

# Elucidate the Impact of Rust Infection on Primary Metabolic Processes in *Phyllanthus emblica*

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## Research Article

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## Abstract

This study investigated the effect of rust infection on primary metabolic processes in *Phyllanthus emblica* (Aonla), a nutritionally important fruit crop known for its exceptionally high vitamin C content and medicinal value. Rust disease, mainly caused by *Ravenelia emblicae*, is one of the most destructive foliar diseases affecting Aonla cultivation and often leads to severe reductions in plant vigor, photosynthetic efficiency, and fruit quality. The experiment was evaluating rust-induced changes in photosynthetic pigments, carbohydrate metabolism, protein and lipid composition, and important fruit quality parameters across different genotypes. Principal Component Analysis (PCA) under healthy conditions revealed that the first two principal components explained 91.80% of the total variation (PC1: 81.75%, PC2: 10.05%), indicating strong genetic differentiation and coordinated metabolic activity among the genotypes. Genotypes G13 (NA-25) and G12 (NA-26) exhibited superior biochemical performance, recording the highest values for vitamin C, total sugars, chlorophyll, carotenoids, and total soluble solids. Strong positive associations among sugars, proteins, fats, fiber, pectin, acidity, and antioxidant pigments indicated efficient photosynthetic activity and stable metabolic balance, contributing to improved fruit quality and nutritional value. Under infection, PC1 and PC2 explained 91.39% of the total variation (PC1: 82.14%, PC2: 9.25%), suggesting that disease stress strongly influenced biochemical variability. Rust infection caused substantial reductions in biochemical traits, including nearly 50% decline in vitamin C, up to 70% reduction in chlorophyll, and about 60% loss of carotenoids, along with decreases in sugars, proteins, fats, fiber, and pectin. These changes indicate metabolic imbalance and deterioration of fruit quality under rust stress.

## Introduction

*Phyllanthus emblica* L., commonly known as Aonla or Indian gooseberry, is a perennial deciduous fruit tree belonging to the family Phyllanthaceae (Morton, 1987; Singh et al., 2011; Kumar et al., 2023). The species is native to the Indian subcontinent and parts of Southeast Asia and has been widely domesticated due to its nutritional and medicinal importance. At present, Aonla is extensively cultivated across tropical and subtropical regions, with major commercial production concentrated in the Indian states of Uttar Pradesh, Gujarat, Maharashtra, Rajasthan, and Himachal Pradesh (Pathak et al., 2011; Gopalan et al., 2007). Aonla fruits are globally recognized for their remarkable nutritional composition, particularly their exceptionally high ascorbic acid (vitamin C) content, which ranges from 400 to 900 mg per 100 g of fresh fruit far exceeding that of most consumed fruits (Bhatia & Bajaj, 2015; Chaphalkar et al., 2017). In addition to vitamin C, the fruit is rich in several bioactive phytochemicals, including hydrolysable tannins such as emblicanin A and B, punigluconin, and pedunculagin, as well as flavonoids like quercetin and kaempferol, and phenolic acids including gallic acid and ellagic acid (Krishnaveni & Mirunalini, 2010; Gul et al., 2022). The pulp also contains significant amounts of dietary fiber (10-15% dry weight) and pectin (3-4%), which contribute to its increasing use as a functional food ingredient (Upadhyay et al., 2018). Modern pharmacological studies have further validated many traditional medicinal claims associated with Aonla, reporting strong antioxidant, anti-inflammatory, hepatoprotective, immunomodulatory, and hypoglycemic properties (Variya et al., 2016; Dasaroju & Gottumukkala, 2014). Despite its adaptability and tolerance to several abiotic stresses such as drought, salinity, and temperature fluctuations, *P. emblica* is highly susceptible to certain fungal diseases, particularly rust, which is considered one of the most economically important foliar diseases affecting Aonla cultivation (Sharma & Gupta, 2003; Meena et al., 2017; Patel et al., 2025). The disease is primarily caused by *Ravenelia emblicae* Syd. and *Phakopsora phyllanthi* (Syd.). Rust infection commonly occurs during the monsoon season (July-September), when environmental conditions such as high relative humidity (>80%), moderate temperatures (20-28°C), and prolonged leaf wetness create a favorable environment for pathogen development and spore germination (Kumar et al., 2019; Kumar et al., 2024; Singh et al., 2025).

Rust infection significantly interferes with the photosynthetic machinery of the plant. Pathogen colonization accelerates the degradation of chlorophyll *a* and *b*, the primary pigments responsible for light absorption, resulting in chlorosis and reduced photosynthetic efficiency (Scholes & Rolfe, 2009; Bastiaans, 1991). This impairment of photosynthesis leads to disruptions in primary metabolic processes, particularly carbohydrate synthesis and translocation. Reduced carbon fixation ultimately

results in lower accumulation of reducing sugars such as glucose and fructose, as well as non-reducing sugars like sucrose, thereby decreasing total soluble sugar content in developing fruits (**Bolton, 2009; Berger et al., 2007**). The metabolic disturbances caused by rust infection also extend to other essential macromolecules, including proteins and lipids. These biochemical alterations adversely affect several fruit quality parameters that are critical for nutritional value, consumer acceptability, and marketability. Ascorbic acid content one of the most valuable nutritional attributes of Aonla is particularly susceptible to oxidative degradation under pathogen-induced stress conditions (**Akram et al., 2017**). Similarly, total soluble solids (TSS), which largely represent the concentration of sugars and organic acids responsible for fruit sweetness and flavor, decline due to impaired carbohydrate metabolism (**Magwaza & Opara, 2015**). Changes are also observed in titratable acidity, primarily determined by organic acids such as citric, malic, and ascorbic acids, thereby influencing taste balance and pH-dependent biochemical reactions within the fruit (**Etienne et al., 2013**).

Consequently, the TSS-to-acidity ratio, an important indicator of fruit quality, consumer preference, and processing suitability, is adversely affected in rust-infected fruits (**Baldwin et al., 2008**). Furthermore, rust infection may influence cell wall metabolism by altering the activity of enzymes such as pectin methyl esterase and by affecting cellulose and hemicellulose synthesis. These changes can modify fruit texture, firmness, and overall processing characteristics (**Hocking et al., 2016; Atmodjo et al., 2013**).

## Method and Materials

This experiment was conducted at the Main Experimental Station, Horticulture Farm, and biochemical analysis worked on the Department of Plant Pathology Laboratory at Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.), India, during the years 2022-23 and 2023-24. Samples from rust-infected and healthy aonla fruits and leaves were collected and subjected to various biochemical assays. In the present study, the biochemical compounds analysed from the Aonla cultivars such as NA-20, NA-10, NA-7, NA-5, NA-6, Anand-1, Chakaiya, NA-26, NA-25, Francis, NA-4, CHES-1, and BSR-1 with traits of Chlorophyll, Carotenoids, Reducing sugar, Non-reducing Sugar, Total Sugar, Protein, Fat, Vitamin-C, Total Soluble Solids, Titratable Acidity, TSS: Acidity ratio, Fibre and Pectin. The methodology, experimental setup, and techniques employed in this research are detailed in the following sections.

| S. No. | Code            | Variety  |
|--------|-----------------|----------|
| 1      | G <sub>1</sub>  | Chakaiya |
| 2      | G <sub>2</sub>  | Anand-1  |
| 3      | G <sub>3</sub>  | CHES-1   |
| 4      | G <sub>4</sub>  | Francis  |
| 5      | G <sub>5</sub>  | NA-4     |
| 6      | G <sub>6</sub>  | NA-7     |
| 7      | G <sub>7</sub>  | NA-6     |
| 8      | G <sub>8</sub>  | NA-5     |
| 9      | G <sub>9</sub>  | NA-10    |
| 1      | G <sub>1</sub>  | NA-20    |
| 11     | G <sub>11</sub> | BSR-1    |
| 12     | G <sub>12</sub> | NA-26    |
| 13     | G <sub>13</sub> | NA-25    |

## Biochemical assays

### Estimate of Chlorophyll (mg/g)

The chlorophyll a, b and total contents of infected and healthy leaves were measured using the dimethyl sulfoxide (DMSO) procedure. The samples were incubated at 65 °C until the leaf disks were entirely colorless. The absorbance of the DMSO-chlorophyll extracts and a blank (pure DMSO) was measured at 645 nm and 663 nm by using a double beam UV visible spectrophotometer (iGENE LABSERVE). Chlorophyll a, b and total were measured using the below formula (**Kumari et al. 2018**).

$$\text{Chlorophyll A (mg/g fresh weight)} = [(12.7 \times A_{663}) - (2.69 \times A_{645})] \times (V/1000 \times W)$$

$$\text{Chlorophyll B (mg/g fresh weight)} = [(22.9 \times A_{645}) - (4.68 \times A_{663})] \times (V/1000 \times W)$$

$$\text{Total Chlorophylls} = (20.08 \times A_{645} + 8.02 \times A_{663}) \times (V/1000 \times W)$$

V = final volume of the chlorophyll extract, and W = Fresh weight of the extracted tissue

### Estimate of Carotenoids (mg/g)

A 0.5 g aliquot of fresh tissue was homogenized in 10 ml of chilled 80% (v/v) acetone using a tissue homogenizer. The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4 °C to separate the supernatant. The absorbance of the resulting extract was measured at 470 nm using a UV-visible spectrophotometer to quantify pigment or metabolite content, following the method described by **Lichtenthaler and Wellburn (1983)**.

### Analysis of Reducing sugar (%)

Total phenolic content was determined using the Folin–Ciocalteu reagent method. Briefly, 0.5 ml of methanolic extract was mixed with 7 ml of distilled water and 0.5 ml of Folin–Ciocalteu reagent. After 3 minutes, 2 ml of 20% sodium carbonate solution was added, and the mixture was thoroughly mixed. The reaction mixture was incubated at 25 °C for 1 hour in a water bath. The absorbance was then measured at 720 nm using a spectrophotometer. The total phenolic content was estimated according to the method described by **Ranganna (2009)**.

