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Original Research Paper

Ultra performance liquid chromatography-mass spectroscopy (UPLC-MS) based carotenoid profiling in Indian avocado (*Persea americana* Mill.)

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ABSTRACT

Avocado (*Persea americana* Mill.), frequently termed 'green gold', experiences high global demand driven by its extensive nutritional and therapeutic benefits. This study evaluated the diversity and profile of total carotenoids across 20 distinct avocado accessions. The evaluated germplasm exhibited substantial variability in total carotenoid content, ranging from 1.6 to 5.8 mg/100 g, with a total of nine specific carotenoids identified. Chromatographic profiling revealed that lutein was the predominant carotenoid (5.25 µg/g), followed sequentially by β-carotene (1.84 µg/g), neoxanthin (1.71 µg/g), and violaxanthin (1.55 µg/g). Principal component analysis captured 70.01% of the total variance across the first three components, highlighting pronounced differentiation among the accessions. Correlation analysis demonstrated highly positive relationship between β-carotene and α-carotene, whereas lutein exhibited a weak negative correlation with chrysanthemaxanthin. Hierarchical cluster analysis categorized the 20 accessions into two primary clusters, confirming significant phenotypic divergence in carotenoid composition. Ultimately, accessions PA-095, PA-078, and PA-069 were identified as superior lines due to their elevated lutein and β-carotene concentrations, marking them as valuable genetic resources for future avocado biofortification and breeding programs.

Keywords: Accessions, avocado, carotenoids, UPLC-MS, β-carotene

INTRODUCTION

Avocado is the only edible fruit species of the family Lauraceae and is now commercially grown in more than 80 countries worldwide. It is capable of thriving in a wide range of agro-climatic conditions from true tropical regions to the warmer parts of the temperate zone depending on the race and cultivar (Muralidhara et al., 2023a). Due to this broad ecological adaptability, the avocado has been widely distributed and cultivated in various parts of the world. Avocados are eaten fresh or used in the preparation many dishes like guacamole, ice creams, salads, soups and sandwiches (Muralidhara et al., 2025). The avocado is highly valued for its rich, creamy texture, distinctive flavor and for being an excellent source of energy, essential fatty acids and vital vitamins (Ford et al., 2023).

Additionally, it is a significant source of sterols, phenolics and pigments such as anthocyanins, chlorophylls and carotenoids (Muralidhara et al., 2023b; Dayi et al., 2025). Carotenoids are crucial for plant photosynthesis and photoprotection (Simkin et al., 2022) and phytohormone biosynthesis (Hossain et al., 2021). Their accumulation adds vibrant colour to plant parts, offering ecological and agronomic value (Gebregziabher et al., 2022; Melendez-Martinez et al., 2023). These carotenoids have significant effects on the prevention and/or reduction of disorders associated with reactive oxygen species (ROS), including some types of cancer, cardiovascular diseases and photosensitivity (Nuroini et al., 2023; Okobi et al., 2023). Lutein being the major carotenoids in avocado minimizes the oxidation of low-density lipoproteins, thereby reducing the risk of atherosclerosis, coronary disease and cerebrovascular disease (Ramos-Aguilar et al., 2019).

The carotenoids content and its composition will vary from genotype to genotype in fruit crops *viz*., avocado (Muralidhara et al., 2025), mango (Bhattacharjee et al., 2023), banana (Munoz et al., 2024), tea (Wang et al., 2010). In India, avocado is mainly grown as homestead crop or mixed crop in Karnataka, Kerala, Tamil Nadu and some parts of North-Eastern states

(Muralidhara et al., 2023a). The wide variability was reported in Indian avocados for morphological and biochemical traits due to natural hybridization (Muralidhara et al., 2023a; Muralidhara et al., 2025). There are few studies carried out in Mexican genotypes to know the variability for carotenoids composition (Mendez-Zuniga et al., 2019). The lutein, β-carotene, neoxanthin, lutein-5,6-epoxide and violaxanthin are the most abundant in the avocado pulp (Ford et al., 2023). Despite its richness in carotenoids, there is limited research on the carotenoid profile of avocados grown in India. To address this gap, the present study investigates the carotenoid profiling of twenty avocado accessions collected from two major avocado growing regions of India.

