natural accessions of common vetch (*Vicia sativa* L.) and selection for improved agronomic performance

Marwa Aouida[1] | Fred Rook[2,3] | Alexandra Bianca Maimann[2,3] | Raquel Sánchez-Pérez[2,3,4] | Ghassen Abid[1] | Marie Laure Fauconnier[5] | Moez Jebara[1]

[1]Laboratory of Legumes, Faculty of Sciences, Center of Biotechnology of Borj Cedria, University of Tunis El Manar, Hammam-Lif, Tunisia

[2]Plant Biochemistry Laboratory, Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Copenhagen, Denmark

[3]Villum Center for Plant Plasticity, University of Copenhagen, Frederiksberg C, Copenhagen, Denmark

[4]Department of Plant Breeding, CEBAS-CSIC, Murcia, Spain

[5]Faculté Gembloux Agro-BioTech, Unite Chimie General et Organique, Université de Liège, Gembloux, Belgium

Correspondence
Moez Jebara, Laboratory of Legumes, Faculty of Sciences, Center of Biotechnology of Borj Cedria, University of Tunis El Manar, Hammam-Lif, Tunisia.
Email: moez.jbara@cbbc.rnrt.tn

Funding information
Robert S. McNamara "Fellowship Program/2016-2017 from the World Bank; UCPH Excellence Program for Interdisciplinary Research

Editor: Wolfgang Link

Abstract

Common vetch (*Vicia sativa* L.) is an important annual forage legume. It is used as a cover crop, green manure, pasture legume and for silage and hay production. Its seeds can be used as a source of highly digestible protein and minerals in animal diets. However, their utilization as a feedstuff for monogastric animals is hindered by the fact that the seeds contain cyanogenic antinutritional factors that reduce their palatability. An effective utilization of *V. sativa* seeds as a successful monogastric feed stuff requires selection for higher protein availability and minimization of the cyanogenic antinutritional factors content. In this study, we selected one natural accession named Mjez Ibeb, from a collection of 25 accessions and cultivars, based on its superior agronomic performance and its naturally occurring genetic variation for cyanogenic traits. We investigated the genetic variation that exists for the cyanogenesis trait in more detail and analysed the seeds of 133 lines derived from accession Mjez Ibeb. Of these, 40 naturally polymorphic lines that showed deficiencies in cyanogenesis and cyano-amino acid content, were subsequently selected for detailed chemical analysis. Cyanogenic glucosides and cyano-amino acid concentrations varied widely in the 40 lines. Multivariate analysis was performed and three lines (L_{16}, L_{21}, L_{18}) with low content of cyanogenic compounds were identified.

KEYWORDS
agronomic performance, cyano-amino acids, cyanogenesis, cyanogenic glucosides (CNglcs), polymorphism, *Vicia sativa* selection

1 | INTRODUCTION

Vicia sativa is a forage legume best adapted to the semi-arid regions of the Mediterranean basin and Australia (Megías, Cortés-Giraldo, Girón-Calle, Vioque, & Alaiz, 2014); is mainly cultivated for green manuring, and as a hay and grazing crop (Chowdhury, Rathjen, Tate, & McDonald, 2004). Their seeds are rich in protein and used as cattle feed but it is also used as a cheap substitute for lentils in human diets (Tate, Rathjen, Delaere, & Enneking, 1999). There is an increasing demand for animal-derived protein for human consumption and consequently for protein-rich animal feed crops. It is estimated that by 2050, between 60% and 70%, more animal products will be needed worldwide (Huang, Gao, Nan, & Zhang, 2017; Makkar et al., 2016). As part of this, world demand for legume protein in animal feed will grow, which would present significant challenges due to factors such as increased land degradation, food-feedstuff competition and the effects and unpredictability of ongoing climate change. Therefore, increasing the animal feed resources base through the recognition

of novel feedstuffs, and improving their utilization efficiency, will be relevant in the development of sustainable animal husbandry (Huang et al., 2017; Makkar et al., 2016).

The increased use of *V. sativa* as a feedstuff is hampered by the fact that the seeds contain the heat stable neurotoxin dipeptide γ-glutamyl-β-cyano-L-alanine (GCA), its precursor amino acid β-cyano-L-alanine (BCA) and the cyanogenic glucosides (CNglcs) vicianin and prunasin (Darre, Minior, Tatake, & Ressler, 1998; Ressler, Nigam, & Giza, 1969; Ressler & Tatake, 2001). Cyanogenesis is a significant social, economic and health problem and can limit the use of particular harvests for human or animal consumption (Takos et al., 2010). The neurotoxic and cyanogenic compounds found in *V. sativa* are part of a biochemically linked pathway of a diverse set of plant chemical defence compounds (Figure 1).

The cyanogenic glucoside prunasin is originally synthesized from the amino acid phenylalanine via R-mandelonitrile, and is found in many plant species (Yamaguchi, Yamamoto, & Asano, 2014). Vicianin is derived from prunasin by an additional glycosylation, and is found in species of the genus *Vicia* belonging to the family of fabaceae. The hydrolysis of vicianin by the action of a specific ß-glycosidase results in the reformation of the chemically unstable hydroxynitrile intermediate R-mandelonitrile and the subsequent release of toxic hydrogen cyanide (Ahn, Saino, Mizutani, Shimizu, & Sakata, 2007). Cyanide can be released as a breakdown product of vicianin and prunasin, but is also generated as co-product in the biosynthesis of ethylene in all plants, and can be detoxified by a cysteine-based protection mechanism (Yi, Juergens, & Jez, 2012). β-Cyanolalanine synthase (β-CAS) catalyses the addition of CN^- to cysteine to yield the neurotoxin β-cyanoalanine that serves as an intermediate in the assimilation of cyanide to asparagine. Such a turnover pathway for prunasin was thought to offer a buffer supply of ammonia, aspartic acid and asparagine, enabling the plants to balance the supply of nitrogen to the developing cotyledons (Sanchez-Perez, Jorgensen, Olsen, Dicenta, & Moller, 2008). However, in *V. sativa*, the subsequent catalysis of BCA to asparagine is thought to be blocked (Fowden & Bell, 1965). Another enzyme, γ-glutamyl transferase, functions to catalyse the formation of GCA from BCA (Figure 1).

The generation of *V. sativa* cultivars low in the neurotoxins BCA and GCA may ultimately be achieved through either blocking the neurotoxin production upstream or enabling the downstream biosynthesis of asparagine. Such lines could be achieved by the development of a robust and efficient transgenic and regeneration system and their use in genetic engineering (Ford, Maddeppungeng, & Taylor, 2009). In considering the present knowledge regarding the biosynthesis of the neurotoxins and their dependence on the cyanogenic compounds, the

