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Morpho-anatomical and physiological characterization of rose genotypes in relation to powdery mildew disease resistance

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Abstract
Powdery mildew remains a significant challenge in rose cultivation, necessitating the development of disease-resistant rose cultivars to reduce fungicide dependency. The identification and characterization of physiological and structural basis of host-plant defense provide a quantifiable framework for screening germplasm and integrating durable resistance into high-yielding rose lines. This study evaluates eight rose genotypes categorized by their disease reactions: Immune (IIHRR 13-4), Resistant (*R. indica*, Arka Nishkant, Knock Out), Moderately Susceptible (IIHRR 4-15-12), and Susceptible (*R. multiflora*, Arka Swadesh, Arka Parimala) to powdery mildew. Analysis reveals that immunity and resistance are closely tied to restricted pathogen entry through thick leaves and regulated gas exchange. Notably, Knock Out combines extreme leaf thickness (190.67 µm) with the lowest observed stomatal conductance (0.26 mol (H_2O) m^{-2} s^{-1}), while IIHRR 4-15-12 though "Moderately Susceptible," presents a valuable breeding base due to its synergistic combination of leaf thickness (134.33 µm) and low stomatal conductance (0.28 mol (H_2O) m^{-2} s^{-1}).

Keywords: Rose genotypes, powdery mildew, disease resistance, morpho-anatomical traits

1. Introduction
Podosphaera pannosa is an obligate biotroph fungus that causes powdery mildew in rose. The disease affects leaves, shoots, and flower buds, leading to reduced photosynthesis, poor plant vigor, and loss of aesthetic value. While chemical control remains a common management practice, frequent fungicide applications raise concerns regarding cost, environmental safety, and development of pathogen resistance. Therefore, host plant resistance offers a sustainable and environmentally friendly alternative. Resistance to powdery mildew is often complex and influenced by a combination of morphological, anatomical, and physiological traits of the host plant. Understanding the relationship between these leaf traits and powdery mildew resistance can provide valuable insights for resistance screening and breeding. Hence, the present investigation was undertaken to study role of structural barriers and gas exchange dynamics and their influence on host-plant interactions across a spectrum of resistance levels.

2. Materials and Methods
2.1 Plant material
Based on the observations made over the years at ICAR-IIHR rose breeding program, the study was conducted using eight distinct rose genotypes having differential response to powdery mildew (Table 1) *viz*, IIHRR 13-4, Knock Out, Arka Nishkant, *R. indica*, IIHRR 4-15-12, Arka Parimala, Arka Swadesh and *R. multiflora*. The experiment was designed in completely randomized design (CRD) with three replications.

Table 1: Resistance category of selected rose genotypes to powdery mildew disease

S. No	Genotype	Disease reaction
1.	IIHRR 13-4	Immune
2.	Knock Out	Resistant
3.	Arka Nishkant	Resistant
4.	*R. indica*	Resistant
5.	IIHRR 4-15-12	Moderately Susceptible
6.	Arka Parimala	Susceptible
7.	Arka Swadesh	Susceptible
8.	*R. multiflora*	Susceptible

2.2 Observations recorded

The following observations were recorded in selected genotypes for characterization of Leaf Morpho-Anatomical Traits

a. **Stomatal characters:** Fully expanded leaves from the middle portion of the canopy were selected for anatomical measurements to ensure uniformity across genotypes. The number of stomata on the abaxial (lower) surface (number/mm^2) was recorded using the leaf impression method (Hamill *et al* 1992) [3]. Stomatal length and breadth, as well as aperture length and breadth were measured as described by Chattopadhyay *et al*., (2011) [1] under the microscope (make OLYMPUS; Light microscope digital camera; DP-22/DP-27) under 40X magnification and values expressed in µm.

b. **Physiological Gas Exchange Parameters:** The data were recorded during peak sunlight hours (09:30 AM to 11:30 AM) in upper most fully expanded leaves using portable photosynthesis system (LCpro+, ADC Bio Scientific limited, UK) (Mamatha *et al*., 2015) [4]. The leaf was held in the chamber for three minutes to obtain stable readings and the gas exchange parameters such as photosynthetic rate (µmol (CO_2) m^{-2} s^{-1}) (P_N), transpiration rate (m mol (H_2O) m^{-2} s^{-1}) (E) and stomatal conductance (mol (H_2O) m^{-2} s^{-1}) (g_s) were recorded.

c. **Leaf Thickness:** Leaf thickness was measured using scanning electron microscope and expressed in µm.