MATERIALS AND METHODS

The study was conducted at Division of Basic Science, ICAR–Indian Institute of Horticultural Research, Bengaluru during 2019-20 and 2020-21. A total of twenty Indian avocado accessions were collected from two avocado growing regions of South India namely, Karnataka (Kodagu and Chikkamagaluru) and Kerala (Wayanad and Idukki). In which, fourteen accessions were collected from Karnataka (PA-058, PA-062, PA-064, PA-068, PA-069, PA-070, PA-071, PA-074, PA-078, PA-082, PA-084, PA-085, PA-102 and PA-103) and six accessions were collected from Kerala (PA-091, PA-092, PA-093, PA-095, PA-097, PA-099).

Sampling and sample preparation

Avocado fruits were sampled and prepared for carotenoid profiling using the procedure given by Lu et al., (2009) with slight modifications. Ten fully mature fruits per accession were collected and kept for uniform ripening under room temperature. Subsequently, the pulp was extracted from five uniformly ripened fruits and 5 g of homogenized fresh pulp was used for estimation of carotenoids in each replication. All the extractions were performed under low-light conditions and the extracts were collected in a beaker. Extracts were transferred to a separating funnel, followed by the addition of 15 mL hexane, 50 mL distilled water and two spatulas of NaCl. The mixture was shaken thoroughly and allowed to stand for a few minutes to allow phase separation. Two phases were formed and upper portion was collected and lower aqueous phase was re-extracted with additional hexane, until the aqueous phase was colourless.

The entire upper layer was collected in a volumetric flask and traces of water was removed by adding anhydrous sodium sulphate (Na_2SO_4) and the final volume was adjusted to 25 mL with hexane. Ten mL of extract was taken and concentrated using vacuum pump and then 5 mL of methanolic potassium hydroxide (15% w/v) was added and saponified for one hour by vortexing using a vortex mixture. One mL of 10% NaCl was added to the saponified solution. Two layers were formed; top hexane layer was collected and washed with distilled water for 2-3 times repeatedly to make it alkali free and water traces were removed by adding anhydrous Na_2SO_4. Then extract was kept for drying at room temperature for 6-8 hours for drying. After complete drying 2 mL of mobile phase was added to it (acetonitrile: methanol: ethyl acetate/53:7:40). The supernatant was filtered with 0.2 µm nylon filter membrane and injected to UPLC-MS system (3.2.14.2 mobile phase solvent - A: acetonitrile: methanol: ethyl acetate (53:7:40) solvent-B: methanol).

LC and MS-MS conditions

Carotenoids profile was carried out by using UPLC method (Serino et al., 2009) with slight modifications. The acquity-UPLC system from the waters consists of a quaternary pump, an auto sampler injector and a PDA detector equipped with an acquity-UPLC+BEH-C18 column (1.7 µm, 2.1 × 50 mm) and a BEH-C18 (1.7 µm, 2.1 × 5 mm) guard column. The mobile phase comprises solvent A - [acetonitrile: methanol: ethyl acetate (53: 7: 40, v/v)] and solvent B - (methanol). For isocratic elution, the ratio of A: B is 95: 5 and a flow rate of 0.2 mL/min was maintained for 10 min, with PDA scanning from 200 to 700 nm. Individual carotenoids were identified on the basis of their diode array spectral characteristics, retention time and relative elution order in comparison to those of the standards and quantified as β-carotene equivalent.