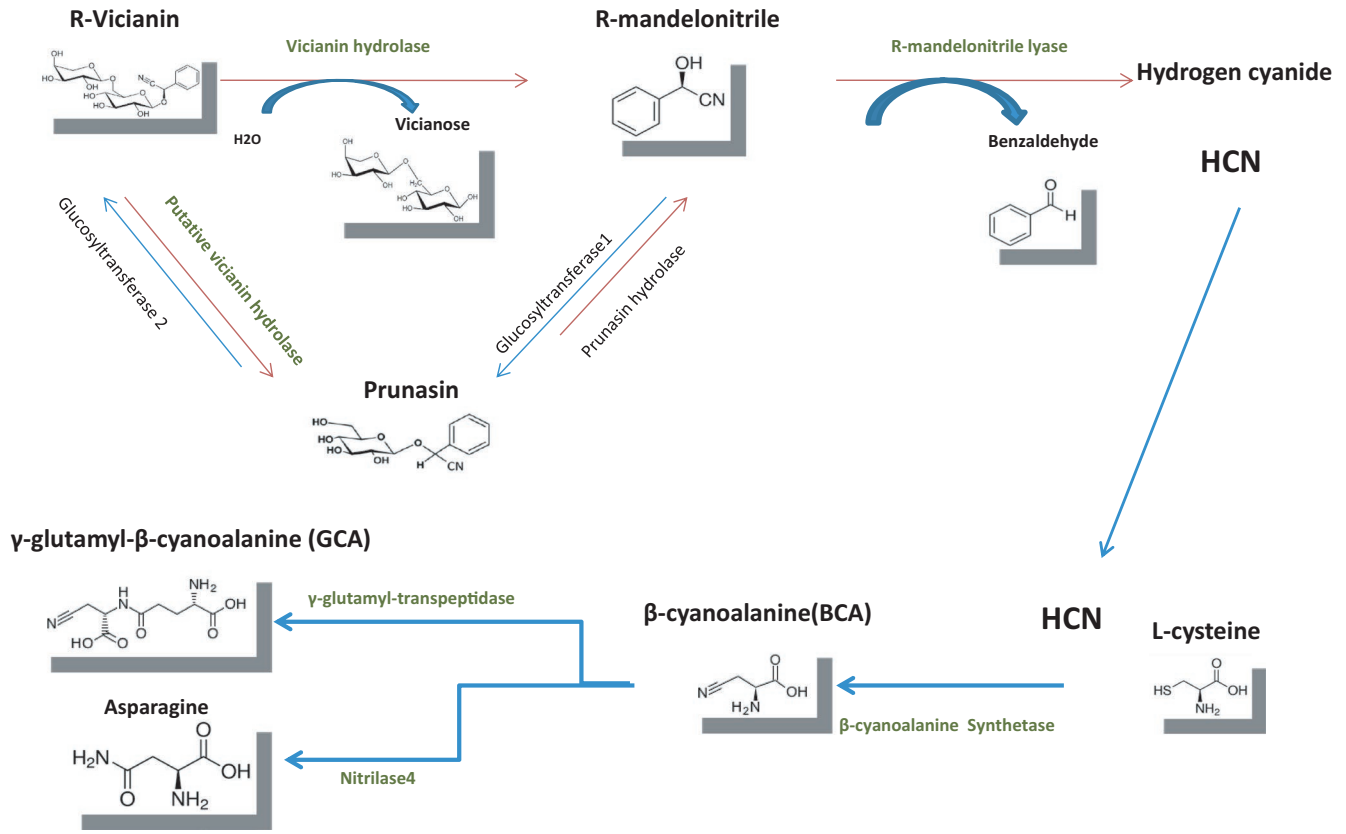

FIGURE 1 The metabolic pathways for the synthesis and catabolism of the cyanogenic glucosides prunasin and vicianin and the cyano-aminoacids BCA and GCA in *Vicia sativa*. R-mandelonitrile, derived from phenylalanine, is chemically stabilized by glycosylation, resulting in the cyanogenic glycosides prunasin and vicianin. These CNglcs are hydrolysed by specific β-glycosidases during a chemical defence response, and the unstable R-mandelonitrile will dissociate with the release of toxic HCN. Endogenous detoxification of HCN is via a cysteine-based pathway, but leads in *Vicia* to the accumulation of the neurotoxin GCA. Synthetic enzymes (Blue lines), catabolic enzymes (Red lines) [Colour figure can be viewed at wileyonlinelibrary.com]

literature suggests that the nutritional value of common vetch seeds can be improved by selective breeding methods, which may be considered as an extremely cost-effective technique in this regard (Huang et al., 2017). Reducing or eliminating the content of both types of antinutritional factors can be achieved by a mutagenesis approach or the use of existing natural variation in the species. For example, variation in cyanogenesis was first noted in populations of white clover (*Trifolium repens*) more than a century ago, and subsequent decades of research have established this system as a classic example of an adaptive chemical defence polymorphism in clover and other legume species (Ballhorn, Kautz, Heil, & Hegeman, 2009; Olsen, Kooyers, & Small, 2014). Mutational approaches have been used to generate acyanogenic plant lines in the monocot *Sorghum bicolor* and in the legume *Lotus japonicus* (Blomstedt et al., 2012; Takos et al., 2010). In this study, we adopted a screening of natural accessions to select low toxins common vetch lines as a safe feed suitable for livestock. The overall goal of this work was to identify natural lines of common vetch (*V. sativa* L.) with low content of CNglcs and neurotoxin compounds, but also with appropriate morphological and phenological characters.

2 | MATERIALS AND METHODS

2.1 | Plant cultivation

The 25 accessions and cultivars used in this study are presented in Table 1. Seeds from 25 accessions of *V. sativa* were germinated in wet filter paper, and 6-day-old uniform size seedlings were transplanted to 5-L pots containing clay loam soil with high lime content and low organic matter. At the stage of flower bud formation, the plants were covered with translucent plastic bags to keep pollinating insects away. Experiments were carried out in a greenhouse of the Center of Biotechnology of Borj Cedria (Tunisia) with a day/night temperature of (14/23°C) and a day/night relative humidity of (70/90%). Pots were randomly moved every week to minimize position effects.

From the 25 accessions, we selected number 15, which is called Mjez Ibab.

The same growing conditions of the 25 accessions were applied for the culture of 133 progeny of the accession Mjez Ibab.

2.2 | Assessment of agronomic traits and line selection

The plant measurement was recorded following the method of Firincioğlu, Tate, Ünal, Doğruyol, and Özcan (2007). A phenological character measured was the number of days from planting to harvesting. Harvest dates were recorded for plants in each genotype as soon as the lower pods were brown and hard enough to crack open. Days to harvesting were calculated by subtracting these dates from the planting date. Plant morphological characters measured were as follows: 1,000-seed weight (g), main stem length (cm), number of pods per plant, number of seeds per pod and dry-matter biomass per plant (g). These plant characters were measured at the harvesting stage for five replicates, randomly selected from each genotype.

TABLE 1 Taxonomy and origin of the studied accessions

Accession number	Taxonomy	Origin
1	*V. sativa 1*[a]	Nabeul Tunisia
2	*V. sativa nigra*	BhiretLbiban Tunisia
3	*V. sativa sativa*	BhiretLbiban Tunisia
4	*V. sativa sativa*	Sangho Zarzis Tunisia
5	*V. sativa nigra*	Jbel Ressas Tunisia
6	*V. sativa sativa*	Jbel Ressas Tunisia
7	*V. sativa nigra*	Jbel Ressas Tunisia
8	*V. sativa*[a]	Jbel Ressas Tunisia
9	*V. sativa nigra*	Tabarka Tunisia
10	*V. sativa sativa*	Tabarka Tunisia
11	*V. sativa*[a]	Jbel Ressas Tunisia
12	*V. sativa sativa*	Jbel Ressas Tunisia
13	*V. sativa nigra*	Borj Cedria Tunisia
14	*V. sativa sativa*	Jbel Ressas Tunisia
15	*V. sativa sativa*	Mjez Ibab Tunisia
16	*V. sativa nigra*	Solb Zarzis Tunisia
17	*V. sativa nigra*	Borj Cedria Tunisia
18	*V. sativa sativa*	Sliman Tunisia
19	*V. sativa c.v cummins*	Selected by a local farmer, South Australia
20	*V. sativa Love3A*	Cultivar
21	*V. sativa Love I*	Cultivar
22	*V. sativa cv Languedoc*	Cultivar originally from Algeria
23	*V. sativa Blanchefleur*	Originally introduced from France
24	*V. sativa Love 2*	Cultivar
25	*V. sativa Jericho White*	Selected by a local farmer Lawrence Jericho, Queensland, Australia

[a]Unknown name of subspecies.

2.3 | Screen for deficient lines in cyanogenic compounds of *V. sativa* with Feigl–Anger paper

In addition to the good performance for agronomic features of accession Mjez Ibeb, it showed natural variation for the cyanogenesis trait using a primary cyanide release test (data not shown).

The seeds of the 133 of *V. sativa* lines derived from the accession Mjez Ibeb were tested for cyanogenesis by homogenization of grinded seed tissues in 300 µl of 20 mM MES buffer, pH 6.5 and placing them in the wells of a 96-well microtiter plate. Cyanide production was assayed by immediately placing a piece of Feigl–Anger

paper between the lid and the microtiter plate, the blue colour developed under 3 hr of incubation and a permanent record was made by scanning the paper (Figure S1). Feigl–Anger paper (Feigl & Anger, 1966) was prepared as described in Takos et al. (2010).

The cyanogenesis variation can be distinguished by metabolite analysis.

2.4 | Metabolites analysis

2.4.1 | High-performance liquid chromatography analysis of BCA and GCA

Ground seed tissues of 133 *V. sativa* lines derived from the accession Mjez Ibeb were stirred in a ratio of 30% methanol: water (3:7 v/v, 1 ml) for 30 min at room temperature and centrifuged at 12,000 rpm for 10 min. Pellets were re-extracted twice more, and the resulting supernatants were collected in glass vials.

Diethyl ethoxymethylenemalonate was added to the samples in 1 M borate buffer, pH 9.0. The solution was thoroughly mixed and incubated at 50°C for 50 min. Samples were filtered through 0.22 µm membranes before injection into the high-performance liquid chromatography (HPLC) system, then were analysed by HPLC on an Agilent HP 1,100 Series instrument equipped with a reversed-phase column (HALO® C18, 4.6 × 75 mm i.d and 2.7 µm particle size) using 0.9 ml/min using a 20 mM phosphoric acid, pH 6.0 (solvent A) and acetonitrile (solvent B) gradient at a flow rate of 1.2 ml/min (injection volume 20 µl). The gradient was as follows: constant 100% (A) at 0–6 min, a linear gradient from 100% to 30% (A) at 6–21 min, a linear gradient from 30% to 0 (A) at 21–24 min, constant 100% (B) at 24–28 min, a linear gradient from 0% to 100% (A) at 28–30 min and constant 100% (A) at 30–35 min. The eluent was monitored by diode array detection at 280 nm. The column was maintained at 18°C. The BCA and GCA were identified by comparing retention times and UV absorption spectra with known standards.

