

Integrated Metabolomics and Principal Component Analysis Uncover Boron-Driven Responses in Groundnut (*Arachis hypogaea* L.)

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Article

Keywords: Groundnut, Boron, GC-MS, Metabolic pathway analysis, PCA

Posted Date: December 8th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-8151077/v1>

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Additional Declarations: No competing interests reported.

Abstract

The strategic application of micronutrients, particularly boron, plays a pivotal role in modulating key metabolic pathways and enhancing physiological traits that contribute to crop productivity. This study investigates the biochemical and physiological responses of groundnut variety Kadiri Lepakshi K1812 to foliar boron application using an integrated approach involving Gas Chromatography–Mass Spectrometry, metabolic pathway enrichment and Principal Component Analysis. Gas Chromatography–Mass Spectrometry profiling at 54 days after sowing identified 43 metabolites among 159 components including amino acids, fatty acids, sugars and secondary metabolites. Boron application enhanced the accumulation of metabolites such as myo-inositol, succinic acid and ferulic acid, which are associated with improved osmotic adjustment, antioxidative defence, carbon and nitrogen metabolism and cellular energy status. Metabolic pathway enrichment analysis revealed significant upregulation of the tricarboxylic acid cycle, amino acid biosynthesis, phenylpropanoid pathway and glycolysis, indicating a coordinated metabolic reprogramming that supports higher energy production, stress resilience and efficient nutrient assimilation. Along with principal component analysis further confirmed that boron-treated plants exhibited distinct separation from the control, with the first two principal components explaining 97.19% of total variation, primarily driven by enhanced chlorophyll content, dry matter accumulation, plant nutrient uptake and pod yield of groundnut. The findings suggest that boron facilitates increased photosynthetic efficiency, carbon partitioning and enhanced source-sink dynamics, which collectively contribute to improved biomass yield and yield attributes of groundnut. This multilayered analysis underscores the role of boron in driving metabolic and physiological enhancements that translate into superior groundnut productivity, offering valuable insights for sustainable crop management.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the most important oilseed crops cultivated in tropical and subtropical regions, valued for its nutritional richness, high oil content, and economic importance. However, its productivity is frequently limited by nutrient imbalances, particularly micronutrient deficiencies that impair growth, development, and yield potential. Among essential micronutrients, boron (B) plays a pivotal role in maintaining pollen tube elongation, cell wall integrity, membrane stability, reproductive development, and metabolic regulation [1] [2] [3] [4]. Boron deficiency in groundnut has been widely associated with impaired flowering, reduced pollen viability, poor pod formation, and inferior seed development, all of which contribute to significant yield loss [5] [6].

According to recent studies, boron affects not only reproductive and structural functions but also essential metabolic pathways that are connected to energy generation, stress tolerance, and nutrient absorption, all of which are vital for increasing yield. It has been demonstrated that sufficient boron availability enhances source-sink relationships, improves assimilate partitioning, and encourages biomass buildup and pod filling, all of which lead to increased yields in groundnut and other crops. For groundnut pod formation and yield augmentation, boron is essential for cell wall synthesis, pollen tube

elongation, flower retention, and seed development [7]. Despite this acknowledged significance, little is known about the exact biochemical processes by which boron affects yield characteristics.

The ability to decipher these biochemical reactions to food manipulations at the molecular level has been made possible by developments in plant metabolomics. A reliable method for untargeted profiling of primary and secondary metabolites in plant tissues is gas chromatography–mass spectrometry (GC-MS) [8] [9] [10] [11]. This method provides a comprehensive picture of metabolic activity and stress responses under various dietary regimes by detecting a wide range of chemicals, including fatty acids, sugars, sugar alcohols, amino acids, organic acids, and phenolic compounds.

In parallel, the identification of certain metabolic pathways affected by boron is made easier by linking GC-MS data with pathway enrichment techniques like KEGG and MetaboAnalyst [12] [13] [14]. Furthermore, in order to identify patterns in high-dimensional datasets and identify important characteristics and metabolites that distinguish treatment responses, multivariate statistical analyses in particular, Principal Component Analysis, or PCA—are being used more and more [15] [16]. Environmental influences have a significant impact on the physiological and yield responses of crops like groundnuts, highlighting the importance of comprehending the underlying metabolic and biochemical pathways in order to design climate-resilient strategies [17].

