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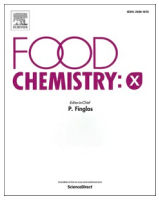

Anti-nutrient factors, nutritional components, and antioxidant activities of faba beans (*Vicia faba* L.) as affected by genotype, seed traits, and their interactions

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ABSTRACT

This study explored how genotype, seed color, and seed weight affect major biochemical components in 95 faba bean accessions. Genotype variation significantly affected convicine, total tannin (TTC), total saponin, and total phenol (TPC) contents. Seed color and weight variations affected several parameters, with their interaction significantly affecting convicine, total vicine-convicine content (TVC), TTC, total polyunsaturated fatty acid (PUFA), and antioxidant activities. Genotype interaction with seed weight and seed color also significantly affected convicine, TVC, TPC, oleic acid, linoleic acid, PUFA, and ferric-reducing antioxidant power. Vicine, dietary fiber, total fat, crude protein, palmitic acid, and stearic acid contents remain unaffected by these factors. Multivariate analysis showed that brown and small beans had distinctive characteristics. Overall, this study demonstrated the connection between biochemical components, genotype, and seed traits in faba beans. Therefore, these factors should be considered when choosing faba bean genotypes for use in the food industry and breeding programs.

1. Introduction

Faba bean (*Vicia faba* L.), also known as broad bean, is a widely cultivated legume known for its nutritional value, desirable agronomic performances, and ability to grow in diverse climatic environments (Crépon et al., 2010; Kamani, Liu, Fitzsimons, Fenelon, & Murphy, 2024; Labba, Frøkiær, & Sandberg, 2021). The seeds of faba beans are rich in vitamins and minerals. They are also good sources of crude and dietary fibers and hence, promote gut health and manage chronic conditions such as diabetes and obesity (Crépon et al., 2010; Sharan et al., 2020). Moreover, the saturated and unsaturated fatty acids of faba bean seeds make them a beneficial option for heart health (Ryu et al., 2017; Sharan et al., 2020). Additionally, faba beans have the potential to serve as a sustainable protein source, particularly as there is a growing demand for plant-based proteins (Kamani et al., 2024; Rahate, Madhumita, & Prabhakar, 2021; Shi, House, Wanasundara, & Nickerson, 2022a). Polyphenolic compounds in faba beans, such as phenolic acids and flavonoids, also have antioxidant properties that reduce oxidative stress and inflammation, offering immense health benefits (Oomah et al., 2011; Siah, Wood, Agboola, Konczak, & Blanchard, 2014).

Although faba beans have several desirable qualities, their utilization in the food industry is restricted because they contain anti-nutritional factors. These substances, also found in other crops, hinder the absorption and bioavailability of nutrients in the human body, making them undesirable for both animal feed and human consumption (Singh, Pandey, Sultan, Singh, & Dar, 2023). Faba beans contain different types of anti-nutrients, with vicine and convicine being the most common. The consumption of faba beans containing these substances has been associated with favism, a condition characterized by hemolytic anemia, mainly in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency (Crépon et al., 2010; Khazaei et al., 2019; Segers et al., 2022). Therefore, faba bean varieties with lower or zero levels of these

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compounds are highly preferred in both the food industry and breeding programs (Khamassi et al., 2013; Khazaei et al., 2019). Likewise, tannins and saponins found in faba bean can affect the digestion and absorption of nutrients. As a result, there is a growing need for faba bean varieties with minimal or no tannins in various sectors (Crépon et al., 2010; Oomah et al., 2011). Overall, in addition to their beneficial metabolites, assessing the possible harmful biochemical compounds found in faba beans is crucial to enhance their utilization (Khazaei et al., 2019; Singh et al., 2023).

The distributions and contents of plant metabolites, in general, are affected by various environmental and genetic factors. Seed-related traits, such as seed color, seed weight, and hilum color, also contribute to the variations in metabolite compositions, especially in legumes (Cho et al., 2013; Lee et al., 2008; Lee et al., 2017; Zhao, Tang, & Yang, 2021). These traits also determine the quality of seeds and influence the preferences made by both consumers and farmers. Moreover, several genes that are involved in the chemical makeup of seeds regulate the diversity of these traits, making them important in breeding efforts (Lippolis et al., 2023; Sharan et al., 2020). Overall, studying the effects of environmental and genetic factors is crucial to understanding the relationships between the biochemical compositions of crops and different variables. Furthermore, such studies assist in selecting suitable crop varieties for utilization in the food industry, conservation, dissemination, and breeding initiatives (Lippolis et al., 2023; Segers et al., 2022; Shi et al., 2022b). In this regard, studying a large collection of genetic materials is crucial as it provides valuable information on the diversity available and several options to select those with desirable qualities (Lippolis, Roland, Bocova, Pouvreau, & Trindade, 2023).

Previously, several studies have explored the influences of different factors on the chemical composition of various types of legumes (Boudjou, Oomah, Zaidi, & Hosseinian, 2003; Kim et al., 2012; Lee et al., 2017; Liu et al., 2024; Zhao et al., 2021). Compared to other legumes, however, there has been limited research on faba beans in this regard (Avramidou et al., 2023; Hendawey & Younes, 2013). To address this gap, this study aimed to statistically investigate the effects of genotype, seed color, seed weight, and their interactions on the levels of major anti-nutrient factors (vicine, convicine, total tannin, total phenol, and total saponin), nutritional components (crude fiber, dietary fiber, crude protein, total fat, stearic acid, palmitic acid, oleic acid, linoleic acid, and linolenic acid), and antioxidant activities (1,1-Diphenyl-2-picrylhydrazyl radical (DPPH$^{\bullet}$) scavenging activity, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium radical cation (ABTS$^{\bullet+}$) scavenging activity, and ferric-reducing antioxidant power (FRAP)) for the first time. This study provides insights into the associations of faba bean biochemicals with genotype, seed color, and seed weight differences. Hence, the results obtained could be applicable in food industries to select faba bean materials with desirable properties and also guide future molecular investigations to develop improved faba bean varieties.

2. Materials and methods

2.1. Faba beans collection, cultivation, classification, and preparation

The seeds of the 95 faba bean accessions were obtained from the gene bank at the National Agrobiodiversity Center, Rural Development Administration (Jeonju, Republic of Korea). The faba bean accessions were cultivated under similar treatment and growth conditions in an experimental farm located at the center (latitude/longitude: 35°49'38.37'' N/127°09'07.78'' E). Specifically, seven seeds were sown for each accession on January 22, 2021 in rows in sandy clay loam soil. The rows were kept 90 cm apart with a seed-to-seed distance of 30 cm. The faba beans were grown under similar conditions and the cultivation period lasted until July of 2021. During the cultivation period, the average monthly precipitation at the growing location was 28.7, 13.7, 95.7, 34.9, 182.2, 145.3, and 255.1 mm in January, February, March, April, May, June, and July, respectively. Additionally, the average monthly temperature was recorded to be 0.5, 4.3, 9.5, 14.4, 17.7, 23.0, and 27.2 °C in January, February, March, April, May, June, and July, respectively. Insecticides containing 2.5 % acetamiprid and 8.0 % etofenprox, along with a mixture of 8.0 % acetamiprid and 5.0 % lufenuron, were applied during the pre-harvest period on June 01, 2021. Matured seeds were hand-harvested and classified into landraces (n = 37) and cultivated varieties (n = 58) based on their genotype. The faba beans were also classified based on their seed coat color as brown (n = 8), green (n = 29), yellow (n = 52), and mixed (n = 6). Moreover, the faba beans were classified as very small (< 50 g 100 seeds^{-1}), small (50–100 g 100 seeds^{-1}), medium (100–150 g 100 seeds^{-1}), large (150-200 g 100 seeds^{-1}), and very large (> 200 g 100 seeds^{-1}) based on their seed weight (Landry, Fuchs, & Hu, 2016). Whole seed samples from each accession were freeze-dried (LP500 freeze dryer, ilShinBioBase, Dongducheon, Korea) in triplicate and ground into powder using an electric grinder. The powdered samples were sieved through 500 μm mesh size and stored at −20 °C until extraction and analysis. General information regarding the names, introduction (IT) number, codes given (FB1-FB95), genotype, seed color, seed weight, and origin of the 95 faba bean accessions is provided in **Table S1** (Supplementary material).

2.2. Chemicals, reagents, and materials

The reagents and chemicals used in this study were of analytical grade. Convicine standard was obtained from Toronto Research Chemicals (Toronto, ON, Canada). Ethanol and sulfuric acid were purchased from Fisher Scientific (Pittsburgh, PA, USA). Other reference standards including vicine, L-ascorbic acid, catechin, diosgenin, Trolox, gallic acid, and individual fatty acids, as well as solvents including acetonitrile and water, and all other reagents, were obtained from Sigma-Aldrich (St. Louis, MO, USA). During the preparation of samples, centrifuge tubes from SPL Life Sciences (Pocheon-si, Gyeonggi-do, Korea) and round bottom glass tubes with screw cups from SciLab (Seoul, Korea) were utilized. A Labogene 1236R centrifuge from Labogene (Seoul, Korea) was used for sample centrifugation at 3134 ×g. Multiple sample assays were performed using 96-well plates ordered from Thermo Fisher Scientific (Waltham, MA, USA). Absorbance measurements were conducted using an Eon Microplate Spectrophotometer (Bio-Tek in Winooski, VT, USA).

