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Full Length Article

Investigation of Macronutrient and Minerals Properties of different Okra (*Abelmoschus esculentus*) Genotypes Grown in Indonesia using Chemometric Analysis

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Abstract

The objective of this study was to determine the carbohydrate, protein, fat and mineral contents (potassium, magnesium and iron) in ten okra genotypes grown in Indonesia. The carbohydrate, protein, and fat contents were analyzed using phenol-sulfuric acid, Biuret, and Soxhlet extraction methods, whereas the mineral content was analyzed using an atomic absorption spectrophotometer. The results revealed that there were significant differences in nutritional content based on the genotype. The highest total carbohydrate content was found in G1 (22.52 ± 0.48 g (100 g)$^{-1}$ DW), while the highest total protein and fat content were found in G2 (6.74 ± 0.13 and 4.59 ± 0.05 g (100 g)$^{-1}$ DW), respectively. In terms of mineral content, genotype G8 had the highest content of potassium (3298.36 ± 129.49 mg (100 g)$^{-1}$ DW), while V1 had the highest content of magnesium (463.05 ± 6.48 mg (100 g)$^{-1}$ DW), and V2 had the highest content of iron (14.04 ± 0.10 mg (100 g)$^{-1}$ DW). Several genotypes showed high potential for other nutrients based on the HCA heatmap, with G2 showing potential for magnesium, V1 for iron, and G8 for potassium. The correlation between macronutrients was significant ($P < 0.05$) in all interactions tested, with a positive correlation shown by the interaction between protein and fat (r = 0.7965). Overall, this study concluded that the nutritional content of okra fruit is highly dependent on genotypic parameters.

Keywords: *Abelmoschus esculentus*; Chemometrics analysis; Genotype; Macrotrients; Minerals; Pearson's correlation
Abbreviation: C = Celsius; DW = dry weight; Fe = iron; g = gram(s); HCA = hierarchical cluster analysis; K = potassium; kg = kilogram(s); L = liter(s); Mg = magnesium; mg = milligram(s); mL = milliliter(s); v/v = volume : volume; v/v/v = volume : volume : volume

Introduction

Functional food, which is part of the human diet that provides adequate nutrition and various health benefits to reduce the risk of disease (Al-Sheraji *et al.* 2013), is similar to conventional food and is derived from fruits and vegetables (Gul *et al.* 2016). Fruits and vegetables are functional food sources containing numerous macronutrients and micronutrients. Macronutrients such as carbohydrates, proteins and fats are required in large quantities to meet human needs, whereas micronutrients, such as vitamins and minerals are required in small amounts (Martirosyan and Singh 2015; Savarino *et al.* 2021). Moreover, as the human population continues to grow, the demand for food including functional foods rich in macronutrients and micronutrients has also increased (Schneider *et al.* 2011). Therefore, research on functional foods is ongoing to discover alternative sources of functional foods, particularly fruits and vegetables that are highly nutritious. Okra has the potential to be developed into functional food source (Dantas *et al.* 2021).

Okra (*Abelmoschus esculentus*), a diploid species with 36 pairs of chromosomes (2n = 2x = 72), was known as a vegetable crop in the Indo-Pak subcontinent (Nwangburuka *et al.* 2011; Dubey and Mishra 2017). It is also known as "lady's finger", "gombo", or "bamje" and has various nutritional contents and health benefits (Gemede *et al.* 2015; Bawa and Badrie 2016; Daliu *et al.* 2020). The parts of the fruit can be used to cook vegetables or eaten directly (Singh *et al.* 2014). Okra pods have a high proximate composition, mineral contents and secondary metabolite contents (Petropoulos *et al.* 2017; Romdhane *et al.* 2020). Furthermore, it contains antinutrients such as phytate, oxalate and tannin (Gemede *et al.* 2016). Traditionally, okra

has been claimed to have functional properties as an antidiabetic and antihyperlipidemic drug in several regions such as India, Nepal and Turkey (Roy *et al.* 2014). Previous studies have also reported several pharmacological activities of the okra plant including antioxidant, anti-fatigue, antibacterial and anticancer activities (Xia *et al.* 2015; Khan *et al.* 2022).

