



RESEARCH ARTICLE

Physiological and biochemical evaluation of peanut (*Arachis hypogaea* L.) varieties in Andijan, Uzbekistan

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Abstract

The study investigated the physiological and biochemical characteristics of peanut (*Arachis hypogaea* L.) varieties cultivated in the Andijan region of Uzbekistan. Analysis results revealed that during the flowering phase, the Chinese-275 variety had the highest chlorophyll "a" content in leaves $(1.66 \pm 0.12 \text{ mg/g})$. In comparison, the Tashkent-112 variety showed the highest chlorophyll "b" content $(0.63 \pm 0.15 \text{ mg/g})$. The Chinese-275 variety also exhibited the highest carotenoid content $(0.65 \pm 0.04 \text{ mg/g})$. In the pod formation phase, the Chinese-275 variety again demonstrated the highest chlorophyll "a" content in plant leaves $(2.39 \pm 0.06 \text{ mg/g})$, the Tashkent-112 variety had the highest chlorophyll "b" content $(1.49 \pm 0.52 \text{ mg/g})$ and the Chinese-275 variety showed the highest carotenoid content $(0.98 \pm 0.01 \text{ mg/g})$. In our experiments examining the protein and fat content of peanut (*Arachis hypogaea* L.) seeds, the Salomat variety exhibited the highest protein content $(26.704 \pm 0.303 \text{ \%})$. The highest fat content was observed in the Mumtoz variety $(0.467 \pm 0.002 \text{ mg/g})$. These findings highlight promising peanut varieties for improved yield and nutritional value under local agroecological conditions.

Keywords: Andijan region; carotenoids; chlorophyll; flowering and podding stages; oil content; peanut; protein content

Introduction

With the growing global population, the demand for highquality food and protein products is also increasing. Therefore, identifying plant varieties with high nutritional value and protein content, increasing their productivity and implementing them into practice is one of today's most pressing issues. Especially considering problems such as drought and low soil fertility, there is a need for new approaches to plant selection and breeding. In this regard, the peanut plant plays a significant role due to its high nutritional value, oil content and drought resistance. Peanuts are one of the plants rich in minerals, vitamins and proteins, with their stems serving as a primary feed in livestock farming and their oil being important in the food industry, particularly in confectionery products. Therefore, the biochemical composition of peanuts, especially its oil and protein content, is a key factor in crop selection. In the arid conditions of Uzbekistan, growing and developing peanuts can improve the efficiency of agricultural practices. The peanut's ability to withstand drought and adapt to soil conditions increases its potential in Uzbekistan.

Recent studies on peanuts' biochemical composition and physiology provide valuable insights into the plant's evolution, oil fatty acid composition and its applications in the food industry. For instance, studies conducted by Turkish researchers have proven the significant impact of planting date on the fatty acid composition of peanut seeds (1). Additionally, research has confirmed the presence of biologically active components in peanuts, such as phenolic compounds, flavonoids and resveratrol and their resistance to abiotic stresses like drought, salinity, heat stress and iron deficiency (2). The high oil content, nutritional quality and widespread use of peanuts in the food industry make it highly promising for Uzbekistan's agricultural development. Regarding peanut breeding, recent studies focus on developing high-yielding, high-quality seed and droughtresistant varieties. Previous research has explored the use of biostimulants to achieve higher yields in peanuts (3). Other studies have examined the plant's resistance to diseases and ability to adapt to various agroecological conditions, which is significant for Uzbekistan's agriculture. Furthermore, research on peanuts' biochemical analysis and photosynthesis processes is crucial. Studies on

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chlorophyll pigments' chemical structure and function provide a better understanding of their role in photosynthesis (4, 5). Chlorophyll pigments and carotenoids play vital roles in protecting plants from adverse environmental factors and participating in photosynthesis by absorbing light and transferring it to chlorophyll.