2.3. Quantification of anti-nutrient factors

2.3.1. Analysis of vicine and convicine

2.3.1.1. Optimization and selection of extraction condition. The two widely used extraction methods, including perchloric acid and water extraction, were evaluated and optimized using randomly selected faba bean samples (Pulkkinen et al., 2015). During perchloric acid extraction, 0.50 g of powdered sample was placed into a 50 mL centrifuge tube, followed by the addition of 7.50 mL of 7.00 % perchloric acid, which was prepared by diluting 70.00 % perchloric acid. The resulting solution was vortex mixed for 1 min and centrifuged for 10 min at 4 °C. The supernatant was collected and the extraction process was repeated once more for the remaining residue. The combined supernatant was then filtered using a 0.45 μm membrane syringe filter and transferred to an injection vial for subsequent analysis. For aqueous extraction, a similar procedure to the perchloric acid extraction method was followed, with the exception being the replacement of the solvent with 7.5 mL of MilliQ-water (Pulkkinen et al., 2015). The extraction was carried out using a 10 mL round-bottom glass tube with a screw cap in a water bath (90 °C) for 3.5 h, with intermittent shaking every 30 min. Following extraction, the mixture was cooled to 25 °C, centrifuged for 10 min, and then 5 mL of the resulting supernatant was combined with 50 μL of 1 N HCl for protein precipitation. After vortex mixing for 1 min, the sample was centrifuged for 10 min at 4 °C. The upper supernatant was filtered

through a membrane syringe filter and prepared for subsequent analysis. Each extraction process was carried out in triplicate and the peak area response was closely monitored. Ultimately, the perchloric acid extraction method was chosen due to its ease of application and clear peak symmetry.

2.3.1.2. UPLC-DAD-QToF/MS analysis and quantification. Identification of vicine and convicine was achieved using an ultra-high performance liquid chromatography-diode array detector system (UPLC-DAD) coupled with quadrupole time-of-flight (QToF) mass spectrometry (SCIEX X500R, SCIEX Co., Framingham, MA, USA). Separation was achieved using CORTECS UPLC T3 column (2.1 × 150 mm, 1.6 μm, Waters Co., Milford, MA, USA) fitted with a CORTECS UPLC Vanguard T3 (2.1 × 50 mm, 1.6 μm, Waters Co.) set at a temperature of 30 °C. The mass analysis was performed using a positive electrospray ionization (+ESI) mode within the *m/z* 100–1200 range. The ion source gas was set at 50 psi, curtain gas at 30 psi, and the ion source temperature at 450 °C. Furthermore, a declustering potential of 80 V, collision energy of 15 ± 10 V, and a spray voltage of 5500 V were applied during the analysis. For quantification, a reverse-phase 1260-Infinity quaternary high-performance liquid chromatography system (Agilent Technologies, Santa Clara, CA, USA) equipped with DAD and an Inertsil ODS-3 (250 × 4.6 mm, 5 μm; GL Sciences, Tokyo, Japan) column maintained at 30 °C was used. The mobile phase was composed of a mixture of water (A) and acetonitrile with 0.1 % formic acid (B) at a flow rate of 0.8 mL/min. The gradient condition started with 100 % solvent A and held isocratic for 15 min. This was followed by a gradual increase of solvent B to 70 % over 7 min and maintained isocratic for an additional 3 min taking a total of 25 min run time. The post-run time was set at 10 min. The sample injection volume was 5 μL, and the chromatogram was monitored at 273 nm using ChemStation software (Agilent Technologies, Santa Clara, CA, USA). Quantification was performed by plotting calibration curves based on peak area responses of vicine and convicine external standards at five concentration levels (0.01–2.00 mg/mL). The results were then reported as mg/g of dried seed weight from triplicate measurements.

2.3.2. Sample extraction for total tannin, saponin, and phenol contents

To determine the contents of total tannin (TTC), total saponin (TSC), and total phenol (TPC), samples were extracted according to a previously reported method (Boudjou et al., 2003). Initially, 0.5 g of powdered sample, in triplicate, was mixed with 5 mL of aqueous ethanol (80 %) in a 15 mL extraction tube. The resulting mixture was thoroughly mixed and then sonicated at 25 °C for 45 min in the dark. After that, the mixture was centrifuged for 10 min, and the upper layer was collected. The remaining residue was extracted for a second round with 2.5 mL of the solvent. The combined supernatant from both extractions was used to quantify total metabolite contents as briefed below. In each case, analysis took place in a 96-well microplate.

2.3.2.1. Determination of TTC. The determination of TTC was carried out using the vanillin-HCl method with some modifications (Price, Van-Scoyoc & Butler, 1978). Specifically, 100 μL aliquot of sample extract was combined with 200 μL of vanillin-HCl reagent. Subsequently, the mixture was incubated in darkness at 25 °C for 20 min, and the absorbance was measured at 500 nm. Catechin, at concentrations ranging from 0.05 to 0.70 mg/mL, was used as a standard for constructing calibration curves ($y = 0.0524\times + 0.0012$, $R^2 > 0.999$). The TTC was determined in milligrams of catechin equivalents per gram of dried seed weight (mg CE/g).

2.3.2.2. Determination of TSC. TSC was determined using the vanillin-sulfuric acid assay (Boudjou et al., 2003). In summary, 25 μL of sample extract was combined with an equal amount of freshly prepared 8 % vanillin in ethanol. Subsequently, 250 μL of 72 % sulfuric acid in water was added, and the mixture was placed in a water bath at 60 °C for 10 min. Then, the mixture was removed and cooled in an ice bath for 15 min before measuring the absorbance at 544 nm. Diosgenin, at concentrations of 0.10–1.50 mg/mL, was used to plot calibration curves ($y = 0.3938\times - 0.0347$, $R^2 = 0.999$). The TSC was then determined as milligrams of diosgenin equivalent per gram of dried seed weight (mg DE/g).

2.3.2.3. Determination of TPC. TPC was determined according to our recently reported protocol without modification (Choi et al., 2023). In summary, TPC was estimated using the Folin-Ciocalteu method and reported as milligrams of gallic acid equivalents per gram of dried seed weight (mg GAE/g).

2.4. Quantification of nutritional components

2.4.1. Crude and dietary fiber contents

The contents of crude fiber and dietary fiber were measured according to the Association of Official Analytical Chemists (AOAC) protocol (AOAC, 1990). To be specific, the crude fiber content was assessed with a Fiber Analyzer (FOSS, Hillerød, Denmark) based on the adapted Henneberg and Stohmann procedure. Likewise, the dietary fiber content was determined through an enzymatic-gravimetric assay utilizing an Analytical Fibertec E-1023 System (FOSS, Hillerød, Denmark). In each case, samples were prepared and analyzed in triplicate. The results were expressed as percentages based on the weight of dried seeds.

2.4.2. Crude protein and total fat contents

The crude protein and total fat contents were determined according to the procedures recommended by the AOAC (AOAC, 1990). Specifically, the crude protein content was determined employing the Kjeldahl method and computed as $N \times 6.25$. The total fat content was determined using the soxhlet extraction method with a Soxtec 8000 extractor (FOSS, Hillerød, Denmark) and n-hexane as the solvent. Once again, samples were prepared and analyzed in triplicate during each assay, and the results were expressed as percentages based on the weight of dried seeds.

2.4.3. Analysis of fatty acid composition using GC-FID

To analyze fatty acids, fatty acid methyl esters (FAMEs) were prepared using a direct methylation method as optimized in our recent study (Choi et al., 2023). Briefly, 0.2 g of powdered sample was mixed with 680 μL of a solvent mixture of methanol, benzene, 2,2-dimethoxypropane, and sulfuric acid (in a ratio of 39:20:5:2) in a 10 mL round bottom glass tube with a screw cap. Then, 400 μL of *n*-heptane was added and the mixture was vortexed. Extraction was conducted in a shaking water bath set at 80 °C for 2 h. The mixture was then cooled to 25 °C and centrifuged for 15 min. The upper *n*-heptane layer containing FAMEs was filtered and analyzed using a QP2010 gas chromatography-flame ionization detector (GC-FID) instrument (Shimadzu, Kyoto, Japan) equipped with an HP-INNOWAX column (30 m × 0.250 mm, 0.25 μm). The injection volume was 1 μL with a split ratio of 50:1. Helium was used as a carrier gas at a flow rate of 1.5 mL/min. Initially, the column temperature started at 100 °C and then increased gradually to 170 °C at a rate of 60 °C/min with a 1 min holding time. Subsequently, the temperature was further increased to 240 °C with a ramp of 6.5 °C/min and held for another 1 min. The complete analysis took 16.4 min to complete. The detector and injection port temperatures were set at 250 °C. LabSolution software (Shimadzu, Kyoto, Japan) was used to manage and analyze the acquired chromatograms. Identification of fatty acids was achieved by comparing retention times with external standards. The percentage of each fatty acid was determined relative to the total fatty acid content based on area peaks.

2.5. Determination of antioxidant activities

The same extraction technique that was utilized for analyzing total tannin, saponin, and phenol contents was also employed for determining antioxidant activities. In each experiment, sample extraction and measurements were carried out in triplicate as briefed below (Choi et al., 2023). The analysis was conducted using a 96-well microplate.

