Biochemical basis for kernel testa pigmentation of groundnut (Arachis hypogaea L.) genotypes under ambient storage

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Research Article

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Abstract

Kernel testa pigmentation in groundnut (*Arachis hypogaea* L.) is a significant quality attribute associated with antioxidant potential, nutritional composition and storage stability of kernels especially during extended periods of storage by farmers before marketing. In this connection, the experiment was conducted during 2024 and 2025 at ANGRAU, Regional Agricultural Research Station, Tirupati, taking six groundnut genotypes in RBD with three replications. Kernel samples were stored and analyzed at harvest, 30 DAH and 60 DAH for phenols, anthocyanins, flavonoids, sugars and protein content. Significant genotypic variability was observed for biochemical metabolites during storage. Among the evaluated genotypes, higher phenols content, sugar content, protein content and oil content are exhibited by K-6 (5.70 mg/g FW), Dharani (0.83 mg /g FW), Dharani (25.64%) and Kadiri Lepakshi (48.8%) respectively. Pearson's correlation analysis revealed positive association between kernel testa-associated metabolites and antioxidant-related compounds suggesting their role in maintenance of pigment stability during storage. The identified genotypes possessing better biochemical stability and progressive decline in phenols, anthocyanins and sugars during subsequent storage phases may be useful for improving post-harvest quality and storage resilience of groundnut under semi-arid tropical environments of Andhra Pradesh.

2. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the major oilseed crops cultivated extensively in tropical and subtropical regions under rainfed agro-ecosystems. India ranks second in Groundnut production with 10.13 million tonnes next to China with 18.38 million tonnes and ranks 12th in productivity with 1847.4 kg per hectare. In India the groundnut is grown in an area of about 5.34 m ha, producing 7.5 mt. Majority of area of peanut cultivation in India is primarily rainfed based and on sandy soils. Andhra Pradesh is in 5th position in terms of area under cultivation with 3.66 lakh ha (crop outlook reports of Andhra Pradesh, 2024).

Groundnut cultivation in Andhra Pradesh is predominantly carried out under semi-arid tropical climatic conditions characterized by elevated temperature, intermittent dry spells and fluctuating relative humidity. The area under cultivation of Groundnut is alarmingly decreasing because of several reasons among which deterioration of seed quality during storage is a significant one. Such environmental conditions not only influence crop productivity but also affect post-harvest seed storability and biochemical stability of kernels during storage. Elevated temperature and relative humidity accelerate oxidative metabolism, lipid peroxidation and degradation of antioxidant-associated metabolites, thereby reducing seed quality and storage life (Bailly, 2020; Walters and Wheeler, 2021). Prolonged storage of kernels prior to marketing often exposes seeds to ambient environmental stress, resulting in progressive deterioration of pigment-associated metabolites and nutritional quality (Kumar et al., 2021).

Biochemical constituents such as phenols, flavonoids, anthocyanins, sugars, proteins and oil content play an important role in determining seed quality, nutritional value and antioxidant potential of groundnut kernels. Phenolic compounds and anthocyanins contribute significantly towards antioxidant defence mechanisms and stabilization of cellular redox balance during storage (Shahidi and Ambigaipalan, 2015) and (Khoo et al., 2017). Similarly, protein and sugar content are important nutritional quality parameters influencing seed vigour, palatability and processing characteristics (Savage and Keenan, 1994). In this connection the current experiment is designed for characterization of biochemical variability among groundnut genotypes, which may facilitate identification of varieties possessing superior nutritional quality and enhanced storage resilience under semi-arid tropical environments (Zhao et al., 2022).

3. MATERIALS AND METHODS

3.1 Environmental conditions during crop growth and storage

During *Kharif* 2024, the prevailing temperature ranged from 21.1 to 34.9°C with morning relative humidity (RH-I) ranging from 75.0 to 81.8% and afternoon relative humidity (RH-II) ranging from 50.0 to 59.5%. Total rainfall received during the crop growth period was 643.4 mm. During *Kharif* 2025, temperature ranged from 16.5 to 35.8°C with RH-I ranging from 69.0 to 84.0 per cent and RH-II ranging from 43.0 to 73.0%. Seasonal rainfall received during the crop period was 587 mm (Table 1 & Fig. 1).