Calibration procedure, validation parameters and quantification strategy

External calibration was performed using β-carotene standards in acetone (1–50 µg/mL; 5 points, each in duplicate). Calibration curves were constructed by plotting peak area versus concentration by linear least-squares regression ($R^2 \geq 0.995$). Carotenoids were quantified as µg β-carotene equivalents/g dry weight using β-carotene calibration curve. Method validation

Table 1 : Variability for total carotenoids and their composition in twenty Indian avocado accessions

Accession	Total carotenoids (mg/100 g)	Lutein (µg/g)	Neoxanthin (µg/g)	Violaxanthin (µg/g)	α-carotene (µg/g)	β-carotene (µg/g)	all trans lutein (µg/g)	Unknown (µg/g)	Neochrome (µg/g)	Chrysanthem axanthin (µg/g)
PA-058	2.13	2.89	0.94	0.83	1.27	1.97	1.46	1.82	1.59	0.86
PA-062	2.28	2.53	1.32	0.73	0.21	1.3	0.92	0.41	0.23	0.01
PA-064	2.14	3.61	1.79	1.78	0.95	1.39	0.79	1.71	1.41	0.62
PA-068	3.3	3.71	1.11	2.25	2.43	1.51	0.46	1.43	0.88	2.05
PA-069	3.35	6.31	3.25	2.74	2.47	2.61	2.09	1.21	2.24	0.81
PA-070	4.34	2.81	1.21	1.04	0.61	1.99	0.65	0.88	0.59	0.34
PA-071	2.61	6.65	1.88	1.91	0.52	2.01	1.9	2.01	1.05	0.2
PA-074	1.97	4.02	0.69	1.48	0.69	2.47	0.66	0.87	0.54	0.35
PA-078	3.43	7.09	4.24	2.21	1.21	2.34	1.56	2.28	1.36	0.47
PA-082	5.86	4.81	0.65	0.49	0.16	0.82	0.55	0.41	0.59	0.47
PA-084	4.61	6.56	2.11	1.78	0.75	1.99	1.28	0.89	1.73	0.62
PA-085	3.09	6.67	3.23	2.53	1.2	1.98	2.17	1.36	1.17	0.47
PA-091	3.46	5.98	3.35	1.5	0.61	2.28	0.67	1.82	0.86	1.27
PA-092	2.39	5.18	0.52	1.57	1.07	2.01	0.39	0.67	1.55	0.62
PA-093	2.37	3.85	0.7	1.02	0.92	2.13	0.29	0.44	0.62	0.49
PA-095	4.72	8.36	1.82	2.1	1.25	2.67	0.57	0.88	0.9	0.68
PA-097	1.93	8.03	0.47	0.91	0.29	1.15	0.43	0.56	0.58	0.13
PA-099	1.6	3.49	1.36	1.02	0.89	1.35	0.66	0.57	0.4	0.37
PA-102	3.24	5.32	2.86	2.54	1.06	1.86	1.44	2.7	1.17	2.4
PA-103	1.73	7.10	0.74	0.63	0.48	1.03	0.28	0.41	0.45	0.68
Mean	3.03	5.25	1.71	1.55	0.95	1.84	0.96	1.16	0.99	0.69
Minimum	1.60	2.53	0.47	0.49	0.16	0.82	0.28	0.41	0.23	0.01
Maximum	5.86	8.36	4.24	2.74	2.47	2.67	2.17	2.70	2.24	2.40
CD at 5%	0.23	0.66	0.201	0.18	0.14	0.31	0.18	0.22	0.13	0.13
CV (%)	4.54	6.03	5.64	5.53	7.05	7.95	9.03	8.81	6.37	8.61

confirmed linearity, intra-day precision (RSD <5%), inter-day precision (RSD <7%) and recovery (93% with RSD <5%). Due to the unavailability of individual standards, quantification relied on a single β-carotene reference, assuming similar molar absorptivity at the detection wavelength. Results are thus expressed as β-carotene equivalents, providing reliable comparative quantification

Statistical analysis

The 20 accessions were replicated twice and analyzed by using WASP 2.0 statistical software (Web Agri Statistical package) with completely randomized design. The principal component analysis (PCA) was done to know the relationships between the collected accessions for carotenoids profile by using GRAPES R statistics software. Cluster analysis of 20 accessions was performed by using 10 characters based on similarity index and dendrogram was constructed by unweighted pair-group method (UPGMA) in PAST statistics software (Hammer et al., 2001). The pearson correlation was analyzed to identify the association between different carotenoids components.

