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Chemometric Modeling Revealed Oleic and Linoleic Acids as Varietal Biomarkers for Six Sesame Varieties—In Vitro and UHPLC Analyses

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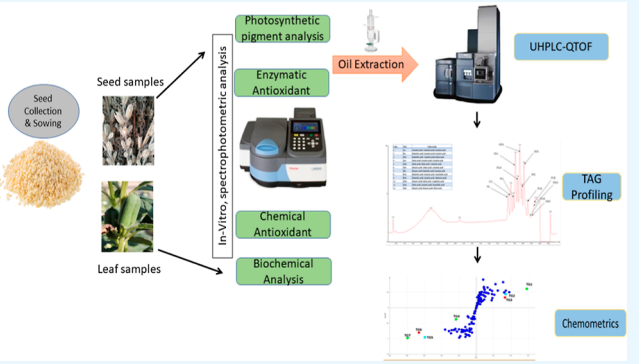
ABSTRACT: Pakistan once considered self-sufficient for edible oil production now became the major importer with 88.6% imports and producing only the minor portion. Scientific negligence in oil seed crops led to a dramatic decrease in edible oil production depending mainly on only the imports. *Sesamum indicum* L., "Queen of Oil seeds" with 50−55% oil, is cultivated in various geographical regions of Pakistan, but farmers are not considering this crop because of insufficient knowledge, poor crop management practices, and low yielding varieties. This study was conducted to check the nutritional, biochemical, antioxidant, and yield potentials of six major varieties, i.e., TS-5, TH-6, Til-18, NIAB-Mil, NIAB-Pearl, and NS-16, and to compare the nutritionals, oil quality, and oil yield potential of these varieties. Field experiment was conducted, and various crop growth biomarkers were analyzed. Chlorophyll content and superoxide dismutase activity were found to be highest in NIAB-Mil followed by NIAB-Pearl and comparable to those of Til-18, while APX, Cat, and GPX activity was found to be highest in Til-18 with 25.6 and 5.9 and 6.02 mg/g, respectively. Seed antioxidant parameters showed a mixed response, but NIAB-Mil, NIAB pearl, and Til-18 were found to be highest in all antioxidant parameters. UHPLC analysis of seed oil resulted in a total of 14 triacylglycerols (TAGs), and principal component analysis and OPLS-Da analysis showed seven TAG biomarkers responsible for the separation of sesame varieties. Til-18 was found to be highest in oil content (53.3%) more abundant with oleic acid, while NIAB-Pearl, NIAB-Mil, and NS-16 were found to be abundant with linoleic acid, both considered as potential TAG biomarkers for sesame oil separation. This study concluded that, in general, Til-18 variety is more resistant with high nutritional status, high antioxidant activity, and oil yielding variety, followed by NIAB-Mil and NIAB-Pearl.



1. INTRODUCTION

The earliest oilseed crop to be cultivated, sesame (*Sesamum indicum*), is prized for its edible seeds and oil that are consumed as food items and have a number of health advantages (Figure 1A−G). From ancient Anatolia to the Bronze Age's Indus Valley Civilization in South Asia, it has been used, and more recently, it has been domesticated in the Indian subcontinent.[1]

Sesame is grown on 14 million hectares of land worldwide, yielding an average of 487 kg/hectare and an annual production of 6.8 million tons (FAOSTAT, 2020). According to FAOSTAT (2020), the gross production value of sesame seeds in 2018 was 3.8 billion US dollars (consistent at prices from 2014 to 2016). Top sesame producers in 2020 included Sudan, Myanmar, Tanzania, India, Nigeria, and China. The sesame seed oil market is projected to expand at a compound annual growth rate of 1.7% on a global scale between 2021 and 2026.[2] Due to higher oil content, it is a potential oil seed crop to meet the rising demand of vegetable oil consumption.

Medicinal and nutraceutical properties of sesame seeds and oil due to the presence of polyunsaturated fatty acids make it a high-quality edible oil. This fatty acid constitution of sesame contributes to many important biochemical pathways and is associated with hypolipidemic, cardioprotective, and anti-atherogenic effects.

As a result, fatty acids made from sesame oil may serve as functional foods and nutraceuticals, providing dietary and

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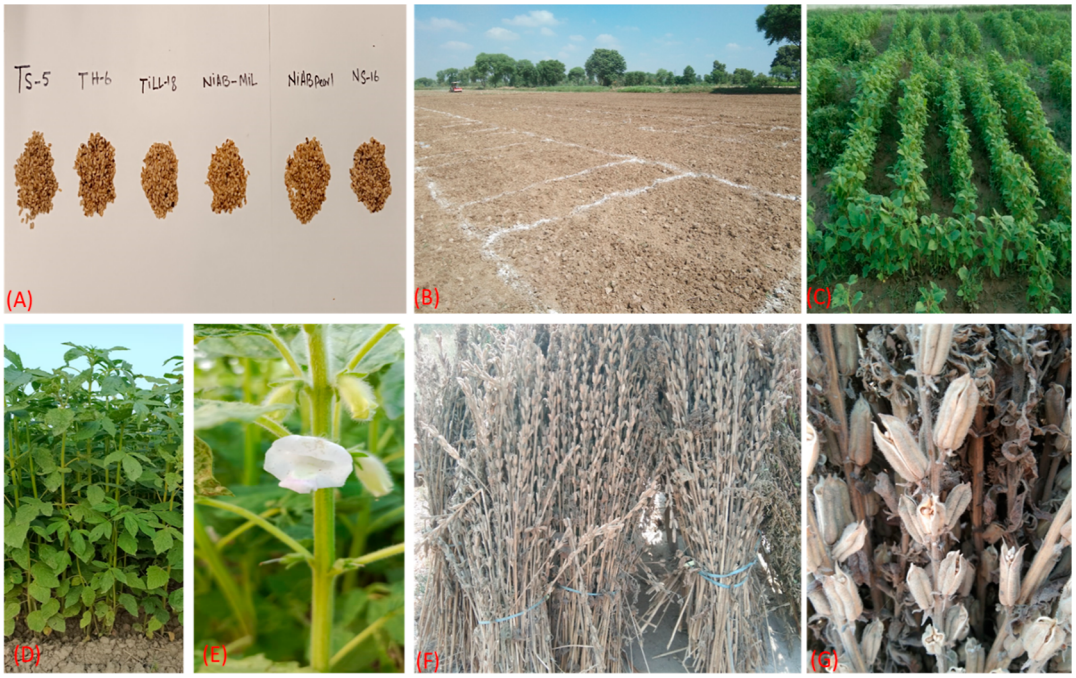

Figure 1. (A) Seeds' six varieties collected from the Ayub Agriculture Research Station and National Agriculture Research Centre (NARC), Pakistan. (B) Land preparation and crop sowing. (C) Crop after 40 days of germination. (D,E) Crop at the flowering stage. (F,G) Crop harvesting and threshing.