2.5.1. $DPPH^{\bullet}$ scavenging activity

Initially, 100 μL of the sample extract, blank solvent, or standard was mixed with an equal volume of freshly prepared $DPPH^{\bullet}$ solution (150 μM). Subsequently, the resulting mixture was incubated in the dark at 25 °C for 30 min, and the absorbance was measured at 517 nm. The $DPPH^{\bullet}$ scavenging activity was determined and reported as milligram ascorbic acid equivalents per gram of dried seed weight (mg AAE/g).

2.5.2. $ABTS^{\bullet+}$ scavenging activity

A 150 μL working solution of $ABTS^{\bullet+}$ was combined with 10 μL of either the sample extract, blank solvent, or standard. Each mixture was then incubated at 25 °C in darkness, and absorbance was measured at 734 nm after 3 min. The $ABTS^{\bullet+}$ scavenging activity was calculated as milligram Trolox equivalents per gram of dried seed weight (mg TE/g).

2.5.3. FRAP

Initially, 60 μL of sample extract was combined with 150 μL of freshly prepared phosphate buffer (pH: 6.6, 0.2 M) and 150 μL of 1 % potassium ferricyanide solution ($K_3Fe(CN)_6$). This mixture was then incubated for 20 min at 50 °C. Subsequently, 150 μL of 10 % trichloroacetic acid was added and the mixture was centrifuged for 10 min. Then, 100 μL of the supernatant was diluted with an equal volume of distilled water and 20 μL of 0.1 % ferric chloride solution. After 10 min of incubation, the absorbance was measured at 700 nm, and the FRAP activity was expressed as mg AAE/g.

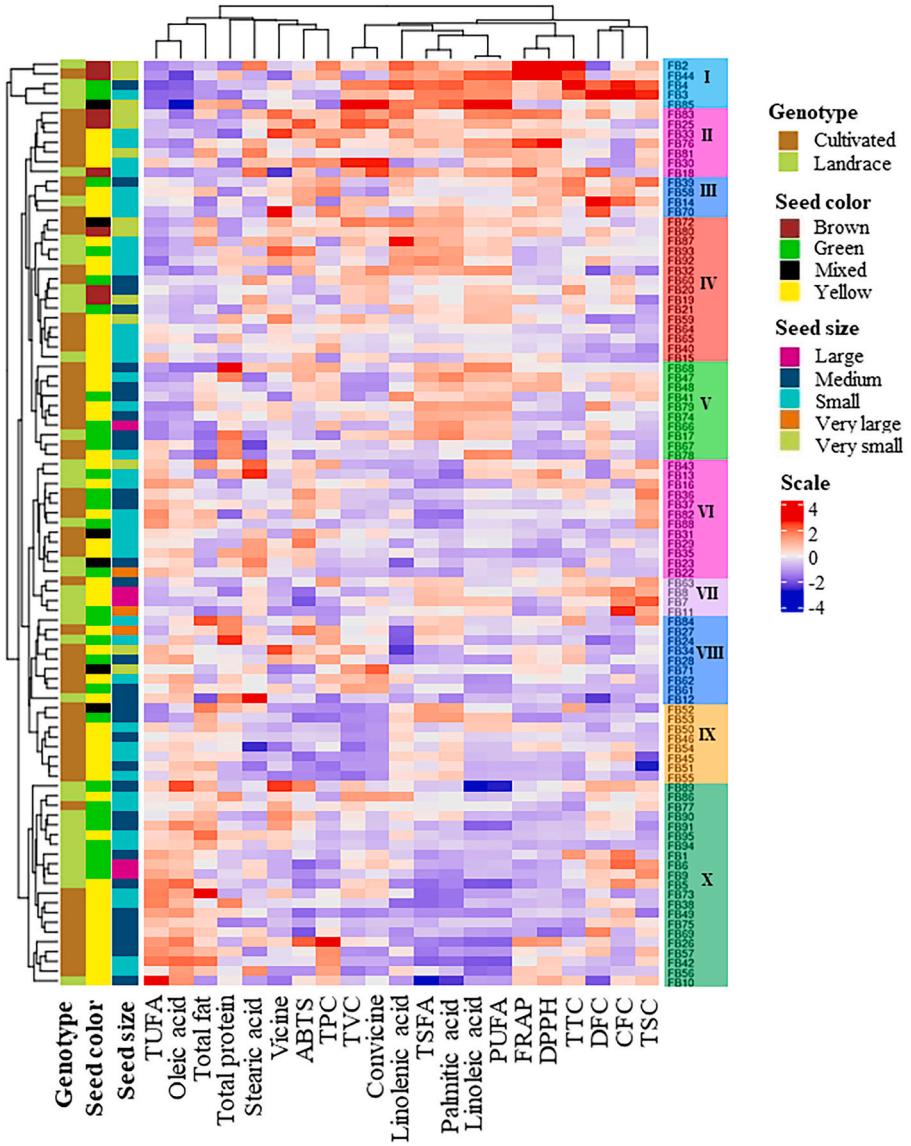

Fig. 1. Heatmap showing the levels of the analyzed parameters among the 95 faba bean accessions and demonstrating two-way hierarchical cluster analysis according to genotype, seed color, and seed weight. ABTS represents $ABTS^{\bullet+}$ scavenging activity; CFC represents crude fiber content, DFC represents dietary fiber content; DPPH represents $DPPH^{\bullet}$ scavenging activity; FRAP represents Ferric reducing antioxidant power; LA represents linoleic acid, LLA represents linolenic acid; OA represents oleic acid; PA represents palmitic acid; PUFA represents total polyunsaturated fatty acid content; SA represents stearic acid; TSFA represents total saturated fatty acid content; TF represents total fat content; TVC represents total vicine-convicine content; TP represents crude protein content; TPC represents total phenolic content; TTC represents total tannin content; TSC represent Total saponin content.

2.6. Statistical analysis

In this study, all measurements and analyses were conducted in triplicates unless specified. Results are expressed as mean ± standard deviation (SD) and were subjected to both univariate and multivariate statistical analyses. Analysis of variance (ANOVA) was applied to statistically determine significant differences between measurements followed by Fisher's least significant difference-multiple comparison test ($LSD_{0.05}$) using XLSTAT software version 2019.2.2 (Lumivero, CO, USA). Principal component analysis (PCA) was performed using JMP software version-17 (SAS, Inc., Cary, North Carolina, USA). A two-way hierarchical cluster analysis (HCA), box plots, and Pearson's correlation analysis were computed using R-software version 4.0.2 (R-project).

3. Results and discussion

3.1. Variations of vicine and convicine contents

The optimized LC conditions effectively separated vicine and convicine with a good peak resolution (**Fig. S1**). Through UPLC-DAD-QToF/MS analysis, both anti-nutrient factors were detected in all studied faba bean accessions. The heatmap in Fig. 1 shows the distribution of vicine and convicine levels in each faba bean accession, revealing wide variations. The vicine and convicine contents were in the ranges of 5.37–15.00 and 1.12–22.93 mg/g, with means of 9.83 and 6.41 mg/g, respectively (Fig. 1, Table 1). The highest vicine content was found in cultivated variety FB70 and the lowest was found in landrace FB18. Likewise, landrace FB85 had the highest convicine content, while cultivated variety FB49 had the lowest. Despite these wide ranges of values, most accessions had vicine and convicine values clustered in the ranges of 6.00–11.00 and 1.50–10.00 mg/g, respectively which agrees with many previous studies. For instance, Pulkkinen et al. (2015) reported a vicine content of 5.16–7.59 mg/g and a convicine content of 2.09–2.76 mg/g. In a more recent study, vicine content in the range of 10.71–15.21 mg/g and convicine content in the range of 3.03–5.45 mg/g were reported (Kowalczyk, Krzyżanowska-Kowalczyk, & Stochmal, 2021). Other studies also reported much higher and/or lower vicine and convicine levels compared to these findings (Arntfield, Ismond, & Murry, 1985; De Silva, Liu, Smith, Vandenberg, & Zhang, 2024). These records indicate a wide variation in the reported contents of vicine and convicine, which could be attributed to disparities in growth environments, genetic factors, and extraction techniques (Rahate et al., 2021; Segers et al., 2022). Moreover, standards for these anti-nutrients, particularly convicine, were not commercially available until recently. Consequently, previous studies had to employ alternative analytical approaches to estimate the concentrations of these components, potentially causing inconsistencies in the reported values (Hendawey & Younes, 2013; Khamassi et al., 2013). To the best of our knowledge, this study is the first to estimate the levels of vicine and convicine in a large collection of faba bean genetic materials using their specific standards. As previously highlighted, the development of faba bean varieties with low and/or zero levels of vicine and convicine is an ultimate research focus (Crépon et al., 2010; Khazaei et al., 2019**)**. In this respect, the cultivated varieties FB49, FB52, FB54, FB55, and FB56 may serve as valuable genetic resources due to their low total vicine and convicine content (TVC). On the other hand, the ratio of vicine to convicine (VC ratio) in the whole faba bean population ranged from 0.33 to 8.41 (**Table S1**). Vicine content was higher than convicine in the majority of faba bean accessions (71.58 %). Interestingly, convicine dominance was observed in about 8.42 % of the faba beans ($n = 8$), resulting in a lower VC ratio. The remaining 18.95 % of the faba beans ($n = 18$) had comparable levels of vicine and convicine, leading to a VC ratio close to 1. Overall, those accessions with a low VC ratio could be valuable resources in the food industry and preferred for individuals with G6PD deficiency (Crépon et al., 2010).