#### **Estimate of Non-Reducing Sugar (%)**

Non-reducing sugars were estimated by deducting the amount of reducing sugars from the total invert sugars and multiplying the difference by a factor of 0.95. The results were expressed as percentage of non-reducing sugars. The estimation was carried out according to the method described by **Ranganna (2009)**.

#### **Estimation of Total sugar (%)**

Extraction of total sugar was carried out using 80% ethanol. Sample extract (0.1 ml) was taken in triplicate in each test tube and anthrone reagent (10 ml) was pipetted in empty test tubes placed in ice-cold water bath. Then early dilutions were added to anthrone reagent and test tubes were placed in boiling water bath for 10 min for colour development. After cooling, absorbance was noted at 625 nm. Standard curve was prepared using the graded (25-250 ppm). concentration of glucose solution, following the method described by **Ranganna (2009)**.

#### **Estimation of Crude protein**

The crude protein estimation followed the method outlined by **A.O.A.C (2000)**. In this procedure, 500 mg of moisture-free samples were digested with 10 mL of concentrated sulfuric acid and 1 g of a digestion mixture (potassium sulfate and copper sulfate in a 9:1 ratio) within Kjeldahl digestion tubes. The mixture was heated until a clear solution formed, then cooled and diluted to 50 mL using double-distilled water. For distillation, 5 mL of the digested sample was combined with 5 mL of 40% NaOH solution and transferred to the distillation assembly. The distilled solution was then titrated against 0.01 M HCl. The nitrogen percentage was calculated and multiplied by 6.25 to determine the crude Protein Content in the sample, ensuring precise quantification.

#### **Estimation of Fat**

Crude fat content was determined using the Soxhlet extraction method as described by **AOAC (2000)**. In this procedure, 5 g of moisture-free sample was placed in a thimble and extracted with petroleum ether (boiling point 40–60 °C) as the solvent. The extraction was carried out in a Soxhlet apparatus for 6 hours to ensure complete lipid extraction. After extraction, the solvent was evaporated, and the extracted fat was dried in a hot air oven at 105 °C for 1 hour to remove residual moisture. The dried fat was weighed, and the crude fat content was calculated as a percentage of the initial sample weight.

#### **Estimation of Vitamin C (Ascorbic acid)**

Ascorbic acid content was estimated following the method of **Jagota and Dani (1982)**. Briefly, 2 g of sample was macerated with an equal volume of 6% metaphosphoric acid to ensure thorough extraction. The homogenate was centrifuged at 5000 rpm for 10 minutes and filtered through Whatman No. 1 filter paper to obtain a clear extract. An aliquot of 0.1 mL of the filtrate was diluted with 3% metaphosphoric acid, and the volume was adjusted to 4 mL with deionized water. Subsequently, 0.4 mL of Folin–Ciocalteu reagent was added to initiate the reaction. The reaction mixture was incubated at room temperature for 10 minutes and then centrifuged at 3000 rpm for an additional 10 minutes. The absorbance of the supernatant was measured at 760 nm using a spectrophotometer to quantify the ascorbic acid content.

#### **Estimation of Total Soluble Solids(<sup>o</sup>Brix)**

Total soluble solids (TSS) in aonla fruits were estimated according to the method described by **AOAC (2000)** using an Abbe's hand refractometer (0–32 °Brix range). Ten fruits were selected from three replications of each cultivar for the analysis. One g of pulp of aonla fruit was macerated in pestle and mortar and was squeezed by putting it on muslin cloth. A drop of juice was taken and was put on Abbe's hand refractometer. Readings were taken in terms of °Brix. Mean values of ten fruit samples were expressed as TSS. The total titratable Acidity was determined by the standard method. One g of fruit pulp was macerated in a pestle and mortar by adding water. The extract was titrated against 0.1 N sodium hydroxide using 1% phenolphthalein as an indicator. The appearance of light pink colour which persists for one minute was taken as end point. The Acidity of fruit was expressed on percent basis.

#### Estimation of Titratable Acidity (%)

Titratable acidity was determined following the method described by **Ranganna (2009)**. Fruit samples were meticulously prepared by macerating them with pestles and mortar to ensure thorough homogenization, yielding a consistent pulp. The extracted pulp was then carefully squeezed through autoclaved muslin cloth to obtain the juice. For Acidity determination, 1 ml of the juice was diluted to 5 ml in a titration flask, followed by the addition of 2 drops of 1% phenolphthalein indicator. Titration was conducted using N/10 NaOH solution until a faint pink coloration appeared, indicating the endpoint. The titer value was recorded, and the percentage of titratable Acidity was calculated, representing the predominant acid content in the sample.

#### Estimation of TSS: Acidity ratio

$$\text{TSS : Acidity Ratio} = \frac{\text{Total Soluble Solids (°Brix)}}{\text{Titrate Acidity (AN) (\%)}}$$

#### Estimation of Crude Fiber

Crude fiber content was determined according to the method described by AOAC (2000). In this procedure, 2 g of defatted samples were initially boiled in 20 mL of 0.26 N sulphuric acid for 30 minutes, then filtered through muslin cloth. The filtrate underwent a second boiling step with 20 mL of NaOH solution for another 30 minutes, followed by filtration through muslin cloth once more. To ensure thorough purification, the filtrate was washed with 1.25% sulphuric acid, then rinsed sequentially with 50 mL of distilled water and 25 mL of alcohol. The processed filtrate was transferred to an ashing dish, dried at 130°C for 2 hours, cooled in a desiccator, and weighed. Subsequently, the sample was ignited in a muffle furnace at 600 ± 15°C for 30 minutes, cooled again in a desiccator, and reweighed. The crude Fiber Content was then calculated and expressed as a percentage of the original sample, ensuring precise quantification of fiber composition.

#### Estimation of Pectin

Pectin was determined following the method described by Ranganna (2009). A 10 g sample was precisely weighed and subjected to boiling for 30 minutes in 60 mL of 0.01 N HCl. After boiling, the residue was carefully collected, thoroughly washed with hot water, and subsequently boiled again for 20 minutes in 20 mL of 0.05 N HCl. The filtrate was then adjusted to a final volume of 100 mL using 0.05 N HCl. For neutralization, 30 mL of the filtrate was treated with 1 N NaOH, with excess NaOH added gradually under constant stirring and left to stand overnight. The next step involved introducing 50 mL of 1 N acetic acid, followed by 25 mL of 1 N calcium chloride solution after a 5-minute interval, ensuring thorough mixing. The mixture was allowed to stand for 1 hour, then boiled for 2 minutes and filtered using pre-weighed Whatman No. 1 filter paper. The precipitate, consisting of calcium pectate, was repeatedly rinsed with boiling water until no chloride

was detected when tested with silver nitrate. Finally, the filter paper containing calcium pectate was dried overnight in a weighing dish at 100°C, cooled in a desiccator, and weighed to determine the pectin content accurately.

### Statistical analysis

ANOVA was performed using Microsoft excel 2021 to assess the effects of aonla variety and infection status on key biochemical traits. Significant differences among treatment means were identified using the LSD at  $P \leq 0.05$ . Pearson correlation, Principal component, heatmap, cluster analysis using RStudio 4.5.0. revealed associations between disease severity and traits such as Chlorophyll, Carotenoids, Reducing sugar, Non-reducing Sugar, Total Sugar, Protein, Fat, Vitamin-C, Total Soluble Solids (TSSN), Titratable Acidity (AN), TSS: Acidity ratio (TSACN), Fibre and Pectin.

## Results and Discussion

The Principal Component Analysis revealed clear differences in biochemical attributes between healthy and infected plants across all genotypes.

### Vitamin-C Content (VCN)

Under healthy condition, Vitamin-C Content (VCN) was markedly higher across genotypes. Genotype G13 (NA-25) recorded the maximum value (528.26 mg/100 g), followed by G12 (NA-26) (498.16 mg/100 g), while G1 (Chakaiya) showed the minimum value (354.12 mg/100 g). In contrast, infected plants exhibited substantially reduced Vitamin-C values, with G12 (270.09 mg/100 g) being the highest and G11 (BSR-1) the second highest (234.41 mg/100 g). The lowest Vitamin-C was observed in G5 (NA-4) (114.19 mg/100 g). Overall, infection resulted in nearly a 50% reduction in Vitamin-C Content (VCN). Similar results were found that the **Kumari (2017)** genotypes were Krishna, Kanchan, NA-10, NA-7, R1-P11, Ananda and R2-11. The Vitamin C levels of these genotypes varied from 343.65mg/100ml to 461.69.

### Reducing Sugar Content (RSN)

Reducing Sugar Content (RSN) in healthy plants remained comparatively higher, with G13 (4.84%) recording the maximum followed by G12 (4.70%), whereas G1 (2.20%) exhibited the minimum value. Under infected condition, Reducing Sugar Content (RSN) values were relatively elevated in G12 (5.32%) and G13 (5.21%), while G4 (Francis) showed the lowest Reducing Sugar Content (RSN) (3.17%). This indicates a noticeable shift in reducing sugar composition under infection stress. **Jamuna et al. 2017** similar findings revealed that there was no significant variation in reducing sugar levels on the 2nd, 4th, and 6th DAS, regardless of packaging treatment. On the 2nd DAS, fruits stored in 100-gauge polyethylene covers without ventilation exhibited the highest reducing sugar content (12.39%), whereas the lowest content (11.84%) was observed in fruits treated with 50- and 200-gauge polyethylene covers having 1% ventilation. Interestingly, by the 4th and 6th DAS, figs in 200-gauge ventilated covers recorded maximum sugar levels (11.79% and 12.98%, respectively),

### Non-Reducing Sugar Content (NRN)

Healthy plants showed higher Non-Reducing Sugar Content (NRN), with G13 (6.14%) as the maximum, followed by G12 (5.62%), and G1 (3.42%) as the minimum. In infected plants, NRN values declined considerably, with G13 (3.27%) and G10 (NA-20) (3.25%) showing the highest values, while G1 (1.17%) recorded the lowest. This reflects a substantial reduction in non-reducing sugar accumulation under disease condition. **Jamuna et al. 2017** similar results revealed that only on the 2nd day after storage (DAS) was there a statistically significant difference in non-reducing sugars. The highest content (2.43%) was observed in the control treatment, which was comparable with fruits stored in 50- and 100-gauge polyethylene covers without ventilation and in 50 gauge covers with 1% ventilation (2.10% each), as well as 200 gauge covers without ventilation (2.04%). However, on the 4th and 6th DAS, the differences in non-reducing sugar levels were found to be statistically non-significant, indicating that packaging type had minimal influence beyond the initial storage period.