In Indonesia, okra fruit remains largely unknown to the public and its production is still low because of the limited supply of okra seeds (Yusuf *et al.* 2023). Although okra have been widely studied in Asia and Africa (Kumar *et al.* 2010; Messing *et al.* 2014; Priyanka *et al.* 2018), there is still a lack of information on the nutritional content and benefits of okra originating in Indonesia (Yora *et al.* 2018). Furthermore, okra fruit must have high nutritional content to be used as a functional food. The selection of a type of okra with a high nutritional content can be based on its genotype. In this study, chemometric analysis (in the form of hierarchical cluster analysis of heat maps) was used to evaluate the relationship between one genotype and another on the nutritional content produced. This method has not been previously used to examine the nutritional content of okra fruit, making it a valuable tool for evaluating the nutritional content of okra fruit in Indonesia. This research will be helpful in filling the knowledge gap regarding the origin of okra from Indonesia, and it is expected to have a practical impact on the cultivation of okra as a functional crop both in Indonesia and around the world. This study aimed to determine the levels of carbohydrates, proteins, fats and minerals (potassium, magnesium and iron) in ten okra genotypes from Indonesia, with the ultimate goal of recommending appropriate genotypes for plant breeding and commercial consumption.

Materials and Methods

Experimental materials and cultivation method: The research was conducted at the IPB University Experimental Garden in Dramaga District, Bogor Regency, West Java, Indonesia, from March to June 2022. Carbohydrates, proteins, and fats were analyzed at the Biochemistry Department Research Laboratory, and the mineral contents (K, Mg and Fe) were analyzed at the Testing Laboratory of the Department of Agronomy and Horticulture. The genetic material used in this study consisted of ten okra genotypes from the collection of the Genetics and Plant Breeding Laboratory, Department of Agronomy and Horticulture, IPB University (Table 1). Cultivation was carried out in a randomized complete block design with three replications, and the fruit was harvested ten days after anthesis.

The process of land preparation involves several steps such as loosening the soil, creating beds, applying manure, lime, mulch, and digging planting holes. Cultivated land is a type of latosol soil that has been treated with fertilizers beforehand. To provide essential nutrients to the soil, 200 kg of urea, 150 kg of SP-36 and 150 kg of KCL per hectare were applied one week prior to planting. In addition, manure was applied at a rate of 20–30 tons per hectare, and agricultural lime at 4–5 tons per hectare.

Plant maintenance requires regular watering, replanting, fertilizing, weeding, pest control and disease control. Watering was done twice daily, while replanting was done for plants that die within one week of planting. Fertilizer was applied weekly using NPK 16-16-16 at a concentration of 10 g L^{-1} and a dose of 250 mL per plant, and poured into each plant hole. Weeding is performed by removing the weeds around the plants using a hoe or scraper. Pest and disease control can be performed manually or chemically. Chemical control involves periodic spraying of insecticides with active ingredients such as profenofos, cypermethrin and methyl eugenol. Manual disease control involves removing affected plants, whereas chemical control uses mancozeb as a fungicide and streptomycin sulfate as a bactericide.

Samples were collected for nutrient analysis, specifically for macronutrients and minerals, using okra fruit seven days prior to anthesis. The fruit was harvested from okra plants that were 2-3 months old and sampling was conducted in triplicate.

Sample preparation

The okra fruit collected from the garden was processed by slicing it into uniform pieces. It was then dried in an oven (EYELA NDO-700) at 60°C for 24 h. Following drying, the samples were ground into powder using a blender (WARING 8010BU) with a mesh size of 60.