2.4.2 | Cyanogenic glucoside quantification using LC-MS/MS analysis

Cyanogenic glucosides were analysed using LC-MS, the seed tissue was boiled with 300 ml 85% methanol in a water bath for 5 min and cooled on ice. The extract was filtered through 45-mm Ultrafree-MC Durapore PVDF filters (Millipore) and stored in glass containers at 4°C prior to analysis. LC-MS/MS was performed on a Dionex UltiMate 3000 Quaternary Rapid Separation UHPLC+ focused system (Thermo Fisher Scientific, Germering, Germany). Separation was achieved on a Kinetex 1.7 µm XB-C18 column (100 × 2.1 mm, 1.7 µm, 100 Å, Phenomenex). For eluting 0.05% (v/v) formic acid in H_2O and methyl cyanide (supplied with 0.05% (v/v) formic acid) were employed as mobile phases A and B, respectively. Gradient conditions were as follows: 0–1 min 10% B; 1–7 min 10%–40% B; 7–20 min 40%–80% B, 20–26 min 80% B, 26–27 min 80%–100%, 27–30 min 100% B, 30–31 min 100%–10% B and 31–34 min 10% B. The flow rate of the mobile phase was 300 µ min^{-1}. The column temperature was maintained at 30°C. UV spectra for each sample were acquired at 205, 210, 250 and 310 nm. The UHPLC was coupled to a Compact microTOF-Q mass spectrometer (Bruker, Bremen, Germany) equipped with an electrospray ion source operated in the positive ionization mode. The ion spray voltage was maintained at 4,500 or 3,900 V in the positive and negative ionization modes, respectively. The vicianin and prunasin content in seed extracts from each mutant line was determined by using the Brucker compass Data analysis program and compared to a prunasin standard and a vicianin crude extract.

2.5 | Chemical analysis

Cyano-amino acids seeds extracts from various lines of *V. sativa* were analysed with HPLC; the peaks were detected and the areas were integrated manually, most of the detected peaks of GCA and BCA have a coefficient of variation (CVs) equal or less than 20% in the three biological replicates (Figure S2). The LC-MS data were analysed using Bruker Data Analysis script 4.0 (Bruker Daltonics).

Principal component analysis (PCA) was used to represent the contribution of GCA and BCA that discriminate between the 133 lines of *V. sativa*. PCA was performed using the XLSTAT-Pro (v.7.5.3) program (Addinsoft, NY).

3 | RESULTS

3.1 | Agro-morphological characterization of the 25 genotypes

A total of 25 accessions, consisting of 18 Tunisian ecotypes from different bioclimatic regions (Figure 2) and seven cultivars, were evaluated in this study based on phenological and morphological traits (Table 2).

The shoot dry weight ranged from 11.60 ± 1.66 g in genotype 6 to 112.28 ± 6.44 g in genotype *Blanche fleur*. Among the Tunisian ecotypes, number 15 from Mjez Ibeb, registered the highest shoot dry weight (74.40 ± 4.39 g); in addition accession 15 is the second genotype after cultivar 'Languedoc' that registered high 1,000 seed weight (87.04 ± 2.77 g) and it has the highest stem length in the collection of Tunisian ecotypes (132.40 ± 7.82 g). Furthermore, it possesses non-shattering pods; these traits are of a valuable importance for legumes selection programme.

3.2 | Accession Mjez Ibeb shows natural variation for cyanogenic traits

As accession 15 from Mjez Ibeb showed beneficial agronomical traits, it was further analysed for the possible existence of genetic variation in antinutritional factors within this population. One hundred and seventy individual seeds from accession Mjez Ibeb were planted and allowed to self-fertilize in order to generate derived lines, numbered L1 to L170, as basis for this analysis. A total of 133 plants survived and produced seeds, forming a set of experimental

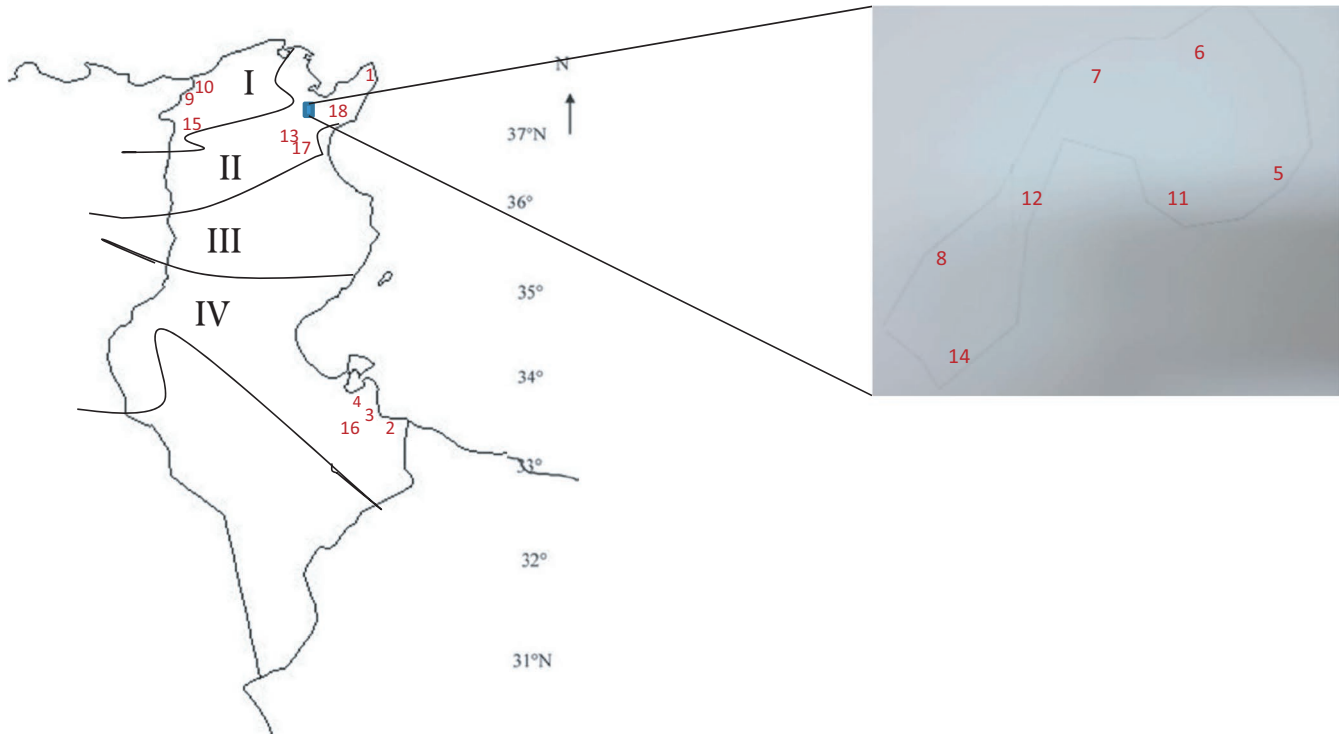

FIGURE 2 Tunisian genotypes of *Vicia sativa* and their bioclimatic regions. The bioclimatic regions are defined on the basis of their annual rainfall (mm): 2, 3, 4 and 16 (IV: arid: 100–400 mm); 1, 5, 6,7, 8, 11,12, 13,14,17 and 18 (II: semi-arid: 400–600 mm); 9, 10 and 15 (I: humid: 800–1,200 mm). The insert in the region II shows the genotypes 5, 6,7,8,11,12 and 14 collected from Jbel Ressas [Colour figure can be viewed at wileyonlinelibrary.com]

lines that reflected the genetic variation present in accession Mjez Ibeb. We initially screened for existing variation in cyanogenesis using a biochemical assay.

A variation of blue colour developed under 3 hr over each microtiter plate well containing seed powder of a single line from accession Mjez Ibeb. The colour intensity was semi-quantitative for the amount of HCN produced. The level of HCN release covered a range from no colour display to a strong dark blue (Figure S1).

Acyanogenesis in a natural population can be the result of a lack of cyanogenic glucosides or lack of the activity of an activating β-glucosidase enzyme.

3.3 | Metabolites analysis

3.3.1 | Cyano-amino acids quantification (β-cyanoalanine and γ-glutamyl-β-cyanoalanine)

As the analysis for cyanogenesis showed that genetic variation for this trait existed within the Mjez Ibeb population, we further analysed the derived lines for variation in the level of the neurotoxins BCA and GCA. The content of BCA varied from 0.8 ppm (7 μM) in line 158 (L_{158}) to 59 ppm (519 μM) in L_{17} and GCA content varied from 28 ppm (115 μM) in L_{158} to 1,500 ppm (6,167 μM) in L_{82} (Figure 3).

