

Impact of genotype and harvest age on the polyphenol content and antioxidant capacity of okra (*Abelmoschus esculentus*) in Indonesia

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Abstract. Liwanda N, Syukur M, Nurcholis W. 2024. Impact of genotype and harvest age on the polyphenol content and antioxidant capacity of okra (*Abelmoschus esculentus*) in Indonesia. *Biodiversitas* 25: 4920-4929. Consuming bioactive compounds like polyphenols and flavonoids is crucial for reducing the risk of diseases due to their antioxidant, antibacterial, and anticancer properties. Okra (*Abelmoschus esculentus*), a widely cultivated vegetable known for its nutritional benefits, remains underutilized in Indonesia. This study aimed to identify the optimal genotype and harvest age of okra to maximize total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity. Ten okra genotypes were evaluated, with a focus on the impact of harvest age on four selected genotypes. The G7 genotype (F7 Clemson × Stripe-3-10-15B) exhibited the highest TPC (0.98 mg gallic acid equivalent/g fresh weight), TFC (0.35 mg quercetin equivalent/g fresh weight), and antioxidant capacity (5.13 μmol Trolox equivalent/g fresh weight). The optimal harvest age was determined to be the 7th day after anthesis, yielding the highest TPC and TFC values, ranging from 0.31 to 0.98 mg GAE/g and 0.19 to 0.35 mg QE/g, respectively. These findings indicate that both genotype and harvest age significantly affect the polyphenol content and antioxidant capacity of okra, with the G7 genotype and the 7th day after anthesis being optimal for maximizing bioactive compounds. This study highlights the potential for developing superior okra varieties in Indonesia.

Keywords: *Abelmoschus esculentus*, genotypes, harvest age, multivariate analysis, two-way ANOVA

Abbreviations: ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); CUPRAC: Cupric Reducing Antioxidant Power; DPPH: 1,1-diphenyl-2-picrylhydrazyl, FRAP: Ferric Reducing Antioxidant Power, fw: fresh weight; GAE: Gallic Acid Equivalent; TE: Trolox Equivalent; TFC: Total Flavonoids Content; TPC: Total Phenolic Content; QE: Quercetin Equivalent

INTRODUCTION

Meeting the body's nutritional needs through the consumption of nutritious food is essential, as it provides both macronutrients and micronutrients necessary for various physiological functions. In addition to these fundamental components, bioactive compounds, particularly those classified as secondary metabolites like polyphenols, play a critical role in enhancing human health and reducing the risk of various diseases (Chandrasekara and Shahidi 2018). Polyphenols, which include phenolic acids and flavonoids, have been widely recognized for their pharmacological effects, such as antioxidant, antibacterial, and anticancer properties (Negro et al. 2003; Roleira et al. 2015; Xu et al. 2016; Adebo and Medina-Meza 2020). These compounds are also involved in protecting the body from oxidative stress, which is a contributing factor to several chronic diseases.

The synthesis of polyphenols in plants is influenced by multiple environmental and agricultural factors. Research has demonstrated that factors such as the age at which a plant is harvested, its geographical conditions, and the availability of soil nutrients significantly affect the levels of

these bioactive compounds (Ishtifaiyyah et al. 2021; Abdillah et al. 2023; Liwanda et al. 2023). Studies by Costa et al. (2016) suggest that the timing of harvest is crucial for maximizing polyphenol content, and Vagiri et al. (2015) found that the phenolic compound levels in plants are significantly influenced by both the plant's maturity and harvest age. Therefore, selecting the optimal harvest age is key to ensuring the highest levels of nutrients and secondary metabolites, which directly enhances the overall nutritional and medicinal value of the plant.

One plant that has garnered attention for its rich nutritional profile and bioactive potential is okra (*Abelmoschus esculentus* (L.) Moench), a member of the Malvaceae family. Commonly known as *lady's finger*, *gombo*, or *bamje*, okra is widely cultivated in tropical and subtropical regions, especially in the Indo-Pak subcontinent (Nwangburuka et al. 2011; Dubey and Mishra 2017). Okra is valued for its high proximate composition, including carbohydrates, proteins, dietary fiber, and essential minerals such as calcium, magnesium, and potassium (Gemedede et al. 2015; Bawa and Badrie 2016; Daliu et al. 2020). Its pods, which are commonly consumed in cooking, are also rich in polyphenols and other secondary metabolites, contributing

to their antioxidant and other health-promoting effects (Petropoulos et al. 2017; Romdhane et al. 2020; Liwanda et al. 2024).

In addition to its numerous health benefits, okra contains antinutrients such as phytates, oxalates, and tannins, which may interfere with nutrient absorption (Gemede et al. 2016). However, despite these antinutritional factors, okra has traditionally been used in various regions, including India, Nepal, and Turkey, as a remedy for conditions such as diabetes and hyperlipidemia (Roy et al. 2014). Recent studies have further confirmed its antioxidant, antibacterial, and anticancer properties, underscoring its potential as a functional food (Xia et al. 2015; Khan et al. 2022).

While significant research on okra has been conducted in regions such as Asia, Africa, and the Mediterranean, studies focusing specifically on the polyphenol content of okra in Indonesia remain limited. Okra is not yet widely recognized in Indonesia, where the public's awareness of its health benefits is minimal. This lack of recognition is largely due to the crop's relatively low production and the limited availability of okra seeds, as well as its small-scale cultivation (Yusuf et al. 2023). In Indonesia, only two hybrid okra varieties, OK 060 (green) and OK 090 (red), have been officially registered, alongside non-hybrid varieties such as Naila (green) and Zahira (red), developed by Bogor Agricultural University (Direktorat Jenderal Hortikultura 2022). Notably, no research has been conducted in Indonesia to evaluate the polyphenol content of these local okra varieties, nor has there been any investigation into the optimal harvest times to maximize bioactive compound content.

This gap in research presents a critical opportunity to explore and optimize the bioactive potential of okra in Indonesia. Given its rich nutritional profile and the potential health benefits associated with its polyphenol content, it is essential to investigate how local varieties can be improved and adapted to enhance their nutritional value. Understanding the impact of harvest age on polyphenol content in Indonesian okra varieties can lead to the identification of superior genotypes that provide higher antioxidant capacities and better health-promoting effects.