Total carbohydrate analysis

The carbohydrate content of the sample was analyzed using the sulfuric acid phenol method, as described by Albalasmeh *et al.* (2013) with some modifications. Briefly, 0.1 g of the sample was mixed with 10 mL distilled water and 13 mL 52% $HClO_4$ (Merck KGaA) in a glass beaker. The mixture was stirred for 20 min by using a magnetic stirrer (SH-2). The filtrate was added to distilled water to obtain a homogeneous solution with a final volume of 250 mL. The solution was then mixed with 5% phenol (Merck KGaA) and 97% H_2SO_4 (Merck KGaA) in a test tube at a ratio of 1:1:5 (v/v/v). The solution was incubated in a water bath at 25°C for 10 min, and the absorbance was measured at 490 nm using a UV-Vis spectrophotometer (PG INSTRUMENT T60). Anhydrous glucose was used as the standard at concentration range of 0–120 mg L^{-1}. The final results are expressed as g $(100\ g)^{-1}$ DW.

Total protein analysis

The protein content of the okra powder was measured using the biuret method as per the protocol described by Yenrina (2015) with some modifications. To prepare the biuret

Table 1: Data on ten genotypes of okra plants

Code	Genotype	Source	Pod Color
V1	Zahira	Variety (parental)	Red
G1	F7 Zah × B291-11-6-1B	Strain (cross)	Red
G2	F7 Zah × MC-13-1-12B	Strain (cross)	Red
G3	F7 Zah × MC-13-7-15B	Strain (cross)	Red
G4	F6 B291 × Zah-2-5B	Strain (cross)	Red
V2	Naila	Variety (Parental)	Green
G5	F7 Clemson × Naila-23-10-1B	Strain (cross)	Green
G6	F7 Clemson × Naila-23-22-9B	Strain (cross)	Green
G7	F7 Clemson × Stripe-3-10-15B	Strain (cross)	Green
G8	F7 Clemson × Stripe-3-23-4B	Strain (cross)	Green

reagent, 0.3 g $CuSO_4.5H_2O$ (Merck KGaA), 0.9 g NaK tartrate (Merck KGaA) and 0.5 g KI (Merck KGaA) were mixed in 100 NaOH 0.2 N (Merck KGaA). The okra powder was macerated in distilled water (1:20 g mL^{-1}) for 24 h. The mixture was then centrifuged and the supernatant was concentrated to 10 mL using a rotary evaporator. The concentrated supernatant was added to 10% trichloroacetic acid (TCA) (Merck KGaA) in a reaction tube at a ratio of 1:1 (v/v). The solution was centrifuged again, and the precipitate was collected. The precipitate was washed with ethyl ether to remove the TCA residue and then dried. Water and Biuret reagent were added to the dry precipitate, and the solution was incubated in a water bath for 10 min at 37°C. The absorbance of the solution was measured at 520 nm using a UV-Vis spectrophotometer. The standard used was bovine serum albumin (BSA), with a concentration range of 0–9000 mg L^{-1}. The final results are expressed in g $(100\ g)^{-1}$ DW units.

Total fat analysis

The total fat content of the okra samples was determined using the Soxhlet method, according to the guidelines provided by AOAC (1999), with some modifications. To determine the fat content, the okra samples were extracted with n-hexane in a 1:150 g mL^{-1} ratio using a Soxhlet device for 2 h at 65°C. The difference between the initial and final weights of the Soxhlet flask was calculated to determine the fat content. The results are expressed in g $(100\ g)^{-1}$ DW.

Evaluation of mineral content (K, Mg and Fe)

The mineral content of the dry okra powder was analyzed using atomic absorption spectrophotometry (AAS) following the method of Lokhande *et al.* (2009) with modifications. First, the powder was ashed using a wet-ashing method. For this, 1 g of okra sample was mixed with 10 mL of 65% HNO_3 (Merck KGaA) and 3 mL of 72% $HClO_4$ (Merck KGaA) in a glass beaker, and the mixture was heated until white crystalline ash was obtained. The ash crystals were then dissolved in 50 mL of distilled water, and the absorbance was measured using AAS (PG INSTRUMENT 990 FG) at wavelengths of 404.4 nm, 202.6 nm, and 252.3 nm to determine the concentration of K, Mg and Fe, respectively. Standards potassium solution, standard magnesium solution and standard iron solution with concentrations of 0–20, 0–10 and 0–5 g mL^{-1}, respectively was used. The results are expressed in mg $(100\ g)^{-1}$ DW units.