3.2 Plant Material

The study was conducted using six cultivated groundnut (*Arachis hypogaea* L.) genotypes, namely K-6, Dharani, Kadiri Lepakshi, Narayani, Himani and Visishta. The experimental seed material was obtained from the section of Breeding, Groundnut and Oilseeds, Regional Agricultural Research Station (RARS), Tirupati, Acharya N.G. Ranga Agricultural University (ANGRAU), Andhra Pradesh, India, where the field experiment was established and maintained. The experimental site is located at 13.63° N latitude and 79.42° E longitude, representing the semi-arid agro-climatic conditions of southern Andhra Pradesh. The study did not involve endangered, protected or wild plant species.

3.3 Experimental material and design

. The experiment was laid out in a Randomized Block Design (RBD) with three replications following recommended agronomic practices for groundnut cultivation under rainfed conditions.

Biochemical analyses of representative sample collected and were performed at harvest, 30 DAH and 60 DAH to assess temporal changes in pigment-associated metabolites and nutritional quality parameters during storage.

Total phenolic content was estimated following the Folin–Ciocalteu colorimetric method described by Singleton and Rossi (1965). Kernel extracts were reacted with Folin–Ciocalteu reagent followed by sodium carbonate solution and incubated for colour development and recorded absorbance at 765 nm and expressed as mg gallic acid equivalents (GAE) g^{-1} FW. Total anthocyanin content was estimated by the pH differential method based on differential absorbance of anthocyanin pigments. Kernel extracts were prepared in appropriate buffer systems and absorbance was measured at 520 and 700 nm and expressed as mg g^{-1} FW. Total flavonoid content was determined using aluminium chloride colorimetric assay. Kernel extracts were reacted with aluminium chloride reagent and the absorbance of the developed colour complex was measured at 415 nm and was expressed as mg quercetin equivalents g^{-1} FW. Total sugars were estimated by the Anthrone method as described by Yemm and Willis (1954). Kernel extracts were reacted with Anthrone reagent under acidic conditions and heated for colour development and recorded absorbance at 620 nm and expressed as mg g^{-1} FW. Protein content was estimated following the method of Lowry et al. (1951). Kernel extracts were reacted with alkaline copper reagent followed by Folin–Ciocalteu reagent and absorbance was measured at 660 nm and expressed in %. Oil content in kernels was estimated using Near Infrared Reflectance Spectroscopy (NIRS) at laboratory of biochemistry, ICAR-Indian Institute of Oilseeds Research (IIOR), Hyderabad following standard calibration procedures. Kernel samples were scanned using NIRS equipment and oil content was expressed on percentage basis. Observations on pod yield, kernel yield and shelling percentage were recorded at harvest following standard agronomic procedures. Pod yield and kernel yield were expressed in kg ha^{-1}.

Experimental data subjected to analysis of variance (ANOVA) appropriate for RBD following standard statistical procedures and tested Significance of treatment differences using critical difference (CD) at 5% probability level. Pearson's correlation coefficients were computed to determine association among kernel testa-associated biochemical metabolites. Linear regression analysis was also performed to study the relationship between total phenols and anthocyanin content among groundnut genotypes.

4. RESULTS AND DISCUSSION

4.1 Variability in kernel testa-associated biochemical metabolites

Significant variability was observed among the evaluated groundnut genotypes for kernel testa-associated biochemical metabolites under ambient storage conditions. Pigmented genotypes exhibited comparatively higher accumulation of phenols, flavonoids and anthocyanins than lightly pigmented genotypes, indicating genotypic differences in synthesis and accumulation of phenylpropanoid-derived metabolites. Similar genotypic variability for testa-associated metabolites in peanut has been reported by Wan et al. (2016), Chen et al. (2021) and Zhao et al. (2022).