RESULTS AND DISCUSSION

Profiling of carotenoids by LCMS

Carotenoid profiling of twenty avocado accessions revealed a wide range of variability in both total carotenoid content and the composition of individual carotenoid compounds (Table 1). Nine major carotenoids were detected by UPLC-MS, namely, lutein, neoxanthin, violaxanthin, α-carotene, β-carotene, all-trans-lutein 5,6-epoxide, neochrome, chrysanthemaxanthin and one unidentified compound. Lutein was detected as predominant carotenoid, followed by β-carotene, neoxanthin and violaxanthin, whereas chrysanthemaxanthin exhibited the lowest concentration among the compounds analysed (Fig. 1).

The values are expressed in fresh weight basis. Among the twenty accessions studied, the content of lutein (2.53 to 8.36 µg/g), neoxanthin (0.47 to 4.24 µg/g), violaxanthin (0.49 to 2.74 µg/g), α-carotene (0.16 to 2.47 µg/g) and β-carotene (0.82 to 2.67 µg/g) varied significantly. Lutein minimizes the oxidation of low-density lipoproteins, thereby reducing the risk of atherosclerosis, coronary disease and cerebrovascular

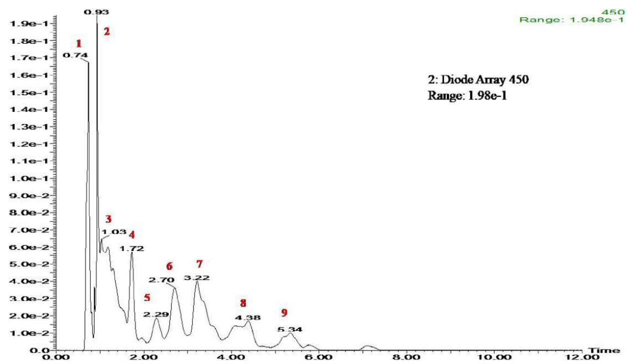

Fig. 1 : LCMS chromatogram of carotenoids profile of avocado accession PA-096 monitored at 450 nm. Peaks numbered according to the elution sequence 1. Lutein 2. Neoxanthin 3. Violaxanthin4. α-carotene 5. β-carotene 7. All trans lutein 5,6 epoxide 7. Unknown 8. Neochrome 9. Chrysanthemaxanthin

disease (Ramos-Aguilar et al., 2019). Lutein content was highest in accession PA-095 (8.36 µg/g), which was statistically on par with PA-097 (8.03 µg/g), followed by PA-103 (7.10 µg/g). In contrast, the lowest lutein content was recorded in accession PA-062 (2.53 µg/g). Similarly, Ford et al. (2023) reported that lutein content in Hass avocado fruit ranged from 1.40 to 8.42 µg/g. The present findings also align with Martinez-Damian et al. (2025), who observed lutein content between 0.78 and 7.13 µg/g across different avocado accessions. Lutein content was comparatively higher (11.2 to 11.7 µg/g) in dried powder of avocado fruit (Hong et al., 2022) and mango pulp (Bhattacharjee et al., 2023). The highest concentration of neoxanthin was observed in PA-078 (4.24 µg/g) and PA-097 showed the lowest content (0.47 µg/g). These values are comparable to those reported by Ford et al. (2023), in Hass variety. However, Hong et al. (2022) reported higher neoxanthin levels, ranging from 6.7 to 8.6 µg/g in dried powder of Hass avocado fruit. Similarly, Cervantes-Paz et al. (2023) reported neoxanthin a (0.80 µg/g DW) and neoxanthin b (1.33 µg/g DW) in Hass avocado.