Therefore, this study aims to integrate GC-MS-based metabolomic profiling, pathway enrichment analysis, and PCA to elucidate the metabolic and physiological mechanisms through which foliar boron application enhances yield in groundnut. By linking metabolite changes to physiological and agronomic trait expression, this multilayered approach seeks to provide novel insights into how boron application can be strategically utilized to optimize physiology growth, yield and promote sustainable groundnut production [18] [19].

MATERIALS AND METHODS

Field and Experimental details

This experiment was conducted during the rabi season 2025 at SRM College of Agricultural Sciences, Baburayanpettai, Chengalpattu (Dt), Tamil Nadu, India. The Groundnut variety “KADIRI LEPAKSHI (K1812)” seeds were sown on 23rd January 2025 with a recommended seed rate of 25 kg kernel/ha at spacing 30 cm × 10 cm. Seeds of the groundnut variety Kadiri Lepakshi (K812) were obtained from the Regional Agricultural Research Station (RARS), Kadiri, Andhra Pradesh, India. The various agronomic practices and other management practices apart from the treatment were performed according to the package and practices of Crop Production Guide given by Tamil Nadu Agricultural University, Coimbatore, India.

The experiment was designed in Randomized Block Design, with nine treatments and 3 replications. The treatments are T₁: 100% RDN + soil application of boron @ 5kg/ha, T₂: 75% RDN + soil application of

boron @ 5kg/ha, T₃: 100% RDN + soil application of molybdenum @ 5kg/ha, T₄: 75% RDN + soil application of molybdenum @ 5kg/ha, T₅: 100% RDN + foliar application of boron @ 0.5%, T₆: 75% RDN + foliar application of boron @ 0.5%, T₇: 100% RDN + foliar application of molybdenum @ 0.3%, T₈: 75% RDN + foliar application of molybdenum @ 0.3%, T₉: control 100% RDN (25:50:75 kg/ha). Growth and yield parameters of the groundnut crop were recorded at 54 DAS.

GC-MS Analysis and Metabolomics Data Processing

Leaf samples from each treatment were collected at 52 DAS (pod formation stage), washed, shade-dried at room temperature, and grinded into a fine powder. For extraction, 1 g of dried powder was mixed with 10 mL methanol, ultrasonicated for 30 minutes, and centrifuged at 10,000 rpm for 10 minutes. The supernatant was filtered through a 0.22 µm syringe filter for GC-MS analysis.

GC-MS was performed using a SHIMADZU QP2010 PLUS system equipped with an HP-5MS capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness).

The raw data were processed using MetaboAnalyst 6.0. Data were uploaded in CSV format, log-transformed, and auto-scaled prior to multivariate analysis. Principal Component Analysis (PCA) was used to visualize treatment-wise clustering and identify key metabolites responsible for separation. Univariate analyses including fold change and volcano plots were conducted to highlight significant metabolites between treatments. Pathway analysis based on the KEGG database was performed to explore the biological relevance of the differential metabolites. Pathway enrichment analysis was performed using KEGG database tools (Kanehisa and Goto, 2000; Kanehisa, 2019; Kanehisa et al., 2025). All analyses and visualizations, including heatmaps and scatter plots, were generated using default settings in MetaboAnalyst 6.0.

RESULTS AND DISCUSSION

GC-MS Analysis

The GC-MS analysis revealed significant differences in metabolite accumulation between treatment 100% RDN + foliar application of boron @ 0.5% and control treatments. A total of 159 metabolites were identified across both treatments, categorized into organic acids, amino acids, sugars, sugar alcohols, fatty acids, and secondary metabolites.

(100% RDN + foliar application of Boron @ 0.5%)

Notably higher abundance of L-aspartic acid, D-glucose, L-serine, palmitic acid, linolenic acid, and succinic acid were observed in 100% RDN + foliar application of boron @ 0.5%. Boron treatment led to elevated levels of stress-responsive compounds such as myo-inositol and glycerol, indicating improved osmotic regulation and stress tolerance. Phenolic acids such as ferulic acid and benzoic acid were also

upregulated, suggesting enhanced antioxidative defence [20]. These findings confirm the essential role of boron in modulating key primary and secondary metabolic pathways [21].