The importance of peanut research for Uzbekistan's agriculture and the impact of biochemical properties on crop selection peanut research is crucial for Uzbekistan's agriculture because the plant's high nutritional value and drought resistance make it well-suited for the country's arid conditions. The biochemical composition of peanuts, particularly its oil and protein content, is an essential factor in crop selection. Growing and developing peanuts in Uzbekistan's arid regions can significantly enhance agricultural productivity. Compared to other countries, Uzbekistan faces unique agroecological challenges, such as low soil fertility and limited water resources. Therefore, when selecting peanut varieties, it is essential to consider drought resistance, pest and disease resistance and adaptability to the local agroecological conditions. Comparing results in Uzbekistan with peanut varieties from other regions and highlighting agroecological issues in Uzbekistan, peanuts are primarily grown in droughtresistant and arid areas. However, unlike other countries, Uzbekistan faces specific agroecological challenges, including poor soil fertility and limited water resources. For example, while research in countries like Turkey and Brazil has focused on high-yielding peanut varieties with superior seed quality, these regions have abundant water resources. In contrast, Uzbekistan's harsh climatic conditions limit peanut yields, making developing special breeding programs essential. In selecting peanut varieties, it is crucial to consider the country's unique agroecological conditions and choose varieties that can adapt to these challenges.

Material and Methods

The study was conducted at the "Xosildor Zamin Obod" farm in Andijan district andijan region, from May to September. The experimental varieties included both local (Lider, Qibray-4, Salomat, Toshkent-125, Mumtoz) and foreign (Xitoy-275, Afrika-411, Hindiston-299, Senegal-685, Vietnam-1126). The varieties mentioned above were selected based on specific criteria, with their main advantages being climate adaptability, high yield potential, quality indicators and resilience. Genetic diversity played a crucial role in developing these varieties - it provided the foundation for combining beneficial traits during breeding. These were planted in three replicates, with each replicate containing 25 plants. The row spacing was 65 cm and plants were spaced 25 cm apart. The environmental conditions during the experiment included an air temperature of 29 °C, soil temperature of 17 °C and soil moisture of 30 %. The selected peanut varieties were characterized based on the soil and climate conditions and appropriate agrotechnical measures were implemented to support their growth.

Fertilization was carried out in three stages: before sowing, during the vegetative phase and up to the ripening

stage. The fertilizers applied were nitrogen (N) at 150 kg/ha, phosphorus (P) at 150 kg/ha and potassium (K) at 100 kg/ha. These rates were consistent with recommendations for optimizing peanut growth. In addition to fertilization, various agronomic practices were followed, including row loosening, moisture control and weed management. The experimental results demonstrated that peanut plants exhibited superior vegetative growth and yield in areas with soil pH levels ranging from 6.8 to 7.0. Notably, the "Andijan 2" variety achieved an average yield of 28.5 centners/ha.

In plots rich in organic matter (2.0 %), fruiting rates were high, with individual nut weights averaging 85-90 g per 10 fruits.

Soil pH level (between 6.8 and 7.3)

Organic matter content (1.4 % - 2.0 %)

Irrigation was performed three times: once after seedling emergence, again during the flowering stage and once more from pod formation to harvest. Weeding and row loosening were carried out four times throughout the growing season to ensure optimal plant development. Scientific studies on peanut fertilization emphasize the importance of nutrient availability for achieving high yields (6, 7). They found that for every 50.8 kg (1 centner) of peanut pods harvested, 4.38 kg of nitrogen, 0.40 kg of phosphorus and 2.60 kg of potassium were required for adequate plant nutrition. Additionally, (8) stated that along with the nutrients mentioned, an additional 1.23 kg of magnesium (Mg) and 4.0 g of zinc (Zn) were necessary per 1 centner of pod yield.

The physiological and biochemical analyses of the peanut varieties were conducted using standard laboratory methods as follows:

Chlorophyll content determination

To determine chlorophyll levels. Leaf samples (50 mg each from three to four plants) were homogenized in 5 mL of 95 % ethyl alcohol. After centrifugation. chlorophyll a, chlorophyll b. and carotenoids were quantified using a UV-Vis spectrophotometer at wavelengths of 664 nm. 649 nm. and 470 nm. respectively. Calculations were based on the following equations 1-3:

$$\begin{aligned} \text{Chl-a} &= 13.36 \text{A}_{664} - 5.19 \text{A}_{649} & \text{(Eqn. 1)} \\ \text{Chl-b} &= 27.43 \text{A}_{649} - 8.12 \text{A}_{664} & \text{(Eqn. 2)} \\ \text{C}_{\text{x+c}} &= & (1000 \text{A}_{470} - 2.13 \times \text{Chl-a} - 97.63 \times \text{Chl-b}) \, / \, 209 \\ & \text{(Eqn. 3)} \end{aligned}$$

Total protein content determination by the Kjeldahl method (Following AOAC Standards)

The total protein content of the plant samples was determined using the Kjeldahl method following the internationally recognized standards set by the Association of Official Analytical Chemists (AOAC) (9). The procedure included the following steps:

- 1.2 g of ground. dried aboveground plant sample (defatted using hexane) was placed into a Kjeldahl digestion flask.
- 2.25 mL of concentrated sulfuric acid was added to rinse down any material adhering to the sides of the flask.