To address these gaps, the current study aims to assess the total phenolic content, flavonoid concentration, and antioxidant capacity of ten different okra genotypes, harvested at four distinct time points. The primary objective is to identify superior genotypes and determine the optimal harvest age for maximizing polyphenol content and antioxidant capacity. By doing so, this study will provide valuable insights into the bioactive properties of okra and offer practical recommendations for enhancing the nutritional and health benefits of this crop. Furthermore, the findings will contribute to bridging the knowledge gap in Indonesian agricultural research, promoting okra as a high-value, functional food crop that can support both public health and local agricultural development.

MATERIALS AND METHODS

Study area

This research was performed between December 2022 and March 2023 at the Research Laboratory, Department of Biochemistry, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Samples for the study were sourced from the Leuwikopo Experimental Garden, Department of Agronomy and Horticulture, Faculty of Agriculture, Institut Pertanian Bogor, Indonesia. The study utilized a randomized complete block design with a single factor, which was genotype. The trial comprised ten genotypes, each replicated three times. Each replication consisted of a single sample. The data for the ten okra fruit genotypes used in the evaluation of superior genotypes are provided in Table 1. The evaluation of okra harvest age utilized four selected genotypes, chosen from the three most superior genotypes and one genotype with low polyphenol content. This harvest age evaluation experiment also employed a randomized complete block design with two factors: genotype and harvest age. The treatment consisted of four genotypes, each replicated three times. Each replication comprised one sample. The four okra fruit genotypes were harvested at three, five, seven, and nine days after anthesis.

Procedures

Extract preparation

Preparation of okra fruit ethanol extract is based on Nurcholis et al. (2022) with adjustments. The fresh okra fruit is washed and cut into equal pieces, and then blended for two minutes. Next, the homogenized sample is extracted using pro analysis ethanol and microwave-assisted extraction for three minutes at 135 W. The extraction process is then continued with continuous shaking in a dark room for 24 hours at room temperature. The extracted sample is filtered with filter paper, and the final volume is returned to 20 mL with solvent (concentration: 0.1 g/mL). The filtrate is stored in a dark bottle at 4°C.

Total phenolic content

The measurement of total phenolic content was carried out based on Nurcholis et al.'s (2022) research, with modifications. To begin, 20 µL of the sample filtrate and 120 µL of a 10% Folin-Ciocalteu reagent (v/v with distilled water) were pipetted into a microplate and incubated for five minutes in a dark room at room temperature (25°C).

Next, 80 µL of a 10% Na₂CO₃ solution (w/v in distilled water) was added to the microplate and the solution was incubated for 30 minutes in a dark room at room temperature (25°C). The absorbance of the solution was then measured at a wavelength of 750 nm using a nanospectrophotometer (SPECTROstarNano BMG LABTECH). The standard used was gallic acid with a concentration variation of 20-300 ppm. The final results were expressed in units of mg GAE (gallic acid equivalent)/g fw (fresh weight).

Table 1. Data on ten okra genotypes as test plant materials

Code	Pedigree	Sources	Phenotypes
V1	Zahira	Variety	Red
G1	F7 Zah×B291-11-6-1B	Lines	Red
G2	F7 Zah×MC-13-1-12B	Lines	Red
G3	F7 Zah×MC-13-7-15B	Lines	Red
G4	F6 B291×Zah-2-5B	Lines	Red
V2	Naila	Variety	Green
G5	F7 Clemson×Naila-23-10-1B	Lines	Green
G6	F7 Clemson×Naila-23-22-9B	Lines	Green
G7	F7 Clemson×Stripe-3-10-15B	Lines	Green
G8	F7 Clemson×Stripe-3-23-4B	Lines	Green

Total flavonoid content

The determination of the total flavonoid content was performed based on the study by Nurcholis et al. (2022) with some adjustments. To achieve this, 120 µL of aquadest was combined with 10 µL of extract filtrate, 10 µL of 10% AlCl₃ (in aquadest), 10 µL of glacial acetic acid, and 50 µL of pro-analysis ethanol (Merck KGaA) in a microplate. The mixture was then incubated in a dark room at room temperature (25°C) for 30 minutes. The absorbance of the solution was recorded at a wavelength of 415 nm using a nanospectrophotometer (SPECTROstarNano BMG LABTECH). The standard used for this analysis was quercetin, with a concentration range of 0-500 ppm. The outcomes of the study were expressed in mg of quercetine equivalent (mg QE)/g of fresh weight (fw).

DPPH antioxidant capacity

The study of Arista et al. (2022) on measuring antioxidant capacity using the DPPH method has been modified. In this process, 100 µL of the extract filtrate was combined with 100 µL of a DPPH reagent (125 µM, w/v in ethanol p.a.) in a microplate. The mixture was then incubated in the dark at room temperature (25°C) for 30 minutes. The absorbance of the solution was measured at a wavelength of 515 nm using a nanospectrophotometer (SPECTROstarNano BMG LABTECH). The trolox standard was used, with a concentration range of 0-50 µM. The final results are expressed in terms of µmol TE (trolox equivalent)/g fw.

FRAP antioxidant capacity

Antioxidant capacity is a topic that has been explored by Arista et al. (2022) through the use of the FRAP method, with certain modifications. This method involves preparing the FRAP reagent by mixing acetate buffer (pH 3.6), TPTZ (10 µM, w/v in 40 mM HCl), and FeCl₃ (20 mM, w/v in distilled water) in a ratio of 10:1:1 (v/v/v). The mixture is then incubated for 30 minutes in the dark at room temperature before being used. To conduct the measurement, 10 µL of the extract filtrate is added to 300 µL of the FRAP reagent in a microplate, and the mixture is incubated for 30 minutes in the dark at room temperature (25°C). The absorbance of the solution is then measured at a wavelength of 593 nm using a nanospectrophotometer (SPECTROstarNano BMG LABTECH). The standard used for this measurement is trolox, with a concentration

ranging from 0-600 µM. The final results are expressed in units of µmol TE/g fw.