Statistical analysis

The nutritional content of okra genotypes was analyzed by ANOVA (α = 5%) and Tukey's test using IBM SPSS Statistics 25. Significant differences were further analyzed using HCA and a heatmap in MetaboAnalyst 5.0. Relationships between macronutrients were analyzed using GraphPad Prism 8.

Results

Nutritional content of okra fruit

The nutritional contents of carbohydrates, proteins, fats and minerals (K, Mg and Fe) in okra fruit are listed in Table 2. According to statistical analysis, the genotype of okra fruit significantly affects the various nutritional contents produced. This effect was demonstrated by categorizing specific genotypes based on similar nutritional content profiles.

Similarity of okra fruit genotypes

The genotypes of okra fruit were analyzed for nutritional content using HCA and the results were visualized as a dendrogram and heatmap (Fig. 1). The analysis revealed three large clusters of genotypes, based on similarities in nutritional content. Genotypes G2 and V1 were grouped together in cluster 1, while genotypes G3, G4, G5, G6, G7 and G8 were grouped together in cluster 2. Finally, genotypes G1 and V2 were grouped together in cluster 3.

Relationship between macronutrients in okra fruit

The correlation between macronutrients in this study was examined using Pearson's correlation test (Fig. 2). The relationship between carbohydrates and proteins, carbohydrates and fat, as well as protein and fat, was

Table 2: Nutritional content in ten genotypes of okra fruit

Genotypes Code	Macronutrient (g (100 g)$^{-1}$ DW)			Mineral (mg (100 g)$^{-1}$ DW)		
	Total carbohydrates	Total protein	Total fat	Potassium	Magnesium	Iron
V1	14.02 ± 0.12e	5.66 ± 0.08c	3.93 ± 0.08b	2951.84 ± 44.68bc	**463.05 ± 6.48a**	9.90 ± 3.98b
G1	**22.52 ± 0.48a**	4.34 ± 0.07g	2.78 ± 0.08d	2407.93 ± 44.56d	432.44 ± 10.22abc	5.00 ± 0.24c
G2	14.04 ± 0.82e	**6.74 ± 0.13a**	**4.59 ± 0.05a**	2793.18 ± 15.37c	449.69 ± 36.28a	6.25 ± 0.50bc
G3	15.85 ± 0.35d	6.45 ± 0.11b	2.86 ± 0.03d	2872.05 ± 177.76c	375.21 ± 5.12cde	4.16 ± 0.48c
G4	18.23 ± 0.90b	5.21 ± 0.09d	3.32 ± 0.10c	2861.90 ± 190.85c	408.22 ± 30.26abcd	4.64 ± 0.09c
V2	18.67 ± 0.43b	4.74 ± 0.12f	2.32 ± 0.05e	2913.64 ± 138.89c	335.08 ± 8.96e	**14.04 ± 0.10a**
G5	18.62 ± 0.16b	4.94 ± 0.13e	2.79 ± 0.08d	3037.53 ± 149.66abc	436.44 ± 11.58ab	4.57 ± 0.15c
G6	16.53 ± 0.29cd	3.99 ± 0.16h	1.30 ± 0.02f	3010.84 ± 72.03abc	356.14 ± 19.31de	4.13 ± 0.22c
G7	17.54 ± 0.24bc	4.80 ± 0.06f	2.25 ± 0.09e	3259.26 ± 113.94ab	388.58 ± 14.27bcde	4.89 ± 0.28c
G8	21.35 ± 0.24a	4.39 ± 0.14g	2.20 ± 0.08e	**3298.36 ± 129.49a**	427.15 ± 16.24abc	4.78 ± 0.28c