Among the evaluated genotypes, K-6 recorded the highest total phenolic content (5.70 mg g^{-1} FW), followed by Himani and Kadiri Lepakshi. Higher accumulation of phenolic metabolites in pigmented genotypes may contribute towards enhanced antioxidant potential and improved biochemical stability during storage. Phenolic compounds are known to play an important role in antioxidant defence and stabilization of cellular redox balance during oxidative stress conditions (Shahidi and Ambigaipalan, 2015). Anthocyanin content differed significantly among the genotypes. K-6 recorded the highest anthocyanin content (0.347 mg g^{-1} FW), followed by Dharani (0.308 mg g^{-1} FW), indicating greater accumulation of pigment-associated metabolites in coloured testa genotypes. Anthocyanins are major flavonoid pigments associated with red and pink seed coat coloration and possess strong antioxidant properties that contribute towards oxidative stress tolerance and pigment stability during storage (Khoo et al., 2017).

Flavonoid content exhibited appreciable variation among the groundnut genotypes, suggesting differential accumulation of antioxidant-associated metabolites under semi-arid tropical conditions. At 60 days after harvest, highest and lowest total flavonoid content was recorded by Visishta (18.6 mg/g FW) and Himani (14.9 mg/g FW) respectively. This observed variability in flavonoid accumulation among genotypes may be attributed to genetic differences in phenylpropanoid metabolism and environmental influence during seed development. Total sugar content was comparatively higher at harvest stage and varied significantly among the genotypes. Dharani recorded the highest total sugar content (0.83 mg g^{-1} FW), followed by K-6 (0.73 mg g^{-1} FW). Sugars play an important role in maintenance of seed vigour, osmotic regulation and stabilization of cellular structures during storage.

Similarly, protein content also varied significantly among the genotypes and ranged from 22.93 to 25.64%, with Dharani recording the highest protein content (25.64%). Kadiri Lepakshi recorded comparatively higher oil content (48.8%), indicating superior kernel quality and nutritional composition among the evaluated genotypes.

4.2 Storage environment-mediated changes in biochemical metabolites and yield:

Progressive decline in phenols, anthocyanins and sugars was observed with advancement of storage duration from harvest to 60 days after harvest (DAH). The observed reduction in pigment-associated metabolites during storage may be associated with storage environment-mediated oxidative degradation under ambient environmental conditions prevailing in the semi-arid tropical region.

Moderate reduction in phenols and anthocyanins was observed at 30 DAH followed by substantial decline at 60 DAH, indicating progressive storage-induced deterioration of pigment-associated metabolites. Similar reductions in antioxidant-related metabolites during storage have been reported in oilseed crops due to oxidative metabolic alterations and seed ageing processes (Bailly, 2020; Kumar et al., 2021). Anthocyanins are particularly susceptible to degradation through oxidation, polymerization and structural modifications during prolonged storage under ambient conditions (Liu et al., 2020). The decline in sugar content during storage may be attributed to respiratory metabolism and utilization of soluble carbohydrates during seed ageing. Protein content exhibited relatively lower variation during storage compared to phenols and anthocyanins, indicating comparatively better stability of storage proteins under ambient conditions. However, slight reductions observed during prolonged storage may be associated with protein denaturation and metabolic deterioration occurring during seed ageing.

The groundnut genotypes studied are differed significantly for pod yield, kernel yield and shelling percentage under semi-arid tropical conditions. Among the genotypes Dharani recorded maximum of total sugars content (0.83 mg/g DW) followed by K-6 (0.73 mg/g DW) and minimum content in Kadiri Lepakshi (0.32 mg/g DW). This can attributed to inherent biochemical and metabolic ability of genotype to produce more sugar content. Visishta recorded superior pod yield (2015.4 kg ha^{-1}) and kernel yield (1396.7 kg ha^{-1}), indicating better yield potential and assimilate partitioning efficiency under prevailing environmental conditions. Whereas Kadiri Lepakshi recorded highest harvest index of 71.7% followed by visishta and himani (69.3%). Kernel oil content is found be highest in Kadiri Lepakshi (48.80%), followed by Visishta (48.51%) and lowest in Himani (45.50%).Genotypic variability in yield traits may be attributed to differential adaptation of genotypes to climatic variability and resource utilization efficiency under rainfed conditions.