ABTS antioxidant capacity

The antioxidant capacity of the ABTS method was measured based on Nurcholis et al.'s (2022) research, with some modifications. To prepare the ABTS reagent, a 2:1 (v/v) mixture of ABTS 7.7 mM (w/v in distilled water) and K₂S₂O₈ 2.4 mM (w/v in distilled water) was used. The reagent was incubated in the dark at room temperature for six minutes. The desired absorbance range of 0.7±0.02 was achieved by adding distilled water. Next, 20 µL of the extract filtrate was combined with 180 µL of the ABTS reagent in a microplate, and the mixture was incubated for six minutes in the dark at room temperature (25°C). The absorbance of the solution was measured at a wavelength of 734 nm using nanospectrophotometer (SPECTROstarNano BMG LABTECH). The trolox standard was used, with concentrations ranging from 0 to 500 µM. The results were reported in units of µmol TE/g fw.

CUPRAC antioxidant capacity

The CUPRAC method was employed to assess antioxidant capacity in accordance with Nurcholis et al.'s (2022) research, with adjustments made. To the microplate, 50 µL of extract filtrate, 50 µL of 0.01 M CuCl₂ (w/v in aquadest), 50 µL of 0.0075 M neocuproine (w/v in aquadest), and 50 µL of pH 7 ammonium acetate buffer were added. The mixture was left to incubate in a dark room at room temperature (25°C) for 30 minutes. The absorbance of the solution was then measured at 450 nm using a nanospectrophotometer (SPECTROstarNano BMG LABTECH). Trolox was used as the standard, with concentrations ranging from 0-500 µM. The final results were expressed in units of µmol TE/g fw.

Data analysis

The data for total phenolic, flavonoid, and antioxidant capacity were analyzed using descriptive statistics, including the F test. If the treatment had a significant effect, a further HSD test was conducted at a 5% significance level. Additionally, multivariate analysis and clustering were performed. This analysis was conducted using the MetaboAnalyst 6.0 platform (Pang et al. 2024). Data analysis also included an orthogonal contrast test to determine the difference in content between red and green okra using IBM SPSS Statistics version 25.

RESULTS AND DISCUSSION

Recapitulation of polyphenol content and antioxidant capacity of okra

The polyphenol content and antioxidant capacity of a plant can be influenced by a variety of factors, one of which is the genotype of the plant (Petropoulos et al. 2018). Genotype refers to the set of unique genes inherited from the parents of an organism and contains instructions for regulating the activity of other genes necessary for the synthesis of specific proteins. These genes have a significant

impact on plant characteristics such as morphology, biochemical traits, and compound content (Halder et al. 2015). The evaluation of okra plant genotypes revealed a significant influence on polyphenol content and antioxidant capacity (Table 2). According to Halder et al. (2015), differences in okra plant genotypes also affect the length, width, and weight of okra fruit.

The content of polyphenols and antioxidant capacity exhibited considerable variations among different okra genotypes (Table 2). Genotype G7 showed the highest total phenolic content (TPC) and flavonoid content (TFC), with values of 0.98 ± 0.04 mg GAE/g fw and 0.35 ± 0.01 mg QE/g fw, respectively. Genotype G6 also exhibited the same TFC content as G7, resulting in no significant difference between the two genotypes in terms of flavonoid content. In contrast, G7 displayed the highest FRAP antioxidant capacity at $5.13 \mu\text{mol TE/g fw}$, although this value was not significantly different from that of genotype G2. Genotype G2 demonstrated the highest antioxidant capacities across DPPH, FRAP, and ABTS assays, with values of $6.33 \pm 0.42 \mu\text{mol TE/g fw}$, $5.13 \pm 0.20 \mu\text{mol TE/g fw}$, and $10.17 \pm 0.84 \mu\text{mol TE/g fw}$, respectively. The highest CUPRAC antioxidant capacity was observed in genotype G1, with a value of $5.49 \pm 0.46 \mu\text{mol TE/g fw}$.

These findings are consistent with previous studies, such as that by Wu et al. (2020), which reported that genotype influences phenolic compound content and antioxidant capacity (DPPH, FRAP, ABTS, and CUPRAC) in okra. Similarly, Petropoulos et al. (2018) found that the phytochemical content varies according to the okra genotype. However, the differences observed between our study and Wu et al. (2020) may be due to variations in the extraction methods. In this study, microwave-assisted extraction was used, which likely enhanced the yield of bioactive compounds compared to the traditional solvent extraction methods employed by Wu et al. (2020). The use of microwave-assisted extraction could explain the higher

antioxidant capacities observed in our study, particularly for flavonoids and phenolics. These variations underscore the importance of considering extraction techniques when comparing polyphenolic profiles and antioxidant activities across studies.

The orthogonal comparison between red and green okra phenotypes revealed significant differences in total flavonoid content (TFC) and antioxidant capacities (DPPH, FRAP, and CUPRAC), while no significant differences were observed in total phenolic content (TPC) and ABTS antioxidant activity (Table 2). The lack of significance in TPC and ABTS may result from the use of crude extracts, which contain a mixture of bioactive and inactive compounds; this variability could mask the effects of specific bioactive components. Nevertheless, these findings are scientifically valuable as crude extracts represent a practical approach for initial screenings, and the results emphasize the need for further fractionation and compound-specific analysis to uncover hidden variations and maximize the detection of bioactivity. The red okra group demonstrated higher DPPH, FRAP, and CUPRAC antioxidant capacities, while the green okra group exhibited higher TFC.

These differences could be attributed to variations in secondary metabolites, such as chlorophyll, carotenoids, and anthocyanins, with red okra generally containing more carotenoids and anthocyanins, and green okra being richer in chlorophyll (Yora et al. 2018). The presence of anthocyanins, which are strongly correlated with TFC, likely enhances antioxidant activity (Nurcholis et al. 2023), while the green okra group's higher chlorophyll content may contribute to its carbohydrate synthesis efficiency (Liwanda et al. 2024). These findings suggest specific phenotypic advantages that could be explored for targeted applications, such as antioxidant activity or glucose regulation (Anjani et al. 2018).