Mean ± standard deviation. Values sharing same letters differ non-significantly ($P > 0.05$)

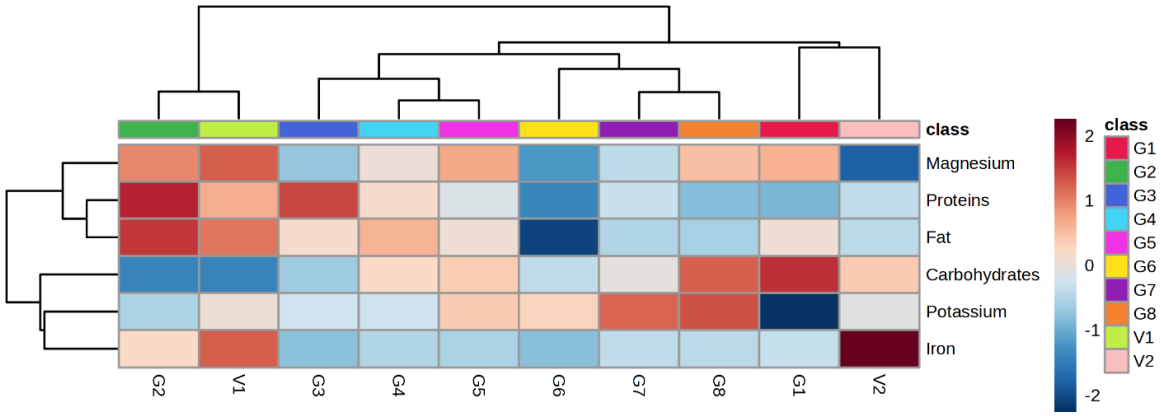

Fig. 1: A heatmap that displays the association between genotype and nutritional value

analyzed. The strength of the relationship was determined using the Pearson's correlation (*r*) value generated from the macronutrient pairings.

Discussion

Macronutrients are substances that humans consume in considerable amounts and consist of carbohydrates, protein, and fat (Savarino *et al.* 2021). These substances play crucial roles in metabolism. Carbohydrates provide immediate energy that is crucial for the human body (Javed and Usmani 2015). Proteins consumed by humans provide essential amino acids that serve as building blocks to create specific proteins (Wu *et al.* 2014). Additionally, fat can generate energy, which is beneficial for the human body. Any excess fat is stored as body fat and used as an energy reserve for the immune response (Zheng *et al.* 2016).

In this study, significant differences in total carbohydrate, protein and fat contents were observed among the genotypes. G1 had the highest total carbohydrate content at 22.52 ± 0.48 g (100 g)$^{-1}$ DW, while V1 had the lowest at 14.02 ± 0.12 g (100 g)$^{-1}$ DW. G2 had the highest total protein content at 6.74 ± 0.13 g (100 g)$^{-1}$ DW, while G6 had the lowest at 3.99 ± 0.16 g (100 g)$^{-1}$ DW. Additionally, G2 had the highest total fat content at 4.59 ± 0.05 g (100 g)$^{-1}$ DW, while G6 had the lowest at 1.30 ± 0.02 g (100 g)$^{-1}$ DW. The total carbohydrate, proteins and fats content in this study were lower than the values recorded in the study of Petropoulos *et al.* (2017) for okra genotypes from Greece, which ranged from 25.3 ± 0.7 to 31.3 ± 0.2 g (100 g)$^{-1}$ DW for carbohydrate, 37.4 ± 0.5 to 40.7 ± 0.3 g (100 g)$^{-1}$ DW for protein, and 24.77 ± 0.03 to 28.89 ± 0.01 g (100 g)$^{-1}$ DW for fat. These findings support the need to improve the quality of okra seeds in Indonesia to increase their nutritional value. Additionally, the results confirm the claims of Yora *et al.* (2018) regarding low okra production in Indonesia due to its low nutritional value. Therefore, several okra genotypes with the highest macronutrient contents in this study are recommended for future development.