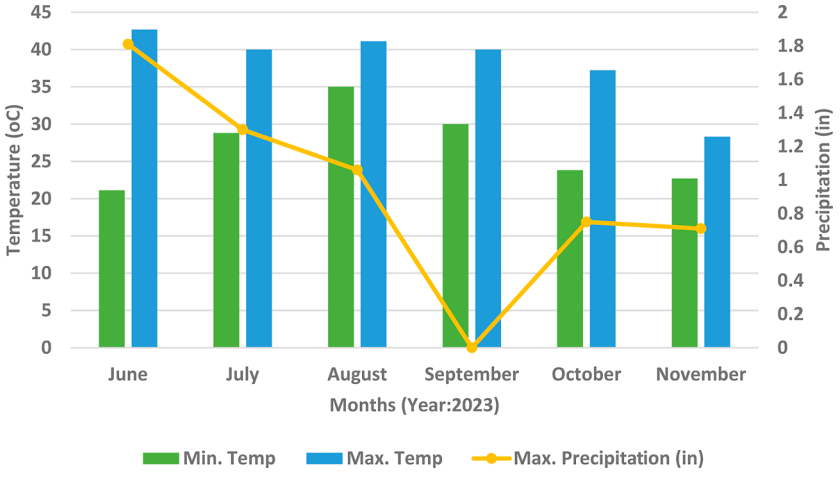

Figure 2. Minimum and maximum temperature and average precipitation during the period of crop growth.

physiological advantages together with pleasing flavor and scent. Sesame oil comprises natural antioxidants such as sesamolin, sesamin, and tocopherol homologues in addition to PUFA.[3−5] Antioxidants scavenge free radicals produced during metabolic processes, protecting cells from a variety of ailments. The food processing industries use a variety of synthetic antioxidants, such as butylated hydroxyl-anisole (BHA) and butylated hydroxyl-toluene (BHT), extensively to minimize food degradation because they can stabilize free radicals. There are studies on the use of olive and sesame oils as natural antioxidants.[4,6]

Pakistan once considered self-sufficient in edible oil production in the 1970s and 1980s is now becoming the third largest importer after China and India by importing 87% of the edible oil requirements, with only 13% of local production. Edible oil is also the third major import after petrochemicals and machinery, costing 3.068 billion USD in 2020. It is projected that 5% increase in edible oil consumption and average 5% price hike in global markets every year will increase the import bill to Rs. 757 billion in 2025, resulting in high pressure on the dangling Pakistan economy.[7]

Current situation has been emphasized to enhance local cultivation of oil seed crops. Rapeseed, mustard, and sunflower are the major oil crops for local use, while sesame has the potential of being locally used as well as great export potential, making it more resistant to market fluctuations. Sesame is being cultivated in both rainfed and irrigated areas in all major districts in Pakistan. However, at the same time, there are some major constraints like less availability of the quality seeds, lack of machinery, vulnerability to disease attack, marginal land cultivation, and poor crop management, which lead to the lower yield of sesame. Out of all these, availability of quality seeds depending upon the area of cultivation (rainfed or irrigated) is the major concern of farmers as either the seeds

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are of low quality or they are very expensive, forcing farmers to not use the approved varieties. Ultimately, crop production and net income depend upon the quality of seeds, which often results in low yield. Pakistan being one of the largest sesame growers and exporters, several varieties are being cultivated in various areas depending upon the geographical region and climatic conditions.[8] With high demand market and adaptation to environmental stresses, sesame production has increased year to year. At the same time, there is a dire need to have a good knowledge about the nutritional profile, yield potential, and stress resistance of the different germplasms to get higher production and profit.[9] Metabolomics deals with the assessment of large data sets, which is difficult to interpret, without employing multivariate and chemometric analyses, which by using mathematical and statistic techniques determines the properties of the extracts, mostly giving the variations among the different samples.[10] This study was employed to enumerate biochemical, physiological, and metabolic profiles coupled with chemometrics to check the metabolic variations among six major sesame varieties cultivated in Pakistan, to get more insights into their potential to be selected by farmers. To the best of our knowledge, this is the first detailed study about the morpho-physiological, biochemical, physiological, antioxidant, and oil metabolic profiles of major sesame varieties grown in Pakistan.

2. MATERIALS AND METHODS

2.1. Experimental Site. Field experiment was performed at the Pakistan Agricultural Research Centre (PARC), Dera Ismail Khan. Soil was ploughed three times followed by rotavator machine one time, making soil more suitable for the crop. A well-drained field was selected with appropriate soil conditions for the crop. Meteorological data during the experiment is given in Figure 2.

2.2. Seed Collection and Crop Husbandry. Six commonly cultivated sesame varieties among farmers, i.e., TS-5, TH-6, Til-18, NIAB-Pearl, NIAB Milenium, and NS-16, in different regions of Pakistan were obtained from the Ayub Agriculture Research Institute and Oil Seed Program, National Agriculture Research Council (NARC), Pakistan. Seeds were surface sterilized with 05% sodium hypochlorite to remove any sort of life on the seeds. A sandy loam soil with a pH of 7.3 was chosen for the crop in a well-drained field. Line-to-line distance was maintained at 55 cm, with a plant-to-plant distance of 15 cm and a block-to-block distance of 3 ft as sowing was done in triplicate in a randomized manner. To achieve a more precise and controlled sowing, the crop was sowed using a hand drill. All of the varieties were sown at the proper time; there was a maximum of 15 days variation between the early and late sowing times for each variety. Irrigation and fertilizer practices were done as recommended for the crop, and data was collected at maturity. NPK fertilizer (15:15:15) was added at the planting stage and was mixed thoroughly to make the soil homogeneous for all the experimental units. Fresh leaf samples were collected in sterilized plastic bags for physiological and antioxidant analyses, and the crop was harvested and threshed at the end of the life cycle. Harvesting and threshing were done with hands to reduce seed loss and ensure accurate measurements. Seeds were stored at appropriate temperature until oil extraction and further analysis (Figure 1).

2.3. Morpho-Agronomic Traits. Data on morphological and agronomic parameters were recorded, including the plant height, the number of capsules per plant, the number of seeds per capsule, and the weight of 1000 seeds. Three batches of 1000 seeds each were counted using a seed counter and weighed on an electrical balance, and the mean weight was determined in order to obtain the weight of 1000 seeds. Batches of bulk seeds were taken.

2.4. Chlorophyll Content. The procedure described by[11] was applied in order to measure chlorophyll *a*, *b* and total chlorophyll. A spectrophotometer was used to measure the absorbance at 645 and 663 nm after 1 g of the freshly crushed leaf material, 20 mL of 80% acetone, and 0.5 g of magnesium carbonate ($MgCO_3$) were added. The 80% acetone solution was used as a blank. Applying the following formula, the amount of chlorophyll *a*, *b* and total chlorophyll content in mg g-1 was calculated.

$$\text{Chlorophyll } a = 12.7\,(A663) - 2.69\,(A645) \times \frac{V}{1000} \times W \quad (1)$$

$$\text{Chlorophyll } b = 22.9\,(A645) - 4.68\,(A663) \times \frac{V}{1000} \times W \quad (2)$$

$$\text{Total Chlorophyll} = 20.2\,(A645) + 8.02\,(A663) \times \frac{V}{1000} \times W \quad (3)$$

where W is the fresh weight of the sample taken for analysis; V the final volume of the chlorophyll extract in 80% acetone; and A the absorbance.

2.5. Plant Extract for Enzymatic Analysis. Freshly harvested leaf samples were crushed and homogenized in 50 mM (pH:07) phosphate buffer solution (5 mL), centrifuged at 12,000 rpm at 4 °C for 20 min, and the supernatant was used for further antioxidant enzymatic analysis.