(Control 100% RDN)

The control treatment showed a relatively lower abundance of amino acids and organic acids. High levels of mannitol and certain fatty acids like oleic acid were recorded, reflecting a baseline metabolic profile without external micronutrient influence. [22]. These changes reflect boron’s role in promoting nitrogen assimilation, carbon fixation, and secondary metabolism, crucial for pod development and physiological resilience [23].

Metabolite Profiling Using GC-MS

GC-MS analysis revealed variations in metabolite composition across different treatments. Several bioactive compounds were identified, as follows in Table 1.

Table 1

Key metabolites identified through GC-MS analysis in groundnut, their biochemical classes and their possible physiological functions contributing to stress adaptation and yield improvement.

Metabolite	Class of metabolite	Possible Function in Plants
Palmitic Acid	Fatty Acid	Energy storage and membrane stability
Linoleic Acid	Fatty Acid	Enhances seed oil quality
Phenol Derivatives	Phenolics	Antioxidant properties and stress resistance
Flavonoids	Secondary Metabolites	Improves plant defence mechanisms
Amino Acids	Proteins	Protein synthesis and stress adaptation

Metabolic Pathway Enrichment

A comparative pathway enrichment analysis revealed distinct alterations in key biochemical pathways under boron treatment Table 2.

Table 2

Overview of significantly enriched metabolic pathways identified through GC-MS-based pathway analysis in groundnut under boron and control treatments, with associated impact levels, statistical significance and key metabolites involved.

Pathway Name	Impact (Boron)	Impact (Control)	P-value (Boron)	P-value (Control)	Key Metabolites Affected
TCA Cycle	High	Moderate	0.004	0.028	Succinic acid, Fumaric acid
Alanine, Aspartate & Glutamate Met.	High	Low	0.002	0.040	Aspartic acid, Glutamic acid
Glycolysis/Gluconeogenesis	Moderate	Low	0.008	0.045	Glucose, Glycerol
Phenylpropanoid Biosynthesis	High	Low	0.001	0.032	Ferulic acid, Benzoic acid
Fatty Acid Biosynthesis	Moderate	Moderate	0.015	0.027	Palmitic acid, Oleic acid

The TCA cycle and amino acid biosynthesis pathways showed higher activity in T₅, indicating enhanced energy metabolism and protein biosynthesis under boron influence [24] [25]. The phenylpropanoid pathway, which is responsible for plant defence and lignin synthesis, was prominently activated in boron-treated plants, aligning with improved Vigor and stress resilience [26].

Functional Interpretation

The upregulation of glycolytic intermediates and TCA cycle metabolites under 100% RDN + foliar application of boron @ 0.5% suggests higher photosynthetic efficiency and better carbon flux. Elevated phenolic compounds under boron application can be attributed to induced defence responses, possibly leading to improved disease resistance (Garcia and Barbas, 2011; Wang *et al.*, 2019). The increased availability of amino acids supports protein synthesis during active vegetative growth and reproductive stages [27].

Implications for Groundnut Productivity

These findings support the hypothesis that foliar application of boron enhances the metabolic competence of groundnut by regulating crucial physiological and biochemical pathways. Improved metabolite profiles are strongly correlated with better chlorophyll content, dry matter accumulation, pod development with overall yield and yield attributes.

Principal Component Analysis (PCA)

To understand the multidimensional variation among physiological, biochemical, and yield-related traits influenced by boron foliar application, Principal Component Analysis (PCA) was carried out using 23 quantitative variables. The first two principal components accounted for 97.19% of the total variance, with PC1 explaining 94.53% and PC2 explaining 2.66%, indicating a strong dominance of PC1 in explaining trait variation. (Fig. 2.)

PC1 was positively and strongly associated with several key traits, including pod yield, haulm yield, dry matter production, chlorophyll content, leaf area index, protein content, oil content, and macro-nutrient uptake like nitrogen, phosphorus and potassium. These variables contributed significantly to the separation of treatments, particularly distinguishing the boron foliar treatment from the control treatment, signifying enhanced physiological and metabolic responses due to boron supplementation.