### Total Sugar Content (TSN)

Total Sugar Content (TSN) was higher under healthy condition, with G13 (10.98%) recording the maximum followed by G12 (10.32%), whereas G1 (5.62%) showed the minimum. Under infected condition, Total Sugar Content (TSN) values decreased, with G12 (8.55%) being the highest, G13 (8.48%) second highest, and G1 (4.35%) the lowest. Similar results were found that the **Devi et al. (2020)**, sugar is a key indicator of sweetness, and the total sugar content increases significantly from the initial stages of maturity to the final fruit harvest. Among the various cultivars, the highest total sugar levels at full maturity were recorded in Kanchan and Krishna (5.75), followed by Balwant (4.90) and Neelum (4.87), while the lowest was observed in desi seedlings (4.25).

### **Protein Content (PN)**

Protein Content (PN) in healthy plants ranged from 3.22% in G10 (NA-20) (maximum) to 2.22% in G2 (Anand-1) (minimum), with G13 (3.18%) ranking second highest. In infected plants, Protein Content (PN) declined, with G13 (2.87%) showing the highest value followed by G12 (2.56%), while G2 (1.42%) recorded the minimum Protein Content (PN).

The Principal Component Analysis revealed distinct anatomical variations between healthy and infected plant tissues across all measured parameters. For Vascular Cambium Number, the healthy condition exhibited substantially higher values with G13 showing the maximum (528.26) followed by G12 (498.16), while G1 recorded the minimum (354.12). In contrast, the infected condition demonstrated considerably reduced Vitamin-C values, with G12 displaying the highest (270.09) and G11 the second highest (234.41), whereas G5 showed the lowest value (114.19). This represents approximately a 50% reduction in vascular cambium development under infected conditions.

Ray Seriation Number patterns showed that healthy tissues maintained higher seriation with G13 achieving the maximum (4.84) and G12 the second highest (4.70), while G1 exhibited the minimum (2.20). The infected condition presented elevated Reducing Sugar Content (RSN) in G12 (5.32) and G13 (5.21), with G4 showing the lowest value (3.17), suggesting altered ray cell organization in response to infection.

For Non-Ray Number, healthy plants demonstrated superior values with G13 recording the maximum (6.14) followed by G12 (5.62) and G1 the minimum (3.42), whereas infected tissues showed G13 with the highest (3.27), G10 second (3.25), and G1 with the lowest (1.17), indicating substantial reduction in non-ray cell production under disease stress.

Total Seriation Number analysis revealed that healthy condition maintained higher overall seriation with G13 exhibiting maximum values (10.98) and G12 second highest (10.32), while G1 showed minimum (5.62). The infected condition displayed reduced Total Sugar Content (TSN) with G12 reaching the highest (8.55), G13 second (8.48), and G1 the lowest (4.35).

Parenchyma Number in healthy tissues showed G10 with maximum (3.22) and G13 with second highest (3.18), while G2 recorded minimum (2.22). Under infected conditions, G13 exhibited the highest PN (2.87) followed by G12 (2.56), with G2 showing the lowest (1.42), demonstrating decreased parenchyma cell formation during infection. Similarly finding **Tewari et al., 2019** among the different cultivars, protein content of fruits was highest in Chakaiya (4.51%) and lowest in NA-10 (3.02%) cultivar.

### **Fat Content (FN)**

Fat Content (FN) analysis indicated that healthy plants exhibited higher values across genotypes, with G13 (NA-25) recording the maximum (0.53%), followed by G12 (NA-26) (0.49%), while G1 (Chakaiya) showed the minimum value (0.21%). Under infected condition, Fat Content (FN) values declined markedly, with G12 (0.43%) showing the highest value and G13 (0.42%) ranking second, whereas G1 (0.12%) recorded the lowest Fat Content (FN), reflecting compromised fat accumulation due to infection. Similar results were found that the **Tewari et al. (2019)** the fat content varied among different aonla fruit cultivars. The lowest values were observed in NA-9 (0.21%), followed by Hathijhool (0.30%), Balwant (0.35%), NA-7 (0.39%), and NA-10 (0.40%). The highest fat content was recorded in the Chakaiya cultivar (0.46%).

### Acidity (AN)

Acidity (AN) followed a similar pattern. Under healthy condition, G13 recorded the maximum Acidity (AN) (2.34%), followed by G12 (2.06%), while G2 (Anand-1) exhibited the minimum value (1.34%). In the infected state, G13 again showed the highest Acidity (AN) (2.36%) followed by G12 (2.05%), whereas G2 recorded the lowest value (1.18%). Similar results were found that the **Saini et al. (2018)** reduce during incubation/storage in the levels of titrable acidity both in healthy and inoculated (disease) fruits. The titrable acidity decreased significantly in susceptible varieties, viz. Chakaiya (healthy fruit, 1.22 % and diseased fruit, 0.98 %) and Banarasi (healthy fruit, 1.40 % and diseased fruit, 1.32 %) as compared to resistant varieties Desi (healthy fruit, 1.50 % and diseased fruit, 1.42 %) and Kanchan (healthy fruit, 1.52 % and diseased fruit, 1.51 %).

### Total Chlorophyll Content (CN)

Total Chlorophyll Content (CN) was higher in healthy plants, with G12 (NA-26) recording the maximum value (2.30 mg g<sup>-1</sup>) followed by G13 (2.25 mg g<sup>-1</sup>), while G1 exhibited the minimum (1.82 mg g<sup>-1</sup>). In infected plants, CN values declined substantially, with G13 (1.88 mg g<sup>-1</sup>) being the highest, G12 (1.86 mg g<sup>-1</sup>) second highest, and G1 (0.55 mg g<sup>-1</sup>) the lowest, indicating severe reduction in chlorophyll content under disease stress. Similar results were found that the **Kumari (2017)** The decline in total chlorophyll content in aonla (*Emblica officinalis* Gaertn.) leaves under rust infection has been well-documented, with consistent observations of pigment degradation across all cultivars from 0 to 15 days of storage. This phenomenon is primarily attributed to the activity of chlorophyllase, the key biochemical enzyme responsible for chlorophyll breakdown during post-harvest storage.

### Carotenoid Content (CRN)

Carotenoid Content (CRN) in healthy plants showed G13 with the maximum value (1.30 mg g<sup>-1</sup>) followed by G10 (NA-20) (1.24 mg g<sup>-1</sup>), whereas G5 (NA-4) recorded the minimum (0.78 mg g<sup>-1</sup>). Under infected condition, G13 again recorded the highest CRN (0.75 mg g<sup>-1</sup>), followed by G12 (0.70 mg g<sup>-1</sup>), while G2 (Anand-1) showed the lowest value (0.28 mg g<sup>-1</sup>), reflecting reduced carotenoid synthesis during infection. Similar results were found that the **Fitriansyah et al. (2018)** carotenoid content in rust-infected aonla (*Phyllanthus emblica*) leaves-Recent biochemical assessments have explored the total carotenoid content in various extracts of *Phyllanthus emblica* (*P. emblica*), revealing notable variation across fruit, leaf, and stem bark samples. Carotenoid levels among different extract codes—BN, BE, BO, DN, DE, DO, KN, KE, and KO—ranged from as low as 0.0004 to a peak of 0.7588 g β-carotene equivalent (BE)/100 g.

### Pectine Content (PEN)

Pectine Content (PEN) under healthy condition was highest in G13 (3.32%), followed by G12 (3.26%), while G1 recorded the minimum value (1.98%). Infected plants showed a marked reduction in Pectine Content (PEN), with G13 (2.68%) and G12 (2.32%) recording the highest values, whereas G1 (0.53%) showed the lowest Pectine Content (PEN). Similar findings align with earlier observations by **Kumari (2017)**, who reported pectin levels ranging between 2.3% and 3.4%, reaffirming the variability based on genetic makeup and maturity stages.

### Fiber Content (FBN)

Fiber Content (FBN) in healthy plants ranged from 10.54% in G13 (maximum) to 10.01% in G12, while G1 showed the minimum value (6.11%). Under infected condition, Fiber Content (FBN) decreased, with G13 (8.89%) and G12 (8.68%) recording the highest values, whereas G1 (5.89%) remained the lowest, indicating impaired fiber accumulation due to disease. According to **Tewari et al. (2019)**, fibre content among the aonla cultivars ranged from 11.68% to 15.98%. Fruits of the NA-7 cultivar exhibited the highest fibre content, followed by NA-9, Hathijhool, Chakaiya, Balwant, and NA-10. The high fibre content in NA-7 justifies the cultivar's notable toughness.