The body requires sufficient quantities of micronutrients, such as vitamins and minerals. These micronutrients act as coenzymes and cofactors in cellular reactions (Chanda *et al.* 2015; Moll and Davis 2017). A deficiency in these micronutrients can result in various diseases (Godswill *et al.* 2020). Macro minerals, such as potassium and magnesium, were found to have higher content than iron in the ten okra fruit genotypes. The highest potassium content was found in G8 (3298.36 ± 129.49 mg (100 g)$^{-1}$ DW), while the lowest content was found in G1 (2407.93 ± 44.56 mg (100 g)$^{-1}$ DW). The magnesium content varied between 335.08 ± 8.96 to 463.05 ± 6.48 mg (100 g)$^{-1}$ DW in V2 and V1, respectively. Meanwhile, the

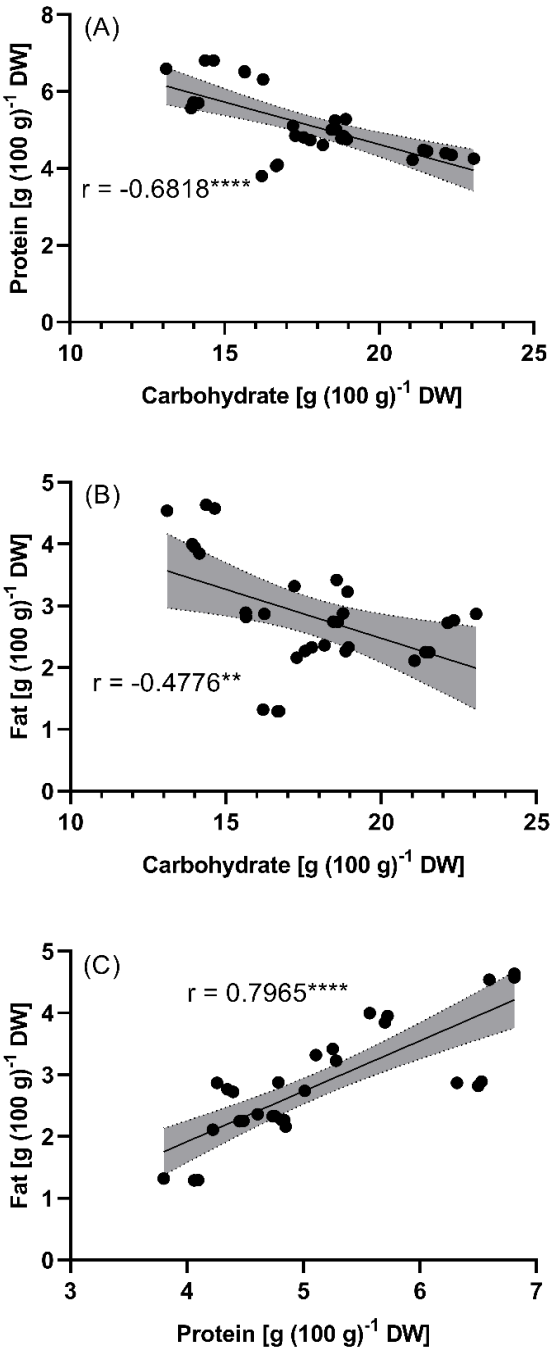

Fig. 2: The scatter plot displays the correlation between macronutrients, specifically the relationship between carbohydrates and proteins (**A**), carbohydrates and fat (**B**) and protein and fat (**C**)

iron content was recorded to be lower than potassium and magnesium, ranging from 4.13 ± 0.22 mg (100 g)$^{-1}$ DW in G6 to 14.04 ± 0.10 mg (100 g)$^{-1}$ DW in V2. These findings are consistent with previous research by Petropoulos *et al.* (2017) and theory, as macrominerals such as potassium and magnesium are expected to have higher content than microminerals such as iron (Nurnadia *et al.* 2013; Hadinoto *et al.* 2021).

