

Comparative Estimation of Ascorbic Acid in Healthy and Rust Infected Aonla (*Phyllanthus emblica* L.) Fruits: Insights into Plant Resistance Mechanisms.

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ABSTRACT

Aonla (*Phyllanthus emblica* Linn.), revered for its exceptional nutraceutical properties and therapeutic value, is a significant fruit crop in India and other tropical regions. Despite its hardiness, the crop is vulnerable to rust disease caused by *Phakopsora phyllanthi*, which adversely affects both yield and post-harvest quality. This study aimed to evaluate the biochemical response specifically Vitamin C (ascorbic acid) content of thirteen aonla cultivars under healthy and rust-infected conditions over two developmental stages (60 and 90 days after infection). Vitamin C content consistently declined under infected conditions, with NA-25, NA-26, and BSR-1 maintaining significantly higher levels compared to other genotypes, highlighting their superior nutritional retention and potential disease resilience. Principal Component Analysis (PCA) revealed that the first component accounted for 95.72% of the variance, confirming its dominant influence. The PCA biplot analysis further illustrated strong associations between rust infection and biochemical degradation, particularly distinguishing between healthy and infected states via PC2. These findings underscore the importance of genotype-dependent variability in disease tolerance and offer valuable insight for breeding disease-resistant, nutritionally superior aonla cultivars.

INTRODUCTION

Aonla, popularly known as Indian gooseberry (*Phyllanthus emblica* Linn.), is a highly esteemed, nutrient-dense fruit native to the Indian subcontinent. Celebrated for its multifaceted therapeutic and medicinal benefits, this botanical gem belonging to the family Euphorbiaceae is often heralded as the "wonder fruit for health" (Bakshi et al., 2015). Widely cultivated in tropical and subtropical climates across India, China, Indonesia, and the Malay Peninsula, India dominates global production. The nation accounts for around 103.55 thousand hectares under cultivation and yields approximately 1.23 million metric tons annually, with leading contributions from Uttar Pradesh, Madhya Pradesh, Maharashtra, and Tamil Nadu (Chandra et al., 2020; Kumar et al., 2004). Aonla has long held a prominent place in traditional systems of medicine, particularly Ayurveda and Unani, forming a key ingredient in revered formulations like *Chyawanprash*, *Triphala*, and *Ashokarishta* (Tripathi et al., 2021; Jat et al., 2020). Its formidable pharmacological profile is attributed to a rich composition of bioactive compounds including ascorbic acid, polyphenols, flavonoids, and tannins. These constituents underlie its broad-spectrum therapeutic actions, such as antiviral, antidiabetic, cardioprotective, anti-inflammatory, and anticancer effects (Priego et al., 2008). Moreover, its potent antioxidant properties make it a powerful agent in combating oxidative stress, with emerging research supporting its role in neuroprotection and skin health (Datta et al., 2010). Given its exceptional

nutraceutical potential, Aonla continues to captivate modern science and traditional wellness alike.

Despite its reputation for resilience, Aonla (*Emblca officinalis*) is vulnerable to numerous biotic stresses, notably diseases such as rust caused by *Phakopsora phyllanthi* as well as anthracnose (*Colletotrichum* spp.) and post-harvest fruit rots triggered by fungi like *Penicillium indicum*, *P. oxalicum*, and *Aspergillus niger* (Rawal, 1993; Singh et al., 2010). Among these, rust poses a particularly severe threat, with potential yield losses reaching up to 30%. A critical factor in this susceptibility lies in the inherent biochemical diversity across aonla cultivars. Genotype-dependent traits including total soluble solids (TSS), pH, titratable acidity, reducing sugars, ascorbic acid, pectin, and phenolic content play a pivotal role in modulating host-pathogen interactions of particular interest are phenolic compounds, renowned for their potent antioxidant activity and their integral role in plant defence. These, along with induced enzymatic responses such as lignin biosynthesis, contribute to the fortification of cellular structures against pathogen intrusion (Liang et al., 2011). Moreover, post-harvest fungal infections exert significant biochemical pressure, altering key nutritional attributes and compromising fruit quality during storage and transit. In light of these challenges, the present investigation was designed to unravel the biochemical mechanisms underpinning resistance in various aonla genotypes against the rust pathogen *Ravenelia emblica*. The ultimate objective is to identify disease-resistant cultivars that support sustainable cultivation while preserving post-harvest integrity and nutritional value.