Accession PA-069 exhibited high levels of violaxanthin (2.74 µg/g) and α-carotene content (2.47 µg/g). The maximum β-carotene content was recorded in accession PA-095 (2.67 µg/g) and which was on par with PA-069 (2.61 µg/g). Similarly, many researchers namely Rozan et al., (2021) with 1.43 µg/g, Martinez-Damian et al. (2025) 0.04-1.38 µg/g and Hong et al. (2022) with 0.6-0.8 µg/g of β-carotene content was reported. Dayi et al. (2025) recorded 0.63 µg/g in the Hass variety, while Cervantes-Paz et al. (2023) reported a lower value of 0.10 µg/g DW. Additionally, Muralidhara et al. (2023b) reported a unique avocado accession PA-026, with α-carotene and β-carotene content of 1.03 µg/g and 3.85 µg/g, respectively, further highlighting the influence of genotype on carotenoid levels. β-carotene aids the maintenance of visual health, healthy skin and mucous membranes as a vital compound in cosmetic and pharmaceutical products (Monge-Rojas & Campos, 2011). The violaxanthin content values are comparable to Ford et al. (2023), who reported 2.02 µg/g in fresh Hass pulp and Cervantes-Paz et al. (2023), who found 2.31 µg/g DW in Hass avocado. In contrast, Hong et al. (2022) reported higher levels, ranging from 4.4 to 4.8 µg/g in dried Hass powder. The accession PA-082 recorded the minimum content of violaxanthin (0.49 µg/g), α-carotene (0.16 µg/g) and β-carotene (0.82 µg/g) among all other twenty avocado accessions. Rozan et al. (2021) reported 0.58 µg/g α-carotene in avocado fruit, which falls within our range.

Among the twenty accessions studied, the contents of all-trans lutein (0.28 to 2.17 µg/g), unidentified compound (0.41 to 2.70 µg/g), neochrome (0.23 to 2.24 µg/g) and chrysanthemaxanthin (0.01 to 2.40 µg/g) exhibited significant variations. The highest all-trans lutein content was recorded in accession PA-085 (2.17 µg/g) and which was statistically on par with PA-069 (2.09 µg/g) and the lowest was reported in accession PA-103 (0.28 µg/g). The highest neochrome content was observed in PA-069 (2.24 µg/g), while PA-102 recorded the highest content of both the unknown compound (2.7 µg/g) and chrysanthemaxan-thin (2.4 µg/g), significantly exceeding all other accessions. Gross et al. (1973) first reported the presence of all-trans lutein, neochrome and chrysanthemaxanthin in avocado fruit. Lu et al. (2009) documented lutein-5,6-epoxide (0.75–5.78 µg/g), neochrome (0.37–1.45 µg/g) and chrysanthe-maxanthin (0.31–2.71 µg/g) in the Ventura variety of avocado. Ramos-Aguilar et al. (2021) reported 0.09 µg/g DW of lutein-5,6-epoxide in Hass avocado. Hong et al. (2022) found neochrome ranging from 1.0 to 1.1 µg/g in dried Hass avocado powder. Muralidhara et al. (2023b) reported 0.61 µg/g of all-trans lutein-5,6-epoxide, 0.44 µg/g of neochrome

Table 2 : *Per cent* contribution of variables on different principal components

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Total carotenoids	1.24	7.15	59.08	0.21	28.58	0.13	0.03
Lutein	2.67	30.17	5.94	0.00	50.47	2.92	1.41
Neoxanthin	14.77	4.75	0.05	10.06	0.31	2.95	13.55
Violaxanthin	18.52	0.10	0.15	0.70	3.31	0.13	9.66
α-carotene	10.19	15.18	0.05	23.25	0.69	3.59	14.73
β-carotene	9.52	1.96	1.19	19.04	0.39	54.75	6.47
All trans lutein	12.14	5.04	13.14	3.95	9.26	8.52	4.26
Unknown	12.69	2.95	0.91	28.98	0.03	2.69	13.66
Neochrome	13.14	0.01	1.39	8.41	2.44	24.28	34.66
Chrysanthemaxanthin	5.12	32.70	18.11	5.40	4.51	0.04	1.58
Eigen values	4.56	1.37	1.06	0.91	0.73	0.58	0.36
Variability (%)	45.64	13.74	10.63	9.14	7.34	5.79	3.60