PC2, though contributing a lesser variance, was influenced by traits such as harvest index, protein content, and root nodule parameters, representing a secondary but relevant axis related to assimilate partitioning and symbiotic nitrogen fixation. The biplot clearly delineates treatment clusters, where T₅ is associated with superior performance traits, while T₉ aligns with lower biomass and yield outputs, indicating its relative inferiority.

The PCA biplot effectively visualized the relationships between treatments and traits, with the first two principal components explaining 94.2% of the total variability in the dataset. [28] These findings are in alignment with previous studies, where GC-MS coupled with multivariate tools such as PCA has been successfully used to characterize metabolomic shifts in plants under micronutrient treatments [29]. In groundnut, similar patterns were observed by [20] and [23], where boron application significantly altered metabolite composition and improved physiological efficiency. PCA analysis in those studies revealed clustering of high-performing treatments along PC1, supporting our current observations.

CONCLUSION

GC-MS and metabolic pathway analysis revealed that boron application at 0.5% foliar concentration significantly alters the metabolic profile of groundnut, promoting energy metabolism, nitrogen assimilation, and defence-related secondary metabolites. These insights justify the inclusion of boron as a critical micronutrient in groundnut cultivation, contributing to sustainable productivity and resource-use efficiency. The GC-MS metabolic pathway comparison between T₅ (Boron 0.5%) and T₉ (Control) demonstrates a marked increase in lipid metabolism, cuticle development, and secondary metabolite biosynthesis in the boron-treated plants. These changes suggest boron stimulates protective cuticle formation, enhances membrane stability and function and supports plant hormonal and stress regulatory mechanisms. The presence of fatty acid elongation exclusively in 100% RDN + foliar application of boron @ 0.5% further implies a boron-induced role in structural lipid enhancement, potentially contributing to improved pod formation overall yield and yield attributes. Thus, PCA confirms the biochemical and agronomic benefits of foliar application of boron at 0.5%, reinforcing its influence on improving carbon assimilation, protein synthesis, and yield-related parameters in groundnut.

Declarations

Author Contribution

All authors contributed to the study conception and design. Conceptualization by Sivakumar K; Methodology by B.T. Suresh Kumar, Sivakumar K; Data curation and analysis was performed by B.T. Suresh Kumar, Chandrasekaran P; Writing original draft: B.T. Suresh Kumar; Vasanth P; Writing, review and editing: Sivakumar K, Jeyajothi R; Supervision: Sivakumar K, Chandrasekaran P. All authors reviewed and approved the final manuscript.

Acknowledgement

Authors express thankful to the Department of Agronomy, SRM College of Agricultural Sciences, SRM Institute of Science and Technology, Baburayenpettai-603201, Chengalpattu, Tamil Nadu, India for providing necessary facilities and assistance in support to conduct the research work.