2.6. Superoxide Dismutase (SOD), Peroxidase (POD), and Catalase (CAT) Activities. SOD activity was assessed following the procedure outlined in ref 12. A mixture consisting of 3 mL, comprising 63 mM nitroblue tetrazolium chloride, 13 mM methionine, 50 mM phosphate buffer, 1.4 mM riboflavin, and 50 mL of enzyme extract, was incubated for 15 min. Absorbance at 560 nm was then measured. The Chance and Maehly method was used to estimate the POD activity.[13] The mixture included an enzyme extract, 40 mM hydrogen peroxide, 50 mM sodium phosphate buffer (7.8 pH), and 20 mM guaiacol. At 470 nm, the absorbance of POD was recorded. For CAT activity determination, a reaction mixture comprising 3 mL of (50 mM PBS, 15 mM H_2O_2) was combined with 50 mL of enzyme extract. The reaction was started upon addition of 100 μL of the extract, and the reduction in H_2O_2 absorbance was monitored at 240 nm.[14]

2.7. Ascorbate Peroxidase (APx) and Glutathione Peroxidase (GSx) Activities and Lipid Peroxidation. APX activity was assessed following the protocol outlined in ref 15. Each leaf sample (1 g) was ground in an extraction solution comprising phosphate-buffered saline (PBS) (50 mM), ascorbate (2 mM), and EDTA (5 mM), vigorously milled to enhance extraction efficiency, and then centrifuged at 13,000 rpm for 15 min. The decrease in the absorbance of ascorbate at 290 nm served as an indicator of APX activity. One unit of APX activity was defined as the enzyme required to catalyze the oxidation of 1 mmol of ascorbate per minute. Glutathione peroxidase activity in sesame leaf samples was determined

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using the method described in ref 15. Lipid peroxidation, a crucial parameter for assessing the integrity and stability of biological membranes, was evaluated by measuring the level of malondialdehyde (MDA) produced via a thiobarbituric acid assay. Approximately 1 g of the plant material was extracted with 5 mL of 1% trichloroacetic acid, followed by centrifugation at 13,000 rpm for 15 min. One mL of the supernatant was mixed with 4 mL of 20% thiobarbituric acid solution. The mixture was then heated at 95 °C for 30 min, and the reaction was halted by cooling on ice for 30 min. Absorbance was measured at 450, 532, and 600 nm, and the MDA content was calculated using the specified formula.[16]

2.8. Seed Antioxidant Analysis. Sesame seeds were milled in a coffee mixer to get a fine powder, and 0.1 mg of the samples was homogenized in 1 mL of methanol and vortexed for 30 min, followed by sonication and centrifugation at 13,000 rpm. The supernatant was filtered with Whatman filter paper to remove any sort of seed material stored at −20 °C until further antioxidant analysis.

2.9. Trolox Equivalent Antioxidant Activity. To quantify the trolox equivalent antioxidant (TEAC) activity, 7 mM $ABTS^{\bullet}+$ was prepared through reaction, 2.45 mM potassium persulfate was added to ABTS stock solution in water, and the mixture was allowed to react for 16 h under dark conditions. After that, 100% ethanol was added to the diluted $ABTS^{\bullet}+$ solution until the absorbance reached 0.70 ± 0.02 at 734 nm. In summary, 20 μL of sesame seed extract and 500 μL of $ABTS^{\bullet}+$ solution were combined, and the mixture was incubated for 6 min at room temperature before the absorbance at 734 nm was measured. To create a standard curve, a series of concentrations of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were made.[17]

2.10. ABTS Activity. A previously synthesized $ABTS^{\bullet}+$ radical was utilized to measure the % radical scavenging activity of sesame seed extract samples. In the experiment, 2.9 mL of $ABTS^{\bullet}+$ solution was combined with 100 μL of seed extract with 100% ethanol acting as the control. Using a Spectramax 250 microplate reader,[18] absorbance was measured at 734 nm after all samples were incubated for 6 min at room temperature in complete darkness. To compute the percent inhibitory activity, the following formula was used.

$$\text{ABTS percent inhibition} = \text{Ac} - \frac{\text{As}}{\text{Ac}} \times 100$$

where Ac is the absorbance of control and As is the absorbance of the sample.

2.11. DPPH Activity. By adapting the procedure of ref 19, the radical scavenging activity of the sesame seed samples was assessed using 0.1 M of DPPH solution. In summary, 275 μL of DPPH solution was combined with around 25 μL of each sample's seed extract, and the mixture was then incubated at 25 °C for 30 min in dark conditions. The absorbance at 517 nm was measured, and the results were reported as a percentage of radical scavenging activity.

2.12. Reducing Power Activity (RPA). About 25 μL of 0.2 M PBS solution with an adjusted pH of 6.6 was combined with around 10 μL of a sesame seed extract from each sample. Next, 25 μL of $C_6N_6FeK_3$ was added, and the mixture was incubated for 20 min at 25 °C. After adding 25 μL of trichloroacetic acid to halt the reaction, 8.5 μL of iron(III) chloride and 85 μL of distilled water were added. For the standard curve, standard solutions with various ascorbic acid concentrations were also prepared. Ascorbic acid equivalent per gram (AAE/g) was the result of measuring the absorbance of all the samples and the standard at 750 nm.[18]

2.13. Total Phenolic Content. The Folin-Ciocalteu reagent method[20] was used to determine the total phenolic content of samples of sesame seeds. 200 μL of deionized water was added to 25 μL of sesame seed extract samples, along with 25 μL of the Folin-Ciocalteu reagent. After 5 min of incubation at 25 °C, 25 μL of 10% sodium carbonate solution was added, and the mixture was then incubated for 60 min in the dark. At 765 nm, the sample solution's absorbance was measured. The standard curve was created using various known concentrations of gallic acid, and total phenolic content (TPC) was expressed as gallic acid equivalent (GAE/g of fresh weight).

2.14. Percent Oil Yield. To obtain sesame oil, 5 mL of hexane was added to 1 g of sesame seed powder. The mixture was then vortexed and placed on a magnetic stirrer at 38 °C for 20 min. Afterward, the solution was centrifuged at 13,000 rpm for 15 min, and the supernatant was dried with nitrogen to obtain the oil fraction. This resulting oil was further centrifuged to eliminate any debris or plant material. Finally, the weight of the oil obtained from 1 g of sesame seed powder was measured, and the oil percentage was calculated using the provided formula.

$$\text{Oil Percentage} = \frac{\text{Weight of Oil}}{\text{Sample Weight}} \times 100$$

2.15. Triacylglycerol Analysis of Sesame Oil Samples. All oil samples underwent triacylglycerol (TAG) analysis via the liquid chromatography-mass spectrometry (LC-MS) method. First, the samples were diluted by a factor of 1 million with n-butanol containing 0.1 μg of tripentadecanoin (TG) as an internal standard and then transferred to LC vials. For TAG profiling, a 5 μL volume of the diluted oil sample was injected into an ultraperformance liquid chromatography (UPLC, Waters) system and separated using a BEH C8 column with dimensions of 2.1 mm × 50 mm and a particle size of 1.7 μm. The mobile phase consisted of two components: A, a mixture of water and acetonitrile in a ratio of 60:40 (v/v) with 10 mmol/L ammonium formate and 0.1% formic acid; and B, methanol with 10 mmol/L ammonium formate and 0.1% formic acid. The separation process was carried out at 60 °C for 10 min. The eluent was introduced into the Xevo-G2-S QTOF for ionization and MS analysis. Ammonium adducts of TAGs were detected in positive mode with an energy setting of 30 V and a cone and capillary voltage of 3 kV for electrospray ionization. The source and desolvation temperatures were set at 120 and 350 °C, respectively. Nitrogen served as both the desolvation and cone gas, with flow rates of 50 and 600 L/h, respectively, while argon was used as the collision gas. To ensure accurate mass measurement, calibration was performed using a sodium formate solution within an m/z range of 50−1500, alongside intermittent injection of lockspray leucine enkephalin with ([M + H] + = m/z 556.2771). For each detected TAG, accurate mass measurement was conducted using an appropriate database, elemental composition analysis, and tandem mass spectrometry (MS/MS) fragmentation with collision energies ranging from 10 to 60 eV. The concentration of all TAG species was determined by calculating their relative abundances in all samples.