This study also investigated the effects of genotype (G), seed color

Table 1
Distributions and variations of anti-nutrient factors, nutritional components, and antioxidant activities across 95 faba bean landraces and cultivated varieties.

Variable	Values	Faba bean genotype		Total
		Cultivated	**Landrace**	
Vicine (mg/g)	Minimum	6.71	5.37	5.37
	Maximum	15.00	14.49	15.00
	Mean	9.85[a]	9.80[a]	9.83
	CV	18.84	18.02	18.53
Convicine (mg/g)	Minimum	1.12	2.49	1.12
	Maximum	17.31	22.93	22.93
	Mean	5.75[b]	7.45[a]	6.41
	CV	68.21	54.26	63.24
TVC (mg/g)	Minimum	8.49	11.77	8.49
	Maximum	28.48	32.91	32.91
	Mean	15.60[a]	17.25[a]	16.24
	CV	31.00	22.85	28.20
Total tannin (mg CE/g)	Minimum	2.04	2.20	2.04
	Maximum	6.30	9.42	9.42
	Mean	3.29[b]	3.89[a]	3.53
	CV	26.08	36.90	32.81
Total saponin (mg DE/g)	Minimum	2.91	4.39	2.91
	Maximum	8.61	9.36	9.36
	Mean	5.94[b]	6.54[a]	6.17
	CV	18.15	19.83	19.51
Total phenol (mg GAE/g)	Minimum	1.85	1.86	1.85
	Maximum	4.74	4.03	4.74
	Mean	3.16[a]	2.49[b]	2.90
	CV	18.38	19.99	22.11
Crude fiber (%)	Minimum	3.28	3.58	3.28
	Maximum	7.41	12.73	12.73
	Mean	5.07[b]	6.13[a]	5.48
	CV	21.42	34.66	30.20
Dietary fiber (%)	Minimum	11.55	10.41	10.41
	Maximum	22.65	24.78	24.78
	Mean	16.38[a]	17.04[a]	16.63
	CV	12.43	19.04	15.60
Crude protein (%)	Minimum	23.51	23.94	23.51
	Maximum	35.71	33.70	35.71
	Mean	27.68[a]	27.63[a]	27.66
	CV	8.24	8.52	8.35
Total fat (%)	Minimum	0.85	0.81	0.81
	Maximum	2.12	1.91	2.12
	Mean	1.34[a]	1.29[a]	1.32
	CV	17.41	20.85	18.87
Palmitic acid (%)	Minimum	15.68	15.12	15.12
	Maximum	18.50	18.92	18.92
	Mean	17.13[a]	17.05[a]	17.10
	CV	4.27	5.49	4.79
Stearic acid (%)	Minimum	1.83	2.12	1.83
	Maximum	2.97	3.27	3.27
	Mean	2.46[a]	2.56[a]	2.50
	CV	9.38	11.27	10.37
Oleic acid (%)	Minimum	21.30	19.25	19.25
	Maximum	38.27	39.45	39.45
	Mean	30.03[a]	30.22[a]	30.10
	CV	12.18	13.98	12.92
Linoleic acid (%)	Minimum	41.54	38.94	38.94
	Maximum	55.33	56.84	56.84
	Mean	47.99[a]	47.74[a]	47.89
	CV	6.32	7.06	6.62
Linolenic acid (%)	Minimum	1.73	1.85	1.73
	Maximum	2.97	3.17	3.17
	Mean	2.39[a]	2.42[a]	2.40
	CV	10.59	13.21	11.72
TSFA (%)	Minimum	18.11	17.34	17.34
	Maximum	20.70	21.24	21.24
	Mean	19.59[a]	19.61[a]	19.60
	CV	3.64	4.59	4.04
PUFA (%)	Minimum	43.62	41.36	41.36
	Maximum	58.15	59.76	59.76
	Mean	50.38[a]	50.17[a]	50.30
	CV	6.24	7.06	6.57
TUFA (%)	Minimum	79.30	78.76	78.76
	Maximum	81.89	82.66	82.66
	Mean	80.41[a]	80.39[a]	80.40
	CV	0.89	1.12	0.98

(*continued on next page*)

Table 1 (*continued*)

Variable	Values	Faba bean genotype		Total
		Cultivated	**Landrace**	
DBI	Minimum	127.58	124.60	214.60
	Maximum	140.41	141.68	141.68
	Mean	133.18[a]	132.98[a]	133.10
	CV	3.83	3.71	2.22
TSFA:TUFA	Minimum	4.52	4.77	3.71
	Maximum	4.11	4.11	4.77
	Mean	4.58[b]	5.76[a]	4.11
	CV	3.83	3.71	5.07
DPPH	Minimum	0.30	0.54	0.30
(mg AAE/g)	Maximum	2.49	2.99	2.99
	Mean	1.00[a]	0.99[a]	1.00
	CV	37.80	43.01	39.88
ABTS	Minimum	2.67	2.67	2.67
(mg TE/g)	Maximum	7.16	6.62	7.16
	Mean	4.84[a]	4.67[a]	4.77
	CV	22.66	20.21	21.86
FRAP	Minimum	0.77	0.85	0.77
(mg AAE/g)	Maximum	3.67	5.18	5.18
	Mean	1.64[a]	1.57[a]	1.61
	CV	34.35	47.68	39.85

Different superscript letters in a row represent significantly different means ($p < 0.05$). AAE represents Ascorbic acid equivalent; ABTS represents $ABTS^{\bullet+}$ scavenging activity; CE represents catechin equivalent; DBI represents double bond index; DPPH represents $DPPH^{\bullet}$ scavenging activity; FRAP represents ferric reducing antioxidant power; GAE represents gallic acid equivalent; PUFA represents total polyunsaturated fatty acids; TE represents Trolox equivalent, TSFA represents total saturated fatty acid; TUFA represent total unsaturated fatty acid and TVC represent total vicine-convicine content.

(C), seed weight (W), and their interactions through statistical analysis. Table 1 shows the effect of genotype variation on the levels of vicine and convicine. Cultivated varieties had the highest average vicine content (9.85 mg/g) and the lowest average convicine content (5.75 mg/g). In contrast, landraces exhibited the lowest average vicine content (9.80 mg/g) and the highest average convicine content (7.45 mg/g). The average TVC was also higher in landraces (17.25 mg/g) than in cultivated varieties (15.60 mg/g). Despite these variations, only the convicine content was significantly affected by genotype difference. Similarly, the variations of vicine and convicine among faba beans of different seed colors and seed weights are illustrated in Fig. 2 and Fig. 3, respectively. The numerical values can be viewed in **Table S2** (Supplementary material), while the statistical results are presented in Table 2. On average, green faba beans had the highest vicine content (10.14 mg/g), while mixed faba beans had the lowest (9.08 mg/g). Mixed faba beans also had the highest convicine content (10.84 mg/g). Among the different seed weights, very small seeds had the highest average vicine content (10.53 mg/g), convicine content (10.29 mg/g), and TVC (20.82 mg/g). Conversely, medium seeds had the lowest average convicine content (5.18 mg/g) and very large seeds had the lowest average vicine content (8.49 mg/g) and TVC (14.05 mg/g). Analysis of variance showed that variations in seed color and seed weight, similar to genotype difference, had significant effects on convicine content but not on vicine content. Seed color and seed weight variations also had significant effects on TVC content (Table 2). These results signify that genotype, seed color, and seed weight could be used as parameters in choosing faba bean varieties with a desirable VC ratio for the food industry (Khamassi et al., 2013; Khazaei et al., 2019). Seed weight and seed color in faba beans are inherited traits, as mentioned earlier, and several genes control their variations (Lippolis et al., 2023; Sharan et al., 2020). Statistical analysis was conducted to assess the effects of the interactions between these traits and with genotype. Accordingly, all two-way (G × C, G × W, and C × W) and three-way (G × C × W) interactions between genotype, seed color, and seed weight affected convicine and TVC levels, but not vicine content (Table 2). Studies that assess the effects of genotype and seed traits on vicine and