Principal component analysis was used to represent the contribution of GCA and BCA content that discriminates between the 133 *V. sativa* lines. The PCA analysis showed that the two factors PC1 and PC2 represented 66%, 89% and 33%, respectively of the variability contained in the original data. The lines that are spatially close to each other are significantly correlated (Figure S2).

The lines containing lower levels of cyano-amino acids are presented negatively by PC1 and PC2 in the group A (Figure S2). Forty lines with the lowest content of GCA (below 1,000 ppm) and BCA (below 40 ppm) of this group were selected for prunasin and vicianin quantification (Figure 3).

3.3.2 | Quantification of prunasin and vicianin

Prunasin and vicianin quantification are expected to provide additional information complementing the cyano-amino acids quantification and to identify the lines with lower content of both cyano-aminoacids and cyanogenic glucosides. We focus here on the 40 lines from the group A with lower levels of cyano-amino acids (Figure S2).

The prunasin content of the 40 lines varied from 0 μM in L_{21}, L_{18}, L_{16} to 13 μM in L_{125} and vicianin content varied from 0 in L_{51} to 250 μM in L_{108} (Figure 4).

The PCA analysis showed that the first two factors explain the highest amount of variance 63.49% across the data set, separating the lines (Figure 5).

The PC1 axis explained 39.31% of the variance and BCA, GCA and vicianin were the main metabolites contributing to the distribution of the lines on the axis PC1. The second axis PC2 explained 24.18% of the variance and separated the lines. Changes in the abundance of prunasin were predominately responsible for lines dispersing along the axis PC2.

TABLE 2 The mean ± standard deviation (*SD*) of the measured traits for the 25 studied genotypes

Genotypes	Days to harvest	1,000-Seed weight (g)	Stem length (cm) plant^{-1}	Number of pods plant^{-1}	Number of seed pod^{-1}	Shoot dry weight (g) plant^{-1}	Pod trait
1	138 ± 3.27	31 ± 1.41	64 ± 6.48	13 ± 2.40	7 ± 1.00	13.20 ± 1.14	NS[a]
2	169 ± 1.51	17.6 ± 1.14	91 ± 8.27	25 ± 4.14	8 ± 0.54	21.10 ± 1.37	S[b]
3	151 ± 2.96	19.4 ± 1.51	77.6 ± 3.16	41 ± 1.81	7 ± 0.83	20.10 ± 2.35	NS
4	141 ± 1.84	31 ± 1.00	81.1 ± 2.89	26 ± 3.19	6 ± 0.83	13.80 ± 1.22	S
5	176 ± 8.49	36.4 ± 1.81	71.1 ± 7.06	19 ± 2.58	7 ± 0.83	19.40 ± 1.11	NS
6	181 ± 1.81	24.6 ± 1.81	123 ± 5.19	21 ± 3.04	6 ± 0.54	11.60 ± 1.66	NS
7	145 ± 1.58	11.6 ± 1.14	50.6 ± 2.6	26 ± 2.23	7 ± 0.54	15.60 ± 0.81	S
8	135 ± 2.58	42.2 ± 2.16	56.8 ± 6.97	17 ± 3.56	7 ± 0.54	13.10 ± 0.62	S
9	103 ± 1.3	32.4 ± 0.54	58.4 ± 2.88	35 ± 1.87	8 ± 0.00	30.84 ± 3.70	S
10	112 ± 3.49	22.8 ± 1.3	63.6 ± 6.1	11 ± 2.48	6 ± 0.54	12.53 ± 0.82	NS
11	104 ± 3.08	42.2 ± 1.92	63.6 ± 2.96	17 ± 3.28	6 ± 0.89	18.80 ± 0.96	S
12	118 ± 1.14	74.2 ± 4.54	64.8 ± 2.77	9 ± 3.63	6 ± 0.54	13.00 ± 1.55	NS
13	122 ± 2.07	12.2 ± 0.83	60.2 ± 1.64	25 ± 0.89	8 ± 0.54	20.30 ± 0.81	NS
14	142 ± 3.36	32.6 ± 1.51	63.8 ± 2.58	26 ± 3.16	7 ± 0.89	23.68 ± 2.33	NS
15	146 ± 8.4	**87.04 ± 2.77**	**132.4 ± 7.82**	23 ± 2.3	6 ± 0.54	**74.40 ± 4.39**	**NS**
16	135 ± 2.19	23.4 ± 2.07	68.2 ± 4.2	18 ± 3.63	7 ± 0.54	14.90 ± 1.23	S
17	159 ± 9.2	17 ± 1.58	67.2 ± 2.68	26 ± 3.83	7 ± 0.44	19.80 ± 1.87	S
18	151 ± 2.4	36.4 ± 1.34	66 ± 5.24	20 ± 1.3	7 ± 0.54	16.30 ± 1.29	S
19	131 ± 1.34	68.2 ± 5.16	134.4 ± 5.17	44 ± 5.26	6 ± 0.54	25.00 ± 1.31	NS
20	185 ± 2.77	54 ± 2.23	153 ± 2.3	22 ± 1.94	7 ± 0.54	36.80 ± 1.22	NS
21	170 ± 2.07	40.6 ± 4.87	142.6 ± 4.87	21 ± 0.89	6 ± 0.83	47.90 ± 3.92	NS
22	138 ± 1.27	91 ± 5.09	160 ± 4.15	33 ± 5.17	7 ± 0.54	46.20 ± 2.74	NS
23	134 ± 11.58	71 ± 1.00	177.96 ± 6.22	43 ± 3.04	5 ± 0.54	112.28 ± 6.44	NS
24	135 ± 3.36	70 ± 6.2	157.4 ± 5.59	39 ± 2.3	5 ± 0.54	26.87 ± 1.65	NS
25	152 ± 2.07	55 ± 2.91	73 ± 4.50	14 ± 1.14	6 ± 0.54	23.40 ± 1.78	NS

The values in bold is to show the highest value recorded in the ecotype 15.
[a]NS: non-shattering pods. [b]S: shattering pods.

The PCA allowed a detailed separation of the lines into three distinct clusters. The samples clustering according to the PCA is reported in Figure 5.

The long distance along PC2 between L_{125} and the lines grouped in the green circle indicated the major differences in the metabolites content between those lines. The relative position of L_{18}, L_{16} and L_{21} indicates that those lines have the lowest content of the four metabolites, while the lines grouped in the opposite direction have the highest content of BCA, GCA and vicianin.

3.4 | Heat map and clustering analysis

The heat map displays the variation of metabolite contents and their similarities between individual samples; each cell represents the differential metabolites content trend indicating higher content in green and lower content in red. In this visualization, columns represent samples and rows represent metabolites (Figure 6).

The clustering analysis was used to group samples based on dissimilarities in auto-scaled values obtained upon comparison of the seeds metabolites content profiles of 40 *V. sativa* lines. It revealed that the samples were separated into three major clusters (Figure 6). The clustering analysis is consistent with the PCA analysis.

The first cluster included the lines with the highest content of BCA, GCA and vicianin presented in the right side of PCA axes (Figure 5), the second cluster included L_{125} and the third cluster presented in the left side of the axes included the rest of lines.

The multivariate analysis PCA, the heat map, the clustering analysis and the Figure S3 showed that the lines (L_{16}, L_{21}, L_{18}) are the three lines with the lower content in both cyanogenic glycosides and cyano-amino acids.

4 | DISCUSSION

4.1 | Natural variation in Mjez Ibeb for agronomic and cyanogenic traits

In this study, we investigated agronomic traits in natural accessions of *V. sativa* L. in order to select lines with beneficial traits for the genetic improvement of this forage legume. This analysis of