Data Availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

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Figures

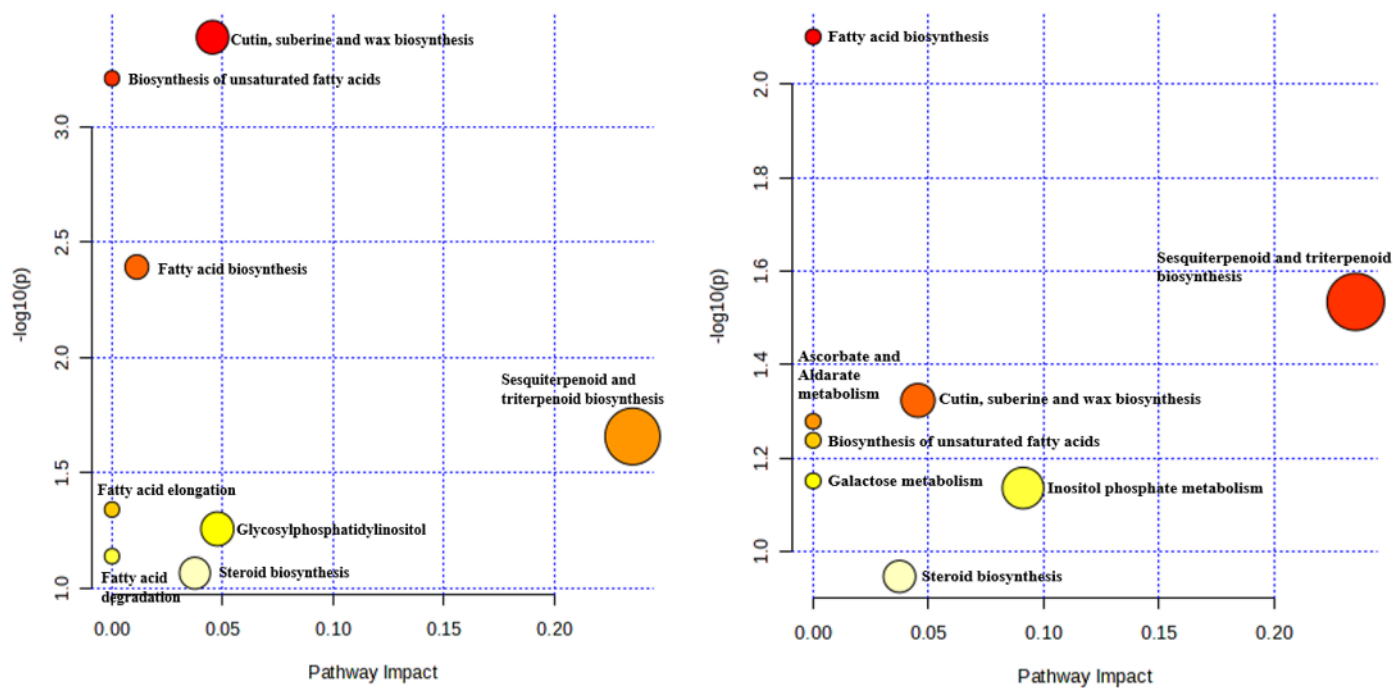


Figure 1

Pathway impact plot comparing enrichment scores for both treatment boron spray and control treatment.