2.16. Statistical Analysis. All the data was collected in triplicate, and the mean values were represented in the

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Table 1. Morphological and Agronomical Traits of Different Sesame Varieties

varieties	plant height	no. of capsules	no. of seeds	1000 seed weight	oil %
TS-5	100.95 ± 3.66	127 ± 4.24	59 ± 4.95	4.66 ± 0.0566	49.1 ± 1.12
TH-6	102.6 ± 5.09	100 ± 1.41	53 ± 5.66	4.335 ± 0.0354	51.1 ± 1.22
TIL-18	110 ± 3.39	188.5 ± 7.78	72 ± 4.95	5.095 ± 0.0212	53.3 ± 1.33
NIAB-MIL	108.45 ± 5.3	142 ± 7.07	54 ± 4.95	4.43 ± 0.0707	50.1 ± 1.94
NIAB-PEARL	109.7 ± 8.63	156 ± 2.12	63 ± 6.36	4.835 ± 0.0354	51.1 ± 2.44
NS-16	100.7 ± 3.54	115 ± 4.95	62 ± 1.41	4.885 ± 0.0354	50.3 ± 2.44

Table 2. Proximate Composition of Six Sesame Varieties

variety	crude fat	crude ash	crude fiber	crude protein	carbohydrate
TS-5	49.1 ± 1.27	4.21 ± 0.031	4.29 ± 0.324	27.81 ± 1.213	14.59 ± 1.221
TH-6	51.1 ± 0.93	3.92 ± 0.312	4.15 ± 0.791	26.55 ± 0.930	14.28 ± 0.0712
TIL-18	53.7 ± 1.27	3.27 ± 0.0391	3.83 ± 0.093	24.73 ± 0.871	14.07 ± 0.093
NIAB-MIL	50.1 ± 1.92	4.62 ± 0.481	4.63 ± 0.527	24.89 ± 1.288	15.74 ± 0.883
NIAB-PEARL	51.1 ± 2.12	4.37 ± 0.093	4.48 ± 0.882	25.57 ± 1.771	14.38 ± 1.29
NS-16	50.3 ± 2.33	4.28 ± 0.119	4.7 ± 0.993	25.27 ± 1.992	15.43 ± 1.661

graphical form with standard deviations. Analysis of variance was performed using Minitab, and mean variations were represented among the groups.

3. RESULTS

3.1. Morphological and Agronomical Traits. The results of morphological parameters indicated that sesame varieties differ significantly in morphological traits, similar to the plant height ranging from 100.7 cm in NS-16 to 110 cm in Til-18; a similar case was observed in a number of capsules/plant: the lowest capsule number (115 capsule/plant) was found in the NS-16 variety, while the highest capsules/plant values were observed in Til-18 with 188 capsules/plant. The number of seeds per capsule also varied significantly as Til-18 was found to have the highest (72) number of seeds/capsules compared to the lowest value (53) noted in TH-6; similar results were obtained for 1000 seed weight, as the highest 1000 seed weight was noted in Til-18 with 5.095 g, suggesting a strong correlation between morphological and agronomical traits, as improved morphological traits result in increased agronomical traits. Furthermore, as sesame is mainly cultivated for its highest oil percentage and superior oil quality, oil percentage also varied between different varieties. It was observed that similar to morphology and agronomical traits, the Til-18 variety showed the highest oil content 53.3%, followed by 51.1, 51.1, 50.8, 50.1, and 49.1% in NIAB-Pearl, TH-6, NS-16, NIAB-Mil, and TS-5, respectively. Oil percentage followed the order Til-18 < NIAB-Pearl < TH-6 < NS-16 < NIAB-Mil < TS-5 (Tables 1 and 2).

3.2. Chlorophyll Content. There is frequently a strong correlation between the amount of chlorophyll in leaves, their photosynthetic ability, and the activity of RuBP carboxylase.[21,22] Low photosynthetic rates are usually correlated with low leaf chlorophyll content, which in turn affects the grain filling process and crop yield. It was noted that the chlorophyll content varied significantly among sesame varieties; the chlorophyll content varied from 9.16 mg/g of F.W in Til-18 to 13.07 mg/g of F.W in NIAB-Pearl. Similarly, chl b was found to be highest in NIAB-Mil with 11.74 mg/g of F.W compared to the lowest values found in NS-16 with 5.0134 mg/g of F.W. Furthermore, the total chlorophyll content was found to be highest in NIAB-Pearl with a content of 21.7076 mg/g of F.W followed by NIAB-Mil with 21.5564. As far as the chlorophyll content is concerned, NIAB-Mil and NIAB-Pearl were found to have higher chl *a*, chl *b*, and total chl content compared to the other varieties, while TS-5 and Til-18 were found to have lesser chl content (Figure 3).

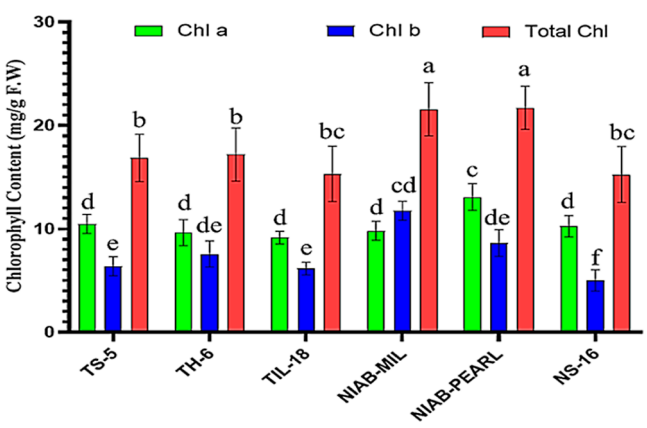

Figure 3. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll content of different sesame varieties analyzed by the spectrophotometric method.

3.3. Malondialdehyde (MDA) Content. For quite some time, researchers have relied on measuring the MDA content as an indicator of lipid peroxidation in studies concerning oxidative stress and redox signaling. This method is particularly prominent in investigations exploring how plants respond to both abiotic and biotic stresses. Higher MDA content means the plant is under oxidative stress, resulting in a lipid peroxidation and higher MDA content. It was noted that the MDA content was highest in NS-16 (16.83 μm/L) followed by NIAB-Mil with 16.74 μm/L, while the lowest concentration of MDA was found in the Til-18 variety with 10.99 hm/L of MDA.

3.4. Antioxidant Potential. Enzymatic antioxidant parameters are widely used to check the antioxidant status of the plant, demonstrating the efficacy to cope with a variety of stresses. It was found that the highest values for SOD activity were found in NIAB-Mil with 4.38 mg/g, followed by 3.92 mg/g in NIAB-Pearl. Similarly, peroxidase activity was found to be highest in NS-16(4.82 mg/g) followed by NIAB-Mil with 4.70 mg/g. Catalase activity was shown to be highest in Til-18