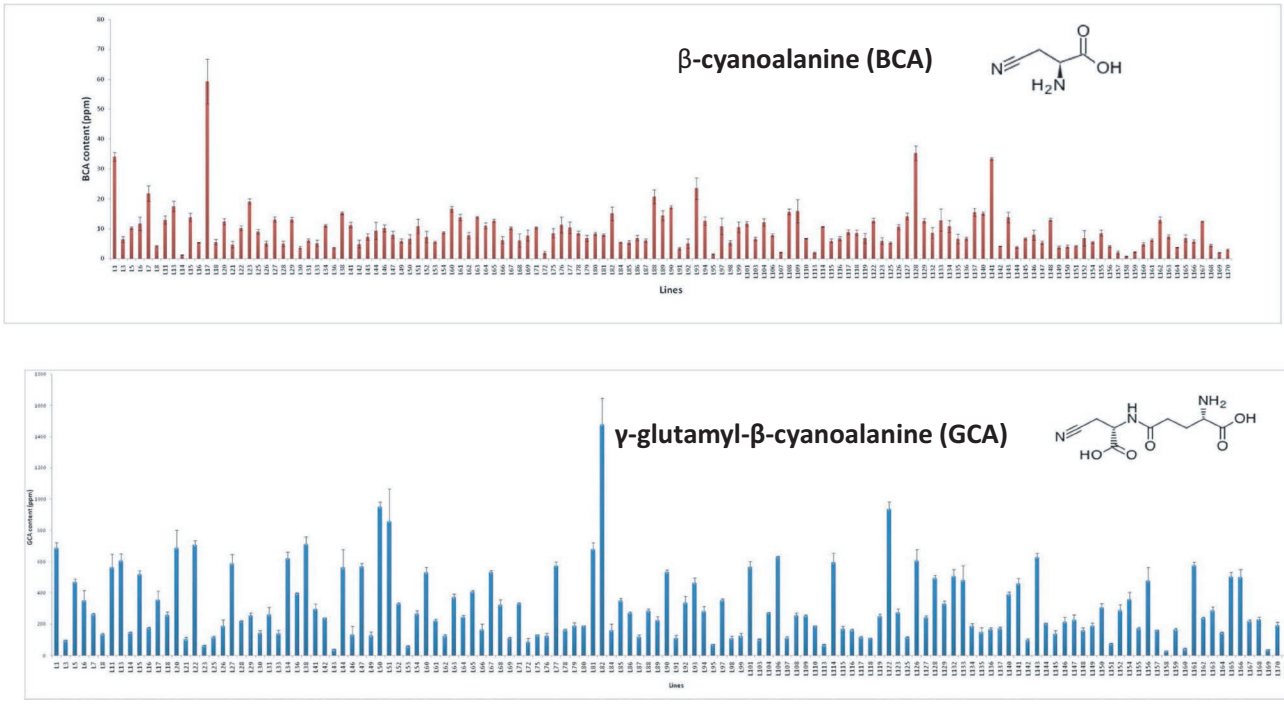

FIGURE 3 Variation in the content (in ppm) of the cyano-amino acids GCA and BCA in the 133 lines of *Vicia sativa*, accession Mjez Ibeb. Values are the mean of three replicates and error bars indicate standard deviation. The chemical structure of each compound is shown [Colour figure can be viewed at wileyonlinelibrary.com]

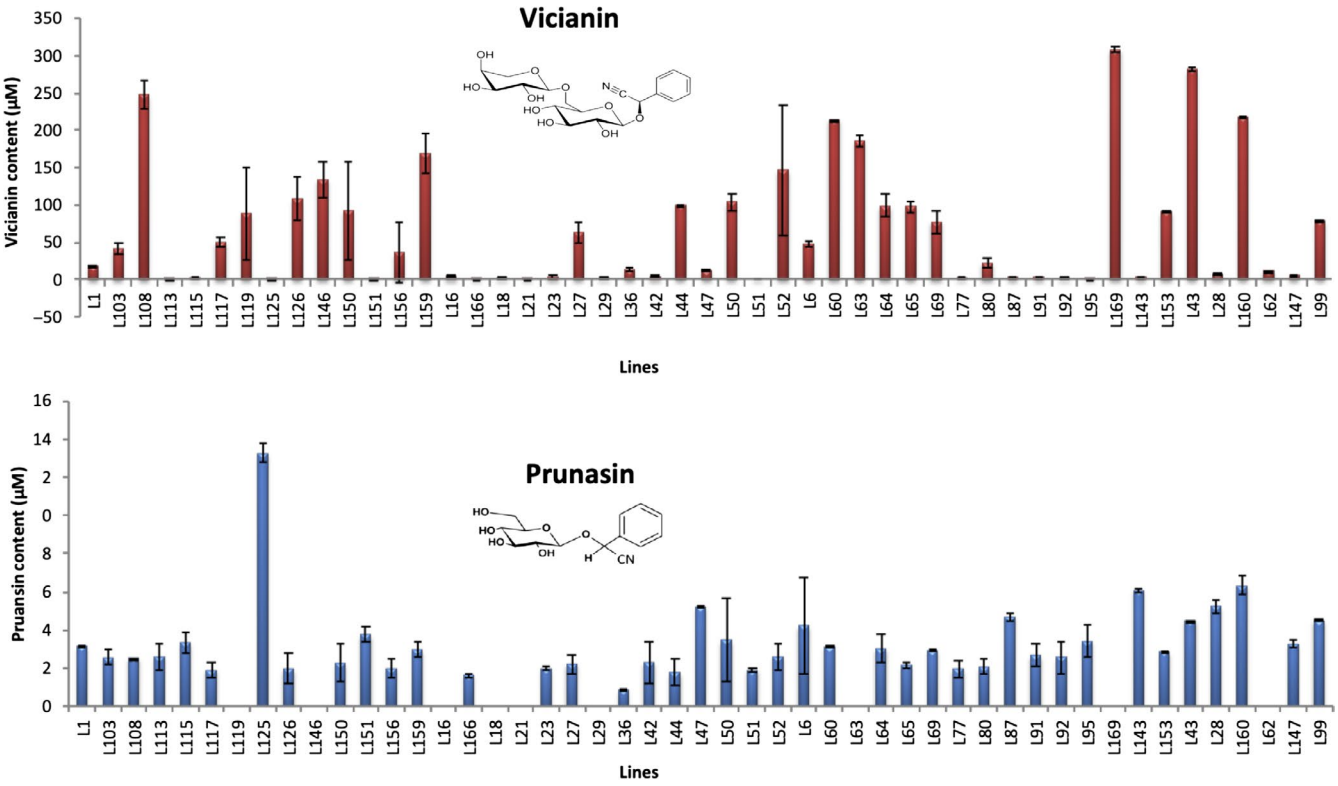

FIGURE 4 Variation in the content of cyanogenic glucosides vicianin and prunasin in the 40 selected lines of *V. sativa*, accession Mjez Ibeb. Values are the mean of three replicates and error bars indicate standard deviation. The chemical structure of each compound is shown [Colour figure can be viewed at wileyonlinelibrary.com]

phenological, morphological and agronomic traits showed that pronounced variation existed among the 25 vetch genotypes in term of shoot dry weight, seed weight and growth performance. This variation may be related to the geographical origin of each genotype (Dong, Jahufer, Dong, Wang, & Liu, 2016). Based on the analysis of several characteristics, we have identified accession Mjez Ibeb as

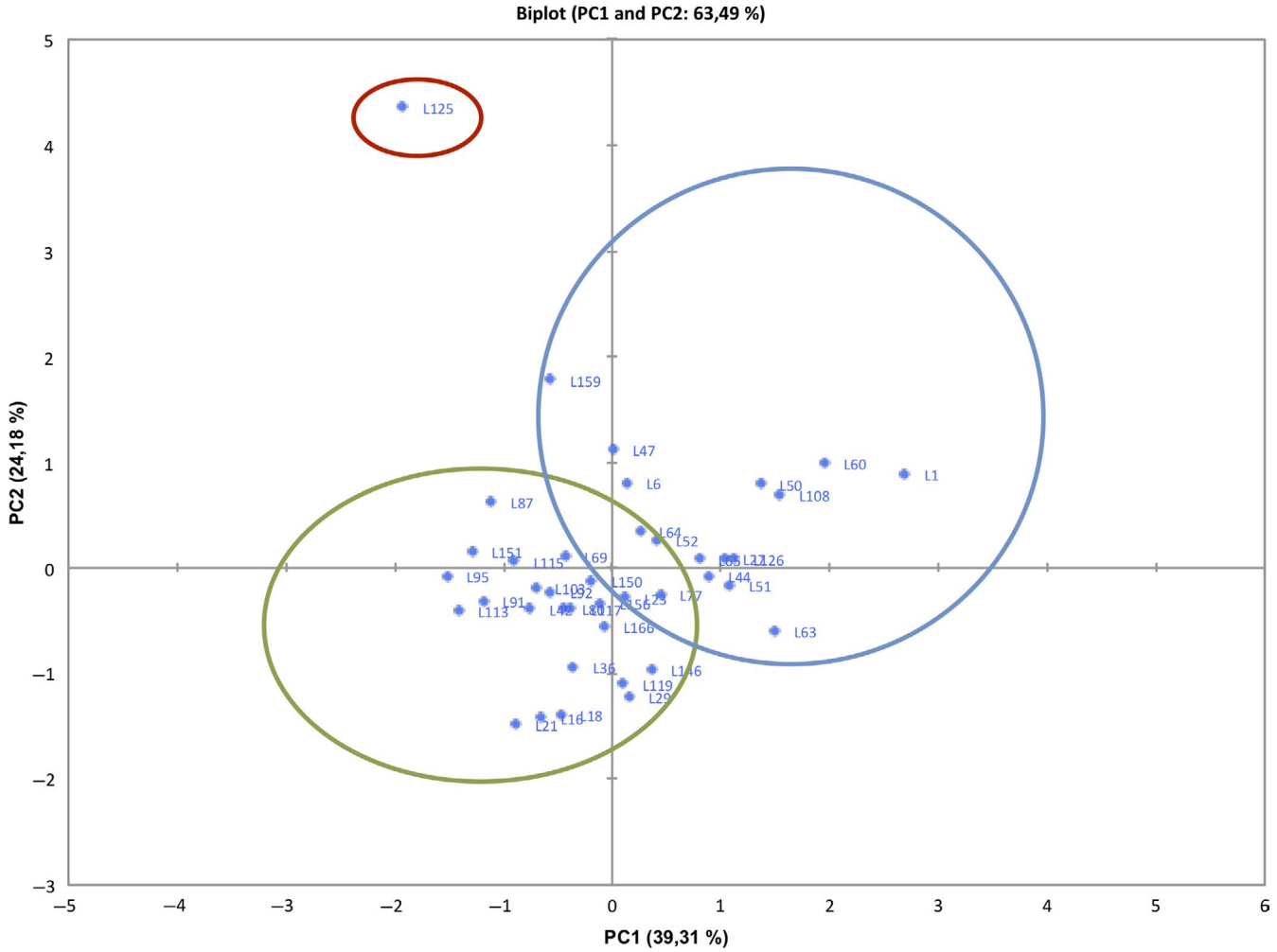

FIGURE 5 Multivariate PCA based on the content of four metabolites GCA, BCA, Vicianin, Prunasin in the 40 selected lines of *Vicia sativa*, accession Mjez Ibeb [Colour figure can be viewed at wileyonlinelibrary.com]