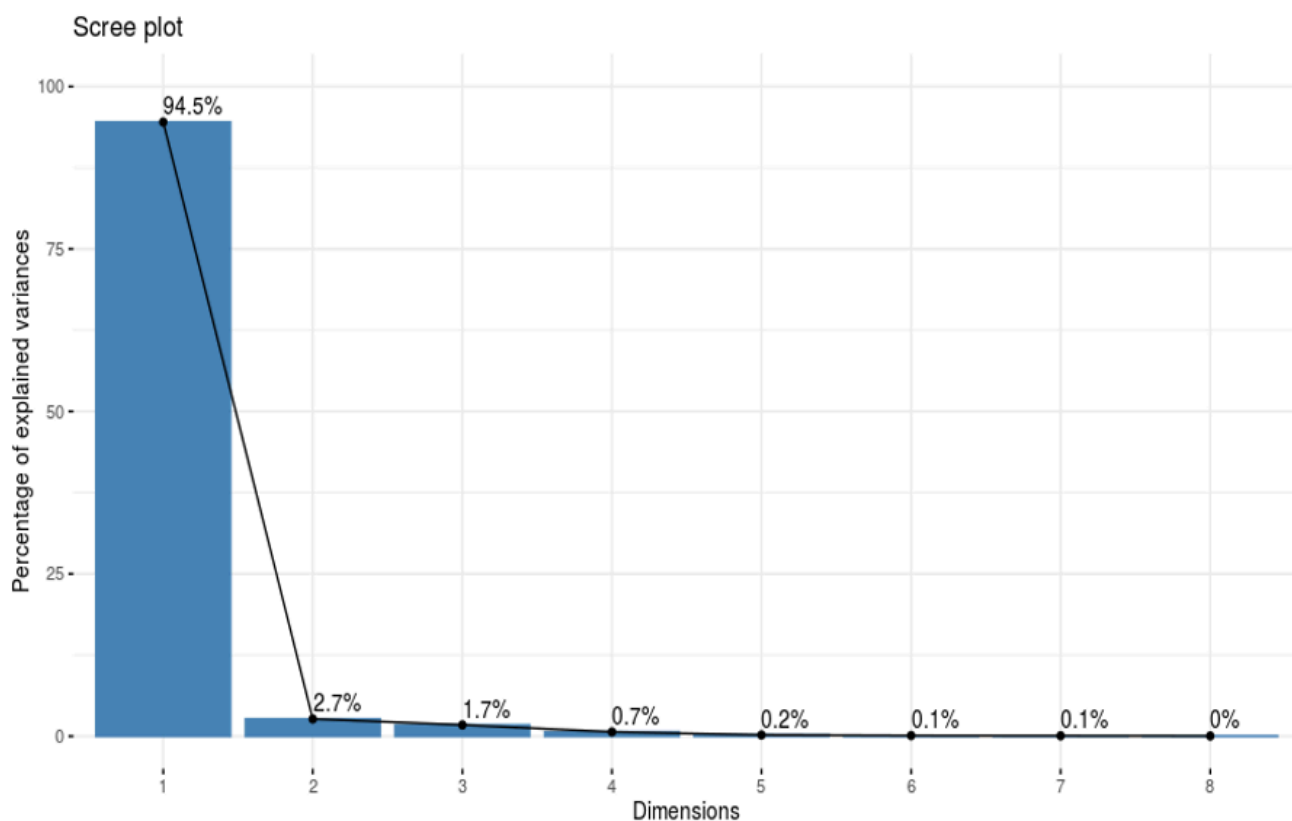


Figure 2

Screen Plot

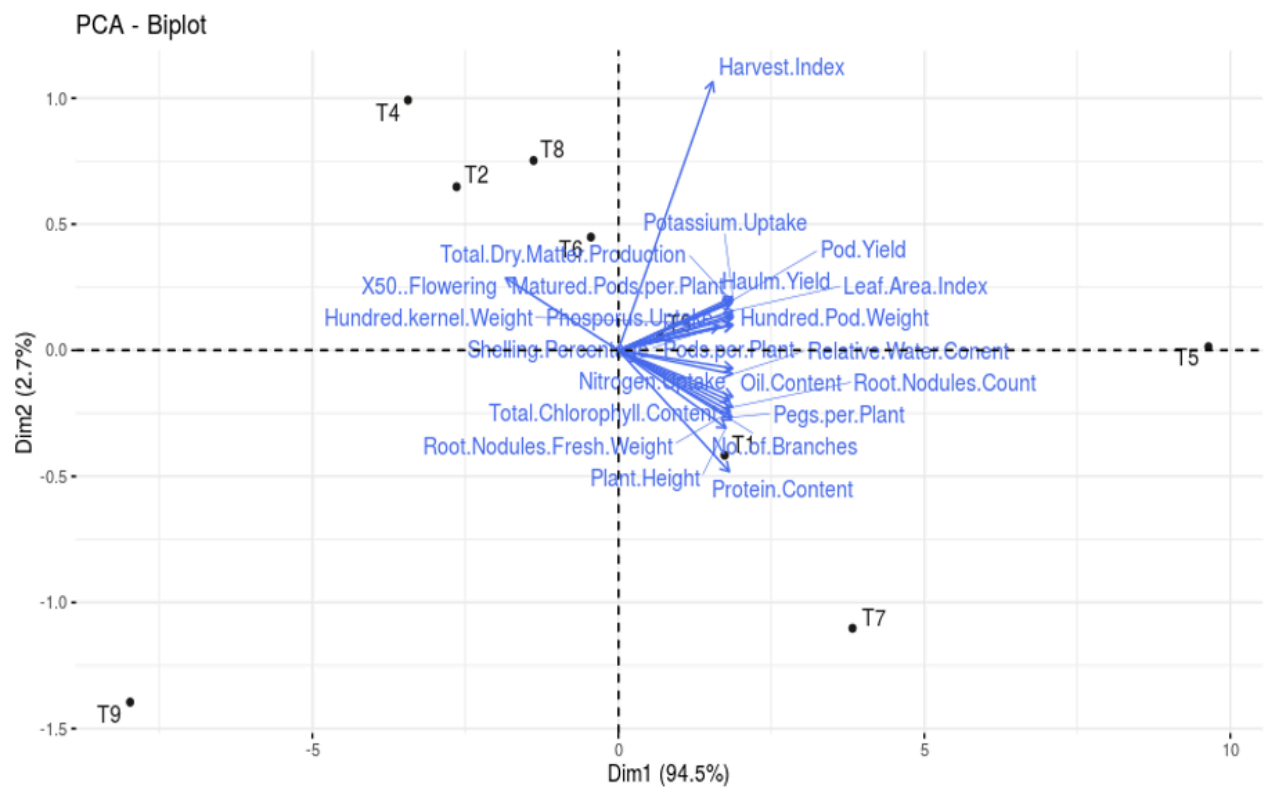