Table 2 Mean values of total phenolic, flavonoid, and antioxidant capacity (DPPH, FRAP, ABTS, and CUPRAC) content of each genotype and further orthogonal contrast test between red and green phenotypes

Genotypes	TPC	TFC	DPPH	FRAP	ABTS	CUPRAC
	(mg GAE/g fw)	(mg QE/g fw)		($\mu\text{mol TE/g fw}$)		
V1	$0.69 \pm 0.05^{\text{cd}}$	$0.26 \pm 0.01^{\text{de}}$	$4.10 \pm 0.49^{\text{def}}$	$4.04 \pm 0.12^{\text{cd}}$	$6.47 \pm 0.16^{\text{cd}}$	$4.20 \pm 0.48^{\text{cde}}$
G1	$0.83 \pm 0.02^{\text{abc}}$	$0.25 \pm 0.02^{\text{e}}$	$5.31 \pm 0.58^{\text{bc}}$	$4.83 \pm 0.20^{\text{ab}}$	$11.06 \pm 1.24^{\text{a}}$	$5.49 \pm 0.46^{\text{a}}$
G2	$0.92 \pm 0.13^{\text{ab}}$	$0.30 \pm 0.02^{\text{bcd}}$	$6.33 \pm 0.42^{\text{a}}$	$5.13 \pm 0.20^{\text{a}}$	$10.17 \pm 0.84^{\text{a}}$	$4.81 \pm 0.30^{\text{abc}}$
G3	$0.78 \pm 0.05^{\text{bc}}$	$0.30 \pm 0.01^{\text{bcd}}$	$3.80 \pm 0.19^{\text{def}}$	$4.81 \pm 0.15^{\text{ab}}$	$8.72 \pm 1.31^{\text{abc}}$	$5.09 \pm 0.08^{\text{ab}}$
G4	$0.62 \pm 0.05^{\text{d}}$	$0.29 \pm 0.02^{\text{cde}}$	$3.31 \pm 0.30^{\text{ef}}$	$3.80 \pm 0.29^{\text{cd}}$	$6.04 \pm 0.39^{\text{d}}$	$4.02 \pm 0.32^{\text{e}}$
V2	$0.82 \pm 0.06^{\text{bc}}$	$0.32 \pm 0.02^{\text{abc}}$	$4.22 \pm 0.33^{\text{de}}$	$4.31 \pm 0.66^{\text{bc}}$	$7.55 \pm 0.13^{\text{bcd}}$	$5.19 \pm 0.54^{\text{ab}}$
G5	$0.62 \pm 0.02^{\text{d}}$	$0.29 \pm 0.02^{\text{cd}}$	$4.53 \pm 0.41^{\text{cd}}$	$3.35 \pm 0.06^{\text{d}}$	$7.28 \pm 0.55^{\text{bcd}}$	$4.07 \pm 0.01^{\text{de}}$
G6	$0.72 \pm 0.06^{\text{cd}}$	$0.35 \pm 0.02^{\text{a}}$	$3.41 \pm 0.35^{\text{ef}}$	$4.03 \pm 0.04^{\text{cd}}$	$7.36 \pm 0.31^{\text{bcd}}$	$4.25 \pm 0.43^{\text{cde}}$
G7	$0.98 \pm 0.04^{\text{a}}$	$0.35 \pm 0.01^{\text{a}}$	$6.04 \pm 0.16^{\text{ab}}$	$5.13 \pm 0.14^{\text{a}}$	$9.67 \pm 0.42^{\text{ab}}$	$4.73 \pm 0.53^{\text{bcd}}$
G8	$0.62 \pm 0.07^{\text{d}}$	$0.34 \pm 0.03^{\text{ab}}$	$3.18 \pm 0.34^{\text{f}}$	$3.93 \pm 0.15^{\text{cd}}$	$7.38 \pm 1.84^{\text{bcd}}$	$3.65 \pm 0.36^{\text{e}}$
HSD 5%	0.16	0.05	0.99	0.73	2.45	0.71
Red vs Green	ns	**	*	**	ns	*
Red	0.77	0.28	4.57	4.52	8.49	4.72
Green	0.75	0.33	4.28	4.15	7.85	4.38

Notes: fw: fresh weight; **: significantly affected at the $\alpha=1\%$ level based on the orthogonal contrast test; *: significantly affected at the $\alpha=5\%$ level based on the orthogonal contrast test; ns: no significant effect, numbers followed by the same letter in the same column are not significantly different based on the 5% level HSD test

Diversity of polyphenol content and antioxidant capacity of okra

The evaluation of polyphenol content and antioxidant capacity in ten okra genotypes using principal component analysis (PCA) revealed significant diversity in antioxidant-related traits across genotypes (Figure 1). These analytical tools were particularly effective in reducing data complexity, explaining 81.5% of the total diversity (PC1 + PC2), which exceeded the 70% threshold typically required for meaningful interpretation (Jolliffe and Cadima 2016). Genotypes V1, G4, G5, G6, and G8 were predominantly distributed in the positive quadrant of PC1, indicating higher values for traits such as TPC, TFC, DPPH, FRAP, ABTS, and CUPRAC, while genotypes V2, G1, G2, G3, and G7 were more dominant in the negative quadrant (Figure 2). These patterns suggest that genotypes in the positive quadrant are likely to possess enhanced antioxidant capacity, making them prime candidates for further exploration in breeding programs aimed at improving nutritional quality.

The practical implications of these findings are significant for breeders, producers, and consumers. For breeders, genotypes such as G6 and V1 in the positive PC1 quadrant represent ideal parental lines for developing cultivars with improved antioxidant traits. These genotypes could serve as the basis for selective breeding programs targeting functional food markets, aligning with the increasing demand for health-promoting crops (Andreanto et al. 2021). Producers could benefit from cultivating these genotypes to enhance marketability, particularly in niche segments such as nutraceuticals or antioxidant-enriched food products. On the other hand, genotypes with lower antioxidant activity, such as G1 and G2, may still hold

value for carbohydrate-rich applications, including starch-based products or industrial biomass. For consumers, the availability of antioxidant-rich okra cultivars could support dietary strategies to mitigate oxidative stress and promote overall health (Nurcholis et al. 2023).

Biologically, the dominance of genotypes in the positive PC1 quadrant is likely driven by genetic differences in secondary metabolite production, particularly the biosynthesis of polyphenols and flavonoids. Genotypes such as G6 and V1 may have more efficient enzymatic pathways or higher expression levels of genes involved in these biosynthetic processes, allowing them to accumulate greater amounts of antioxidant compounds. Environmental factors, such as soil type, light intensity, and temperature, may also influence the observed diversity. Conversely, genotypes in the negative quadrant, such as G3 and G7, may prioritize other traits, such as carbohydrate metabolism or yield stability, which could explain their relatively lower antioxidant levels.