the best performing genotype and this accession can be a valuable genotype in a future breeding programme. Mjez Ibeb for instance shows some of the highest 1,000-seed weight and shoot dry weight values. Seed size, maturity and 1,000-seed weight were previously identified as indicative traits for selection of *V. sativa* (Firincioglu et al., 2009). A big seed size is suggested to be related to greater planting depth in agricultural systems, with larger seeds producing more vigorous seedlings (Abbo, Mesghenna, & Oss, 2011).

Furthermore, accession Mjez Ibeb is one to the accessions that possesses non-shattering pods. The loss of seed shattering has been a fundamental characteristic under selection in most legume grain crops in order to facilitate seed harvesting, and speculatively Mjez Ibeb may have undergone previous human selection (Abd El-Moneim, 1993). The selection for the non-shattering trait would have occurred automatically as a result of the harvesting process that favoured non-shattering natural mutants in harvested populations that were subsequently resown (Smýkal et al., 2017). Seed shattering is not favoured by domestication, resulting in the reduced occurrence of the natural explosive seed pod opening mechanism of wild legumes (Abbo, Lev-Yadun, Heun, & Gopher, 2013).

Antinutritional factors in the seeds of *V. sativa* include the cyanogenic glucosides prunasin and vicianin, which release HCN upon their hydrolysis by a specific β-glycosidase. Cyanogenesis is a two component chemical defence system present in a wide range of plant species including several other legumes (Ballhorn, Heil, & Lieberei, 2006; Olsen et al., 2014; Takos et al., 2010). Although a mutational approach has been used to generate an acyanogenic *Sorghum* variety (Blomstedt et al., 2012), the existence of natural variation in the cyanogenic trait has been established for several legumes species. For example, in white clover (*T. repens*) adaptive polymorphisms in cyanogenesis recurrently develop as climate associate clines related to temperature and even aridity (Kooyers & Olsen, 2013). This natural variation in cyanogenesis independently arises through segregating polymorphisms for the presence/absence of the two required cyanogenic components, cyanogenic glucosides and their hydrolysing enzyme (Kooyers, Gage, Al-Lozi, & Olsen, 2014). For use as feed, only biosynthetic mutants lacking the cyanogenic compounds are considered safe for animal consumption as hydrolysis of CNglcs in the digestive tract may otherwise occur. In our study, we characterized the cyanogenic potential of 133 progeny lines derived from

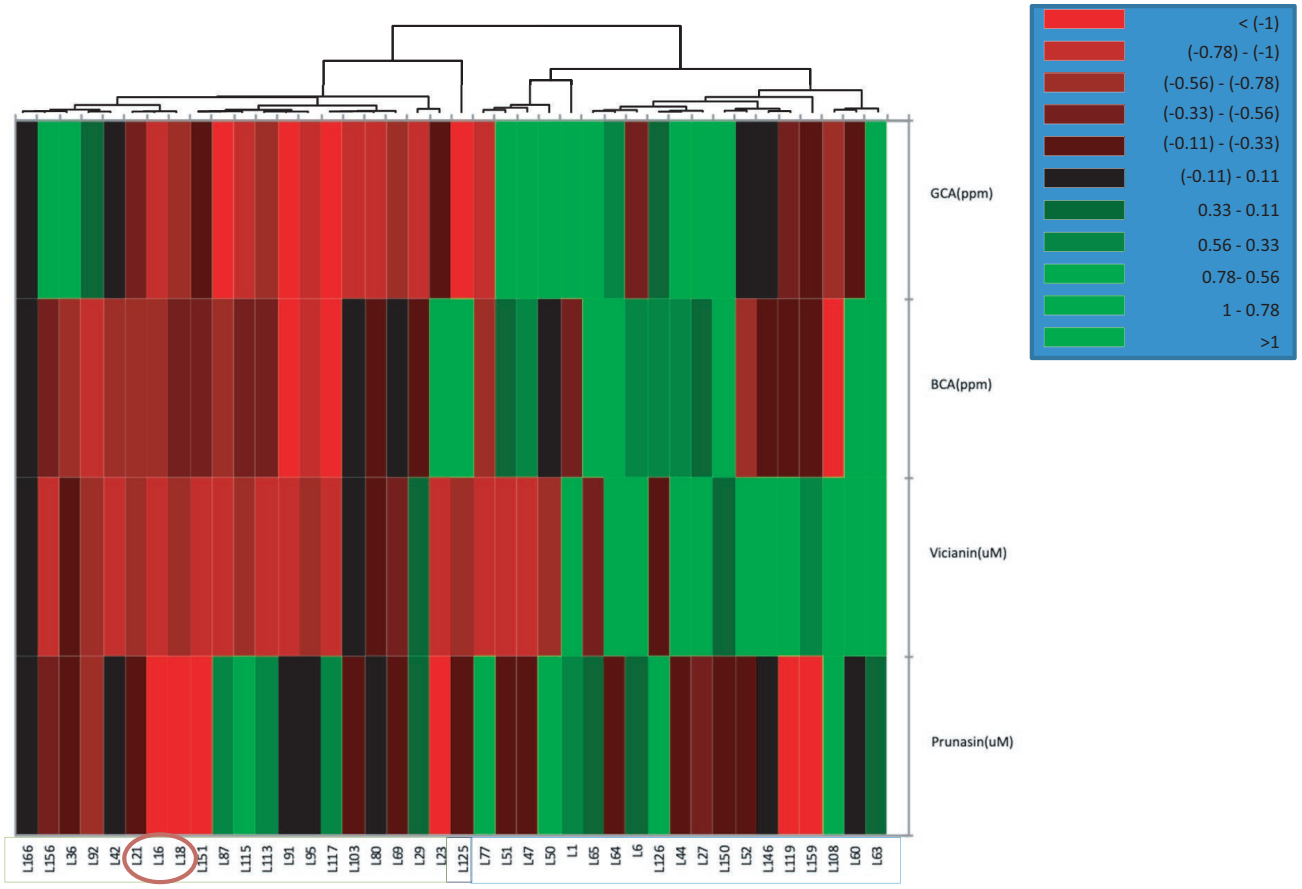

FIGURE 6 Heat map illustrating and hierarchical clustering analysis based on the content variation of four metabolites GCA, BCA, Vicianin, Prunasin in 40 lines of *Vicia sativa*. The red circle includes the lines L_{21}, L16 and L_{18}. The colour scale indicates the content of metabolites (green: high content; Black: medium content; Red: low content). Heat map was created using XLSTAT-Pro (v.7.5.3) program [Colour figure can be viewed at wileyonlinelibrary.com]

accession Mjez Ibeb using a semi-quantitative biochemical assay (Takos et al., 2010). The variability of the blue intensity of the Feigl–Anger paper demonstrated the existence of different phenotypic classes and the natural occurrence of acyanogenic plants in the Mjez Ibeb population. Based on the presence of high seed weight, non-shattering pods and the existence of natural variation in cyanogenesis, accession Mjez Ibeb was selected for further metabolite analysis.

4.2 | Cyano-amino acids and cyanogenic glucosides variation in Mjez Ibeb

In addition to cyanogenic glucosides, the seeds of *V. sativa* also contain the neurotoxic amino acids BCA and GCA, which result from the detoxification of HCN and are thus metabolically linked to the cyanogenic glucosides (Figure 2). Using metabolite analysis, we determined that accession Mjez Ibeb also showed considerable variation in the content of these amino acids. The PCA based on the cyano-amino acids content allowed the identification of a group that includes 40 lines with lower content of GCA and BCA (Figure S2). Vicianin and prunasin were analysed in the seeds of the 40 Mjez Ibeb lines selected based on low GCA and BCA content and on cyanogenesis variation.