### Total Soluble Solids (TSSN)

Total Soluble Solids (TSSN) were higher under healthy condition, with G13 (13.22 °Brix) showing the maximum value followed by G12 (12.88 °Brix), while G1 recorded the minimum (8.24 °Brix). In infected plants, Total Soluble Solids (TSSN) declined considerably, with G13 (9.35 °Brix) being the highest, G12 (8.50 °Brix) second highest, and G1 (5.01 °Brix) the lowest. Similar results were found that the **Saini et al. (2018)** reduction in TSS (°Brix) under rust infection across mango genotypes aligns with broader patterns of disease-induced physiological disruptions in fruit crops. changes in the level of TSS in inoculated and healthy fruits at 5 and 10 days after inoculation. The data in the table revealed significant reduction in TSS due to pathogenesis. TSS content was higher in healthy and diseased fruits of susceptible varieties then resistant ones. Susceptible var. Chakaiya fruits showed 10.830 and 13.660 Brix TSS content in healthy and diseased fruits, respectively. Another susceptible cv Banarsi had 11.27° and 14.32° Brix TSS in the two categories respectively.

### TSS: Acidity ratio (TSACN)

The TSS: Acidity ratio (TSACN) showed a distinct pattern. Under healthy condition, G6 (NA-7) recorded the maximum value (7.56), followed by G8 (NA-5) (7.24), while G13 exhibited the minimum ratio (5.65). Under infected condition, G8 (5.08) showed the highest TSS: Acidity ratio (TSACN), followed by G6 (4.91), whereas G10 (NA-20) recorded the lowest value (3.82), indicating genotype-specific responses of sugar-acid balance under infection.

Overall, the PCA-based comparative analysis demonstrated that G12 (NA-26) and G13 (NA-25) consistently exhibited superior biochemical quality traits under both healthy and infected conditions, while G1 (Chakaiya) frequently recorded the lowest values. Infection stress caused pronounced reductions in chlorophyll, carotenoids, sugars, fiber, pectine, fat, and TSS-related parameters, with approximately 40-60% decline observed in most biochemical attributes across genotypes.

**Table 1: Mean performance of healthy Aonla lines**

| Genotype | VCN    | RSN  | NRN  | TSN   | PN   | FN   | AN   | CN   | CRN  | PEN  | FBN   | TSSN  | TSACN |
|----------|--------|------|------|-------|------|------|------|------|------|------|-------|-------|-------|
| Chakaiya | 354.12 | 2.2  | 3.42 | 5.62  | 2.38 | 0.21 | 1.42 | 1.82 | 1    | 1.98 | 6.11  | 8.24  | 5.80  |
| Anand-1  | 375.22 | 2.6  | 3.73 | 6.33  | 2.22 | 0.23 | 1.34 | 2.03 | 0.88 | 2.06 | 6.79  | 8.91  | 6.65  |
| CHES-1   | 376.13 | 2.5  | 3.73 | 6.23  | 2.46 | 0.24 | 1.64 | 2.05 | 1    | 2.18 | 6.66  | 10.23 | 6.24  |
| Francis  | 398.34 | 2.8  | 4.34 | 7.14  | 2.56 | 0.27 | 1.62 | 2.07 | 1    | 2.29 | 6.7   | 10.58 | 6.53  |
| NA-4     | 400.34 | 3.2  | 4.21 | 7.41  | 2.78 | 0.29 | 1.66 | 2.09 | 0.78 | 2.52 | 6.83  | 10.61 | 6.39  |
| NA-7     | 450.22 | 3.31 | 4.48 | 7.79  | 2.67 | 0.29 | 1.42 | 2.1  | 1    | 2.98 | 6.98  | 10.74 | 7.56  |
| NA-6     | 456.34 | 3.4  | 4.38 | 7.78  | 2.28 | 0.3  | 1.68 | 2.13 | 0.89 | 3.02 | 7.09  | 10.88 | 6.48  |
| NA-5     | 463.14 | 3.5  | 4.71 | 8.21  | 2.78 | 0.31 | 1.58 | 2.15 | 1    | 3.01 | 7.89  | 11.44 | 7.24  |
| NA-10    | 472.11 | 3.64 | 4.62 | 8.26  | 2.92 | 0.3  | 1.98 | 2.15 | 1    | 3.08 | 7.69  | 11.63 | 5.87  |
| NA-20    | 478.13 | 4    | 5.22 | 9.22  | 3.22 | 0.35 | 1.96 | 2.18 | 1.24 | 3.17 | 9.26  | 11.82 | 6.03  |
| BSR-1    | 486.31 | 4.2  | 5.41 | 9.61  | 3.01 | 0.38 | 1.98 | 2.2  | 1.23 | 3.25 | 9.72  | 11.86 | 5.99  |
| NA-26    | 498.16 | 4.7  | 5.62 | 10.32 | 3.06 | 0.49 | 2.06 | 2.3  | 1.24 | 3.26 | 10.01 | 12.88 | 6.25  |
| NA-25    | 528.26 | 4.84 | 6.14 | 10.98 | 3.18 | 0.53 | 2.34 | 2.25 | 1.3  | 3.32 | 10.54 | 13.22 | 5.65  |

**Table 2: Mean performance of Infected Aonla lines**

| Genotype | VCN    | RSN  | NRN  | TSN  | PN   | FN   | AN   | CN   | CRN  | PEN  | FBN  | TSSN | TSACN |
|----------|--------|------|------|------|------|------|------|------|------|------|------|------|-------|
| Chakaiya | 192.15 | 3.18 | 1.17 | 4.35 | 1.51 | 0.12 | 1.27 | 0.55 | 0.32 | 0.53 | 5.89 | 5.01 | 3.94  |
| Anand-1  | 171.91 | 3.72 | 1.25 | 4.97 | 1.42 | 0.12 | 1.18 | 0.78 | 0.28 | 0.64 | 6.29 | 5.46 | 4.63  |
| CHES-1   | 115.92 | 3.68 | 1.43 | 5.11 | 1.66 | 0.14 | 1.53 | 0.70 | 0.34 | 0.73 | 6.19 | 6.14 | 4.01  |
| Francis  | 126.09 | 3.17 | 1.67 | 4.84 | 1.73 | 0.16 | 1.50 | 0.76 | 0.33 | 1.07 | 6.31 | 6.19 | 4.13  |
| NA-4     | 114.19 | 3.97 | 2.10 | 6.07 | 1.68 | 0.18 | 1.54 | 0.89 | 0.50 | 1.28 | 6.88 | 6.73 | 4.37  |
| NA-7     | 135.35 | 4.32 | 2.13 | 6.45 | 1.78 | 0.24 | 1.28 | 0.87 | 0.52 | 1.62 | 6.49 | 6.29 | 4.91  |
| NA-6     | 158.54 | 4.35 | 2.24 | 6.59 | 1.86 | 0.26 | 1.58 | 1.00 | 0.48 | 1.65 | 6.65 | 6.99 | 4.42  |
| NA-5     | 229.89 | 4.32 | 2.38 | 6.70 | 1.98 | 0.28 | 1.38 | 1.20 | 0.56 | 1.76 | 7.79 | 7.01 | 5.08  |
| NA-10    | 234.31 | 4.36 | 2.29 | 6.65 | 2.28 | 0.27 | 1.94 | 1.78 | 0.54 | 1.88 | 7.55 | 8.09 | 4.17  |
| NA-20    | 170.23 | 5.12 | 3.25 | 8.37 | 2.35 | 0.32 | 1.91 | 1.72 | 0.64 | 2.08 | 8.09 | 7.29 | 3.82  |
| BSR-1    | 234.41 | 5.18 | 3.10 | 8.28 | 2.38 | 0.32 | 1.96 | 1.82 | 0.60 | 2.26 | 8.17 | 8.26 | 4.21  |
| NA-26    | 270.09 | 5.32 | 3.23 | 8.55 | 2.56 | 0.43 | 2.05 | 1.86 | 0.70 | 2.32 | 8.68 | 8.50 | 4.15  |
| NA-25    | 205.89 | 5.21 | 3.27 | 8.48 | 2.87 | 0.42 | 2.36 | 1.88 | 0.75 | 2.68 | 8.89 | 9.35 | 3.96  |

## Principal component analysis in healthy and infected

### Principal Component Analysis under Healthy Condition (90 DAS)

Under healthy condition at 90 days after sowing, Principal Component Analysis revealed that PC1 explained 81.75% of the total variation, followed by PC2 contributing 10.05%, together accounting for 91.80% of the total variability among genotypes.

In the PCA biplot, most biochemical traits including Total Sugar, Non-Reducing Sugar, Reducing Sugar, Fiber, TSS, Vitamin-C, Fat, Protein, Pectine, Total Chlorophyll, Carotenoids and Acidity (AN) exhibited strong positive loadings along PC1, indicating a high degree of positive association among these quality and yield-related traits. The close clustering and similar directional orientation of these vectors suggested coordinated biochemical expression and balanced metabolic activity under healthy conditions.

The second principal component (PC2) was mainly influenced by Acidity (AN) and Carotenoid Content (CRN), which showed relatively higher positive loadings along this axis. In contrast, TSS: Acidity ratio (TSACN) exhibited a strong negative loading on PC2, indicating an inverse relationship with most biochemical and yield-associated traits under non-stress conditions.

Among the genotypes, G10 (NA-20), G11 (BSR-1), G12 (NA-26), and G13 (NA-25) were positioned on the positive side of PC1 and in close proximity to major trait vectors, indicating a strong association with superior biochemical quality traits under healthy condition. Conversely, G6 (NA-7) and G8 (NA-5) were located away from the main cluster and closer to TSS: Acidity ratio (TSACN), suggesting comparatively weaker associations with key quality attributes (Figure 1).

### Principal Component Analysis under Infected Condition (90 DAS)

Under infected condition at 90 days after sowing, Principal Component Analysis revealed that the first two principal components explained a substantial proportion of the total variability among genotypes. PC1 accounted for 82.14% of the total variation, while PC2 explained 9.25%, together contributing 91.39% of the cumulative variance.