4.3 Linear regression analysis was performed to study the relationship between total phenols and anthocyanin content and shown a weak positive association between total phenols and anthocyanins. This may be attributed to genotype-specific differences in metabolite partitioning, anthocyanin biosynthesis and storage environment-mediated degradation of pigment-associated metabolites under ambient conditions. These observations were in alignment with reports of Chen et al. (2021) and Zhao et al. (2022). with the regression equation: $y = 0.0068x + 0.2448$ (where y represents anthocyanin content and x represents total phenolic content). The coefficient of determination ($R^2 = 0.014$) indicated relatively low linear dependence between total phenols and anthocyanin accumulation among the genotypes.

Pearson’s correlation analysis revealed differential association among biochemical metabolites associated with kernel testa pigmentation and storage stability. Positive association was observed among phenols, flavonoids and anthocyanins, indicating coordinated accumulation of phenylpropanoid-derived metabolites associated with kernel testa pigmentation. Positive relationship among antioxidant-associated metabolites indicates their possible role in maintenance of biochemical stability and cellular redox balance under ambient storage conditions. Protein content exhibited negative association with pre-harvest phenols, suggesting differential metabolic allocation among groundnut genotypes. Similar positive relationships between testa colour intensity and antioxidant metabolites have been reported in peanut genotypes by Zhao et al. (2022). Higher accumulation of phenols and anthocyanins in pigmented genotypes may contribute towards stabilization of cellular redox balance during storage, thereby reducing degradation of pigment-associated metabolites. The results indicated that genotypes possessing higher antioxidant metabolite accumulation may exhibit comparatively better storage stability and post-harvest quality under semi-arid tropical environments.

4.4 Principal Component Analysis (PCA)

Principal component analysis (PCA) was performed to elucidate the multivariate relationships among biochemical and storage-related traits of groundnut showed that, first two principal components (PC1 and PC2) together explained 81.9% of the total variation, indicating adequate representation of components in variability among the evaluated genotypes. The PCA biplot clearly differentiated the genotypes based on their biochemical composition and storage behaviour.

K-6 was positioned closer to phenol and anthocyanin vectors, indicating its association with higher antioxidant metabolite accumulation. Dharani was associated with higher protein and sugar content, suggesting superior nutritional quality. K. Lepakshi showed a strong association with kernel oil content, while Narayani exhibited greater proximity to anthocyanin-related traits, indicating comparatively better pigment stability during storage. The observed separation among genotypes demonstrates substantial biochemical diversity and suggests that antioxidant-associated metabolites contribute significantly to storage stability in groundnut kernels. These findings highlight the potential of K-6, Dharani and Narayani as valuable genetic resources for improving nutritional quality and post-harvest storage resilience in groundnut.

5. CONCLUSION

Significant genotypic variability was observed for kernel testa-associated biochemical metabolites among the evaluated groundnut genotypes under semi-arid tropical conditions. K-6 exhibited higher phenols (5.70 mg g^{-1} FW) and anthocyanin content (0.347 mg g^{-1} FW), Dharani recorded higher sugar (0.83 mg g^{-1} FW) and protein content (25.64%), while Kadiri Lepakshi showed higher oil content (48.8%). Progressive decline in phenols, anthocyanins and sugars during storage indicated storage environment-mediated deterioration of antioxidant-associated metabolites under ambient conditions. Positive association among phenols, flavonoids and anthocyanins suggested their role in maintenance of kernel testa pigmentation and biochemical stability during storage. Among the evaluated genotypes, Visishta recorded superior pod yield (2015.4 kg ha^{-1}) and kernel yield (1396.7 kg ha^{-1}), whereas K-6, Dharani and Narayani exhibited comparatively better biochemical stability. The identified genotypes may be useful for improving post-harvest quality and storage resilience of groundnut under semi-arid tropical environments of Andhra Pradesh.

Declarations

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Funding Declaration:

There was no external funding received to conduct the trail and the entire experiment is conducted from financial and logistic support from ANGRAU.

Ethics Approval, Consent to Participate and Consent to Publish

Not applicable. This study did not involve human participants, human data, human tissues, animals, or any procedures requiring ethical approval.