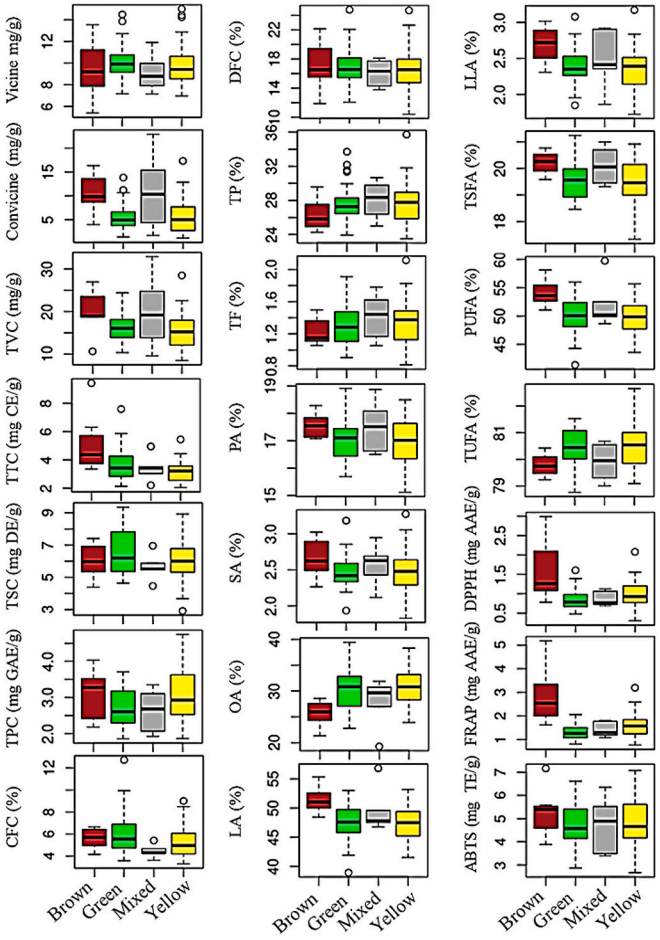

Fig. 2. Variation of anti-nutrient factors, nutritional components, and antioxidant activities in seeds of faba bean accessions according to seed color. The representations of the abbreviations are similar to those described in Fig. 1.

convicine levels in faba beans are scarce, with most studies comparing individual varieties (Hendawey & Younes, 2013; Kowalczyk et al., 2021). Some studies have also examined the levels of vicine and convicine in different genotypes of faba beans compared to varieties with high or low levels (Pulkkinen et al., 2015; Zhang, Purves, Warkentin, & Vandenberg, 2023). In general, this study revealed that the convicine level in faba beans is significantly affected by the differences in genotype and seed traits. Therefore, these factors need to be taken into account when selecting faba bean varieties according to their convicine and TVC levels. These findings could serve as a foundation for future studies and promote the development and utilization of faba bean varieties that have reduced levels of anti-nutrients (Labba et al., 2021).

3.2. *Variations of total tannin, saponin, and phenol contents*

The TTC, TSC, and TPC were in the ranges of 2.04–9.42 mg CE/g, 2.91–9.36 mg DE/g, and 1.85–4.47 mg GAE/g in the whole population, with approximately five, three, and two-fold variations, respectively (Table 1). The highest TTC was found in landrace FB2, while the highest TSC was in FB3. FB26, a cultivated variety, had the highest TPC. On the other hand, the lowest TTC was observed in landrace FB69, while the lowest TSC was found in landrace FB51. Landrace FB53 had the lowest TPC (Fig. 1). Previously, Labba et al. (2021) reported TPC and TSC ranging from 1.4 to 5.0 mg GAE/g and from 18.3 to109.0 μg/g, respectively with the former being comparable with the results obtained in this study. On the other hand, Corzo-Ríos et al. (2022) reported a much higher TSC ranging from 13.67 to 15.96 mg DE/g and a

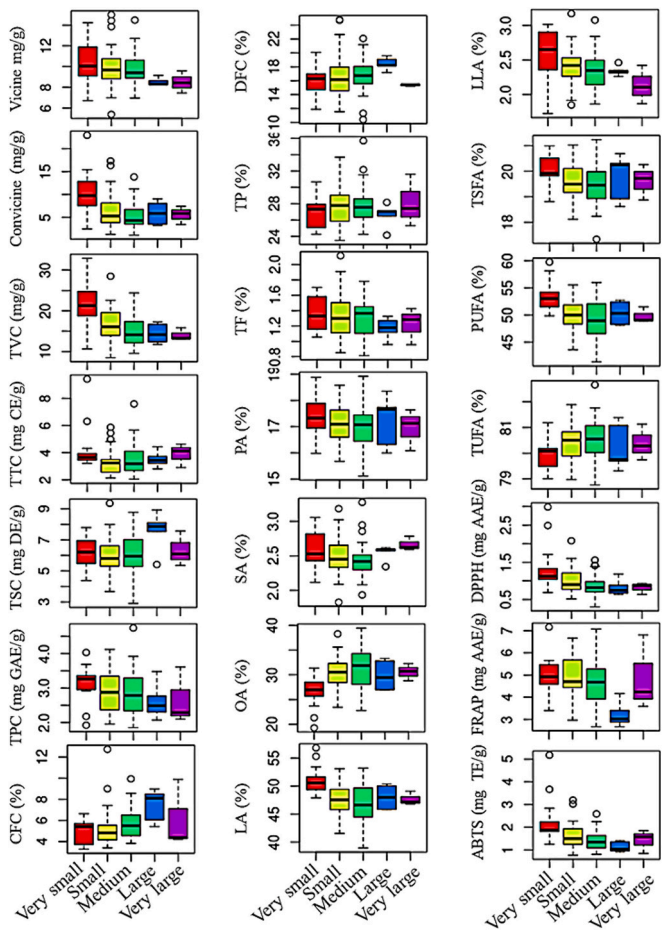

Fig. 3. Variation of anti-nutrient factors, nutritional components, and antioxidant activities in seeds of faba bean accessions according to seed weight. The representations of the abbreviations are similar to those described in Fig. 1.

comparable TPC ranging from 1.35 to 1.83 mg GAE/g. Oomah et al. (2011) also reported a TTC range of 0.50–5.23 mg CE/g, which was lower than the TTC range observed in this study. Several other studies have also reported wide-ranging results, which once again could be attributed to discrepancies in genetic and environmental factors, and post-harvest handling procedures (Martineau-Côté et al., 2023; Shi et al., 2022a). Although tannins, saponins, and phenols have health advantages, they are viewed as anti-nutritional elements because they can reduce nutrient absorption and bioavailability (Singh et al., 2023). Moreover, the unpleasant tests of faba beans are associated with polyphenols and saponins (Lippolis et al., 2023). In this regard, those faba bean accessions containing small quantities of these metabolites may serve as important genetic materials for the food industry (Fig. 1). Specifically, the cultivated varieties FB51 and FB55, as well as landrace FB15 simultaneously exhibited low levels of TTC (< 2.50 mg CE/g), TSC (< 5.00 mg DE/g), and TPC (< 2.10 mg GAE/g), making them valuable resources.

Previous studies have reported varied distributions of phenols, tannins, and saponins in different types of legumes, emphasizing the effects of genotype and seed-related trait variations. For instance, differences in the levels of these metabolites have been documented in legumes with varying seed coat colors (Desta et al., 2022; Lee et al., 2017; Zhao et al., 2021). Moreover, smaller legume seeds have been found to possess elevated metabolite levels due to their high surface area-to-volume ratio (Kim et al., 2012; Lee et al., 2008). A study on faba beans also demonstrated that colored varieties tended to have higher phenolic content (Siah et al., 2014). Likewise, tannins have been recognized as the predominant polyphenols in faba bean seed coats, with their distribution possibly differing based on seed coat color variation (Rahate et al., 2021; Sharan et al., 2020). This study also conducted statistical analyses to examine how genotype, seed color, seed weight, and their interactions affect the levels of TPC, TTC, and TSC. As shown in Table 1, landraces demonstrated higher average TTC and TSC compared to cultivated varieties. In contrast, cultivated varieties exhibited higher average TPC than landraces (Table 1). Among the different seed colors, the average TTC decreased in the order of brown (5.05 mg CE/g) > green (3.72 mg CE/g) > mixed (3.43 mg CE/g) > yellow (3.20 mg CE/g) faba beans (Fig. 2**, Table S2**). Brown faba beans also displayed the highest average TPC (3.08 mg GAE/g), while green faba beans had the highest average TSC (6.58 mg DE/g). Conversely, mixed faba beans showed the lowest average TSC (5.63 mg DE/g) and TPC (2.63 mg GAE/g). Fig. 3 shows the variations of TPC, TTC, and TSC among faba beans of different seed weights, and the numerical values can be viewed in **Table S2** (Supplementary material). Remarkably, very small faba beans exhibited the highest average TTC (4.28 mg GAE/g) and TPC (3.13 mg GAE/g). These findings were consistent with previous results in soybeans and could be attributable to their high surface area-to-volume ratio (Kim et al., 2012; Lee et al., 2008). On the other hand, large seeds had the highest average TSC (7.57 mg DE/g) and the lowest average TPC (2.62 mg GAE/g), while small seeds displayed the lowest average TTC (3.25 mg CE/g) and TSC (6.02 mg DE/g). Analysis of variance indicated that genotype variation significantly affected all parameters. In contrast, difference in seed color affected TTC, while seed weight variation did not have a significant effect on any of these parameters (Table 2). Interaction analysis revealed that all interactions, except for G × W, had significant effects on TTC. Similarly, all interactions, except for C × W, significantly affected the TPC level. In contrast, the TSC level remains unaffected by any of the interactions (Table 2). Overall, the results of this study revealed the variable effects of genotype, seed color, seed weight, and their interactions on total metabolite levels in faba beans. In particular, the significant effect of seed color variation on TTC, both individually and in combination with seed weight and genotype, signify that it could be a useful indicator of tannin level in faba beans (Lee et al., 2008; Lee et al., 2017; Sharan et al., 2020).