In the PCA biplot, most biochemical and quality-related traits including Total Sugar, Non-Reducing Sugar, Reducing Sugar, Fiber, TSS, Vitamin-C, Fat, Protein, Pectine, Total Chlorophyll, Carotenoids and Acidity (AN) were oriented towards the negative side of PC1, indicating a strong negative association with this principal component. The close grouping and similar direction of these trait vectors suggested that these biochemical attributes were collectively suppressed under infection stress, reflecting overall deterioration in fruit quality and metabolic performance.

In contrast, TSS: Acidity ratio (TSACN) exhibited a strong positive loading along PC2 and a negative association with PC1, indicating that this parameter behaved independently and contributed distinctly to variability under infected conditions. The pronounced divergence of TSS: Acidity ratio (TSACN) from other trait vectors highlights a physiological imbalance in sugar-acid regulation induced by infection stress.

With respect to genotype distribution, G1 (Chakaiya), G3 (CHES-1), and G4 (Francis) were positioned on the positive side of PC1 and were distantly located from major trait vectors, indicating a comparatively weaker association with biochemical quality traits under infected condition. Meanwhile, G6 (NA-7) and G8 (NA-5), located in the lower quadrants and closer to TSS: Acidity ratio (TSACN), reflected greater sensitivity to infection stress. Overall, the PCA pattern under infected condition clearly demonstrated disrupted trait interrelationships and dominance of stress-driven variation (Figure 2).

### **Eigenvalue Distribution under Healthy Condition (90 DAS)**

Under healthy condition, the scree plot showed a similar pattern of eigenvalue distribution. The first principal component (PC1) recorded the highest eigenvalue (10.6), followed by PC2 with an eigenvalue of 1.3. The eigenvalues of PC3 (0.4) and subsequent components were well below unity, indicating minimal contribution to total variance (Figure 3).

Based on Kaiser's rule, PC1 and PC2 were retained for interpretation under healthy condition. The steep drop in eigenvalues after the second principal component suggested that the first two components effectively summarized the majority of variability among the genotypes under non-stress conditions.

### **Eigenvalue Distribution under Infected Condition (90 DAS)**

The scree plot of the infected condition revealed that the first principal component (PC1) had the highest eigenvalue (10.7), explaining the majority of the total variation among genotypes. The second principal component (PC2) had an eigenvalue of 1.2, while the third principal component (PC3) showed a much lower eigenvalue (0.6). Subsequent components (PC4 onwards) had eigenvalues less than 1 and contributed negligibly to the total variation.

According to Kaiser's criterion (eigenvalue > 1), only PC1 and PC2 were considered significant under infected condition. A sharp decline in eigenvalues after PC2 indicated that most of the variability was captured by the first two principal components, while the remaining components represented noise or minor variation (Figure 4).

### **Trait-PC Correlation under Healthy Condition (90 DAS)**

Under healthy condition, the correlation heatmap revealed a balanced and well-structured pattern of biochemical trait contributions across the principal components. The first principal component (PC1) showed moderate positive correlations ( $\approx 0.27$ - $0.30$ ) with almost all traits, including TSS: Acidity ratio (TSACN), TSS, Fiber, Pectine, Carotenoids, Total Chlorophyll, Acidity (AN), Fat, Protein, Total Sugar, Non-Reducing Sugar, Reducing Sugar and Vitamin-C. This pattern indicates that PC1 represented overall biochemical quality and metabolic performance under healthy condition.

The second principal component (PC2) exhibited a strong negative correlation with TSS: Acidity ratio (TSACN) ( $-0.80$ ), while the remaining traits showed weak associations with this axis. The third principal component (PC3) was primarily influenced by Carotenoid Content (CRN) ( $-0.74$ ), whereas PC4 showed a strong negative correlation with Protein Content (PN) ( $-0.77$ ).

Several quality-related traits displayed strong positive correlations with intermediate principal components. Fiber Content (FBN) showed notable positive correlations with PC7 (0.55) and PC9 (0.58), while Total chlorophyll was positively correlated with PC6 (0.48) and PC7 (0.45). The thirteenth principal component (PC13) exhibited a strong positive correlation with Total Sugar Content (TSN) (0.82), indicating its distinct and isolated contribution to variability (Figure 5).

Overall, the correlation pattern under healthy condition reflected coordinated and stable biochemical trait expression across principal components, highlighting well-regulated metabolic activity in non-stress conditions.

### **Trait-PC Correlation under Infected Condition (90 DAS)**

Under infected condition, the correlation heatmap revealed that the contribution of biochemical traits to different principal components varied markedly. The first principal component (PC1) exhibited moderate negative correlations with most traits, including TSS, Fiber, Pectine, Carotenoids, Total Chlorophyll, Acidity (AN), Fat, Protein, Total Sugar, Non-Reducing Sugar, Reducing Sugar and Vitamin-C, indicating a general decline in biochemical quality attributes under infection stress.

The second principal component (PC2) showed a strong negative correlation with TSS: Acidity ratio (TSACN) (-0.87), while other traits displayed weak to moderate associations with this axis. The third principal component (PC3) was strongly influenced by Vitamin-C (-0.89), suggesting that Vitamin-C Content (VCN) contributed substantially to variability along this component under infected condition.

Higher-order principal components (PC4 to PC13) exhibited scattered and comparatively weaker correlations. Notably, Fiber showed a strong negative correlation with PC6 (-0.74), Carotenoid Content (CRN) with PC8 (-0.80), and Total Sugar Content (TSN) with PC13 (-0.81), indicating that specific biochemical traits exerted isolated influence on later components during infection stress (Figure 6).

Overall, the correlation pattern under infected condition reflected fragmented trait contributions and stress-induced redistribution of biochemical variability, highlighting disruption of normal metabolic coordination due to infection.

## **Conclusion**

The Principal Component Analysis of *Phyllanthus emblica* genotypes at 90 days after sowing clearly demonstrated a pronounced contrast between healthy and rust-infected conditions in terms of biochemical diversity and metabolic performance. Under healthy condition, the first two principal components explained 91.80% of the total variation (PC1: 81.75%, PC2: 10.05%), reflecting strong genetic differentiation and well-coordinated metabolic activity among genotypes. Genotypes G13 (NA-25) and G12 (NA-26) consistently exhibited superior biochemical quality, recording maximum values for vitamin C (528.26 and 498.16 mg/100 g), total sugars (10.98% and 10.32%), total chlorophyll (2.25 and 2.30 mg g<sup>-1</sup>), carotenoids (1.30 and 1.24 mg g<sup>-1</sup>), and Total Soluble Solids (TSSN) (13.22 and 12.88 °Brix). Strong positive associations among reducing sugars, non-reducing sugars, proteins, fats, fiber, pectine, Acidity (AN), and antioxidant pigments indicated harmonized primary metabolism, efficient photosynthetic activity, and stable biochemical homeostasis conducive to superior fruit quality and nutritional value. In contrast, rust-infected plants exhibited marked metabolic disruption, with PC1 and PC2 together explaining 91.39% of the total variation (PC1: 82.14%, PC2: 9.25%), indicating dominance of disease stress over genetic variability. Infection caused 40-60% reductions in most biochemical parameters, including an approximate 50% decline in vitamin C, up to 70% reduction in total chlorophyll, and nearly 60% loss of carotenoids, accompanied by decreased sugars, proteins, fats, fiber, and pectine. The shift from positive to negative trait loadings along PC1, fragmented trait correlations, and altered TSS: Acidity ratio (TSACN)s revealed profound metabolic reorganization, impaired sugar-acid balance, and deterioration of fruit quality under pathogen pressure. Although G12 and G13 retained comparatively better biochemical profiles even under infection, substantial quality losses highlight the vulnerability of nutritionally superior genotypes to rust stress. Overall, the combined PCA analysis emphasizes the importance of G12 and G13 as elite genotypes under healthy conditions, while simultaneously underscoring the urgent need for rust-resistant

cultivar development and integrated disease management strategies to safeguard the exceptional nutritional and commercial value of *Phyllanthus emblica*.

## Declarations

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### Conflict of Interest

The authors declare that there is no conflict of interest

### Author's Contribution

All the authors substantially contributed to the conception, design, analysis and interpretation of data, checking and approving final version of manuscript.

### Funding

None

### Data Availability

All the datasets analysed during the study are included in manuscript.