Materials and Methods

The present investigation was carried out during the 2022-23 and 2023-24 cropping seasons at the Main Experimental Station, Horticulture Farm, and the Department of Plant Pathology Laboratory, Acharya Narendra Deva University of Agriculture and Technology, Ayodhya (Uttar Pradesh), India. For the purpose of Vitamin-C assessment, mature fruits were systematically collected from a diverse range of aonla (*Phyllanthus emblica*) cultivars. Thirteen cultivars- 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 were subjected to Vitamin-C analysis.

Estimation of Vitamin- C (Ascorbic acid)

The determination of ascorbic acid content followed the method described by Jagota and Dani (1982). In this procedure, 2 g of sample was macerated with an equal volume of 6% metaphosphoric acid, ensuring thorough extraction. The mixture was then subjected to centrifugation at 5000 rpm for 10 minutes, followed by filtration through Whatman No. 1 filter paper to obtain a clear extract. Next, 0.1 mL of the filtrate was diluted with 3% metaphosphoric acid, and the final volume was adjusted to 4 mL using deionized water. To initiate the reaction, 0.4 mL of Folin Ciocalteu reagent was added. The test tubes containing the mixture were incubated at room temperature for 10 minutes, then centrifuged at 3000 rpm for an additional 10 minutes. Finally, the absorbance of the supernatant was measured at 760 nm, providing an accurate quantification of ascorbic acid content.

Statistical analysis

Table 1: Various quantity of ascorbic acid of different Aonla genotypes.

Cultivars	Vitamin-C (mg/100gm)			
	After 60DAI		After 90DAI	
	H	I	H	I
Chakaiya	396.31	300.21	354.12	192.15
Anand-1	406.09	328.13	375.22	171.91
CHES-1	406.49	334.15	376.13	115.92
Francis	416.13	348.26	398.34	126.09
NA-4	421.55	368.44	400.34	114.19
NA-7	461.01	400.42	450.22	135.35
NA-6	466.33	412.32	456.34	158.54
NA-5	485.89	428.16	463.14	229.89
NA-10	491.25	437.22	472.11	234.31
NA-20	496.55	432.64	478.13	170.23
BSR-1	506.11	440.18	486.31	234.41
NA-26	525.99	448.36	498.16	270.09
NA-25	541.55	460.12	528.26	205.89
CD =0.5%	12.83	10.98	11.26	4.95
SEm±	4.45	3.81	3.91	1.72

Principal component analysis

Principal component analysis (PCA), which is a size reduction method using the data set of the studied agricultural characteristics, applied.

All of the total variation has been derived from two principal component axis and Eigenvalues, Variability values (%) and

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 $P \leq 0.05$. Person correlation, Principal component, heatmap, cluster analysis using *RStudio* 4.3.2 revealed associations between rust disease severity and ascorbic acid.

Results

Estimating the impact of Rust on biochemical properties of aonla

Estimation of Vitamin-C (Ascorbic acid)

The ascorbic acid (Vitamin C) content in various Aonla cultivars (Table 1) at 60 and 90 days after initiation (DAI) under two conditions i.e. Healthy (H) and Infected (I). Overall, Vitamin C content decreases from 60 to 90 DAI across all cultivars, with a more pronounced reduction under condition I. Among the cultivars, NA-25, NA-26, and BSR-1 exhibit the highest Vitamin C levels at both time points, particularly under condition H, suggesting better retention and potential for nutritional use (Davey, M. W. et al., 2007). In contrast, cultivars like CHES-1 and NA-4 show the lowest Vitamin C levels at 90 DAI under condition I. The consistent superiority of the H condition in preserving Vitamin C indicates it is more favourable for maintaining nutritional quality (Giannakourou, M. C. et al., 2021). Statistical parameters such as CD (0.5%) and Sem ± confirm that the observed differences are significant and reliable.

Cumulative values (%) showed that Table2. The first principal component had 95.722% of the total covariation (PC1). The second principal component (PC2) explained 4.278% of the total variation.

Table 2. Eigenvalues and percentage of variation in for different principal components in thirteen cultivars of aonla.