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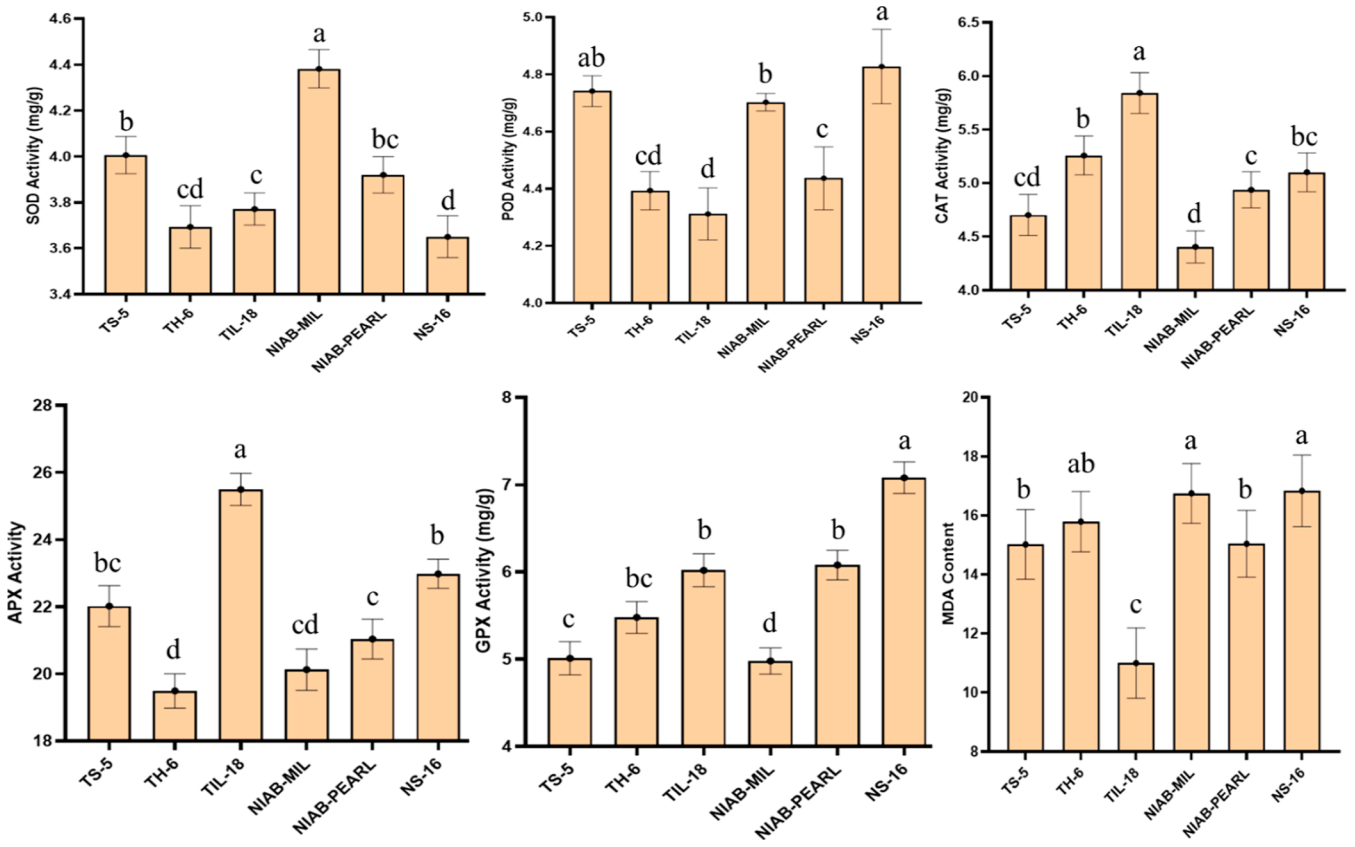

Figure 4. Enzymatic antioxidant potential of the leaf extract of six sesame varieties cultivated in Pakistan.

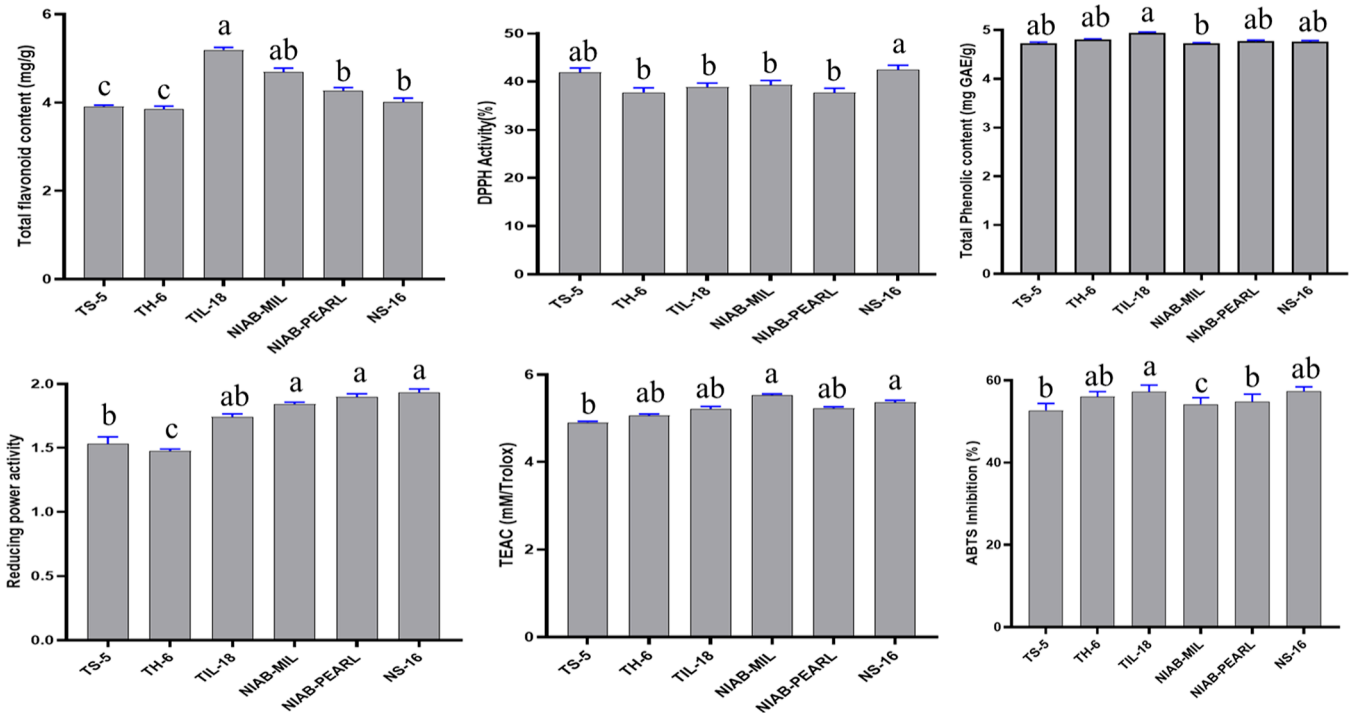

Figure 5. Seed antioxidant potential of six sesame varieties cultivated in Pakistan.

with 5.84 mg/g, followed by TH-6 (5.25), NS-16 (5.10), NIAB-Pearl (4.93), NIAB-Mil (4.40), and TS-5 (4.70). Similar trend was shown by APX activity as the highest APX activity was shown in Til-18 with 25.49 mg/g and lowest in TH-6 with 19.48 mg/g. This trend in enzymatic activities showed that different varieties have different levels of antioxidant enzymes, which, more probably, may be the cause of the ability of these varieties to cope against the stress. NIAB-Mil, NIAB-Pearl, and Til-18 are widely cultivated in the warmer areas of Pakistan