These findings also hold promise for future cultivar development. Breeding programs can utilize genotypes like G6 and V1 to enhance phenolic and flavonoid content, while genotypes such as G3 and G7 can be explored for traits related to stress tolerance or biomass production. Additionally, understanding the relationships among antioxidant traits, as indicated by vector lengths in the PCA biplot, could guide the selection of genotypes that exhibit favorable combinations of traits for diverse applications. The integration of advanced genomic tools with PCA and HCA analyses could further accelerate the development of targeted cultivars, ensuring sustainable agricultural practices and market competitiveness (Roughani et al. 2018).

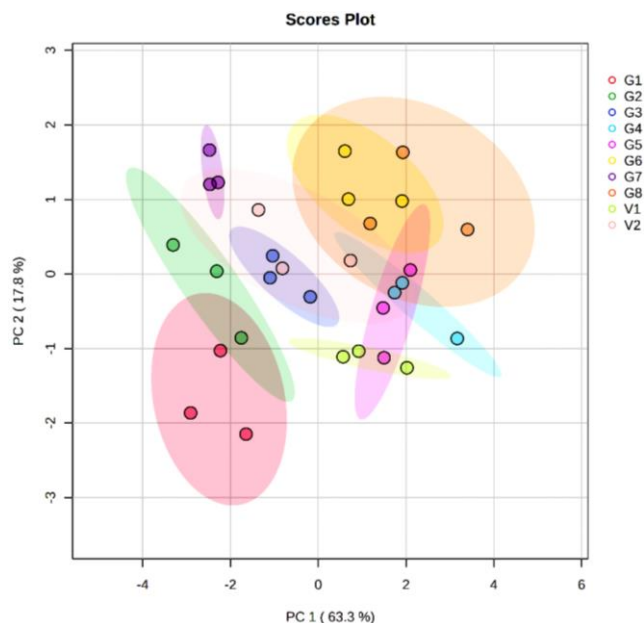


Figure 1. Plot of scores of principal component analysis (PCA) results of ten okra genotypes

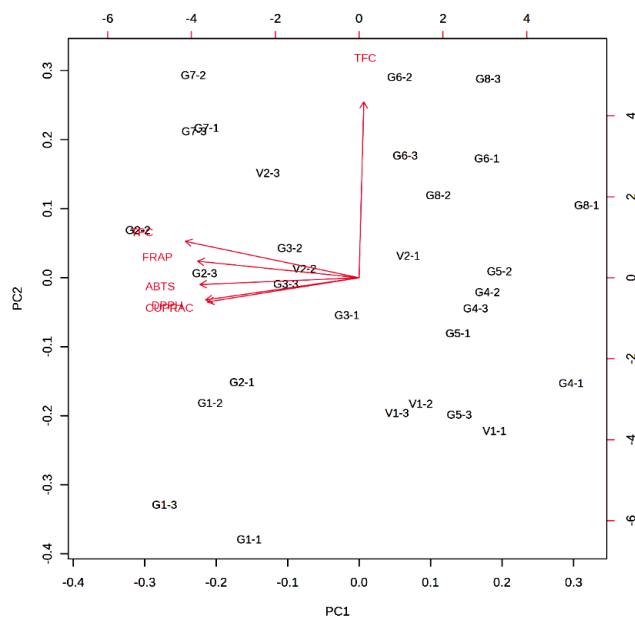


Figure 2. Biplot results of principal component analysis (PCA) of ten okra genotypes

Hierarchical cluster analysis (HCA) is a method that complements principal component analysis (PCA) by providing a different perspective on the relationships among genotypes. While PCA reduces dimensionality by summarizing the variation across multiple variables into principal components (Jolliffe and Cadima 2016), HCA clusters genotypes based on their similarity across these variables (Roughani et al. 2018). These methods serve distinct purposes: PCA highlights patterns in the data by creating orthogonal components, while HCA organizes genotypes into groups based on the closeness of their responses to the studied traits. By integrating both approaches, this study provides a comprehensive analysis, where HCA was applied to cluster genotypes based on the antioxidant-related variables highlighted by PCA, visualized as a heatmap matrix of average response values (Figure 3). The heatmap colors indicate the abundance level of each genotype's response, with positive colors representing high abundance and negative colors indicating low abundance.

The HCA results revealed that genotypes G7, G2, and G1 exhibited a high level of response abundance across most traits, except for the CUPRAC response in G7 (0.35). Genotype G2 showed relatively high abundance for DPPH (1.57), FRAP (1.25), TPC (1.18), and ABTS (1.20), but lower abundance for CUPRAC (0.49) and TFC (-0.21). Genotype G1 demonstrated strong responses for ABTS (1.61), CUPRAC (1.48), DPPH (0.85), FRAP (0.82), and TPC (0.60), but had the lowest abundance for TFC (-1.69). Conversely, G8 consistently showed low responses across most traits, including TPC (-1.16), DPPH (-1.23), and CUPRAC (-1.61), while G5 and G4 exhibited the lowest abundance for FRAP and ABTS responses, respectively. These findings highlight the potential of combining PCA and HCA to provide actionable insights into genotype

diversity. PCA identifies the key traits contributing to variability, while HCA groups genotypes based on similarity, offering valuable tools for breeding programs aiming to select genotypes with superior antioxidant profiles or specific trait combinations (Andreanto et al. 2021).

Evaluation of optimum harvest age of okra

The ANOVA analysis outcomes revealed that the polyphenol compounds content and antioxidant capacity were considerably impacted by genotype and harvest age as shown in Table 3. Both genotype, harvest age, and the interaction between them had a substantial influence on the total phenolic content (TPC), total flavonoids (TFC), and antioxidant capacities of DPPH, FRAP, ABTS, and CUPRAC. The coefficient of variation (CV) value demonstrated a relatively low percentage, ranging from 4.94% to 12.43%.