- 3.1 g of copper sulfate and 5 g of potassium sulfate were added as catalysts.
- 4. The mixture was heated gently at first. Then vigorously until the solution turned from dark brown to clear blue. Indicating complete digestion. A funnel was inserted into the flask to reduce fume formation.
- 5. After cooling, the digest was diluted with a small amount of water and transferred to the distillation apparatus. The digestion flask was rinsed twice with water and the rinses were added to the distillation flask.
- $6.100~\mathrm{mL}$ of 50~% sodium hydroxide was added to the distillation flask.
- 7. A receiving flask containing 40-50 mL of 0.1 N sulfuric acid and 4-5 drops of indicator (methyl orange or methyl red) was placed at the condenser outlet. Ensuring immersion of the outlet tube.
- 8. The mixture was distilled until two-thirds of the liquid was transferred. The condenser tip was rinsed. and distillation continued until the medium tested alkaline with a universal indicator.
- 9. The remaining sulfuric acid in the receiving flask was titrated with 0.1 N sodium hydroxide. Observing the indicator's colour change from red to yellow.

Nitrogen content was calculated using the following formula:

$$\%$$
 N = ((VH₂SO₄ - VNaOH) × 0.0014 × 100) / m (Eqn. 4) Where:

- VH_2SO_4 : Volume (mL) of 0.1 N sulfuric acid used to absorb NH_2
- VNaOH: Volume (mL) of 0.1 N sodium hydroxide used in back titration
- m: Mass of the dry sample in grams
- 0.0014: Conversion factor for sulfuric acid to nitrogen

Protein content was calculated using the following equation:

$$\%$$
 protein = $K \times \% N$ (Eqn. 5)

Where K = 5.70 for plant samples with low fat content.

Determining the oil content of seeds

Plant seeds are dried until they reach a uniform weight and then ground in a mortar. They are then placed into paper packets weighing 5-10 g. The packets should be sized to fit comfortably within the Soxhlet apparatus. Subsequently, the packets are dried in an oven at 90-100 °C for 2 hrs and then allowed to cool before being placed in the extractor.

Ether is poured into a pre-weighed flask, filled to 2/3-3/4 of its volume and then connected to the extractor. The water bath connected to the condenser is switched on. The water bath temperature should be maintained at a level that allows for the complete washing of the plant material with ether 8-10 times per hour. Typically, the water bath temperature is around 45-50°C. The extraction process, which depends on the oil content of the material, takes 6-10 hours. Once the oil is completely extracted, the flask is removed from the apparatus, the ether is distilled and the

flask is dried in a drying cabinet at 90-100°C. The flask is then weighed again to determine the oil content.

$$X = \frac{(A-B) * 100}{V}$$
 (Eqn. 6)

X - Crude oil content, expressed as a percentage, A - Weight of the oil flask, gr. V - Weight of plant material, gr.

Statistical analysis

The statistical analysis of the obtained data was carried out using Microsoft Excel 2016 and StatView software. Analysis of variance (ANOVA) was performed to assess differences between groups. Results were expressed as mean \pm standard deviation (SD) and differences were considered statistically significant at p < 0.05.

Results and Discussion

Trends in chlorophyll, protein and oil content and their relevance for groundnut breeding