	PC1	PC2
Eigenvalue	1.900	0.100
Variability %	95.722	4.278
Cumulative %	95.722	100

Scree Plot Analysis

Scree Plot (Graphical representation of Eigenvalues) was given in Fig. 1. Eigenvalues were 1.90 for PC1 and 0.10 for PC2 respectively. If the eigenvalues are above 1, it indicates that the

evaluated PC weight values are reliable. On the other, reported that if the eigenvalues value is >1, it is more informative than the original variable (Grossman, G. D. et al., 1991).

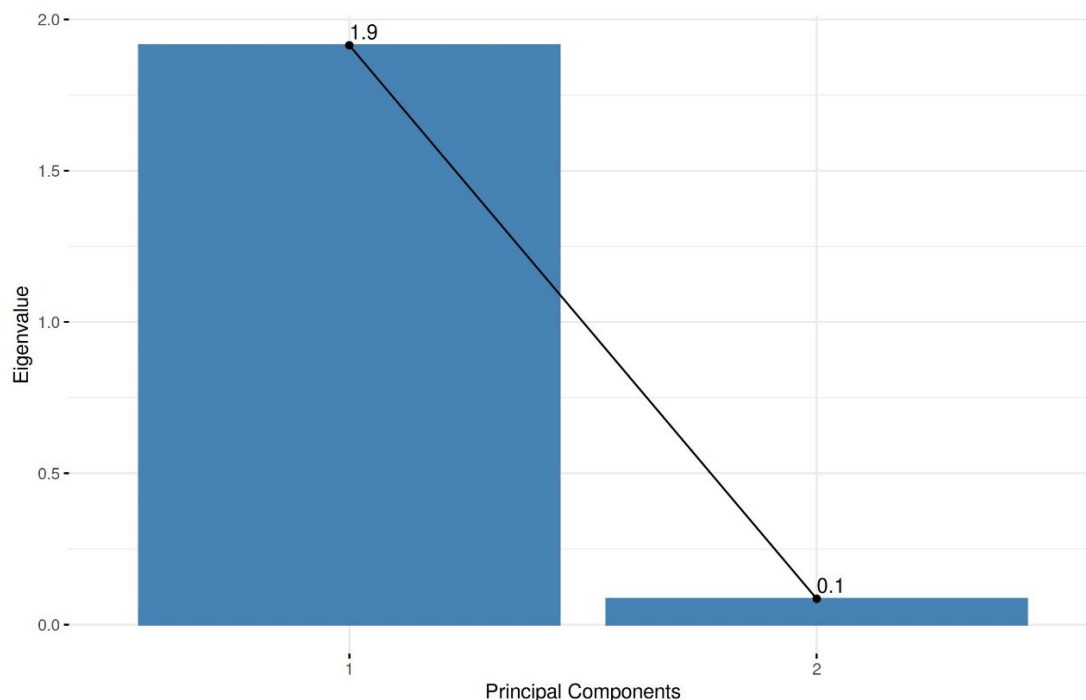


Figure 1. Scree plot showing variation for different principal component values.

Interpretation of PCA Biplot

This Principal Component Analysis (PCA) biplot is a statistical tool used to visualize how different samples relate to each other based on underlying variables. The plot maps samples like 'Francis', 'Chakaiya', 'NA-20', and 'Anand-1' along two principal axes i.e. PC1 and PC2, which together explain 100% of the variance in the dataset (95.722% by PC1 and 4.278% by PC2). Points closer together, such as 'NA-10' and 'NA-5', likely share similar

characteristics, while those farther apart, such as 'Francis' and 'Chakaiya', differ markedly. The arrows represent the contribution and direction of variables influencing the distribution, showing which traits push samples in certain directions. This visualization is particularly useful for identifying clusters, outliers, and patterns of similarity or divergence, making it a powerful method for comparative studies in fields like plant breeding, genetics, or trait-based evaluation.