The integrated data from metabolites content GCA and BCA together with the content of vicianin and prunasin were investigated by multivariate analysis. The PCA and the clustering analysis showed that the 40 selected lines were grouped into three distinctive clusters. The heat map analysis and Figure S3 synchronized patterns of change in metabolite abundance and allowed the identification of the lines L_{21}, L_{16} and L_{18} with a low content of both cyano-amino acids and cyanogenic glucosides. These lines are of most interest to improve common vetch as a feed crop, showing both beneficial agronomical traits and low levels of antinutritional factors.

It should be noted that the observed variation in the cyanogenic glucosides is not always related to the content of the cyano-amino acid compounds, despite their biochemical link. In plants, cyanide (CN^-) is a naturally occurring molecule produced through biological and environmental processes and this metabolic poison requires detoxification (Machingura, Salomon, Jez, & Ebbs, 2016; Yi et al., 2012). The cyanide in higher plants is produced by several catabolic reactions, such as the biosynthesis of ethylene, degradation of cyanogenic glycosides, glucosinolates, the oxidation of amino acids and the metabolism of hydroxylamine (Sánchez-Pérez, Jørgensen, Motawia, Dicenta, & Møller, 2009). To enable maintaining CN at non-toxic levels, plants have developed

various biochemical pathways such as the β-cyanoalanine synthase pathway (β-CASP), the thiocyanate pathway, the formamide hydrolase pathway and the natural release of CN through volatilization. The β-CASP is a well-studied detoxification pathway in almost all higher plants and operates as a bridge between the physiology of plants and ecology of the environment. In *V. sativa*, the cyanide detoxification pathway is interrupted, resulting in the accumulation of the cyanoalanine-based neurotoxic compounds, specifically GCA. Because of its neurotoxic effect, GCA may have evolved as an alternative chemical defence compound biochemically linked to cyanogenic glucosides. Such evolution of alternative chemical defence compounds was previously reported for *L. japonicus* where a bifurcation of the CNglcs biosynthetic pathway results in the production of the non-cyanogenic rhodiocyanosides, illustrating the ongoing "arms-race" between plants and their natural enemies (Takos et al., 2011).

The genetic factors responsible for the observed variation in CNglcs and GCA content in *V. sativa* remain to be identified, but the individual lines of the Mjez Ibeb population provide useful experimental tools.

4.3 | Cyanide detoxification and the breakdown of cyanogenic glycosides into cyanoalanine products may serve as storage compounds of reduced nitrogen

A non-defence role for CNglcs as storage compounds for reduced nitrogen has also been proposed. Clausen et al. (2015) stated that the major qualitative and quantitative changes in the accumulation and disappearance of cyanogenic glucosides at different stages of plant ontogeny would imply that cyanogenic glycosides are subject to endogenous turnover. Given that the cyanogenic glucoside concentration is a function of not only the rate of biosynthesis but also the rate of endogenous turnover, it is possible that genes affecting the latter process account for some of the variation in cyanogenic potential (Picmanova et al., 2015). The natural variation of cyanogenic glucosides content between the lines of *V. sativa* may be due to the variation in such a proposed turnover pathway.

In analogy, glucosinolates are another class of chemical defence metabolites well known for their role in plant resistance to insects and pathogens, and their synthesis and activation are similar to the biosynthesis of vicianin and prunasin. Aromatic glucosinolates are synthesized from an amino acid precursor (Sørensen, Neilson, & Møller, 2017) and hydrolysed by specific thioglycosidases (myrosinases) upon tissue disruption in a process releasing numerous toxic constituents including isothiocyanate (Burow & Halkier, 2017). Glucosinolates may serve as storage compounds of reduced carbon, nitrogen and perhaps especially sulphur, but this process is not fully understood because the endogenous turnover pathways remain to be identified (Sørensen et al., 2017).

5 | CONCLUSIONS

In contrast to previous studies, which have focused exclusively on cyano-amino acids as antinutritional factors in common vetch seeds, our findings include cyanogenic glucosides characterization and the consideration of factors involved in the polymorphism of cyanogenic potential in *V. sativa* seeds.

The considerable variation in cyanogenesis capacity in the lines of *V. sativa* provides an excellent system for investigating the genetic factors involved in these polymorphisms. The results of the study enabled us to identify three promising lines L_{21}, L_{16} and L_{18} with low cyanogenic content with nutritional, environmental, or other benefits that farmers can directly appreciate. Findings from this work give insight to feed security programme and sustainable agriculture, by improving the protein-rich legume crop common vetch as a feedstuff for monogastric animals and consequently for protein-rich animal feed crops. Production of low or free toxin varieties of *V. sativa* will encourage farmers to grow it for animal feed as it offers them several economic advantages.

ACKNOWLEDGEMENTS

The authors thank Birger Lindberg Møller (University of Copenhagen) for his valuable advice, Mohammed Saddik Motawia (University of Copenhagen) for providing the chemical standards of prunasin and linamarin, Lasse Nielsen (University of Copenhagen) for developing the Bruker Data Analysis script to analyse large LC-MS data sets, Max Tate and Dirk Enneking from the University of Adelaide for their valuable advice, providing GCA and BCA standards and the accessions of *Vicia sativa*. We are indebted grateful to Thomas Bertrand (University of Liège), Elizabeth Neilson and Bruna Marques dos Santos (University of Copenhagen) for valuable advice in HPLC and LC-MS/MS.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Marwa Aouida, Fred Rook, Raquel Sánchez-Pérez and Moez Jebara conceived the idea and designed the experiments. Marwa Aouida carried out laboratory work. Marwa Aouida and Ghassen Abid carried out the greenhouse work and the agro-morphological traits analysis. Marwa Aouida, Alexandra Bianca Maimann and Raquel Sánchez-Pérez carried out the LC-MS analysis and analysed data. Marwa Aouida and Marie Laure Fauconnier carried out the HPLC analysis. Marwa Aouida, Fred Rook and Moez Jebara drafted the manuscript. All authors read and approved the final manuscript.

ORCID

Moez Jebara https://orcid.org/0000-0002-2054-3892

REFERENCES

Abbo, S., Lev-Yadun, S., Heun, M., & Gopher, A. (2013). On the "lost" crops of the neolithic Near East. *Journal of Experimental Botany*, *64*(4), 815–822. https://doi.org/10.1093/jxb/ers373

Abbo, S., Mesghenna, Y. T., & Van Oss, H. (2011). Interspecific hybridization in wild Cicer sp. *Plant Breeding*, *130*(2), 150–155. https://doi.org/10.1111/j.1439-0523.2010.01838.x

Abd El-Moneim, A. M. (1993). Selection for non-shattering common vetch, *Vicia sativa* L. *Plant Breeding*, *110*(2), 168–171. https://doi.org/10.1111/j.1439-0523.1993.tb01231.x

Ahn, Y. O., Saino, H., Mizutani, M., Shimizu, B. I., & Sakata, K. (2007). Vicianin hydrolase is a novel cyanogenic b-glycosidase specific to b-vicianoside (6-O-a-L-arabinopyranosyl-b-D-Glucopyranoside) in seeds of Vicia angustifolia. *Plant and Cell Physiology*, *48*(7), 938–947. https://doi.org/10.1093/pcp/pcm065

Ballhorn, D. J., Heil, M., & Lieberei, R. (2006). Phenotypic plasticity of cyanogenesis in lima bean Phaseolus lunatus-activity and activation of beta-glucosidase. *Journal of chemical ecology*, *32*(2), 261–275. https://doi.org/10.1007/s10886-005-9001-z

Ballhorn, D. J., Kautz, S., Heil, M., & Hegeman, A. D. (2009). Cyanogenesis of wild lima bean (Phaseolus lunatus L.) is an efficient direct defence in nature. *PLoS ONE*, *4*(5), 1–7. https://doi.org/10.1371/journal.pone.0005450

Blomstedt, C. K., Gleadow, R. M., O'Donnell, N., Naur, P., Jensen, K., Laursen, T., … Neale, A. D. (2012). A combined biochemical screen and TILLING approach identifies mutations in *Sorghum bicolor* L. Moench resulting in acyanogenic forage production. *Plant Biotechnology Journal*, *10*(1), 54–66. https://doi.org/10.1111/j.1467-7652.2011.00646.x

Burow, M., & Halkier, B. A. (2017). How does a plant orchestrate defense in time and space? Using glucosinolates in Arabidopsis as case study. *Current Opinion in Plant Biology*, *38*, 142–147. https://doi.org/10.1016/j.pbi.2017.04.009

Chowdhury, D. M. S., Rathjen, J. M., Tate, M. E., & McDonald, G. (2004). Genetics of colour traits in common vetch (*Vicia sativa* L.). *Euphytica*, *136*(3), 249–255. https://doi.org/10.1023/B:EUPH.0000032703.02526.6d

Clausen, M., Kannangara, R. M., Olsen, C. E., Blomstedt, C. K., Gleadow, R. M., Jørgensen, K., … Møller, B. L. (2015). The bifurcation of the cyanogenic glucoside and glucosinolate biosynthetic pathways. *Plant Journal*, *84*(3), 558–573. https://doi.org/10.1111/tpj.13023

Darre, M. J., Minior, D. N., Tatake, J. G., & Ressler, C. (1998). Nutritional evaluation of detoxified and raw common vetch seed (*Vicia Sativa* L.) using diets of broilers. *Journal of Agricultural & Food Chemistry*, *46*(11), 4675–4679. https://doi.org/10.1021/jf980931i

Dong, R., Jahufer, M., Dong, D., Wang, Y., & Liu, Z. (2016). Characterisation of the morphological variation for seed traits among 537 germplasm accessions of common vetch (*Vicia sativa* L.) using digital image analysis. *New Zealand Journal of Agricultural Research*, *59*(4), 422–435. https://doi.org/10.1080/00288233.2016.1229682

Feigl, F., & Anger, V. (1966). Replacement of benzidine by copper ethylacetoacetate and tetra base as a spot-test reagent for hydrogen cyanide and cyanogen. *Analyst*, *91*, 282–284.