Declaration of Generative AI Use

During the preparation of this manuscript, the authors taken help of OpenAI to assist with language editing, grammar correction, formatting and improvement of scientific writing. The authors reviewed, edited, and take full responsibility for the content of the manuscript. No generative AI tool was used for data generation, interpretation of results or drawing scientific conclusions.

Further, all the data in the article is result of field and laboratory experimentation for two years period. The data is original and we authors can provide the raw data if required.

Ethics and consent to participate

The study involved cultivated groundnut (*Arachis hypogaea* L.) genotypes and did not involve endangered or protected plant species. Collection and use of plant materials of the cultivated Groundnut genotypes complied with institutional and national guidelines. All experimental procedures were conducted in accordance with the regulations of Acharya N.G. Ranga Agricultural University (ANGRAU), India. The plant materials were collected exclusively from the institutional experimental oilfields where the study was conducted as per the technical programme evaluated and approved by the university. Therefore, no additional permits, collection licences or governmental approvals were required for collection or use of the cultivated plant material.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Tables

Table 1: Environmental conditions prevailing during *Kharif* 2024 and 2025 under semi-arid tropical conditions at RARS, Tirupati.

Month/Year	Max. Temp. (°C)	Min. Temp. (°C)	RH-I (%)	RH-II (%)	Sunshine hours	Rainfall (mm) [Rainy days]
Kharif 2024						
Jul-24	34.7	23.9	75	50.9	2.9	132.8 (9)
Aug-24	34.9	23.8	75.8	51	5.1	130.0 (7)
Sep-24	34.9	22.7	75	50	4.7	149.8 (7)
Oct-24	33.2	21.1	81.8	59.5	5.8	230.8 (9)
Rabi 2024–25						
Dec-24	29.1	18.7	86.1	67.7	4.4	286.4 (9)
Jan-25	29	14.7	85	56.6	6.3	29.6 (2)
Feb-25	33.6	14.4	83.9	38.2	9	0.0 (0)
Mar-25	36.5	18.7	79.4	34.8	8.6	0.0 (0)
Apr-25	38.4	22.4	69.8	34.5	8.2	2.0 (0)

RH= Relative Humidity mm= milli meter ^{0}C= degrees centigrade

S.No.	Canopy Temperature (^{0}C)	Soil Temperature (^{0} C)
1	29.0	30.4
2	29.4	32.8
3	28.7	31.0
4	28.8	30.2
5	28.5	30.5
6	28.4	32.0
Mean	29.0	30.4
SEm(±)	0.48	0.3
CD (P=0.05)	1.57	0.99
CV (%)	2.89	1.69

Table 2: Variability in kernel testa-associated phenols, anthocyanins and flavonoids among groundnut genotypes during pre harvest and post harvest storage periods.

S.No.	Variety	Testa colour	Total phenols (mg GAE/g FW)				Total Flavonoids (mg/g FW)			Anthocyanins (mg/g FW)		
			Pre harvest (leaves)	30 DAH (kernels)	60 DAH (kernels)	Retention (%)	Pre harvest (leaves)	60 DAH (kernels)	Retention (%)	Pre harvest (leaves)	30 DAH (kernels)	Retention (%)
1	Himani	White	5.35	3.9	0.71	18.2	22.9	14.9	65.1	0.230	0.106	46.1
2	K-6	Rose	5.70	4.5	0.69	15.3	13.9	17.6	79.0	0.347	0.111	32.0
3	Visishta	Light pink	4.11	4.4	0.63	14.3	24.2	18.6	76.9	0.231	0.101	43.7
4	K Lepakshi	Light pink	5.22	3.7	0.66	17.8	19.2	18.5	96.4	0.260	0.121	46.5
5	Dharani	Light pink	3.65	4.7	0.75	16.0	18.9	15.3	81.0	0.308	0.107	34.7
6	Narayani	Light red	4.29	4.9	0.88	18.0	20.3	17.2	84.7	0.284	0.228	80.3
	Mean		4.72	4.4	0.72	16.4	19.9	17.0	85.4	0.277	0.129	46.6
	SEm (±)		0.189	0.215	0.221		0.004	0.004		0.296	0.193	
	CD (P=0.05)		0.732	0.678	0.719		0.012	0.011		0.932	0.608	
	CV (%)		28.91	31.22	30.17		2.67	4.68		2.64	1.96	

GE= Gallic acid equivalents Ha= hectare DAH = Days After Harvest; FW = Fresh Weight

Table 3: Variability in total sugars, protein and oil content among groundnut genotypes during storage under ambient conditions.