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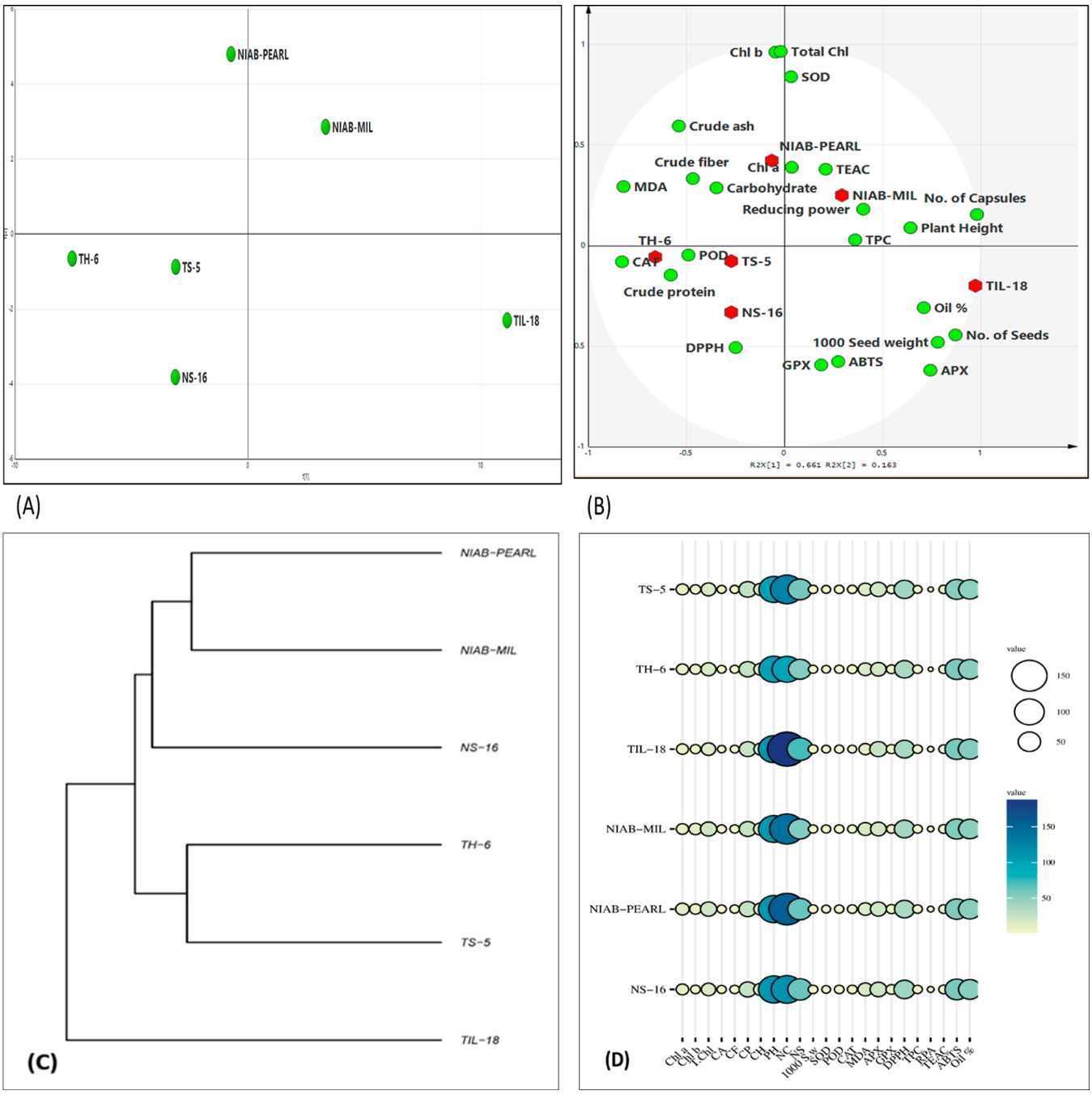

Figure 6. (A) Score scatter plot of six sesame varieties obtained by principal component analysis (PCA), (B) biplot of all growth parameters and sesame varieties, (C) phylogram showing clustering of sesame varieties depending upon pigments and leaf and seed antioxidant parameters, and (D) balloon plot showing comparative differences in chlorophyll content and morpho-agronomic trait leaf and seed antioxidant analysis of six sesame varieties.

and are therefore considered resistant against high temperature (Figure 4).

3.5. Seed Antioxidant Parameters. *3.5.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ABTS Activity.* Popular, rapid, simple, and reasonably priced method for measuring antioxidant qualities is 2,2-diphenyl-1-picrylhydrazyl (DPPH), which uses free radicals to determine whether a material has the ability to act as a hydrogen provider or a free-radical scavenger. Evaluating sesame's DPPH activity gives us information about its antioxidant capacity, which is important for preventing oxidative stress and maintaining cellular viability. Comprehending this exercise facilitates the investigation of sesame's medicinal and nutritional advantages, emphasizing its function in enhancing general health. DPPH activity was noted to be highest in the NS-16 variety with 42.45% scavenging activity followed by 41% in TS-5 with 41.94%, while Til-18, NIAB-Pearl, and NIAB-Milenium showed relatively similar percentages of ROS scavenging, while the lowest values were noted in the TH-6 variety. Further, ABTS radical scavenging activity was noted to be highest in Til-18 with 57.35% activity, followed by NS-16 with 57.19%. Lowest ABTS radical scavenging was noted in TS-5 with 50.68% radical scavenging (Figure 5).

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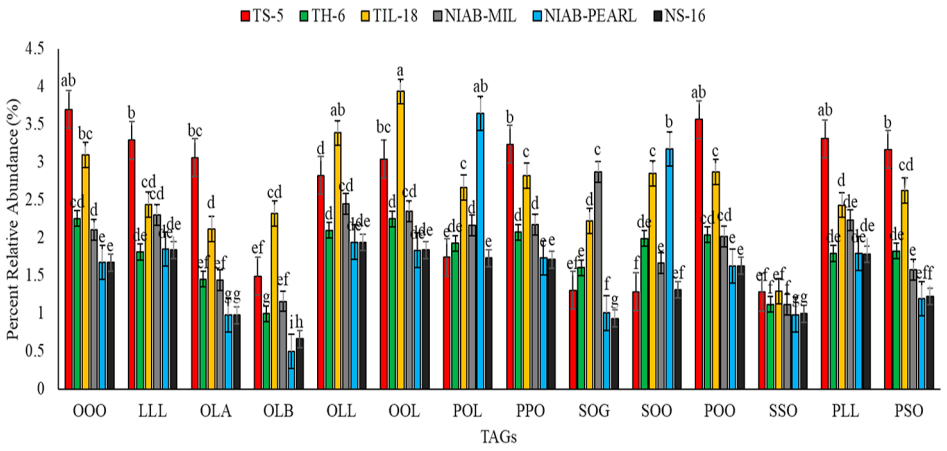

Figure 7. Relative abundance of major TAG species identified from six sesame varieties by UHPLC analysis.