The measure of diversity, or the coefficient of diversity, is used to gauge the level of accuracy and precision in the conclusions of an experiment (Bedeian and Mossholder 2000). When the coefficient of diversity value is low, it signifies a high degree of accuracy and precision because the level of diversity can be minimized. In this study, the coefficient of determination (R^2) value ranged from 0.72 to 0.98, which is considered high and indicates a strong relationship between the independent and dependent variables. The R^2 value, which ranges from 0 to 1, measures how well the model can explain the diversity of dependent variables (Saunders et al. 2012). The CV and R^2 values obtained in this study suggest that the data obtained has high validity, allowing further testing without the need for data transformation.

Table 3. Analysis of polyphenol content variation and antioxidant capacity of okra based on genotype and harvest age

Variables	Genotypes			Harvest age	Interaction	CV (%)	R^2
	(G) ^a	(U) ^a	(G×U) ^a				
TPC	**	**	**	**	**	8.54	0.96
TFC	*	**	*	*	*	12.43	0.72
DPPH	**	**	**	**	**	10.21	0.95
FRAP	**	**	**	**	**	4.94	0.98
ABTS	**	**	**	**	**	7.86	0.97
CUPRAC	**	**	**	**	**	8.42	0.97

Notes: CV: Coefficient of Variation; R^2 : Coefficient of determination; **: Significantly influential at the $\alpha=1\%$ level; *: significantly influential at the $\alpha=5\%$ level

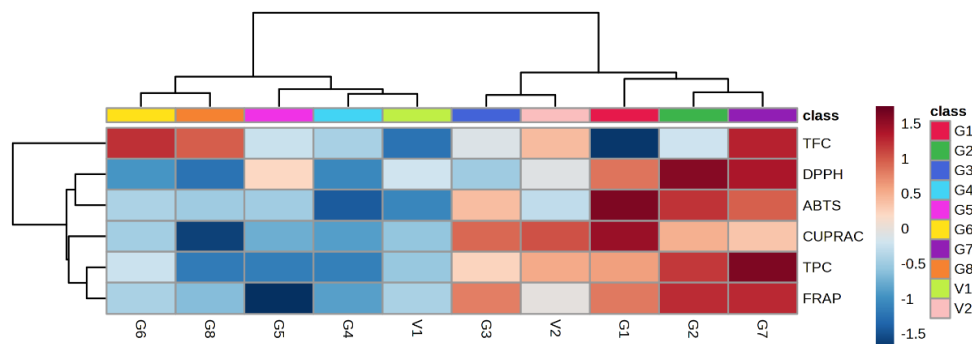


Figure 3. Heatmap of the results of hierarchical cluster analysis (HCA) of ten okra genotypes

The relationship between genotype and harvest age demonstrates that harvesting okra on the 7th day after anthesis results in the highest average TPC, TFC, DPPH, FRAP, ABTS, and CUPRAC (Figure 4). Genotype G7 excels in TPC and TFC content, while G2 demonstrates the highest DPPH antioxidant capacity, and G1 produces the highest values for ABTS and CUPRAC. These results suggest that both genotype and harvest age influence the synthesis of polyphenols and antioxidant capacity. However, it is important to note that this study does not account for

the potential effects of environmental factors, such as soil type, climate, and agricultural practices, which are known to influence the levels of polyphenols and antioxidants in plants (Halder et al. 2015). Variations in these conditions could contribute to differences observed among genotypes and harvest ages. Future research should address these variables to provide a more comprehensive understanding of the interaction between genotype, environment, and harvest timing.