S.No.	Variety	Kernel Protein (%)	Total Sugars mg/g DW		Kernels Oil Content (%)
			Pre harvest (leaves)	30 DAH (Kernels)	
1	Himani	23.92	0.62	0.43	45.50
2	K-6	22.93	0.73	0.32	46.45
3	Visishta	24.96	0.43	0.39	48.51
4	K Lepakshi	23.85	0.32	0.30	48.80
5	Dharani	25.64	0.83	0.53	47.03
6	Narayani	23.47	0.71	0.28	46.87
	Mean	24.1	0.60	0.40	47.19
	SEm (±)	0.2	0.02	0.014	0.37
	CD (P=0.05)	0.64	0.064	0.043	1.17
	CV (%)	1.47	4.85	5.23	1.34

Table 4: Yield and yield-related traits of groundnut genotypes evaluated under semi-arid tropical conditions.

S.no	Genotype	Pod yield (kg/ha.)	Kernel Yield (Kg /Ha)	Shelling %
1	Himani	1143.3	792.3	69.3
2	K-6	1068.8	731.0	68.4
3	Visishta	2015.4	1396.7	69.3
4	K Lepakshi	1016.9	729.1	71.7
5	Dharani	1337.9	892.4	66.7
6	Narayani	1326.3	855.4	64.5
	Mean	1330.3	927.2	68.3
	SEM	108.6	83.4	
	CD (5%)	228.1	175.2	
	CV (%)	34.6	38.2	