## References

1. Akram, N. A., Shafiq, F., & Ashraf, M. (2017). Ascorbic acid-A potential oxidant scavenger and its role in plant development and abiotic stress tolerance. *Frontiers in Plant Science*, 8, 613. <https://doi.org/10.3389/fpls.2017.00613>
2. AOAC. 2000. Official Methods of Analysis. 17th ed. Gaithersburg, Association of Official Analytical Chemists. 2200 p.
3. Atmodjo, M. A., Hao, Z., & Mohnen, D. (2013). Evolving views of pectin biosynthesis. *Annual Review of Plant Biology*, 64, 747–779. <https://doi.org/10.1146/annurev-arplant-042811-105534>
4. Baldwin, E. A., Bai, J., Plotto, A., & Dea, S. (2008). Electronic noses and tongues: Applications for the food and pharmaceutical industries. *Sensors*, 11(5), 4744–4766. <https://doi.org/10.3390/s110504744>
5. Bastiaans, L. (1991). Ratio between virtual and visual lesion size as a measure to describe reduction in leaf photosynthesis of rice due to leaf blast. *Phytopathology*, 81(6), 611–615. <https://doi.org/10.1094/Phyto-81-611>
6. Berger, S., Sinha, A. K., & Roitsch, T. (2007). Plant physiology meets phytopathology: Plant primary metabolism and plant-pathogen interactions. *Journal of Experimental Botany*, 58(15–16), 4019–4026. <https://doi.org/10.1093/jxb/erm298>
7. Bhatia, A., & Bajaj, R. K. (2015). Antioxidant capacity and nutritional composition of *Emblica officinalis* (Amla) fruits. *International Journal of Pharmaceutical Sciences Review and Research*, 33(1), 196–199.
8. Bolton, M. D. (2009). Primary metabolism and plant defense—Fuel for the fire. *Molecular Plant-Microbe Interactions*, 22(5), 487–497. <https://doi.org/10.1094/MPMI-22-5-0487>
9. Chaphalkar, R., Apte, K. G., Talekar, Y., Ojha, S. K., & Nandave, M. (2017). Antioxidants of *Phyllanthus emblica* L. bark extract provide hepatoprotection against ethanol-induced hepatic damage: A comparison with silymarin. *Oxidative*

10. Dasaroju, S., & Gottumukkala, K. M. (2014). Current trends in the research of *Emblica officinalis* (Amla): A pharmacological perspective. *International Journal of Pharmaceutical Sciences Review and Research*, 24(2), 150–159.
11. Devi, I., Kaur, S., Singh, N.P. and Singh, S. 2020. Physico-chemical changes during fruit growth and maturation in aonla cultivars. *Indian J. Agric. Sci.* 90(5): 943-946
12. Etienne, A., Génard, M., Lobit, P., Mbéguié-A-Mbéguié, D., & Bugaud, C. (2013). What controls fleshy fruit Acidity (AN)? A review of malate and citrate accumulation in fruit cells. *Journal of Experimental Botany*, 64(6), 1451–1469. <https://doi.org/10.1093/jxb/ert035>
13. Fitriansyah, S.N., Aulifa, D.L., Febriani, Y. and Sapitri, E. 2018. Correlation of total phenolic, flavonoid and carotenoid content of *Phyllanthus emblica* extract from Bandung with DPPH scavenging activities. *Pharmacogn. J.* 10(3)
14. Gopalan, C., Rama Sastri, B. V., & Balasubramanian, S. C. (2007). *Nutritive value of Indian foods*. National Institute of Nutrition, Indian Council of Medical Research.
15. Gul, M., Liu, Z. W., Iqbal-Ul-Haq, Rabail, R., Faheem, F., Walayyet, A., Nawaz, A., Shabbir, M. A., Muneke, P. E. S., Lorenzo, J. M., & Aadil, R. M. (2022). Functional and nutraceutical significance of Amla (*Phyllanthus emblica* L.): A review. *Antioxidants*, 11(5), 816. <https://doi.org/10.3390/antiox11050816>
16. Hocking, B., Tyerman, S. D., Burton, R. A., & Gilliam, M. (2016). Fruit calcium: Transport and physiology. *Frontiers in Plant Science*, 7, 569. <https://doi.org/10.3389/fpls.2016.00569>
17. Jagota, S.K. and Dani, H.M. 1982. A new colorimetric technique for the estimation of vitamin C using Folin phenol reagent. *Anal. Biochem.* 127: 178-182
18. Jamuna, K.S., Ramesh, C.K. and Paramesha, M. 2017. Quantitative analysis of natural antioxidants and free radical scavenging activities of Cruciferae vegetables. *Asian J. Pharm. Clin. Res.* 10: 470-476
19. Krishnaveni, M., & Mirunalini, S. (2010). Therapeutic potential of *Phyllanthus emblica* (Amla): The Ayurvedic wonder. *Journal of Basic and Clinical Physiology and Pharmacology*, 21(1), 93–105. <https://doi.org/10.1515/JBCPP.2010.21.1.93>
20. Kumar, A., Singh, A., Singh, S., & Yadav, H. K. (2019). Effect of rust disease on photosynthetic pigments and biochemical parameters in Aonla (*Emblica officinalis* Gaertn.). *Journal of Pharmacognosy and Phytochemistry*, 8(3), 3214–3217.
21. Kumar, J., Singh, H.K., Rahul, S.N., Singh, A. and Singh, A., (2023). Germplasm evaluation of aonla (*Emblica officinalis* Gaertn.) against rust (*Ravenelia emblicae* Syd.) for resistance. *The Pharma Innovation Journal*, 12, 1423-1426.
22. Kumar, J., Singh, H.K., Singh, A., Patel, S. and Raghuvanshi, V.V., (2024). Epidemiological studies on aonla rust: a novel weather-based model for predicting *Ravenelia emblicae* Syd. infection in aonla fruits. *Environment and Ecology*, 42(3B), 1342-1348.
23. Kumari R, Ashraf S, Bagri GK, Khatik SK, Bagri DK, Bagdi DL (2018) Extraction and estimation of chlorophyll content of seed treated lentil crop using DMSO and acetone. *J Pharmacogn Phytochem* 7(3):249–250
24. Kumari, P. 2017. Evaluation of chlorophyll and cellulose content in different varieties of aonla during room temperature storage. *Chem. Sci. Rev. Lett.* 6(21): 59-63
25. Lichtenthaler, H.K., & Wellburn, A.R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, 11(6), 591-592. <https://doi.org/10.1042/bst0110591>
26. Magwaza, L. S., & Opara, U. L. (2015). Analytical methods for determination of sugars and sweetness of horticultural products—A review. *Scientia Horticulturae*, 184, 179–192. <https://doi.org/10.1016/j.scienta.2015.01.001>
27. Meena, M., Swapnil, P., Zehra, A., Aamir, M., Dubey, M. K., Goutam, J., & Upadhyay, R. S. (2017). Beneficial microbes for disease suppression and plant growth promotion. In D. P. Singh, H. B. Singh, & R. Prabha (Eds.), *Plant-microbe interactions in agro-ecological perspectives* (Vol. 2, pp. 395–432). Springer. [https://doi.org/10.1007/978-981-10-6593-4\\_16](https://doi.org/10.1007/978-981-10-6593-4_16)
28. Morton, J. F. (1987). *Fruits of warm climates*. Julia F. Morton.

29. Patel, S., Singh, H.K., Kumar, J., Maurya, M.K. and Singh, S.K., (2025). Tracking Aonla rust: climate cues, cultivar resilience, and field-based management. *Indian Phytopathology*, 78(3), 615-625.
30. Pathak, R. K., Ram, R. A., & Pradan, S. (2011). *Aonla production technology*. National Research Centre for Aonla, ICAR.
31. **Ranganna, S. 1986.** Handbook of analysis and quality control for fruit and vegetable products. 2nd ed. New Delhi, Tata McGraw-Hill. 1112 p.
32. Saini, A.K., Sharma, S.K., Kumar, A., Kumar, P. and Singh, D. 2018. Analysis of biochemical basis of resistance mechanism of aonla (*Emblica officinalis*) varieties against blue mould rot disease (*Penicillium islandicum*). *Indian J. Agric. Sci.* 88: 1770-1778
33. Scholes, J. D., & Rolfe, S. A. (2009). Chlorophyll fluorescence imaging as tool for understanding the impact of fungal diseases on plant performance: A phenomics perspective. *Functional Plant Biology*, 36(11), 880–892.  
<https://doi.org/10.1071/FP09145>
34. Sharma, I. M., & Gupta, J. N. (2003). Effect of fungicides on the control of rust (*Ravenelia emblicae*) disease of Aonla (*Emblica officinalis* Gaertn.). *Plant Disease Research*, 18(1), 42–44.
35. Singh, H.K., Kumar, J., Pandey, A.K., Patel, S., Yadav, A., & Singh, A.K. (2025). Aonla rust, its causes, epidemiology and management- A review. *Indian Journal of Arid Horticulture*. 6(1), 1-20
36. Singh, I. S., Pathak, R. K., Dwivedi, R., & Singh, R. (2011). *Aonla (Emblica officinalis Gaertn.) germplasm: A compendium*. National Research Centre on Aonla, ICAR.
37. Tewari, R., Kumar, V. and Sharma, H.K. 2019. Physical and chemical characteristics of different cultivars of Indian gooseberry (*Emblica officinalis*). *J. Food Sci. Technol.* 56: 1641-1648
38. Upadhyay, R., Sehwaag, S., & Singh, S. P. (2018). Nutritional, therapeutic and processing aspects of Aonla (*Emblica officinalis* Gaertn.): An overview. *Journal of Postharvest Technology*, 6(3), 1–18.
39. Variya, B. C., Bakrania, A. K., & Patel, S. S. (2016). *Emblica officinalis* (Amla): A review for its phytochemistry, ethnomedicinal uses and medicinal potentials with respect to molecular mechanisms. *Pharmacological Research*, 111, 180–200. <https://doi.org/10.1016/j.phrs.2016.06.013>