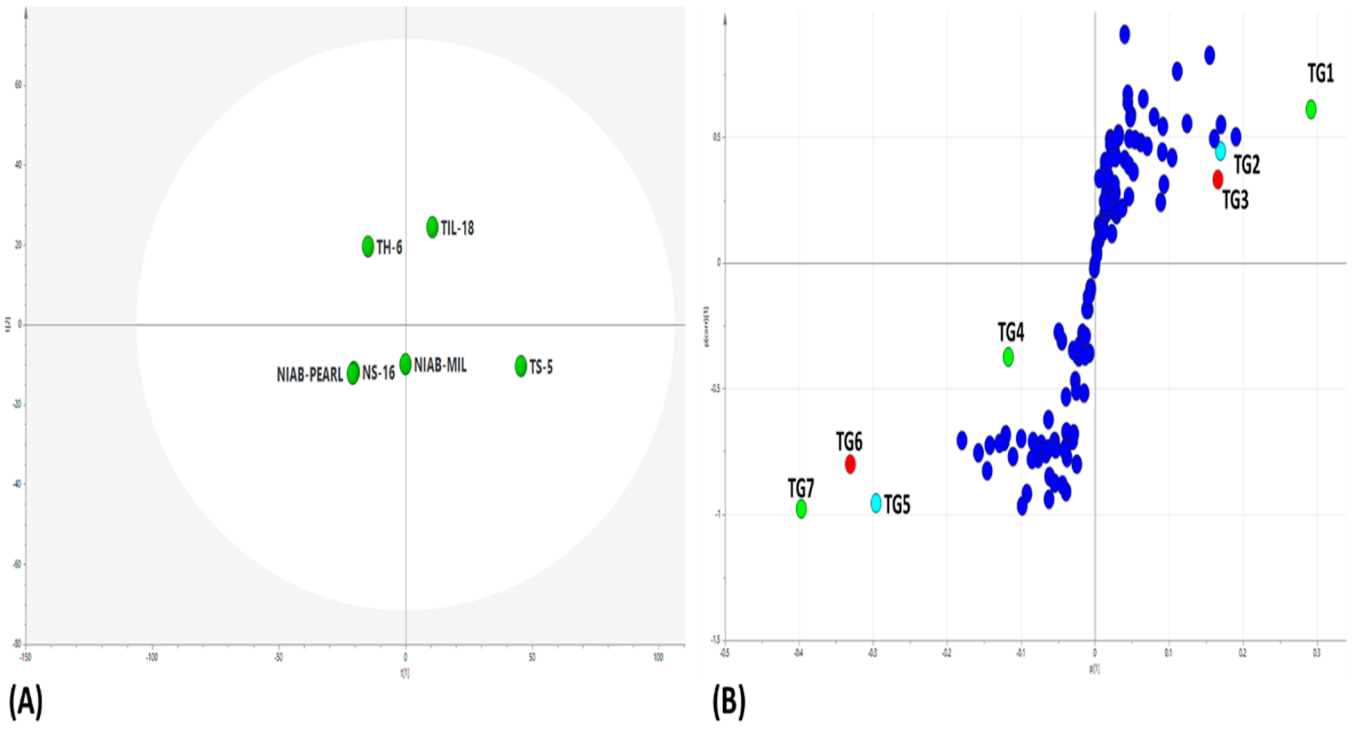

Figure 8. (A) PCA of six sesame varieties. (B) S-plot obtained from OPLS-DA of sesame varieties showing TAG biomarkers responsible for varietal separation.

3.5.2. Total Phenolic Content. In reaction to stress, plants store phenolic chemicals, such as phenolic acids, to fend off oxidative stress and shield themselves from reactive oxygen species. Phenolic acids are involved in defense, development, and plant growth, among other things. They also serve as precursors to other important bioactive compounds that are frequently employed in the food, cosmetic, and pharmaceutical industries. The TPC content ranged from 4.72 mg GAE/g in NIAB-Mil to 4.93 mg GAE/g in Til-18; TH-6, a first single stem variety, showed 4.80 mg GAE/g of TPC and TS-5 with 4.73 mg GAE/g. The total phenolic content differed between varieties, but no significant difference was noted among the varieties, suggesting that the varieties are almost the same in TPC content (Figure 5).

3.5.3. Reducing Power Activity and Trolox Equivalent Antioxidant Activity. The reducing power assay (RPA), a widely used antioxidant in vitro assay to check the reduction potential of sample plant extract, relies on the concept that compounds with reduction potential undergo a reaction with potassium ferricyanide (Fe^{3+}), yielding potassium ferrocyanide (Fe^{2+}). This ferrocyanide then interacts with ferric chloride to produce a ferric−ferrous complex with a peak absorption at 700 nm. Similar to other antioxidant assays, the RPA was highest in NS-16 (1.93) followed by NIAB-Pearl (1.89) and NIAB-Mil with 1.84. Moreover, compared to other individual antioxidant assays, the TEAC assay evaluates the total antioxidant capacity of a specimen by assessing its capability to counteract the stable radical cation of 2,2′-azino-bis (3-ethylbenzthiazolin-6-sulfonic acid) (ABTS). This radical cation, produced through ABTS oxidation, exhibits coloration and interacts with antioxidants, resulting in color reduction. The TEAC activity of sesame seed samples ranged from 4.90 mM/Trolox in TS-5 to 5.52 mM/Trolox in NIAB-Mil (Figure 5). This activity may be attributed to individual higher

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Table 3. Major TAG Species Contributing to the Separation of Sesame Varieties

ID	formula	TAG composition	measured mass of $[M + NH_4]^+$	calculated mass	mass deviation (ppm)
TG1	$C_{57}H_{104}O_6$	OOO	902.8160	902.8177	0.0017
TG2	$C_{55}H_{102}O_6$	POO	876.8006	876.8020	0.0014
TG3	$C_{57}H_{106}O_6$	SOO	900.7999	900.8020	0.0021
TG4	$C_{55}H_{100}O_6$	POL	874.7848	874.7864	0.0016
TG5	$C_{55}H_{98}O_6$	PLL	872.7690	872.7707	0.0017
TG6	$C_{57}H_{100}O_6$	OLL	898.7844	898.7864	0.002
TG7	$C_{57}H_{98}O_6$	LLL	896.7688	896.7707	0.0019

antioxidant properties, which led to the higher total antioxidant status (Figure 6A−D).

3.5.4. Chemometric Analysis of the Oil TAG Profile. Chemometric modeling of the oil of six sesame seed varieties was conducted to check the differences in the TAG profile. LC-MS analysis followed by PCA and OPLS-Da analysis revealed that all three varieties, i.e., NIAB-Mil, NIAB-Pearl, and NS-16, from the Nuclear Institute of Agriculture and Biology (NIAB) all grouped together, stating that they have the same TAG profile, while Til-18 serrated from all other varieties. The S-Plot, obtained from OPLS-Da modeling, suggested seven (07) TAG biomarkers separating these varieties. It was noted that Til-18 was mainly abundant in TAG species containing oleic acid; for example, three TAG biomarkers (TG2, TG2, and TG3) were identified as OOO, POO, and SOO, suggesting oleic acid abundance, while the other four TAG biomarkers (TG5-TG7) were POL, PLL, OLL, and LLL highly abundant in linoleic acid. Both the fatty acids are considered good for health purposes, but oleic acid is monounsaturated omega-9 fatty acid, while linoleic acid is polyunsaturated omega-6 fatty acid with immense ability to reduce total and LDL cholesterol (Figure 8A,B and Table 3).

3.5.5. Relative Abundance of TAG's in Sesame Varieties. Significant variation was found in the relative abundance of TAG in sesame varieties. Three major TAG species, OOO, LLL, and OLA, were found to be highest in TS-5, followed by Til-18, suggesting the abundance of these species with oleic acid, while the abundance of OLL was found to be highest in Til-18. These results further confirmed the chemometric analysis results, which conferred that TS-5 and Til-8 varieties are high in oleic acid content compared to the rest of the varieties. Furthermore, it was noted that linoleic acid containing TAG species, such as POL, was found to be highly concentrated in NIAB-Pearl, NIAB-Mil, and NS-16 varieties. It was also found that some of the minor TAG species like OLB were also detected and found to be highest in Til-18 followed by TS-5, suggesting the diversity of the TAG profile of TS-5 and Til-18. Therefore, from these results, conclusion can be drawn that TS-5 and Til-18 varieties are more diverse in TAG profile along with high content of oleic acid, while three NIAB varieties (NIAB-Mil, NIAB-Pearl, and NS-16) were abundant in linoleic acid with less content of other TAG species (Figure 7).