According to the results of this study, significant variations were observed in the content of chlorophyll "a", chlorophyll "b", total chlorophyll and carotenoids during the flowering and pod formation stages among groundnut varieties. Notably, the local varieties Mumtoz and Lider and varietv China-275 showed foreign concentrations of these photosynthetic pigments, indicating superior photosynthetic activity and biomass production potential. High levels of photosynthetic pigments such as chlorophyll and carotenoids reflect a plant's capacity to efficiently capture light energy, which contributes to enhanced metabolic activity during critical growth stages. In particular, higher chlorophyll content is associated with improved nitrogen metabolism, which is essential for protein biosynthesis (10). Furthermore, carotenoids act as antioxidants that protect plants from oxidative stress, indirectly supporting seed oil synthesis (11). Interestingly, the varieties with higher pigment content were also found to have elevated protein and oil levels, which underscores the physiological link between pigment accumulation and the synthesis of major nutritional compounds. This relationship is significant in groundnut breeding, as protein and oil content are primary determinants of commercial and nutritional value (12). These findings suggest that in groundnut breeding programs, attention should be paid to agronomic traits and biochemical markers such as chlorophyll, protein and oil content. Primarily, genotypes with high chlorophyll and carotenoid levels can serve as valuable genetic resources for the development of nutritionally superior varieties.

The research included local peanut varieties *Lider*, *Salomat*, *Mumtoz*, *Toshkent-112* developed at the Uzbekistan Institute of Plant Science and foreign peanut varieties introduced to Uzbekistan from India, Pakistan, China, Russia, Turkey, Japan and other countries. These varieties, which are currently grown on a limited scale in Uzbekistan, were planted and grown in dense soil conditions in the Andijan region.

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For the research, the chlorophyll "a" chlorophyll "b," total chlorophyll and carotenoid content in the leaves of selected peanut varieties were studied during the flowering and podding stages. During the flowering stage, the highest chlorophyll "a" content was observed in *Lider*, *Mumtoz* and *Xitoy-275* (1.53 \pm 0.12 mg/g, 1.58 \pm 0.12 and 1.66 \pm 0.12 mg/g, respectively). The lowest values were found in *Salomat*, *Vetnam-1126* and *Senegal-685* (1.22 \pm 0.11 mg/g, 1.23 \pm 0.11 mg/g and 1.1 \pm 0.07 mg/g, respectively) (Table 1).

The leaves' chlorophyll "b" content was analyzed in the same stage. The highest values were observed in Toshkent-112 (0.63 ± 0.15 mg/g) and *Mumtoz*, *Lider* and *Xitoy -275* varieties. In contrast, the lowest values were found in Vetnam-1126, *India* and *Senegal* varieties (0.4 ± 0.02 mg/g, 0.41 ± 0.01 mg/g and 0.38 ± 0.02 mg/g, respectively).

During the flowering stage, the highest carotenoid content in the leaves was observed in *Xitoy-275* (0.65 ± 0.04 mg/g) among the foreign varieties and the lowest in *Senegal* (0.48 ± 0.03 mg/g). Among the local varieties, the highest level was observed in *Lider* (0.64 ± 0.03 mg/g) and the lowest in *Salomat* (0.55 ± 0.03 mg/g).

In terms of total chlorophyll content during the flowering stage, the local varieties Mumtoz and Lider (2.14 \pm 0.14 mg/g and 2.07 \pm 0.08 mg/g, respectively) exhibited the highest levels. In comparison, Salomat had the lowest (1.7 \pm 0.13 mg/g). Among the foreign varieties, the highest total chlorophyll content was observed in Xitoy-275 (2.2 \pm 0.09 mg/g) and the lowest was found in Senegal-685 (1.48 \pm 0.08 mg/g).

It was observed that the chlorophyll "a" pigment content increased in all samples during the podding stage compared to the flowering stage. The highest levels of this pigment were found in the foreign varieties $\it Xitoy-275$ and $\it Hindiston-299$ (2.39 \pm 0.06 mg/g and 2.31 \pm 0.06 mg/g. respectively). While the lowest level was found in Senegal-

685 (1.947 \pm 0.19 mg/g). Among the local varieties. the highest levels were observed in *Lider* and *Mumtoz* (1.838 \pm 0.13 mg/g. 1.943 \pm 0.12 mg/g). with the lowest level in *Toshkent-112* (1.58 \pm 0.13 mg/g) (Table 2).

During this period. an examination of the leaves' chlorophyll "b" content showed that the highest levels were found in the local varieties *Toshkent-112* and *Salomat* (1.49 \pm 0.52 mg/g and 0.99 \pm 0.45 mg/g. respectively). At the same time, the lowest level was in *Qibray-4* (0.59 \pm 0.08 mg/g). Among the foreign varieties. The highest levels were observed in *Xitoy-275*. *Vetnam-1126*. and *Hindiston-299* (0.85 \pm 0.01 mg/g. 0.82 \pm 0.05 mg/g. and 0.83 \pm 0.03 mg/g. respectively), while the lowest level was observed in *Senegal-685* (0.71 \pm 0.08 mg/g). The results obtained for the total chlorophyll content were similar to those for chlorophyll "a" and chlorophyll "b".