The heatmap in Fig. 1 shows that some genotypes, such as G2 and V1, were closely clustered based on their nutritional content. These genotypes are relatively high in protein, fat, and magnesium contents. Heatmaps are used in multivariate analysis to group and cluster data based on their level of similarity, making it easier to visualize and annotate data (Zhao *et al.* 2014). The colors on the heatmap indicate the relationship between okra fruit genotype and nutritional content, with darker red indicating a higher relationship and darker blue indicating a lower content (Arista *et al.* 2022). Based on the heatmap, several okra genotypes with high nutritional content potential, including G2, V1, G3, G7, G8, G1 and V2, were identified (Fig. 1). After comparing the nutritional content data (Table 2), five genotypes were superior to the other genotypes, namely V1, G1, G2, V2 and G8. Genotypes V1, G1, V2 and G8 had advantages in magnesium, total carbohydrate, iron, and potassium content. G2 was superior in terms of total protein and fat content.

The final step in the study was a Pearson correlation analysis to evaluate the associations between macronutrients, specifically carbohydrates, proteins, and fats. These compounds were chosen for the correlation analysis because they are directly synthesized by plants (Chapman *et al.* 2013; Lastdrager *et al.* 2014; Li *et al.* 2016) and stored in the fruit, a storage organ (Li *et al.* 2015). The analysis revealed a significant negative correlation between carbohydrates and proteins (r = -0.6818) (Fig. 2A) and between carbohydrates and fat (r = -0.4776) (Fig. 2B). These results suggest that proteins and fats strongly influence the synthesis of carbohydrate compounds in plants. Macronutrient analysis of okra fruit (Table 2) supports this hypothesis. For instance, G1 genotype had the highest carbohydrate content but the lowest protein and fat content. Conversely, the G2 genotype had the highest protein and fat content but produced low carbohydrate content values. The interaction between protein and fat showed a significant positive correlation with a value of r = 0.7965 (Fig. 2C). These results indicate that the higher the total protein content in okra fruit, the higher is the total fat produced. On the other hand, finding superior genotypes in all types of nutrition is quite tricky, considering that the results of the Pearson correlation analysis obtained in this study were only partially positive. However, okra can be developed as a functional food by optimizing the dominant nutritional content of each genotype. All types of nutrition are essential for the human body, especially growth (Savarino *et al.* 2021).

Conclusion

Genetic makeup of okra fruit has a considerable impact on its nutritional value. Specifically, G1 genotype had the highest total carbohydrate contents, whereas genotype G2 had the highest protein and fat content. Additionally, G8,

V1 and V2 produced the highest levels of K, Mg and Fe, respectively. Moreover, the heatmap indicates that G2 may have the potential for magnesium, V1 for iron and G8 for potassium. Interactions between macronutrients were examined, and a significant negative correlation was found between carbohydrates and protein as well as between carbohydrates and fat. In contrast, a positive correlation was observed between the protein and fat content.

Limitation

In this study, we acknowledge that the data presented are currently limited because the research was conducted only during the rainy season. Nevertheless, there is a significant opportunity to further examine the okra genotypes involved in this study, with the aim of generating more comprehensive data. By cultivating these genotypes in the dry season, additional information, such as various macro- and micronutrients, as well as the genetic diversity of okra fruits can be obtained. This would result in more accurate data and enhanced scientific understanding.

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Author Contributions

WN and MS planned the experiments; NL collected samples in the field; NL performed the analysis; NL, WN, and MS interpreted and discussed the results; NL statistically analyzed the data; NL, WN, and MS wrote and revised the text.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Data Availability

The data presented in this study are available upon request from the corresponding author.

Ethics Approval

Not applicable to this study.

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