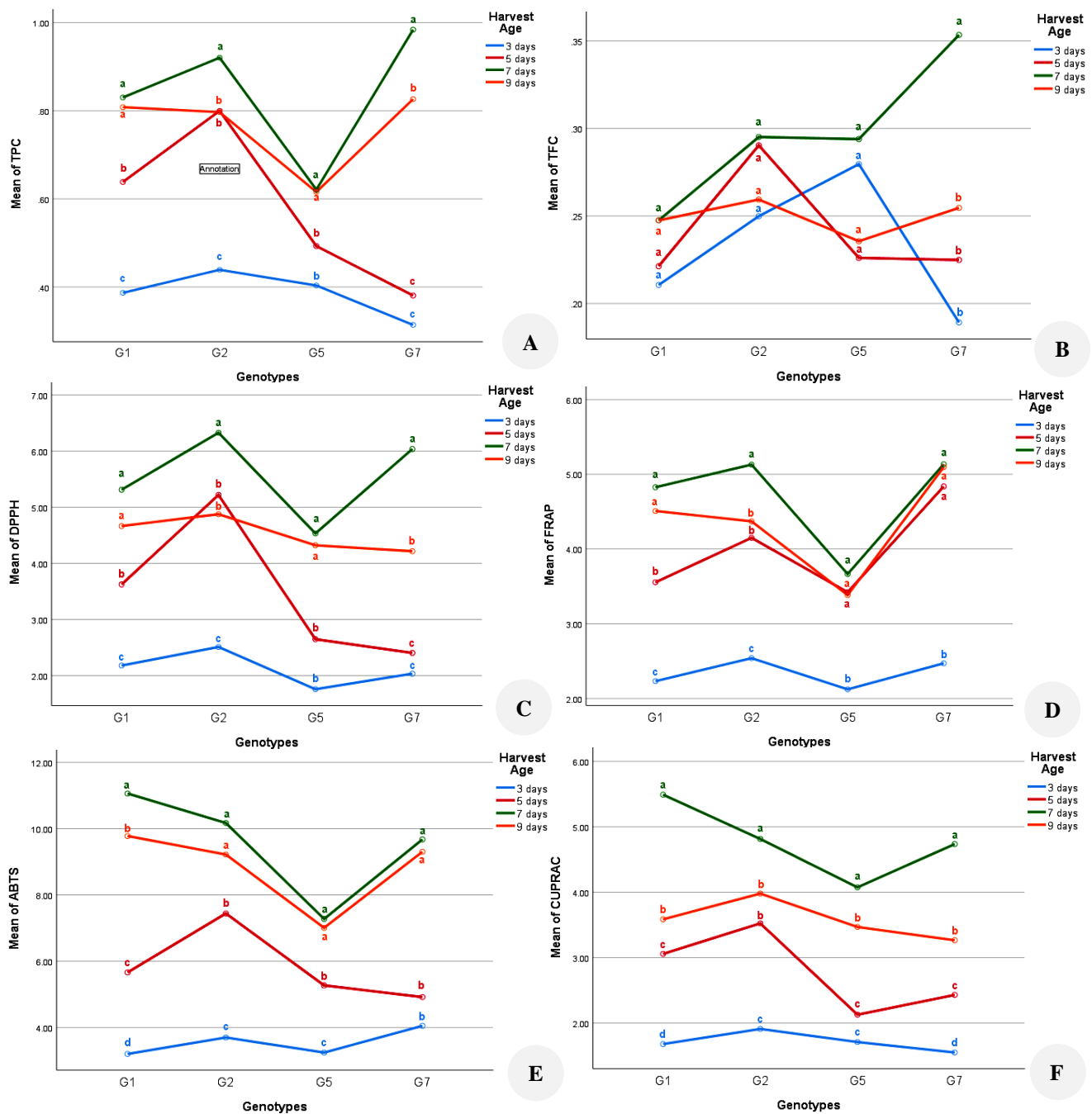


Figure 4. Interaction curves of genotype-harvest age on test variables. A. Total phenolic content; B. Total flavonoid content; C. DPPH antioxidant capacity; D. FRAP; E. ABTS; F. CUPRAC. G1, F7 Zah × B291-11-6-1B; G2, F7 Zah × MC-13-1-12B; G5, F7 Clemson × Naila-23-10-1B; G7, F7 Clemson × Stripe-3-10-15B. Different letters on each genotype indicate significant differences at each harvest age with $p < 0.05$

The content of polyphenols was assessed by evaluating the types of phenolic and flavonoid compounds. Total phenolic content (TPC) was found to be higher than total flavonoid content (TFC) (Figure 5). On the 7th day after anthesis, the TPC content was highest for the four genotypes analyzed, with values of 0.83 ± 0.02 , 0.92 ± 0.13 , 0.62 ± 0.02 , and 0.98 ± 0.04 mg GAE/g fw for G1, G2, G5, and G7, respectively. Conversely, the lowest TPC values were observed on the 3rd day after anthesis, with values ranging from 0.31 ± 0.05 to 0.40 ± 0.02 mg GAE/g fw. Similarly, the lowest TFC values on day 3 were 0.21 ± 0.03 , 0.25 ± 0.02 , 0.28 ± 0.02 , and 0.19 ± 0.05 mg QE/g fw for G1, G2, G5, and G7, respectively. By day 7, TFC increased significantly, reaching values of 0.25 ± 0.02 , 0.30 ± 0.02 , 0.30 ± 0.02 , and 0.35 ± 0.01 mg QE/g fw for G1, G2, G5, and G7. These results underline the importance of timing harvest to maximize polyphenol and flavonoid synthesis, although the environmental context in which these genotypes are grown must be considered to generalize these findings effectively.

The age at which fruits are harvested has a direct impact on the compounds that are produced. Choosing different harvesting times can lead to variations in the biochemical profiles of the fruit. This is because the process of fruit ripening involves changes in the biochemical components of secondary metabolite anabolism (Zuhdi et al. 2018).

In okra, these secondary metabolite compounds are found in the form of phenolic and flavonoid groups. These compounds are produced through the shikimic acid pathway, which uses simple sugar precursors and aromatic amino acids (Ghasemzadeh and Jaafar 2011). Ngangbam and Jahangir (2011) reported that the Sinnova and Arka Anamika okra cultivars from India contained the highest levels of ascorbic acid six days after anthesis. This and other studies suggest that the optimal age for harvesting okra plants can vary depending on the genotype or type of okra being grown and the specific compounds being evaluated.

The levels of antioxidant capacity in okra, as assessed by different analysis techniques, varied with the age of harvest (Figure 6). The ABTS method produced the highest

antioxidant capacity, with an average range of 3.21 ± 0.29 to 11.10 ± 1.24 $\mu\text{mol TE/g fw}$. In comparison, the DPPH, FRAP, and CUPRAC methods produced average ranges of 1.76 ± 0.18 to 6.33 ± 0.42 $\mu\text{mol TE/g fw}$, 2.12 ± 0.09 to 5.13 ± 0.14 $\mu\text{mol TE/g fw}$, and 1.55 ± 0.05 to 5.49 ± 0.46 $\mu\text{mol TE/g fw}$, respectively. The study found that the 3rd day after anthesis resulted in the lowest antioxidant capacity in all methods. However, the antioxidant capacity increased on the 5th day and peaked on the 7th day. On the 9th day, the antioxidant capacity decreased based on all antioxidant analysis methods. The four genotypes evaluated in this study showed that the 7th day after anthesis was the optimal harvest time to achieve the highest antioxidant capacity, which was consistent with the highest TPC and TFC content produced on the 7th day after anthesis.

The relationship between okra plants and antioxidant capacity is closely linked to the presence of polyphenolic compounds, specifically phenolics and flavonoids. Phenolic compounds, which are simple in nature, possess phenol groups in their structure. The antioxidant properties of phenolic compounds are attributed to their high redox potential, allowing them to act as hydrogen atom donors, reducing agents, and neutralizers of radical oxygen atoms. Additionally, the resonance effect of the phenolic group's structure rearranges the electrons in the aromatic ring and restabilizes the phenol structure after the stabilization of free radicals (Chandra and Arora 2017).

Flavonoids are a class of chemical compounds that are derived from phenolic compounds and possess multiple phenol groups in their structure (González et al. 2011). Their basic structure consists of C6-C3-C6, featuring two types of aromatic carbon atoms, namely benzopyran and benzene. These compounds are grouped into various smaller complex categories based on their degree of oxidation and hydroxyl content, such as anthocyanidins, flavones, flavonones, flavonols, isoflavones, monomeric flavanols, and polymeric flavanols (Kim et al. 2014). The antioxidant properties of flavonoid compounds are similar to those of phenolic compounds, including the delocalization effect (rearrangement) of aromatic rings and resonance effects (Speisky et al. 2022).