3.3. Variations of CFC, DFC, crude protein, and total fat contents

Fig. 1 once again displays the variations of CFC, DFC, crude protein, and total fat contents among the studied faba bean accessions. CFC and DFC ranged between 3.28 and 12.73 and 10.41–24.78 %, with means of 5.48 and 16.63 %, respectively. Previous studies have reported DFC in the range of 11.37–16.59 % and CFC in the range of 4.61–6.91 %, which fall within the ranges observed in this study (Hendawey & Younes, 2013; Labba et al., 2021). Similarly, the crude protein content ranged from 23.51 to 35.71 %, with a mean of 27.66 %, while the total fat content ranged from 0.81 to 2.12 %, with a mean of 1.32 %. The crude protein and total fat contents observed in this study were consistent with previously reported values (Akgun & Canci, 2023; Hendawey & Younes, 2013; Labba et al., 2021). Among the faba bean accessions, landrace FB3 had the highest CFC and DFC simultaneously. Conversely, cultivated variety FB59 had the lowest CFC, while landrace FB12 had the lowest DFC. Additionally, cultivated varieties FB33 and FB68 showed the lowest and the highest crude protein content, respectively. The total fat content was the lowest in landrace FB12 and the highest in cultivated variety FB73. Legumes containing high levels of fiber play a crucial role in providing various health benefits, including lowering the risk of type 2 diabetes, obesity, and heart disease. Faba beans have also been utilized in combination with other ingredients to increase the fiber content in food items (Liu et al., 2024; Rahate et al., 2021). This makes faba bean varieties rich in fiber a valuable genetic resource for the food industry. In this regard, landraces FB3, FB4, and FB14 could potentially be excellent sources of fiber due to their high DFC (> 22.00 %) and CFC (≥ 9.00 %) levels. Similarly, faba beans are well-known for their high protein content and have been added to commonly consumed crops to enhance the

Table 2
Analysis of variance on the effects of genotype, seed color, seed weight, and their interactions on the levels of anti-nutrient factors, nutritional components, and antioxidant activities in 95 faba bean accessions.

Factor	Convicine	Vicine	TVC	Total tannin	Total saponin	Total phenol	Dietary fiber
Genotype (G)	*	NS	NS	*	*	****	NS
Seed color (C)	***	NS	**	***	NS	NS	NS
Seed weight (W)	**	NS	**	NS	NS	NS	NS
G × C	***	NS	*	***	NS	****	NS
G × W	**	NS	**	NS	NS	***	NS
C × W	****	NS	**	*	NS	NS	NS
G × C × W	***	NS	**	*	NS	*	NS
	Crude fiber	Total fat	Crude protein	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid
Genotype (G)	**	NS	NS	NS	NS	NS	NS
Seed color (C)	*	NS	NS	NS	NS	**	**
Seed weight (W)	*	NS	NS	NS	NS	**	**
G × C	*	NS	NS	NS	NS	*	*
G × W	*	NS	NS	NS	NS	*	*
S × C	NS	NS	NS	NS	NS	NS	NS
G × C × W	NS	NS	NS	NS	NS	NS	NS
	Linolenic acid	TSFA	TUFA	PUFA	DPPH	ABTS	FRAP
Genotype (G)	NS	NS	NS	NS	NS	NS	NS
Seed color (C)	*	*	*	**	****	NS	****
Seed weight (W)	NS	NS	NS	**	**	**	****
G × C	NS	NS	NS	*	NS	NS	****
G × W	NS	NS	NS	*	NS	**	**
S × C	**	NS	NS	*	**	*	****
G × C × W	**	NS	NS	NS	NS	NS	***

ABTS represents $ABTS^{\bullet+}$ scavenging activity; DPPH represents $DPPH^{\bullet}$ scavenging activity; FRAP represents ferric reducing antioxidant power; PUFA represents total polyunsaturated fatty acids; TSFA represents total saturated fatty acid; TUFA represents total unsaturated fatty acid and TVC represents total vicine-convicine content. NSNot significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

protein quality of food products (Johnston, Ying, Teape, Liesaputra, & Oey, 2023; Kamani et al., 2024). Therefore, faba bean accessions with elevated protein levels could be valuable materials for utilization in the food industry. Specifically, the cultivated variety FB68 and landraces FB24 and FB17 exhibited crude protein levels exceeding 32.00 %, making them potential protein resources.

Table 1 also shows the effect of genotype variation on the levels of CFC, DFC, crude protein, and total fat. Likewise, the box plots in Fig. 2 and Fig. 3 illustrate the variations of these parameters among faba beans with varying seed color and seed weight. The numerical values can be viewed in **Table S2** (Supplementary material). On average, landraces exhibited higher CFC (6.13 %) and DFC (17.04 %), while cultivated varieties contained higher levels of crude protein (27.68 %) and total fat (1.35 %), and vice versa. However, only the CFC level was significantly affected by genotype difference. Regarding seed color variation, brown faba beans displayed the highest average DFC (17.13 %), but the lowest average crude protein (26.30 %) and total fat (1.22 %) contents. In contrast, mixed faba beans exhibited the lowest average DFC (16.10 %) and CFC (4.43 %), but the highest average crude protein (28.09 %) and total fat (1.42 %) contents. Green faba beans had the highest average CFC (6.14 %). In terms of seed weight, large seeds had the highest average DFC (18.44 %) and CFC (7.41 %), but the lowest average crude protein (25.60 %) and crude fat (1.17 %) contents. In contrast, very small faba beans displayed the lowest average CFC (4.95 %) as well as the highest average total fat (1.36 %) content. Very large faba beans had the lowest average DFC (15.35 %) and the highest average crude protein (28.11 %) contents. Despite these variations, seed color and seed weight variations had significant effects only on CFC level, similar to genotype variation (Table 2). Interaction analysis also showed that only G × C and G × W interactions had significant effects on CFC, while the other interactions did not show significant effects on the remaining parameters (Table 2). A few studies have reported the effects of seed-related traits on the levels of nutritional components in legumes, mainly in soybeans. Supporting our findings, a previous study noted no significant effect of seed color variation on the levels of total protein and total fat in soybeans (Cho et al., 2013). In another study, Lee et al. (2017) also found no significant effects of seed color, seed weight, and their interactions on the levels of crude protein and total oil. Overall, the results of this study suggest that genotype, seed weight, and seed color might not be reliable criteria for selecting faba beans based on dietary fibers, crude protein, and total fat contents. Therefore, it is important to evaluate individual faba bean varieties in this context.

3.4. Variations of fatty acid compositions and contents

The fatty acid analysis was conducted using GC-FID, and the results demonstrated that all the faba bean accessions contained the five targeted fatty acids, each with varying contents (Fig. 1). The contents of palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid were in the ranges of 15.68–18.50, 1.83–3.97, 21.30–38.27, 41.54–55.33, and 1.73–3.17 %, respectively (Table 1). Palmitic acid and linoleic acid were the most dominant fatty acids in their respective groups. Previous studies have also reported similar findings, indicating the dominance of these two fatty acids in different faba bean varieties (Ryu et al., 2017; Yoshida, Saiki, Yoshida, Tomiyama, & Mizushina, 2009). Among all the faba bean accessions examined, landraces FB10 and FB4 exhibited the lowest and the highest palmitic acid content, respectively. Likewise, cultivated variety FB54 had the lowest stearic acid content, while landrace FB12 had the highest. In terms of unsaturated fatty acids, landrace FB85 exhibited the lowest oleic acid and the highest linoleic acid contents simultaneously, whereas landrace FB89 exhibited the opposite trend. Moreover, cultivated variety FB34 and landrace FB87 had the lowest and the highest linolenic acid content, respectively. Many of the faba bean accessions demonstrated contrasting levels of oleic acid and linolenic acid, potentially due to the activity of fatty acid desaturase (FAD) enzymes that regulate fatty acid biosynthesis in legumes (Simopoulos, 2002). In terms of total fatty acid contents, all faba bean accessions exhibited a lower level of TSFA compared to MUFA and TUFA levels which was consistent with previous observations (Ryu et al., 2017; Yoshida et al., 2009). Consequently, the ratio of TUFA to

TSFA ranged from 3.71 to 4.77, with the double bond index (DBI) value varying from 124.60 to 141.68. Studies suggest that consuming oils with a low ratio of ω6 (linoleic acid) to ω3 (linolenic acid) could have a disease-preventive effect (Simopoulos, 2002). Therefore, those accessions with a lower ω6:ω3 ratio could be valuable sources of healthy fats. On the other hand, high levels of polyunsaturated fatty acids may reduce the shelf life and storage stability of legume oil, emphasizing the importance of accessions with a lower DBI (**Table S1**).