The highest carotenoid content in peanut leaves was observed during the podding stage in the foreign varieties Xitoy-275. Vetnam-1126. and Hindiston-299 (0.98 \pm 0.01 mg/g. 0.93 \pm 0.04 mg/g. and 0.93 \pm 0.02 mg/g. respectively). The lowest carotenoid content was found in the local variety Qibray-4 (0.59 \pm 0.08 mg/g). Among the local varieties. the highest level was observed in Salomat (0.77 \pm 0.14 mg/g). In the experiment conducted. The highest oil content in the seeds of the local peanut varieties was found in Mumtoz (0.467 \pm 0.002 mg/g). while the lowest content was observed in Toshkent-112 (0.441 \pm 0.012 mg/g). Among the foreign varieties, the highest oil content was in Afrika-411 (0.468 \pm 0.001 mg/g) and the lowest was in Senegal-685 (0.427 \pm 0.001 mg/g) (Table 3).

Protein content of peanut varieties

Peanuts as a legume. can fix atmospheric nitrogen through a symbiotic relationship with nitrogen-fixing bacteria. Nodules formed on the plant's roots are crucial in this nitrogen fixation. Peanuts rank second only to soybeans in terms of

Table 1. Chlorophyll content (mg/g) at full flowering stage

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Varieties	Chlorophyll "a" (mg/g)	Chlorophyll "b" (mg/g)	UXM (mg/g)	Carotenoids (mg/g)
Lider	1.53 ± 0.12	0.53 ± 0.03	2.07 ± 0.08	0.64 ± 0.03
Qibray	1.42 ± 0.10	0.43 ± 0.04	1.91 ± 0.07	0.57 ± 0.03
Mumtoz	1.58 ± 0.12	0.56 ± 0.03	2.14 ± 0.14	0.65 ± 0.04
Salomat	1.22 ± 0.11	0.47 ± 0.02	1.7 ± 0.13	0.55 ± 0.03
Toshkent-112	1.27 ± 0.07	0.63 ± 0.15	1.9 ± 0.15	0.56 ± 0.04
Afrika-411	1.25 ± 0.10	0.43 ± 0.05	1.7 ± 0.08	0.53 ± 0.04
Vetnam-1126	1.23 ± 0.11	0.41 ± 0.02	1.64 ± 0.12	0.51 ± 0.04
Xitoy-275	1.66 ± 0.12	0.53 ± 0.06	2.2 ± 0.09	0.65 ± 0.04
Indiya	1.25 ± 0.05	0.41 ± 0.01	1.67 ± 0.03	0.49 ± 0.01
Senegal-685	1.10 ± 0.07	0.38 ± 0.02	1.48 ± 0.08	0.48 ± 0.03
	Lider Qibray Mumtoz Salomat Toshkent-112 Afrika-411 Vetnam-1126 Xitoy-275 Indiya	Lider 1.53 ± 0.12 Qibray 1.42 ± 0.10 Mumtoz 1.58 ± 0.12 Salomat 1.22 ± 0.11 Toshkent-112 1.27 ± 0.07 Afrika-411 1.25 ± 0.10 Vetnam-1126 1.23 ± 0.11 Xitoy-275 1.66 ± 0.12 Indiya 1.25 ± 0.05	Lider 1.53 ± 0.12 0.53 ± 0.03 Qibray 1.42 ± 0.10 0.4 3 ± 0.04 Mumtoz 1.58 ± 0.12 0.56 ± 0.03 Salomat 1.22 ± 0.11 0.47 ± 0.02 Toshkent-112 1.27 ± 0.07 0.63 ± 0.15 Afrika-411 1.25 ± 0.10 0.43 ± 0.05 Vetnam-1126 1.23 ± 0.11 0.41 ± 0.02 Xitoy-275 1.66 ± 0.12 0.53 ± 0.06 Indiya 1.25 ± 0.05 0.41 ± 0.01	$\begin{array}{cccccccccccccccccccccccccccccccccccc$