Firincioglu, H. K., Erbektas, E., Dogruyol, L., Mutlu, Z., Ünal, S., & Karakurt, E. (2009). Phenotypic variation of Autumn and Spring-sown vetch (*Vicia sativa* ssp.) populations in central Turkey. *Spanish Journal of Agricultural Research*, *7*(3), 596. https://doi.org/10.5424/sjar/2009073-444

Firincioğlu, H. K., Tate, M., Ünal, S., Doğruyol, L., & Özcan, I. (2007). A selection strategy for low toxin vetches (*Vicia sativa* spp.). *Turkish Journal of Agriculture and Forestry*, *31*(5), 303–311.

Ford, R., Maddeppungeng, A. M., & Taylor, P. W. J. (2009). Vetch. In C. Kole & T. C. Hall (Eds.), *Compendium of transgenic crop plants*. Oxford: John Wiley. https://doi.org/10.1002/9781405181099.k0309

Fowden, L., & Bell, E. A. (1965). Cyanide metabolism by seedlings. *Nature*, *206*(4979), 110–112. https://doi.org/10.1038/206110a0

Huang, Y. F., Gao, X. L., Nan, Z. B., & Zhang, Z. X. (2017). Potential value of the common vetch (*Vicia sativa* L.) as an animal feedstuff: A review. *Journal of Animal Physiology and Animal Nutrition*, *101*, 1–17. https://doi.org/10.1111/jpn.12617

Kooyers, N. J., Gage, L. R., Al-Lozi, A., & Olsen, K. M. (2014). Aridity shapes cyanogenesis cline evolution in white clover (*Trifolium repens* L.). *Molecular Ecology*, *23*(5), 1053–1070. https://doi.org/10.1111/mec.12666

Kooyers, N. J., & Olsen, K. M. (2013). Searching for the bull's eye: Agents and targets of selection vary among geographically disparate cyanogenesis clines in white clover (*Trifolium repens* L.). *Heredity*, *111*(6), 495–504. https://doi.org/10.1038/hdy.2013.71

Machingura, M., Salomon, E., Jez, J. M., & Ebbs, S. D. (2016). The β-cyanoalanine synthase pathway: Beyond cyanide detoxification. *Plant Cell and Environment*, *39*, 2329–2341. https://doi.org/10.1111/pce.12755

Makkar, H. P. S., Tran, G., Heuzé, V., Giger-Reverdin, S., Lessire, M., Lebas, F., & Ankers, P. (2016). Seaweeds for livestock diets: A review. *Animal Feed Science and Technology*, *212*, 1–17. https://doi.org/10.1016/j.anifeedsci.2015.09.018

Megías, C., Cortés-Giraldo, I., Girón-Calle, J., Vioque, J., & Alaiz, M. (2014). Determination of β-cyano-L-alanine, γ-glutamyl-β-cyano-L-alanine, and common free amino acids in *Vicia sativa* (Fabaceae) seeds by reversed-phase high-performance liquid chromatography. *Journal of Analytical Methods in Chemistry*, *2014*, 1–5. https://doi.org/10.1155/2014/409089

Olsen, K. M., Kooyers, N. J., & Small, L. L. (2014). Adaptive gains through repeated gene loss: Parallel evolution of cyanogenesis polymorphisms in the genus Trifolium (Fabaceae). *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1648), 20130347–20130347. https://doi.org/10.1098/rstb.2013.0347

Picmanova, M., Neilson, E. H., Motawia, M. S., Olsen, C. E., Agerbirk, N., Gray, C. J., … Bjarnholt, N. (2015). A recycling pathway for cyanogenic glycosides evidenced by the comparative metabolic profiling in three cyanogenic plant species. *The Biochemical Journal*, https://doi.org/10.1042/BJ20150390

Ressler, C., Nigam, S. N., & Giza, Y. H. (1969). Toxic principle in vetch. Isolation and identification of gamma-L-glutamyl-L-beta-cyanoalanine from common vetch seeds. Distribution in some legumes. *Journal of the American Chemical Society*, *91*(10), 2758–2765. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=5784950

Ressler, C., & Tatake, J. G. (2001). Vicianin, prunasin, and β-cyanoalanine in common vetch seed as sources of urinary thiocyanate in the rat. *Journal of Agricultural and Food Chemistry*, *49*(10), 5075–5080. https://doi.org/10.1021/jf010343w

Sánchez-Pérez, R., Jørgensen, K., Motawia, M. S., Dicenta, F., & Møller, B. L. (2009). Tissue and cellular localization of individual β -glycosidases using a substrate-specific sugar reducing assay. *Plant Journal*, *60*, 894–906. https://doi.org/10.1111/j.1365-313X.2009.03997.x

Sanchez-Perez, R., Jorgensen, K., Olsen, C. E., Dicenta, F., & Moller, B. L. (2008). Bitterness in almonds. *Plant Physiology*, *146*(3), 1040–1052. https://doi.org/10.1104/pp.107.112979

Smýkal, P., Coyne, C. J., Ambrose, M. J., Maxted, N., Blair, M. W., Berger, J., … Besharat, N. (2017). Legume crops phylogeny and genetic diversity for science and breeding. *Critical Reviews in Plant Sciences*, *34*(1–3), 43–104. https://doi.org/10.1080/07352689.2014.897904

Sørensen, M., Neilson, E. H. J., & Møller, B. L. (2017). Oximes: Unrecognized chameleons in general and specialized plant metabolism. *Molecular Plant*, *11*(1), 95–117. https://doi.org/10.1016/j.molp.2017.12.014

Takos, A., Lai, D., Mikkelsen, L., Abou Hachem, M., Shelton, D., Motawia, M. S., … Rook, F. (2010). Genetic screening identifies cyanogenesis-deficient mutants of *Lotus japonicus* and reveals enzymatic specificity

in hydroxynitrile glucoside metabolism. *The Plant Cell*, *22*(5), 1605–1619. https://doi.org/10.1105/tpc.109.073502

Takos, A. M., Knudsen, C., Lai, D., Kannangara, R., Mikkelsen, L., Motawia, M. S., … Rook, F. (2011). Genomic clustering of cyanogenic glucoside biosynthetic genes aids their identification in *Lotus japonicus* and suggests the repeated evolution of this chemical defence pathway. *The Plant Journal: for Cell and Molecular Biology*, *68*(2), 273–286. https://doi.org/10.1111/j.1365-313X.2011.04685.x

Tate, M. E., Rathjen, J., Delaere, I., & Enneking, D. (1999). Covert trade in toxic vetch continues. *Nature*, *400*(6741), 207. https://doi.org/10.1038/22198

Yamaguchi, T., Yamamoto, K., & Asano, Y. (2014). Identification and characterization of CYP79D16 and CYP71AN24 catalyzing the first and second steps in l-phenylalanine-derived cyanogenic glycoside biosynthesis in the Japanese apricot, Prunus mume Sieb. et Zucc. *Plant Molecular Biology*, *86*(1–2), 215–223. https://doi.org/10.1007/s11103-014-0225-6

Yi, H., Juergens, M., & Jez, J. M. (2012). Structure of soybean β-cyanoalanine synthase and the molecular basis for cyanide detoxification in plants. *The Plant Cell*, *24*(6), 2696–2706. https://doi.org/10.1105/tpc.112.098954

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Aouida M, Rook F, Maimann Bianca A, et al. Polymorphisms in cyanogenic glucoside and cyano-amino acid content in natural accessions of common vetch (*Vicia sativa* L.) and selection for improved agronomic performance. *Plant Breed*. 2019;138:348–359. https://doi.org/10.1111/pbr.12691