This study once again examined how all three factors and their interactions influenced the levels of both individual fatty acids and total fatty acids. Regardless of genotype, seed color, or seed weight variations, palmitic acid, and linoleic acid were still the dominant fatty acid in their groups (Ryu et al., 2017). On average, cultivated varieties had the highest palmitic acid and linoleic acid contents, while the remaining individual fatty acid levels were the highest in landraces (Table 1). However, genotype variation had no significant effect on the levels of any of the fatty acids, including total fatty acid contents. In terms of seed color, brown faba beans exhibited the highest average palmitic acid (17.55 %), stearic acid (2.66 %), linoleic acid (51.40 %), linolenic acid (2.69 %), and TSFA (20.22 %), but the lowest average oleic acid (25.70 %) and TUFA (79.79 %). Yellow faba beans displayed the opposite pattern to brown faba beans in every aspect, except for the level of stearic acid (Fig. 2**, Table S2**). The average stearic acid content was the lowest in green faba beans (2.46 %). In terms of seed weight, very small faba beans had the highest average palmitic acid (17.42 %), linoleic acid (51.06 %), linolenic acid (2.56 %), and TSFA (20.02 %), but the lowest average oleic acid (26.35 %) and TUFA (79.98 %) (Fig. 3**, Table S2**). Very large seeds exhibited the highest average stearic acid (2.67 %), but the lowest average palmitic acid (16.95 %) and linolenic acid (2.13 %) contents. Medium-sized faba beans had the highest average oleic acid (31.42 %) and TUFA (80.54 %) while having the lowest average stearic acid (2.45 %), linoleic acid (46.77 %), and TSFA (19.46 %). Unlike genotype variation, seed color variation exhibited significant effects on all individual unsaturated fatty acids, TSFA, and TUFA, while the levels of saturated fatty acids remained unaffected (Table 2). Seed weight variation also had a significant effect on oleic acid, linoleic acid, and PUFA. Moreover, G × C and G × W interactions showed significant effects on oleic acid, linoleic acid, and PUFA. S × C interaction also had a significant effect on PUFA. Previous studies also demonstrated the influence of seed color and seed weight variations on fatty acid levels in legumes, particularly soybeans, and reported variable findings. For instance, Cho et al. (2013) did not find a significant effect of seed color variation on any of these fatty acids in soybeans. In our recent study, we also exhibited no significant variations of fatty acids, except for palmitic acid, among soybeans of different seed colors (Desta et al., 2022). Conversely, Lee et al. (2017) noted significant effects of seed color and seed weight variations on all five fatty acids, except for linolenic acid, which was affected only by seed color variation. The same study reported a significant effect of C × W interaction on all five fatty acids except for palmitic acid. This study is the first to examine how genotype, seed color, and seed weight affect fatty acid levels in faba beans. The findings suggest that differences in seed color and seed weight could help in selecting faba bean varieties based on their levels of unsaturated fatty acids such as oleic acid and linoleic acid.

3.5. Variations of antioxidant activities

The antioxidant activities of each faba bean accession were assessed using three independent assays, including DPPH$^{\bullet}$ scavenging activity, ABTS$^{\bullet+}$ scavenging activity, and FRAP (Fig. 1). The DPPH$^{\bullet}$ scavenging activity ranged from 0.30 to 2.99 mg AAE/g, showing approximately a ten-fold variation. Furthermore, the FRAP ranged from 0.77 to 5.18 mg AAE/g, with an approximately seven-fold difference. Additionally, the ABTS$^{\bullet+}$ scavenging activity ranged from 2.67 to 7.16 mg TE/g, showing a difference of more than two times (Table 1). Previous studies have applied different analysis conditions and reporting methods which resulted in wide-ranging antioxidant activity values in different faba bean genotypes (Oomah et al., 2011; Siah et al., 2014). Among the accessions studied, landrace FB2 showed the highest DPPH$^{\bullet}$ scavenging activity and FRAP levels, while the cultivated variety FB69 had the lowest DPPH$^{\bullet}$ scavenging activity and ABTS$^{\bullet+}$ scavenging activity simultaneously. The cultivated varieties FB35 and FB25 exhibited the lowest FRAP and the highest ABTS$^{\bullet+}$ scavenging activity, respectively. Landrace FB2 also had the highest TTC and the third highest TPC (4.03 mg GAE/g), whereas FB69 had the lowest TTC value. Overall, accessions with high phenol content tended to show increased antioxidant activities (Fig. 1). These findings suggest that faba bean polyphenolic compounds also play a significant role in neutralizing reactive radicals (Boudjou et al., 2003; Siah et al., 2014). In this regard, the cultivated varieties such as FB26, FB30, FB33, FB39, FB58, and FB80 which simultaneously showed high levels of DPPH$^{\bullet}$ scavenging activity, ABTS$^{\bullet+}$ scavenging activity, and FRAP activities could be ideal genetic resources (Fig. 1**, Table S1**).

Statistical analysis revealed that all factors, except for genotype variation, had significant effects on one or more of the antioxidant activities. As shown in Table 1, cultivated varieties exhibited higher average antioxidant activities for each assay than the landraces. However, genotype variation had no significant effect on any of the antioxidant activities. In terms of seed color, brown faba beans exhibited the highest average values for all antioxidant activities, including DPPH$^{\bullet}$ scavenging activity (1.58 mg AAE/g), ABTS$^{\bullet+}$ scavenging activity (5.23 mg TE/g), and FRAP (2.82 mg AAE/g) (Fig. 2**, Table S2**). On the other hand, green faba beans showed the lowest FRAP (1.36 mg AAE/g) and ABTS$^{\bullet+}$ scavenging activity (4.70 mg TE/g), while mixed faba beans showed the lowest DPPH$^{\bullet}$ scavenging activity (0.86 mg AAE/g). Among the different seed weights, very small faba beans showed the highest average DPPH$^{\bullet}$ scavenging activity (1.38 mg AAE/g), ABTS$^{\bullet+}$ scavenging activity (5.05 mg TE/g), and FRAP (2.33 mg AAE/g) (Fig. 3**, Table S2**). Conversely, very large faba beans exhibited the lowest DPPH$^{\bullet}$ scavenging activity (0.82 mg AAE/g) and large seeds showed the lowest ABTS$^{\bullet+}$ scavenging activity (3.23 mg TE/g) and FRAP (1.34 mg AAE/g). In contrast to genotype variation, both seed color, and seed weight differences showed significant effects on DPPH$^{\bullet}$ scavenging activity and FRAP (Table 2). Seed weight difference also had a significant effect on ABTS$^{\bullet+}$ scavenging activity. The fact that very small seeds and brown faba beans showed high levels of antioxidant activities reaffirms the role of faba bean polyphenols in controlling reactive radicals (Boudjou et al., 2003; Siah et al., 2014). Analysis of variance results also showed that all interactions between these factors had significant effects on FRAP. On the other hand, G × W and S × C interactions exhibited significant effects on ABTS$^{\bullet+}$ scavenging activity, while S × C interaction had a significant effect on DPPH$^{\bullet}$ scavenging activity (Table 2). The significant effect of seed color variation in legumes has been widely documented (Cho et al., 2013; Desta et al., 2022). Overall, the results of this study signify that seed traits could be important parameters for classifying faba bean materials based on the levels of their antioxidant activities (Desta et al., 2022; Kim et al., 2012).

3.6. HCA, PCA, and correlation analyses

The entire biochemical dataset was analyzed using HCA, PCA, and Pearson's correlation analysis to further explore the distribution of the faba bean accessions and their relationships with the parameters studied (Segers et al., 2022; Shi et al., 2022b). The HCA demonstrated distinct groupings of the faba bean accessions depending on their biochemical levels (Fig. 1). Statistical analysis also supported this observation (**Table S3**). For instance, accessions in cluster X were characterized by high levels of oleic acid and TUFA, and low levels of LLA, LA, PA, and TSFA. On the other hand, cluster I included accessions with contrasting characteristics to those in cluster X. Similarly, accessions with contrasting levels of convicine and TVC were grouped in clusters IX and II. Despite these unique characteristics, the HCA did not clearly classify the

faba bean accessions based on genotype, seed color, or seed weight. However, the HCA showed that cultivated varieties dominated in clusters II, V, VII, and IX, while landraces were dominant in clusters I and VIII. Yellow faba beans were popular in clusters I and IX, while brown faba beans were mostly found in clusters I to IV. In terms of size, small seeds dominated clusters VI and IV. These observations signify the importance of genotype, seed color, and seed weight in classifying faba bean varieties based on specific parameters significantly affected by each factor (Akgun & Canci, 2023; Lee et al., 2017). The PCA confirmed the findings of the HCA. The analysis was conducted on the first two principal components (PC1 and PC2), accounting for approximately 44.57 % of the total variance (**Table S4**). Similar to the HCA results, the PCA did not clearly separate the faba bean accessions based on their genotype (Fig. 4A), seed color (Fig. 4B), or seed weight (Fig. 4C). Nevertheless, most of the brown faba beans and many very small faba beans tended to cluster on the negative side of PC1. Consistent with the HCA findings, the faba bean accessions were grouped based on their cluster (**Fig. S2**). Factor loading (FL) analysis revealed that palmitic