4. DISCUSSION

Since most native plants and seeds are high in vital nutrients and have a variety of therapeutic uses, there has been a rise in the demand for vegetable oil.[23] Due to its high labor demand for growing and harvesting, sesame (*S. indicum* L.), an oilseed herbaceous crop of the Pedaliaceae family, is only grown by a small number of developing nations worldwide. Asia and African nations contribute over 96% of the world's sesame crop. One significant annual oilseed crop that is grown for human food oil, meal, and animal feed is sesame.[24,25] Sesame seed oil has unsaturated and saturated fatty acids, proteins, and lignans, which include sesamin, sesamol, sesamolin, and tocopherols, along with other minor nutrients such as vitamins and minerals.[26−28] Iron, magnesium, copper, calcium, vitamin B1 (thiamine), vitamin E (tocopherol), and phytosterols are also abundant in the seeds.[24,29,30] Because it can withstand prolonged exposure to air, sesame seed oil is well-known for its stability.[31]

Our results suggested that as far as the morphology is concerned, there is no significant difference between varieties, but it was noted that morphology is somehow affecting the agronomical attributes. It was noted that the Til-18 variety has shown the highest value for all morphological and agronomic traits, which ultimately resulted in the highest oil content of 53.3%, compared to the lowest oil percentage in TS-5 (49.15), suggesting an interaction between morphology crop growth and oil yield. But it is important to note that these characters were comparable to all three varieties obtained from NIAB, NIAB-Pearl, NIAB-Mil, and NS-16. It was imperative to note that the chlorophyll content was highest in NIab-Mil and NIAB pearl compared to that in Til-18 and all other varieties. It was also noted that the chlorophyll content was highest in NIab-Mil and NIAB-Pearl, which might have also resulted in higher crop growth, yield, and oil content. Chlorophyll is the primary pigment responsible for the photosynthetic efficacy along with net primary production and carbon budgeting,[32] due to which chl content is also an important indicator of plant growth and nutritional status, which can ultimately determine the yield potential of the crop. Furthermore, there is also a strong correlation between the nitrogen (N) content and the chlorophyll status of the plant. These sesame varieties might have different levels of N absorption efficacy, which lead to the differences and chlorophyll content and ultimate increase and decrease in crop yield.[33,34] According to ref 35, the most important factor influencing the grain yield and biomass was leaf photosynthesis. On the other hand, in order to create a biomass or yield model, not much research has been done on the connection between a crop's photosynthetic capacity and biological photosynthetic components such as leaf chlorophyll or carotenoid levels.36 Chlorophyll, an essential component of the Calvin−Benson cycle, is responsible for photosynthesis, which produces electron excitation that powers the synthesis of nicotinamide adenine dinucleotide phosphate and adenosine triphosphate, a chemical energy source.[37]

Our study also showed that sesame varieties have different levels of antioxidant status, but all the varieties showed a mixed response as far as different enzymatic parameters are concerned. For example, SOD activity was found to be highest in NIAB-Mil, highest POD and GPX activity in NS-16, and highest CAT and APX activity in Til-18. Further, the levels of

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MDA content were found to be lowest in Til-18, suggesting a lower level of lipid peroxidation and hence more resilience to external stresses. Literature suggests excessive ROS production leading to oxidative stress which results in diminished crop growth and yield. Hence, improved antioxidant efficacy is linked with greater crop growth and yield. When exposed to stress, plants initiate a cascade of antioxidant processes aimed at minimizing the buildup of excessive reactive oxygen species. Research has demonstrated that in response to a variety of stressors, the enzyme activity of SOD, CAT, APX, and POX increases quickly and dramatically.[38,39] Plenty of literature is available discussing the role of antioxidant systems for improved yield. According to ref 40, physiological processes at several growth stages, particularly the jointing stage, affected the grain yield, for example, plant photosynthetic efficiency may be enhanced by appropriate nitrogen (N) fertilization, which may also raise antioxidant enzyme levels and chlorophyll content; consequently, this encouraged the buildup of dry matter and increased grain yield.[41,42] The study conducted by[43] suggested that antioxidant defense activity and osmolyte accumulation were different in three cultivars of maize, ultimately affecting its yield potential. It was imperative to note that antioxidant activities were positively correlated with drought stress resistance and ultimate yield response, further confirming that the improved antioxidant level results in higher yield. In practice, now genetically improved crops with a high antioxidant status have been made through molecular manipulation with antioxidant enzymes and transcription factors to increase overall improvement in plants.

Various studies have been conducted to check the antioxidant potential and metabolic profiles of different colored sesames, and the differences are mainly attributed to the differences in coat color. In the present study, we also noted that the color of these sesame varieties was different from white to yellow to light brown, suggesting differences in their seed coat. Seed antioxidant analysis suggested that antioxidant parameters were comparable in all six varieties, and no significant difference was observed. Further, still there was an observable difference between seed antioxidant analyses, for example, the ABTS percent radical scavenging activity was found to be highest in Til-18, and TEAC was found to be highest in NIAB-Mil and reducing power ability in NS-16. Moreover, it was also important to note that total phenolic and flavonoid contents were found to be highest in Til-18, collectively giving it a higher antioxidant potential. These differences in antioxidant activities might be due to slight variation in seed coat color as it ranged from white to yellow to light brown. According to the study conducted in ref 44, the differences were checked in the metabolic profiles of white, yellow, brown, and black sesames, suggesting that different colored sesames have totally different colored metabolic profiles. For example, a total of 169 significantly different metabolites were found between white and black sesame, 178 compounds between yellow and black sesame, and 130 compounds between black and brown sesame. Similarly, black sesame was found to have higher flavonoids, amino acids, and terpenoid content. Further, DPPH radical scavenging, ferric ion reducing power (FRAP) activity, and ABTS activity were also found to be highest in black color sesame and was in the order: black sesame < brown sesame < yellow sesame < white sesame. This phenomenon can simply be explained in a way that the darker the color of the sesame seed, the more the diversity in its metabolic profile along with high antioxidant activity. It was important to note that regulation of many metabolic processes in these different colored sesames was also different conferring them variation in overall metabolic profile. Further, the effect of germplasm and different environmental factors are very well characterized in rice45 and soybean.[46] Plant secondary metabolites, particularly flavonoids, play a pivotal role in determining the colors of flowers and seeds.[47,48] In sesame seeds, the primary classes of flavonoids identified are flavones, flavonols, and isoflavones. Through a differential metabolite analysis, it is observed that white sesame seeds predominantly contain uncolored pigments (flavones), whereas yellow, brown, and black sesame seeds exhibit higher concentrations of flavonols, isoflavones, and anthocyanins. These findings suggest that variations in the abundance of colored flavonoids likely contribute to the diverse coat colors observed in sesame seeds with an improved metabolic and antioxidant profile. For instance, the yellow hue in sesame seed coats may be attributed to the elevated levels of flavonols, as observed in peanuts.[49]

5. CONCLUSIONS

This study was conducted to check the growth and yield potential of six major sesame varieties cultivated in Pakistan. It is concluded that Til-18, NIAB-Pearl, and NIAB-Milenium are better performing varieties with high oil content, antioxidant potential, and resistance to environmental stresses. It was also noted that TS-5 and TH-6 showed some better antioxidant characters but did not perform well overall with regard to yield potential. These varieties were introduced in 2009 and 2011, which might be the reason that with passage of time and changing climate, these varieties are not performing well with regard to oil and yield potential, compared to Til-18 and other varieties released by the NIAB. Further, molecular, genomic, and proteomic research is needed to explore the underlying cause for oil production, helping to introduce better performing sesame varieties with high stress resistance and oil yield.

■ ASSOCIATED CONTENT

Data Availability Statement

All data generated or analyzed during this study are included in this manuscript Plant guideline statement: Sesame is a cultivated plant and not in the category of threatened species around the world. However, IUCN recommendations were followed during sample collection and throughout the experiment.

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Notes

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