d. **Epicuticular Wax Content:** ECW content was estimated by following the procedure suggested by Ebercon *et al.* (1977) [2] with some modifications. Fully expanded and matured leaves collected from the selected genotypes were surface cleaned using cotton to remove dust particles. Leaf segments (3 cm^2) were prepared and immersed in a test tube containing 10 ml chloroform and shaken on an electronic shaker for 30 seconds. The resulting suspension was transferred to another test tube and allowed to evaporate completely. Five ml of acidic potassium dichromate ($K_2Cr_2O_7$) (10 g in 20 ml distilled water and volume adjusted to 500 ml with distilled water) was added to that test tube after complete evaporation and the contents were heated in boiling water bath for 30 minutes. After cooling, the final volume was adjusted to 12 ml with distilled water and optical density of resulting solution was read at 590 nm using UV-VIS spectrophotometer (T_80+ UV/VIS, PG Instrument Ltd., UK). The absorbance values were calculated using carbowax as standard. The wax content was calculated applying following formula and expressed as µg cm^{-2}.

$$\text{ECW (µg cm}^{-2}) = \frac{(OD_{590nm} \times \text{Standard value})}{(\text{Area of sample})^2}$$

2.3 Statistical analysis

Data analysis was performed using SAS software (version 9.3). Analysis of Variance (ANOVA) was employed to detect statistical differences, with Fisher's Least Significant Difference (LSD) test used for mean comparisons at $P \leq 0.05$. Additionally, Pearson's correlation coefficient (r) was calculated to determine the linear relationship between the studied factors and disease resistance.

3. Results and Discussion

The results obtained from present investigation were presented in Table 2.

3.1 Stomatal characters

Significant differences were observed among genotypes for stomatal density on the abaxial leaf surface. Susceptible genotypes such as *R. multiflora* recorded a high number of stomata (34.48/mm^2), whereas immune and resistant genotypes such as IIHRR 13-4 and Knock Out exhibited significantly lower stomatal density as 16.56 and 15.63/mm^2 respectively. Stomatal length, breadth, and aperture dimensions were generally larger in susceptible genotypes. In contrast, resistant genotypes exhibited comparatively smaller stomatal apertures, which may restrict the formation of a favorable microenvironment for conidial germination and penetration by *P. pannosa*.

3.2 Physiological Gas Exchange Parameters

a. **Stomatal Conductance (mol (H_2O) $m^{-2}s^{-1}$):** Highest stomatal conductance was observed in susceptible genotype Arka Swadesh (0.60) and the resistant genotype Knock Out recorded lowest stomatal conductance (0.26), followed by another resistant genotype *R. indica* (0.28) and moderately susceptible IIHRR 4-15-12 (0.28). The immune genotype IIHRR 13-4 recorded stomatal conductance as 0.33.

b. **Transpiration Rate (m mol (H_2O) $m^{-2}s^{-1}$):** The susceptible genotype *R. multiflora* has recorded highest transpiration rate (8.04) and Arka Parimala recorded the lowest (4.42). The immune genotype IIHRR 13-4 (5.42) and moderately susceptible IIHRR 4-15-12 (5.44) has recorded significantly lower transpiration rates than susceptible genotypes.
The regulation of gas exchange plays a secondary role in preventing the spread of infection. The powdery mildew spores are disseminated by wind and they are paradoxically sensitive to liquid water, which can be lethal to them. However, they necessitate high relative humidity for germination (Gubler and Koike 2009) [5]. In this context, the lower stomatal conductance which regulates moisture exit may maintain a drier leaf boundary layer, hindering the high-humidity requirements of the pathogen, thereby creating a microenvironment less conducive to spore germination.

c. **Photosynthetic Rate (µmol (CO_2) $m^{-2}s^{-1}$) (P_N):** Photosynthetic rate varied significantly among the genotypes. Arka Nishkant (Resistant) showed the highest photosynthetic rate (19.41), while IIHRR 4-15-12 showed the lowest (12.93) suggesting a possible metabolic trade-off for its structural defenses. The immune genotype IIHRR 13-4 and resistant genotype Knock Out recorded the photosynthetic rates as 15.46 and 13.54 respectively.