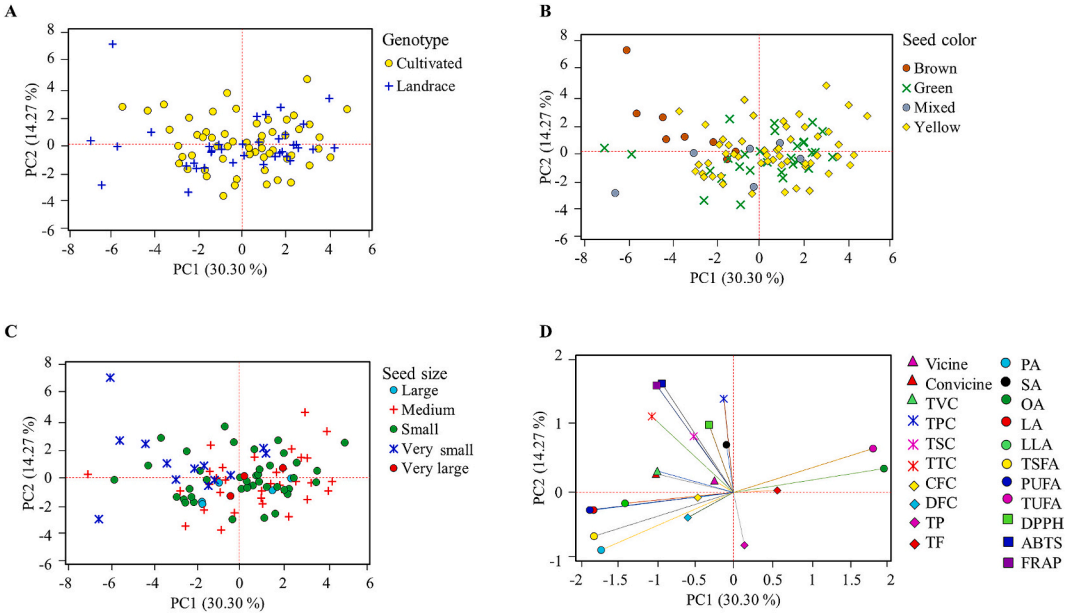

Fig. 4. Score plot of faba bean accessions according to genotype (A), seed color (B), and seed weight (C), and loading plot of variables (D) along the first two principal components. The representations of the abbreviations are similar to those described in Fig. 1.

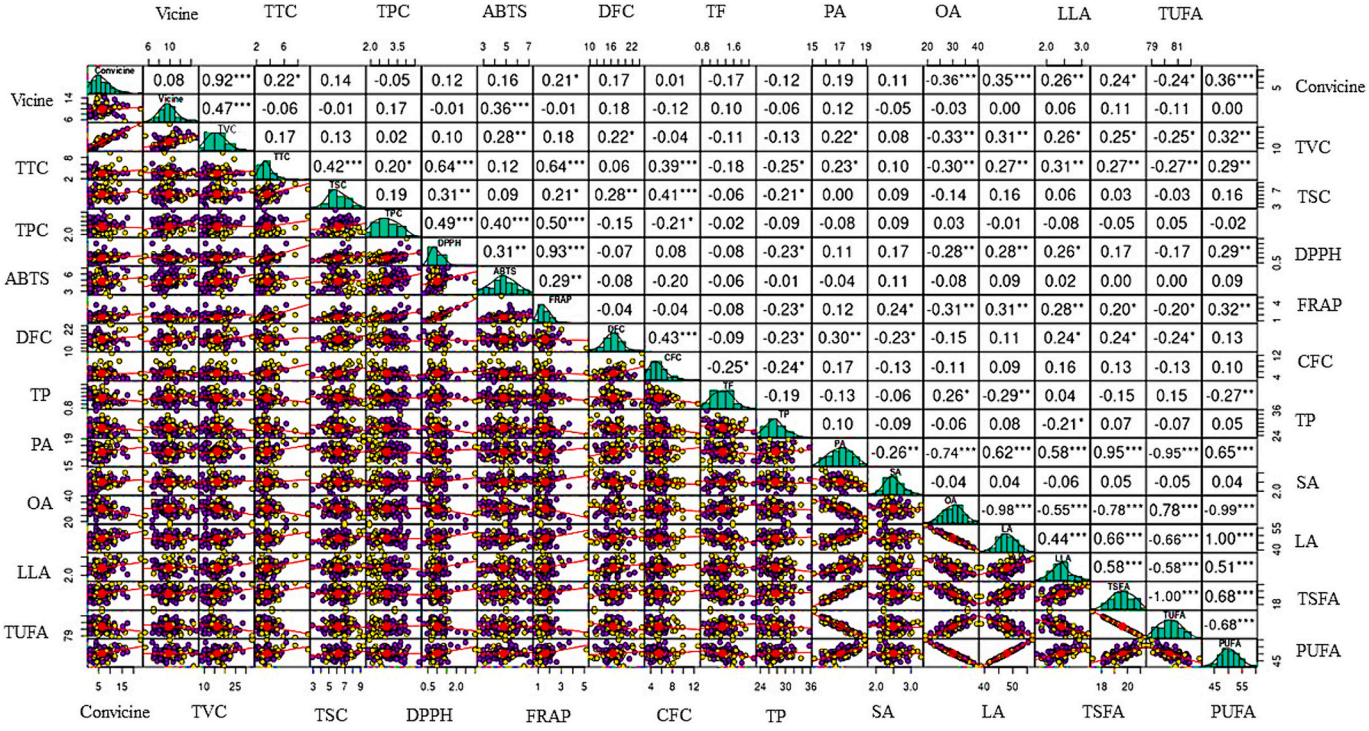

Fig. 5. Pearson's correlation matrix showing pair-wise association of analyzed parameters in faba bean accessions. Yellow dots represent landraces, and purple dots represent cultivated varieties. ***Significant at $p < 0.001$, **Significant at $p < 0.01$, *Significant at $p < 0.05$. The representations of the abbreviations are similar to those described in Fig. 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

acid, oleic acid, linoleic acid, linolenic acid, and total fatty acid levels were the main contributing factors to the variance observed in PC1, with FLs $\geq \pm 0.50$ (Fig. 4D, **Table S4**). Their contributions ranged from 10.41 % to 13.42 %. On the other hand, TPC, TTC, DPPH$^{\bullet}$ scavenging activity, and FRAP were identified as the major contributors in PC2, with contributions varying from 9.54 % to 19.57 %. Vicine showed comparable contributions along PC1 and PC2, while convicine had a higher contribution in PC1 than in PC2. Pearson's correlation analysis was computed to assess the degree of correlation among the parameters examined (Fig. 5). Accordingly, a strong and significant correlation was observed between convicine and vicine ($r = 0.92$, $p < 0.001$). Similarly, TTC and TPC showed strong and positive correlations with DPPH$^{\bullet}$ scavenging activity ($r = 0.64$ and 0.49, respectively) and FRAP (r = 0.64 and 0.50, respectively), each being significant at $p < 0.001$ (Fig. 5). These findings once again support the role of phenolic compounds in controlling harmful radicals (Boudjou et al., 2003; Siah et al., 2014). Antioxidant activities exhibited strong correlations with each other at various levels of significance which corroborate previous observations (Desta et al., 2022; Siah et al., 2014). The DFC and CFC showed a strong and positive correlation with each other ($r = 0.43$, $p < 0.001$). In contrast, TF and TP had a weak and negative correlation with each other ($r = -0.19$) and with DFC and CFC at different levels of significance. These inverse associations could be explained by differences in biosynthesis pathways (Yoshida et al., 2009). The negative and strong correlation of OA with LA ($r = -0.98$, $p < 0.001$) and LLA ($r = -0.55$, $p < 0.001$) can be explained by their contradictory levels due to the actions of FAD enzymes (Ryu et al., 2017; Simopoulos, 2002). These unsaturated fatty acids also showed strong correlations with convicine and TTC at various levels of significance. In summary, the correlation analysis revealed important relationships between the analyzed parameters and supported the observations from the HCA (Fig. 1) and PCA (Fig. 4D).

4. Conclusion

This study investigated the various anti-nutrient factors, nutritional components, and antioxidant activities of faba bean genetic materials. It also examined the effects of genotype, seed color, and seed weight variations and their interactions on these parameters. The study revealed wide variations in all the parameters analyzed among the faba beans, offering a range of options for selecting desirable varieties. Statistical analysis revealed that genotype, seed color, seed weight, and their interactions significantly affected different parameters. In general, cultivated varieties showed high levels of total vicine-convicine, total phenol, palmitic acid, linoleic acid, and antioxidant properties, with significant differences in the first two components. Conversely, landraces had higher levels of total saponin, crude fiber, dietary fiber, stearic acid, oleic acid, and linolenic acid, with significant variations in the first two parameters. Brown faba beans, when compared to green, yellow, and mixed-colored faba beans, had significantly higher levels of oleic acid, total unsaturated fatty acid, and antioxidant activities, making them valuable genetic resources. Similarly, small faba beans could be important because of their high levels of linoleic acid, linolenic acid, total saturated fatty acid, and antioxidant properties, despite containing high levels of anti-nutrients. In conclusion, this study highlighted how genotype, seed color, and seed weight play a crucial role in determining the levels of important biochemical components in faba beans for the first time. The results signified that several factors need to be considered when selecting faba bean varieties with desirable qualities for use in the food industry and breeding programs. Moreover, the findings of this study could lead to further studies in metabolomics, molecular research, and the development of faba bean varieties with reduced anti-nutritional factors and increased beneficial compounds.

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CRediT authorship contribution statement

Yu-Mi Choi: Project administration, Methodology, Funding acquisition. **Myoung-Jae Shin:** Supervision, Resources. **Sukyeung Lee:** Project administration, Conceptualization. **Hyemyeong Yoon:** Methodology, Conceptualization. **Jungyoon Yi:** Project administration. **Xiaohan Wang:** Resources, Methodology. **Heon-Woong Kim:** Software, Investigation, Formal analysis. **Kebede Taye Desta:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All the data are included in the manuscript and supplementary material.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101780.

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