Figures

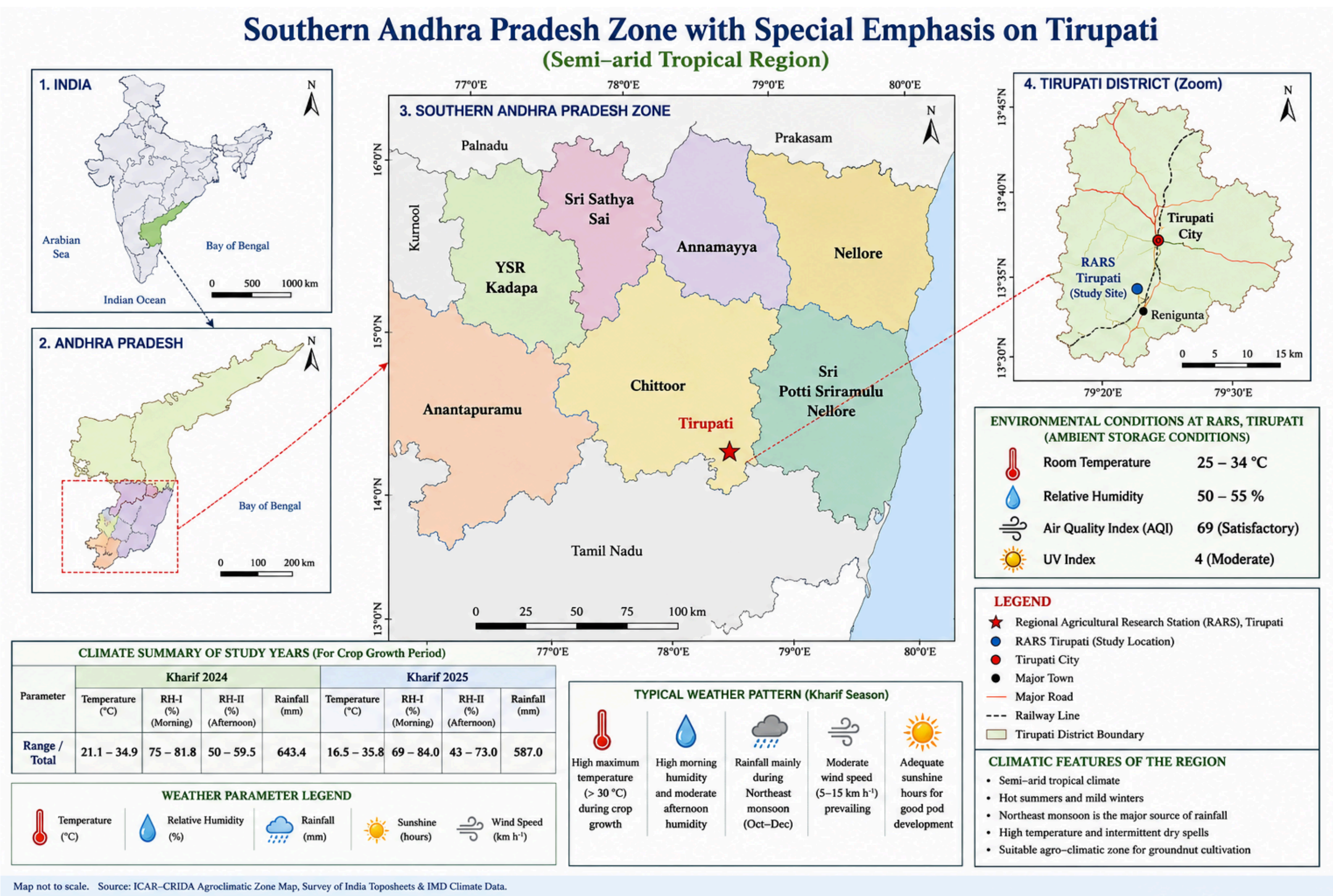

Figure 1

Study area and prevailing environmental conditions of RARS, Tirupati under semi-arid tropical conditions.

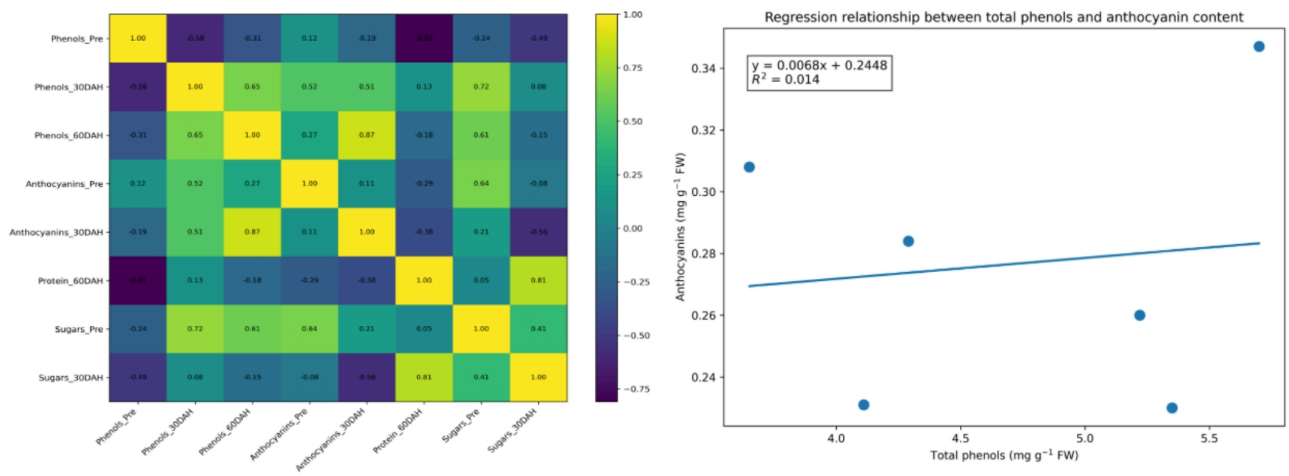

Figure 2

Pearson correlation heatmap showing association among biochemical metabolites in groundnut genotypes. And regression relationship between total phenols and anthocyanin content among groundnut genotypes