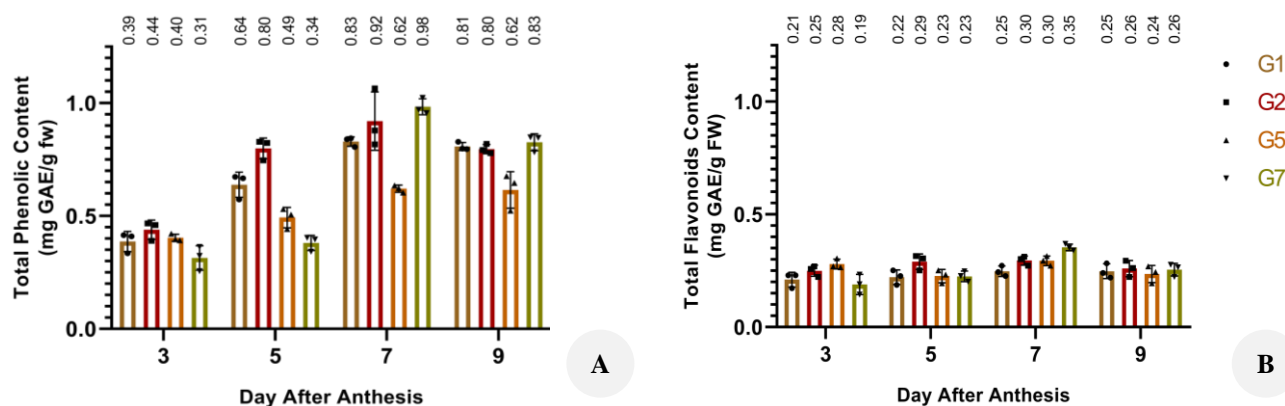


Figure 5. Polyphenol content of okra based on harvest age. A. Total phenolic content; B. Total flavonoid content. Each value is presented as mean \pm standard deviation. GAE, gallic acid equivalent; QE, quercetin equivalent; fw fresh weight

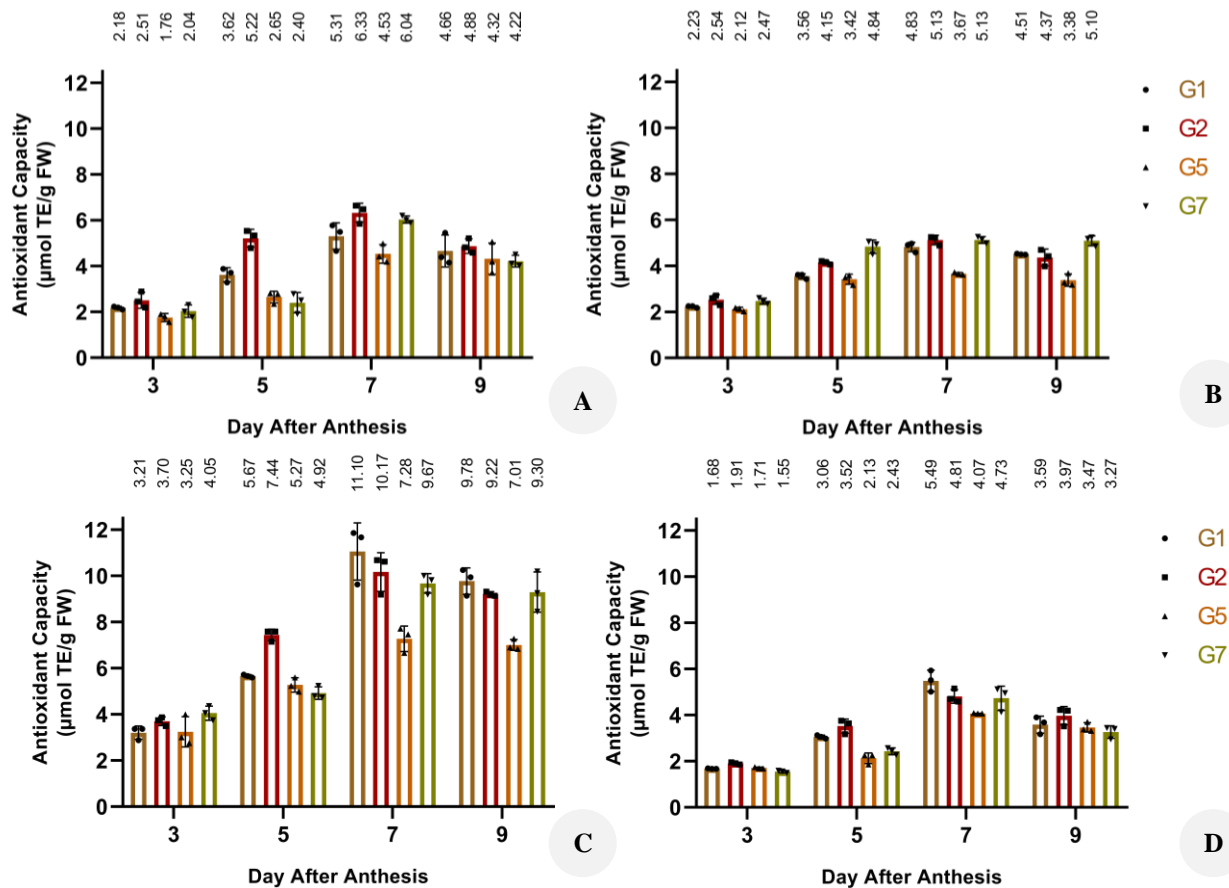


Figure 6. Antioxidant capacity of okra based on harvest age. A. DPPH method; B. FRAP; C. ABTS; D. CUPRAC. Each value is presented as mean \pm standard deviation. TE: Trolox Equivalent; fw: fresh weight

This study assessed the total phenolic content, flavonoids, and antioxidant capacity of ten different okra genotypes at four distinct harvest times. The findings indicate that genotype and harvest age significantly influence the bioactive compound content and antioxidant properties of okra. Among the genotypes tested, G7 (F7 Clemson \times Stripe-3-10-15B) consistently exhibited the highest levels of total phenolic and flavonoid content, as well as antioxidant capacity across multiple assays. The 7th day after anthesis was identified as the optimal harvest time to maximize these bioactive compounds. These results underscore the potential of selecting specific genotypes and harvest times to enhance the nutritional and health benefits of okra. Consequently, the G7 genotype and harvesting at the 7th day after anthesis represent promising targets for developing superior okra varieties, particularly in regions like Indonesia where the crop remains underutilized. Future research should focus on expanding these findings to other growing conditions and further exploring the mechanisms underlying these genotype and harvest age effects.

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