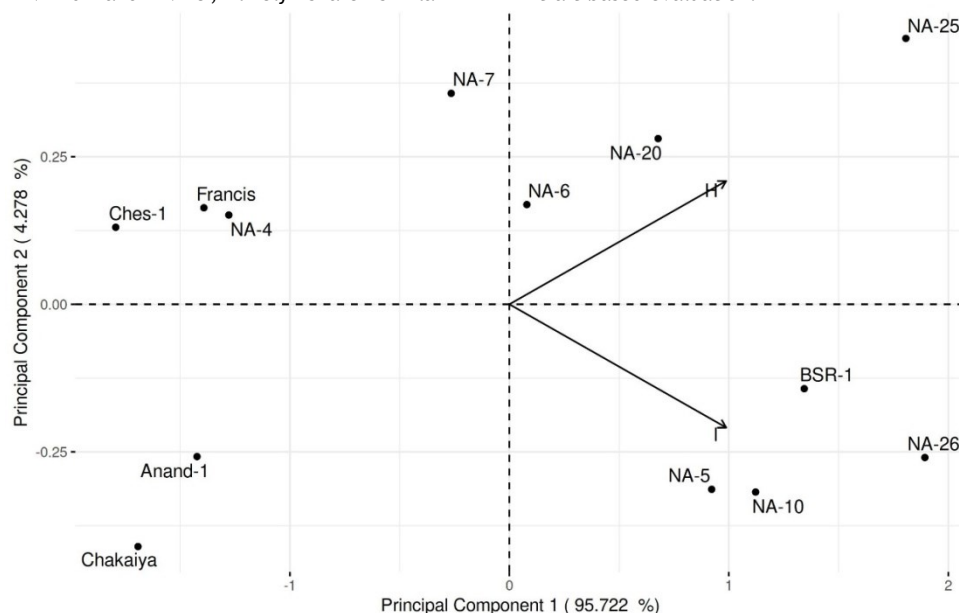


Figure 2. Interpretation of PCA Biplot

Correlation analysis of PCA

The heatmap visualizes the correlation between Conditions H and I with Principal Components PC1 and PC2, based on their loading values. Both H and I show a strong positive correlation with PC1 (0.71), indicating that PC1 represents a shared component or pattern in both conditions. However, they differ markedly in their correlation with PC2: H has a strong positive correlation (0.71), while it has a strong negative correlation (-0.71). This contrast

suggests that PC2 effectively separates or distinguishes between the two conditions, capturing variance unique to each. The colour gradient in the heatmap emphasizes this pattern, with red shades indicating positive correlations and blue shades showing negative ones, centered around zero. This plot is useful for interpreting how each condition contributes to or is represented by the underlying principle components (Houle, D. et al., 2002).

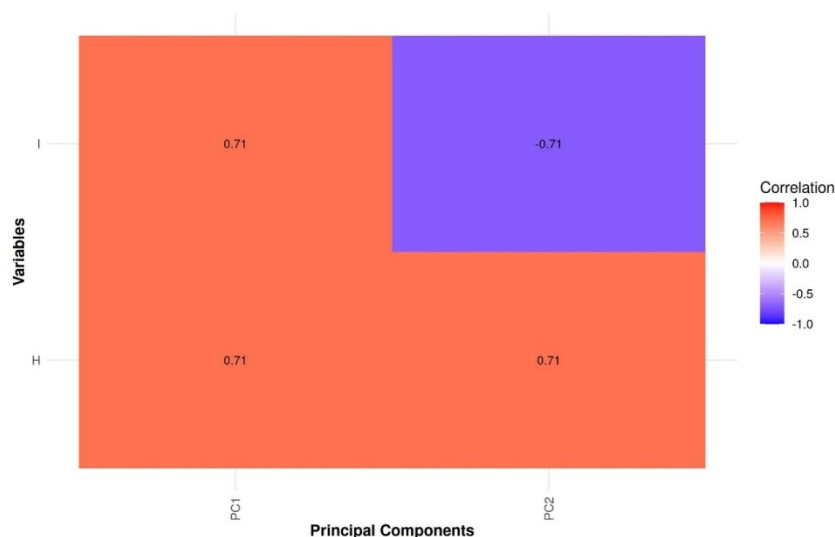


Figure 3. Correlation analysis of PCA

CONCLUSION

The present study demonstrated that rust infection significantly compromises the Vitamin C content in aonla fruits, with variation in susceptibility and biochemical resilience observed across genotypes. Cultivars such as NA-25, NA-26, and BSR-1 consistently exhibited higher ascorbic acid levels, even under infection pressure, suggesting their potential utility in breeding programs aimed at improving disease resistance and nutritional stability. The PCA analysis effectively identified the main drivers of variability, while the biplot and correlation heatmap emphasized the differential impact of infection on fruit biochemistry. Overall, the research highlights the crucial role of biochemical profiling in understanding host-pathogen interactions and supports the development of sustainable aonla cultivation strategies rooted in genotype selection.

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