## Figures

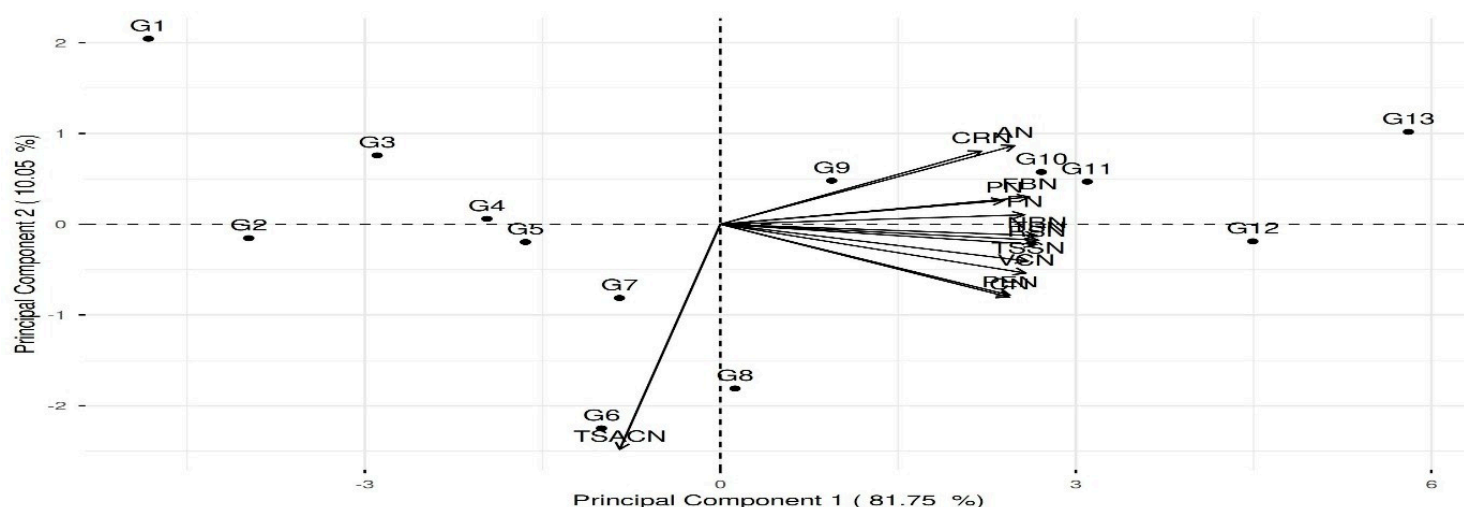
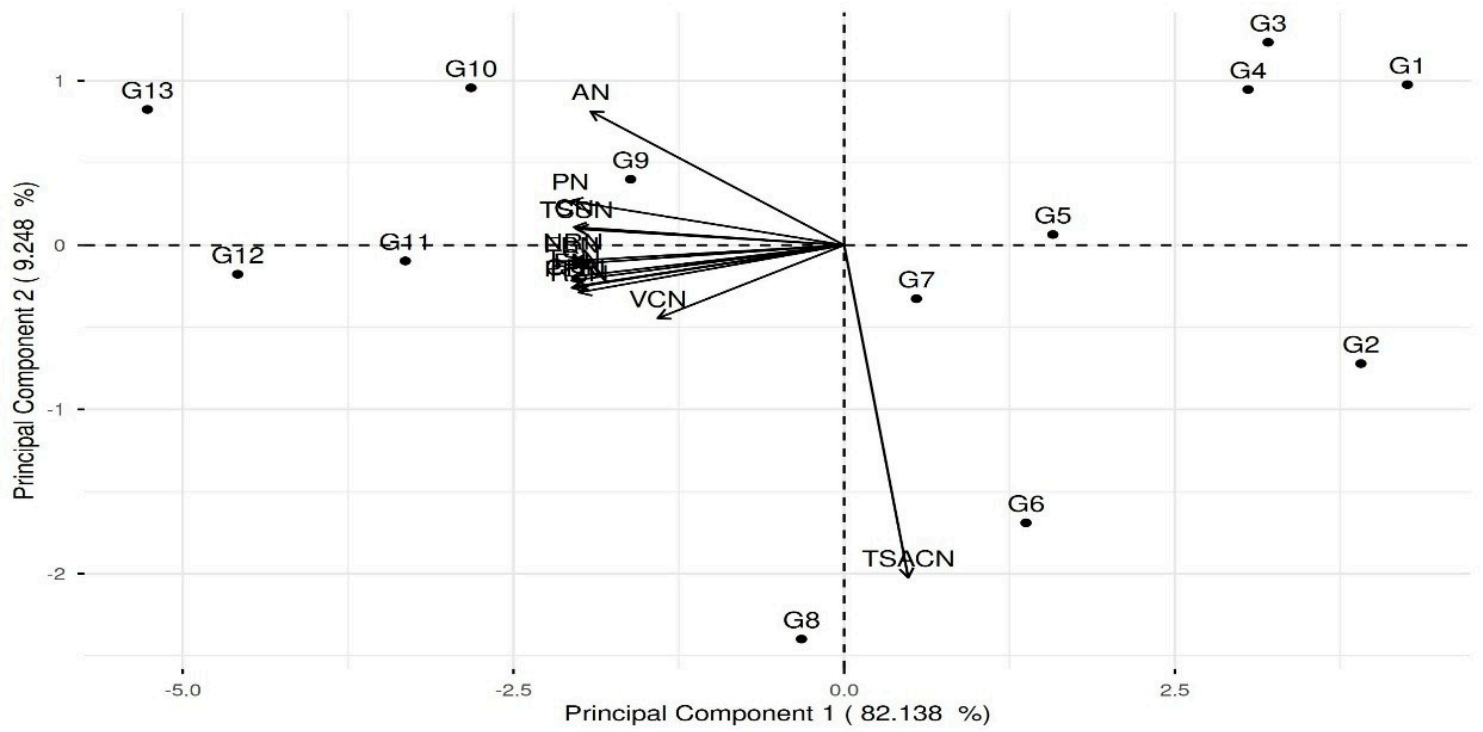
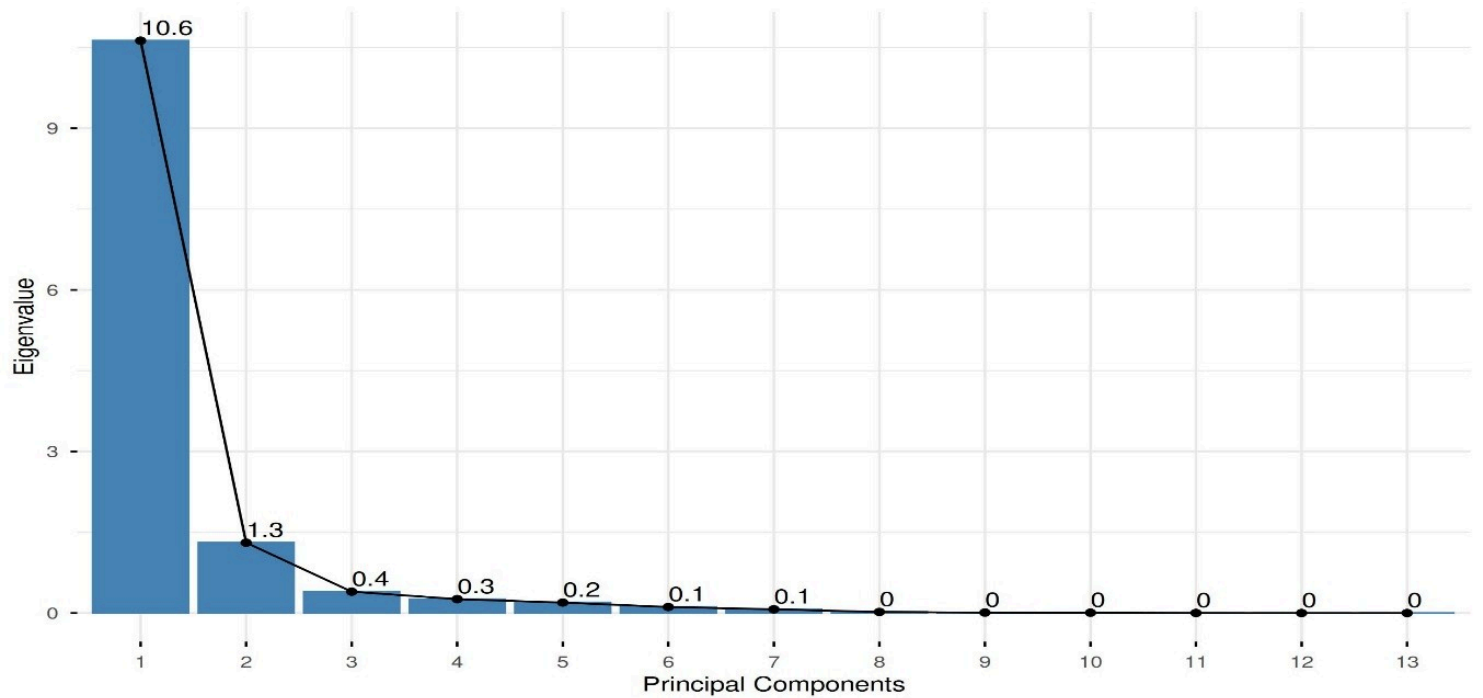