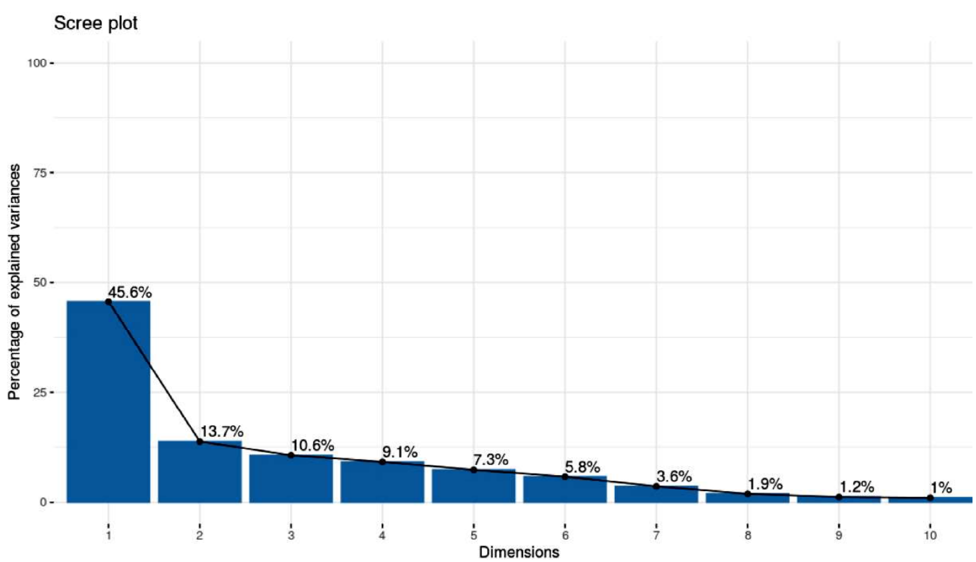

Fig. 2 : Scree plot of eigen values for carotenoids in Indian avocado accessions

and 0.15 µg/g of chrysanthemaxanthin in unique yellow avocado accession. In contrast, PA-103, PA-082 and PA-062 reported the lowest content of the unknown compound (0.41 µg/g). PA-062 also exhibited the lowest neochrome (0.23 µg/g) and chrysanthemaxanthin (0.01 µg/g) contents among all accessions.

Principal component analysis

PCA was performed using 10 traits of 20 avocado accessions collected from two major growing regions. In this study, first three components showed more than 1.0 eigen values (Fig. 1) The results revealed that the first three PCA components accounted for 70.01% of the total variation. This indicates a substantial variation among the studied accessions for various carotenoids (Table 2). The first component had an eigen value of 4.56, accounting for 45.64% of the total variation and was primarily associated with traits such as violaxanthin, neoxanthin, neochrome and all-trans lutein (Fig. 2). The second component had an eigen value of 1.37, accounting for 13.74% of the total variation and was mainly reported in chrysanthemaxanthin, lutein, α-carotene and an unknown compound. The third component had an eigen value of 1.06, accounting for 10.63% of the total variation, notably involving traits such as total carotenoids, chrysanthemaxanthin, all-trans lutein and lutein.