Figure 3

PCA-Biplot

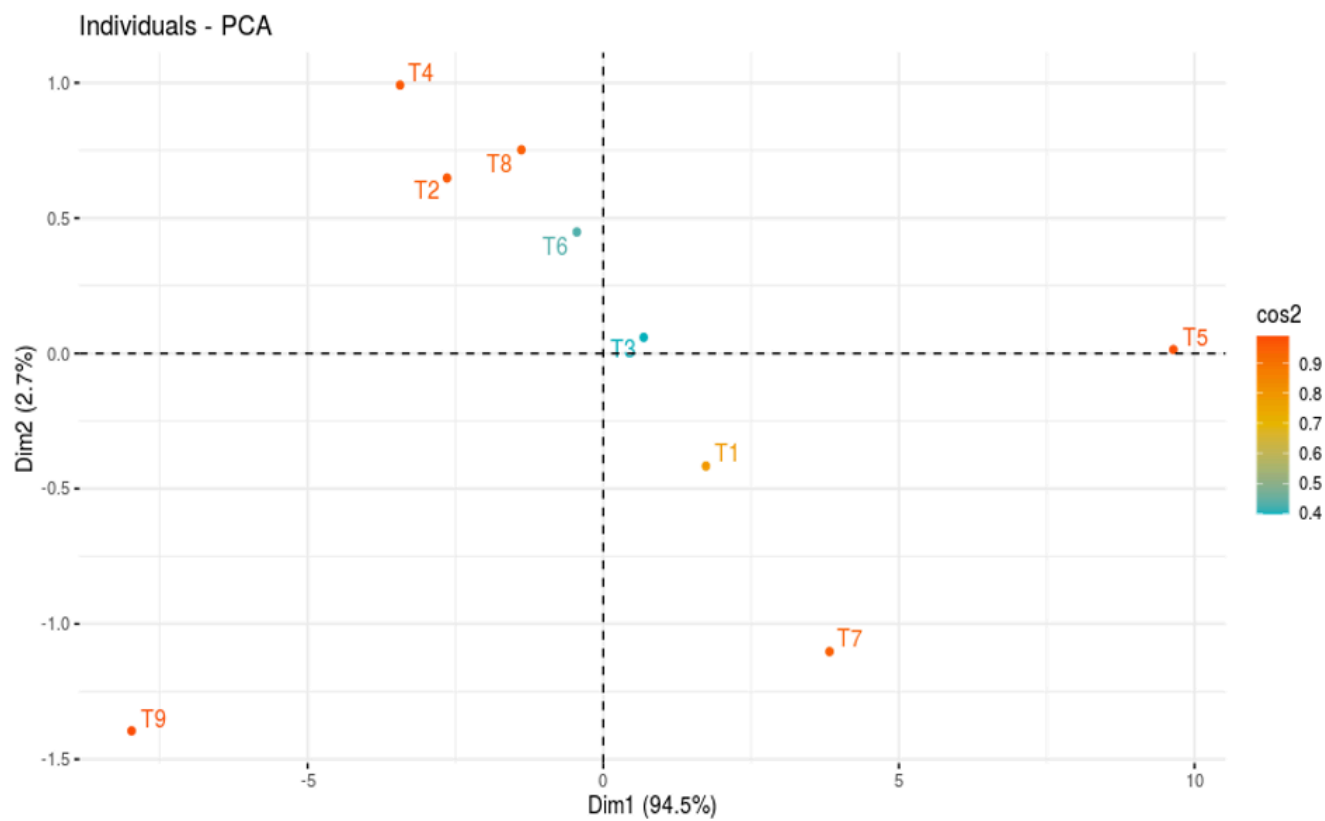


Figure 4

Individuals PCA