Table 2. Chlorophyll content in mg/g during the podding stage

Sr. No.	Varieties	Chlorophyll "a" (mg/g)	Chlorophyll "b" (mg/g)	UXM (mg/g)	Carotenoids (mg/g)
1	Lider	1.83 ± 0.13	0.65 ± 0.07	2.49 ± 0.15	0.76 ± 0.05
2	Qibray-4	1.81 ± 0.27	0.59 ± 0.08	2.4 ± 0.17	0.71 ± 0.08
3	Mumtoz	1.94 ± 0.12	0.64 ± 0.05	2.58 ± 0.08	0.79 ± 0.04
4	Salomat	1.68 ± 0.34	0.99 ± 0.45	2.67 ± 0.56	0.77 ± 0.14
5	Toshkent-112	1.58 ± 0.13	1.49 ± 0.52	3.08 ± 0.39	0.58 ± 0.16
6	Xitoy-275	2.39 ± 0.06	0.85 ± 0.01	3.24 ± 0.03	0.98 ± 0.01
7	Vetnam-1126	2.18 ± 0.06	0.82 ± 0.05	3 ± 0.05	0.93 ± 0.04
8	Afrika-411	2 ± 0.15	0.74 ± 0.05	2.74 ± 0.1	0.84 ± 0.04
9	Indiya-299	2.31 ± 0.06	0.83 ± 0.03	3.14 ± 0.07	0.93 ± 0.02
10	Senegal-685	1.95 ± 0.19	0.71 ± 0.08	2.66 ± 0.13	0.84 ± 0.06

Table 3. Oil and protein content in the seeds of local and foreign peanut varieties

Sr. No.	Varieties	Oil content in mg/g	Total protein content (%)
1	Lider	0.462 ± 0.003	25.333 ± 0.496
2	Qibray-4	0.456 ± 0.003	23.256 ± 0.571
3	Mumtoz	0.467 ± 0.002	25.365 ± 0.481
4	Salomat	0.463 ± 0.003	26.704 ± 0.303
5	Toshkent-112	0.441 ± 0.012	23.453 ± 0.292
6	Xitoy-275	0.464 ± 0.004	26.295 ± 0.377
7	Vetnam-1126	0.459 ± 0.001	25.732 ± 0.575
8	Afrika-411	0.468 ± 0.001	26.245 ± 0.475
9	Indiya-299	0.454 ± 0.001	24.584 ± 0.627
10	Senegal-685	0.427 ± 0.001	26.363 ± 0.523

protein content. Their shells contain up to 12 % protein. They are nutritionally comparable to other legumes. Peanut and soybean proteins are nutritionally equivalent to meat and egg proteins, making them beneficial for human growth and health.

When analyzing the protein content of peanut seeds. The lowest levels were observed in the local varieties Mumtoz and Salomat (25.365 \pm 0.481 % and 26.704 \pm 0.303 %. respectively). While Qibray-4 (23.256 \pm 0.571 %) had the lowest. Among the foreign varieties. the highest protein content was found in Xitoy-275 (26.295 \pm 0.377 %). while Indiya-299 (24.584 \pm 0.627 %) exhibited the lowest (Table 3).

Conclusion

The comparative analysis of local and foreign peanut (Arachis hypogaea L.) varieties under Andijan's agro-climatic conditions revealed distinct differences in chlorophyll content, protein and oil accumulation. Notably, the varieties Mumtoz and Xitoy-275 exhibited superior physiological and biochemical performance, highlighting their potential as donor genotypes in future breeding programs to enhance nutritional quality and environmental adaptability. The increased chlorophyll concentration during the podding phase suggests enhanced photosynthetic capacity likely driven by intensified symbiotic nitrogen fixation. These physiological trends, consistent with prior studies, underscore the importance of integrating biochemical traits in selection criteria. To reinforce varietal improvement strategies, future research should prioritize the identification and application of molecular markers linked to chlorophyll biosynthesis and nutrient accumulation. This will facilitate marker-assisted selection for developing high-performing, climate-resilient peanut cultivars suited to diverse soil and climatic conditions.

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Authors' contributions

GDA conceptualized the study, conducted data analysis, and drafted the manuscript. HXM contributed to the study design, data collection and manuscript revision. NIM assisted with data interpretation and statistical analysis. STY provided critical feedback on the manuscript and contributed to the final revisions.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interest to declare.

Ethical issues: None

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