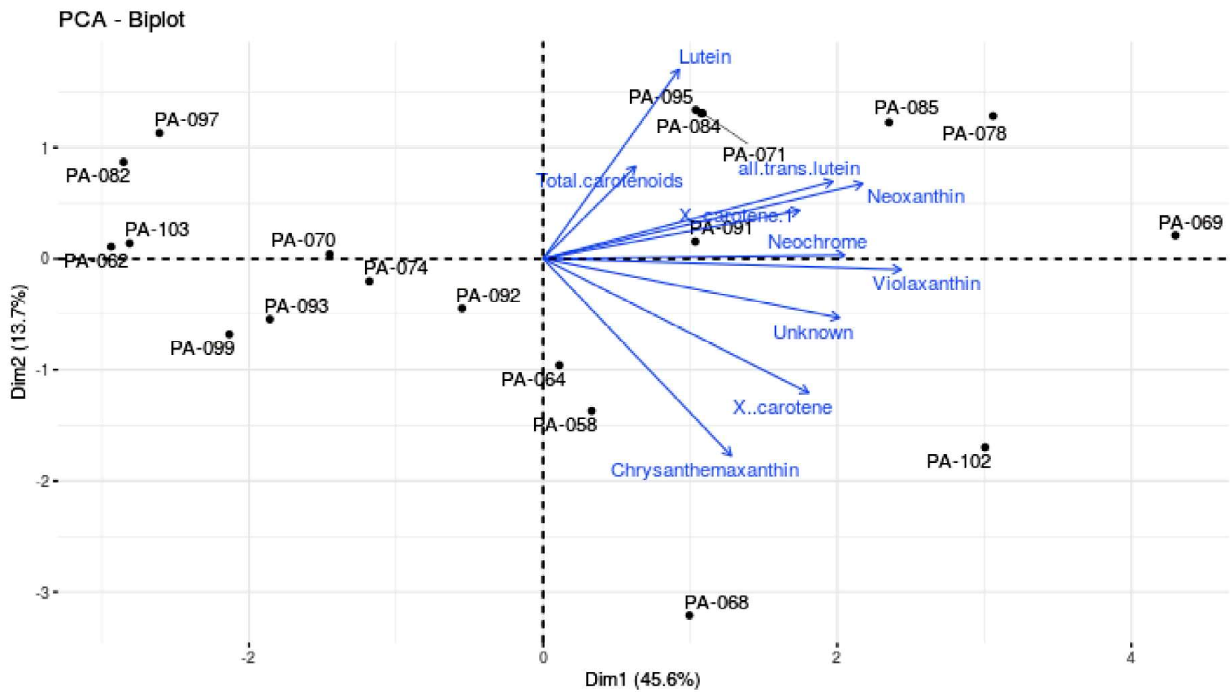

Fig. 3. Principal component analysis biplot of carotenoids in Indian avocado accessions

PCA biplot revealed clear distinctions among the accessions, as evidenced by their dispersion across all four quadrants of the plot (Fig. 3). The accession PA 069, positioned farthest from the centre of the biplot, was identified as the most distinct among the evaluated accessions. It showed promising levels for several traits, including violaxanthin, α-carotene, neochrome, β-carotene, all-trans lutein and neoxanthin. Among the assessed traits, chrysanthemaxanthin, lutein, violaxanthin and neoxanthin exhibited relatively long vector length in the biplot, indicating their substantial contribution to the observed diversity among the analysed avocado accessions. Cervantes-Paz et al. (2023) similarly reported that the first two PCA components explained 78.9% of carotenoid-related variation in avocado, while Martinez-Damian et al. (2025) found carotenoids like lutein and β-carotene critical in clustering genotypes alongside other nutritional traits in avocado.

Correlation studies

Highly significant positive correlations were observed within the evaluated accessions (Table 3). The findings revealed an intricate network of relationships among the carotenoids, with significant correlations offering valuable insights into their physiological interactions. The traits such as violaxanthin and neoxanthin, α-carotene and violaxanthin, β-carotene and violaxanthin, all trans lutein and neoxanthin, β-carotene and α-carotene have showed highly significant positive correlation. Similarly, Sodedji et al. (2022) reported significant variation and strong positive correlations among carotenoids (lutein, zeaxanthin and β-carotene), with accessions clustering into three groups based on their carotenoid profiles and C044 exhibiting the highest carotenoid content in cowpea sprouts.

Cluster analysis

The dendrogram, constructed from 10 quantitative traits, classified the accessions into two main clusters (Fig. 4). Cluster I was further divided into two subgroups, with subgroup I-A contained two accession (PA-097 and PA 103) and subgroup I-B consisting of eight accessions namely, PA 102, PA 069, PA 078, PA 085, PA 071, PA 091, PA 084 and PA 095. Cluster II was further divided into two subgroups, with subgroup II-A contained nine accessions *viz*., PA 070, PA 074, PA 093, PA 099, PA 062, PA 064, PA 058, PA 092 and PA 082. Subgroup II-B was found to consist of only one accession namely, PA 082. This highlights genetic relationships among accessions, aiding trait identification for future plant breeding studies. These finding aligns with the findings of Martinez-Damian et al. (2025), who reported that carotenoids particularly, lutein and β-carotene significantly