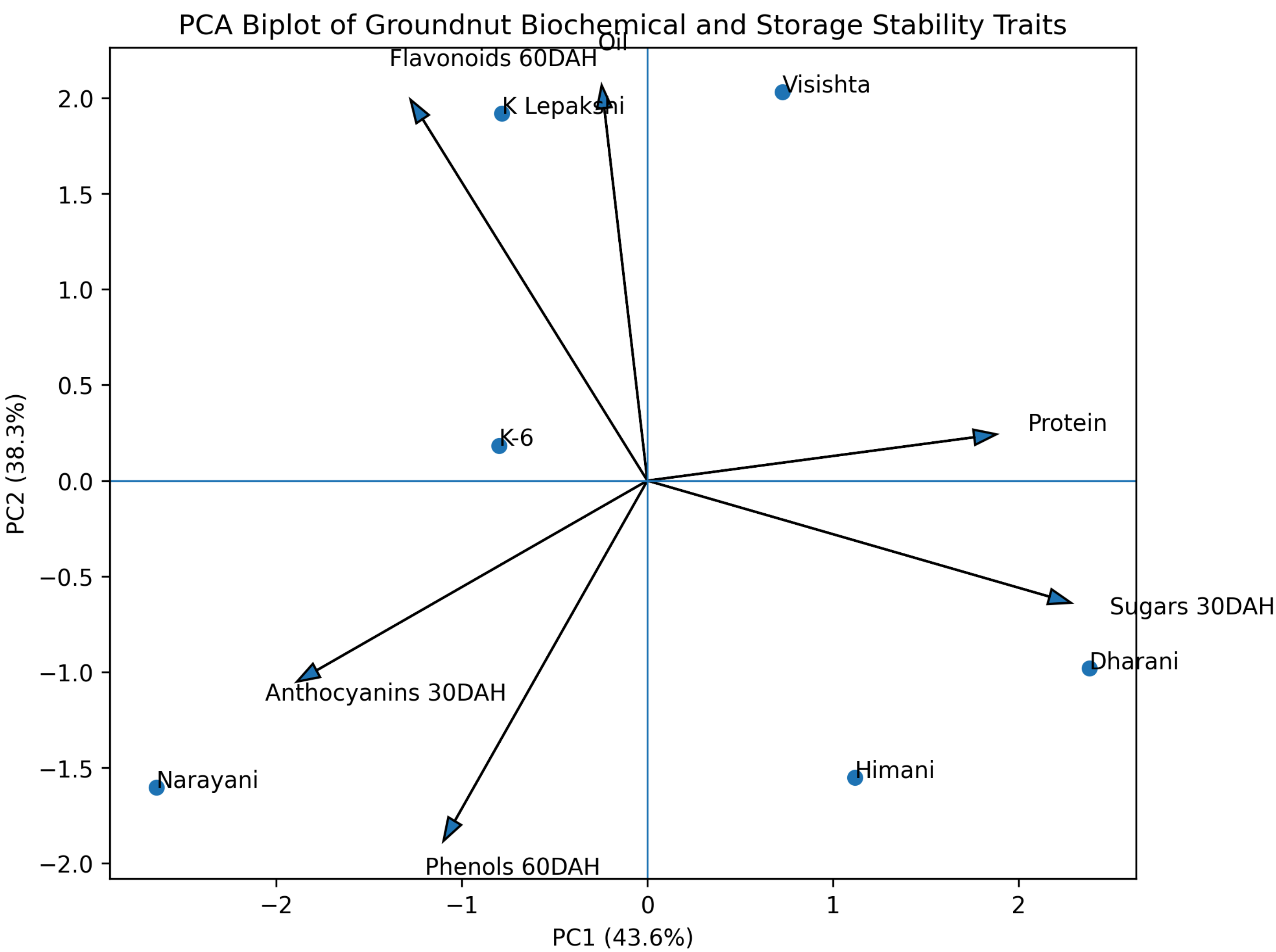

Figure 3

Principal component analysis (PCA) Bi plot displaying association among storage stability and biochemical quality traits of Groundnut under pre and post harvest conditions.

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