Figure 1

Biplot: PCA analysis in healthy condition in 90 days



**Figure 2**  
**Biplot: PCA analysis in infected condition in 90 days**



**Figure 3**  
**Eigenvalue Distribution under Healthy Condition (90 DAS)**

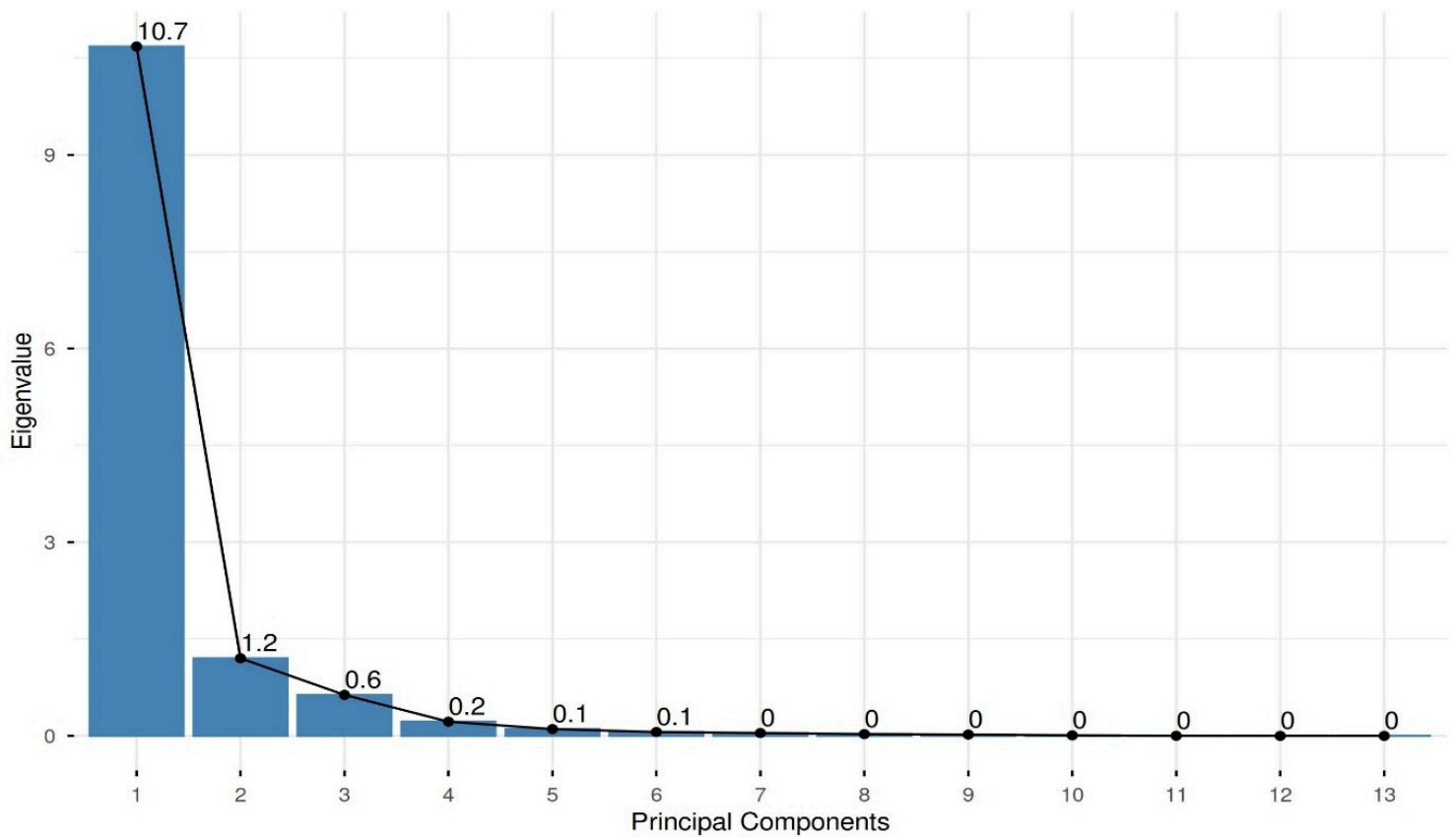


Figure 4

Eigenvalue Distribution under Infected Condition (90 DAS)

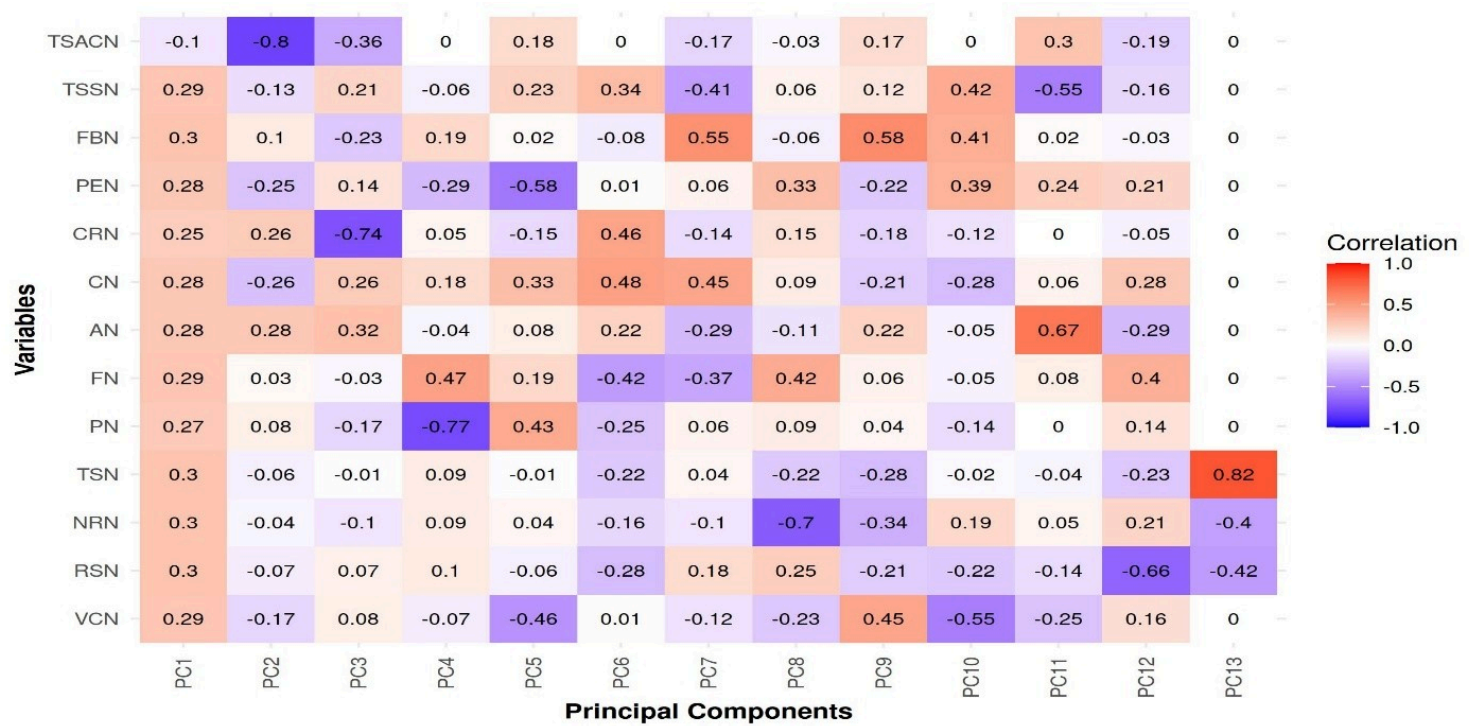


Figure 5

Heatmap Correlation under Healthy Condition (90 DAS)

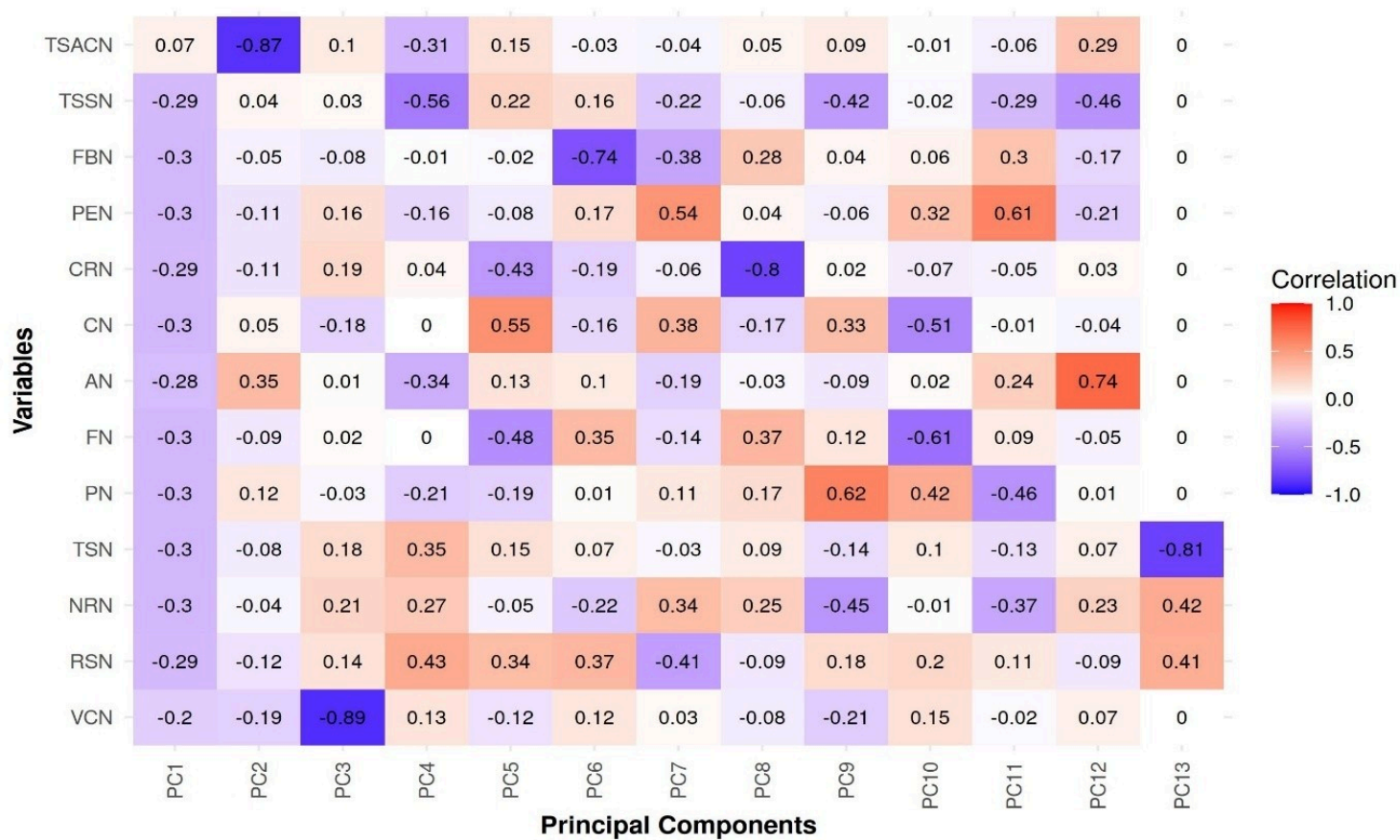


Figure 6

Heatmap Correlation under Infected Condition (90 DAS)