Table 3 : Correlation matrix of various carotenoids in Indian avocado accessions

Variable	Total carotenoids	Lutein	Neox anthin	Violax anthin	α-carotene	β-carotene	all translutein	Un known	Neo chrome	Chrysanthe-maxanthin
Total carotenoids	1.0									
Lutein	0.21	1.0								
Neoxanthin	0.25	0.37	1.0							
Violaxanthin	0.18	0.37	0.71***	1.0						
α-carotene	0.04	0.01	0.34	0.69***	1.0					
β-carotene	0.04	0.01	0.34	0.69***	1***	1.0				
all trans lutein	0.09	0.21	0.70***	0.62**	0.33	0.33	1.0			
Unknown	0.04	0.09	0.63**	0.61**	0.29	0.28	0.54*	1.0		
Neochrome	0.16	0.23	0.48*	0.63**	0.60**	0.60**	0.61**	0.42	1.0	
Chrysanthemaxanthin	0.15	-0.04	0.26	0.47*	0.52*	0.52*	0.04	0.65**	0.25	1.0

*** Correlation is significant at 0.001 level (two tailed); ** Correlation is significant at 0.01 level (two tailed); * Correlation is significant at 0.05 level (two tailed)

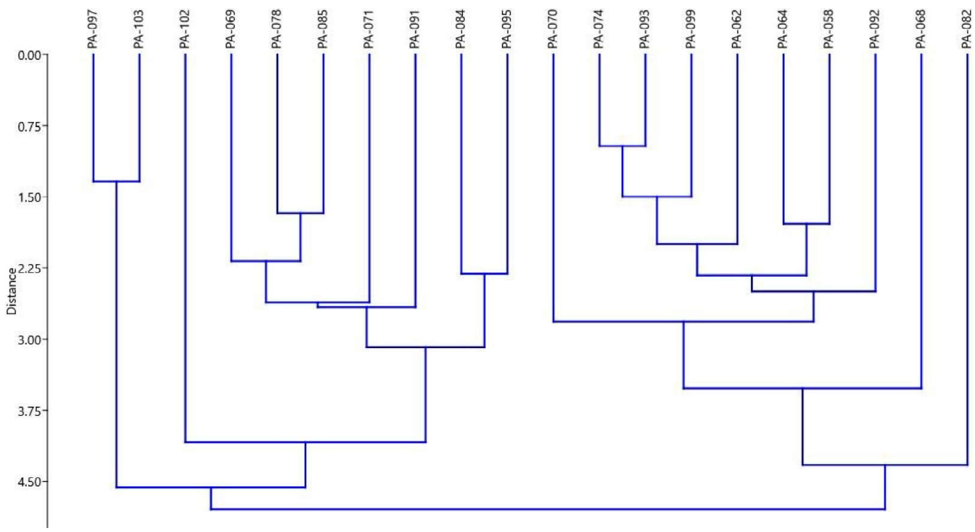

Fig. 4 : UPGMA dendrogram clustering of the studied 20 Indian avocado accessions

contributed to principal component variation and were more prominent in S2 genotypes, underscoring their role in cluster differentiation in avocado.

CONCLUSION

In present study, the carotenoid profiles of twenty Indian avocado accessions were analyzed using UPLC-MS, revealing their high nutritional value. The results showed high levels of lutein, followed by β-carotene, neoxanthin, violaxanthin and chrysanthemaxanthin. Among the accessions, the highest lutein (8.36 µg/g) and β-carotene (2.67 µg/g) were found in PA-095; PA-078 had the highest neoxanthin (4.24 µg/g); PA-069 recorded the highest violaxanthin (2.74 µg/g), α-carotene (2.47 µg/g) and neochrome (2.24 µg/g); PA-085 showed the highest all-trans lutein (2.17 µg/g); and PA-102 had the highest chrysanthemaxanthin (2.40 µg/g). These findings demonstrate substantial variation in carotenoid composition across accessions. This variation can guide the selection of nutritionally rich avocado varieties. The study offers valuable insights for breeding and promoting health-focused avocado consumption.

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