3.3 Epicuticular Wax Content

Epicuticular wax content varied significantly among genotypes and ranged from 50.63 µg cm^{-2} being found in Arka Nishkant to 305.63 µg cm^{-2} in Arka Parimala. Resistant genotypes such as Knock Out (254.13 µg cm^{-2}), *R. indica* (203.38 µg cm^{-2}) and Moderately susceptible IIHRR 4-15-12 (168.75 µg cm^{-2}) recorded significantly higher ECW content. IIHRR 13-4

recorded ECW content as 88.13 µg cm^{-2}. Increased wax accumulation in resistant genotypes likely reduced spore adhesion and surface wetness, thereby limiting fungal establishment. Further, the presence of high epicuticular wax in susceptible genotypes, such as Arka Parimala, suggests that wax content in isolation is insufficient to confer resistance.

3.4 Leaf Thickness

Leaf thickness was significantly higher in resistant genotypes, particularly in Knock Out (190.67µm) and moderately susceptible IIHRR 4-15-12 (134.33 µm) compared to susceptible genotypes. Thicker leaves may provide enhanced structural resistance and reduce successful haustorial penetration. These results partially align with the findings of Qiu *et al.* (2015) [6], who observed that immunity and high resistance to powdery mildew in rose species were associated with specific morphological markers, such as thickened, lustrous leaf surfaces or rugose textures with prominent venation.

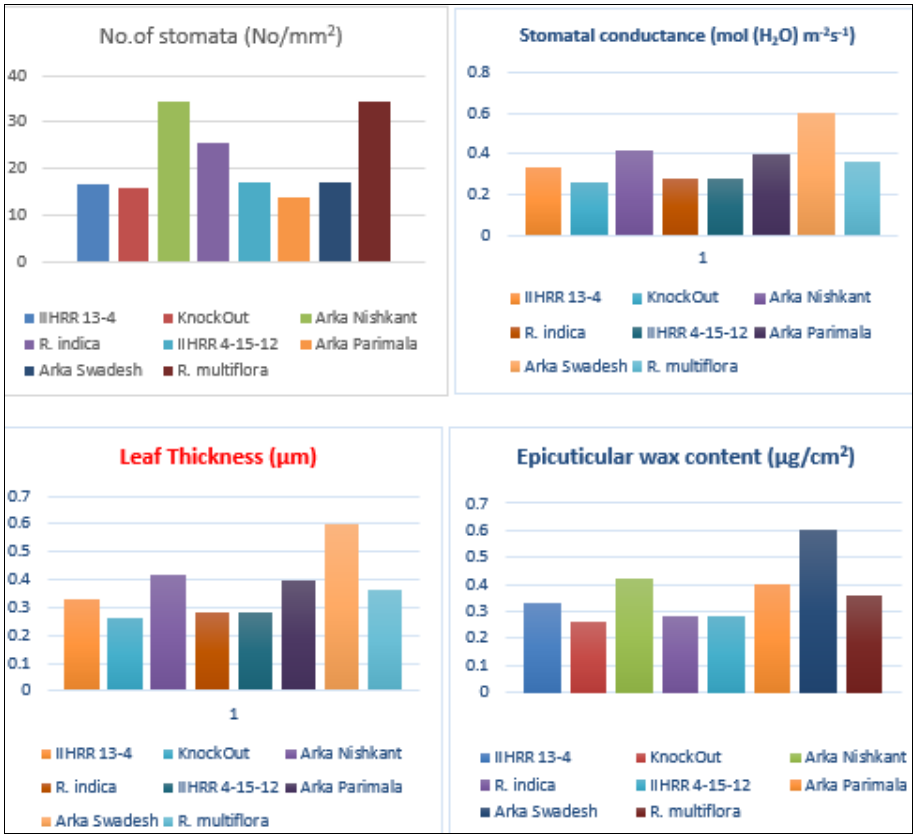

Fig 1: Stomata number, stomatal conductance, leaf thickness and epicuticular wax content of different rose genotypes

Table 1: Leaf Morpho-Anatomical and physiological Traits of rose genotypes

S. No.	Genotype and disease reaction	No. of stomata/mm²		Stomata length (µm)	Stomata breadth (µm)	Aperture length (µm)	Aperture breadth (µm)	Gas exchange parameters			Epicuticular wax (ECW) content (µg/cm²)	Leaf thickness (µm)
		Adaxial surface	Abaxial surface					Transpiration rate (m mol (H_2O) $m^{-2}s^{-1}$) (E)	Stomatal conductance (mol (H_2O) $m^{-2}s^{-1}$) (g_s)	Photosynthetic rate (µmol (CO_2) $m^{-2}s^{-1}$) (P_N)		
1	IIHRR 13-4 (I)	0	16.56 cd	53.75	34.32	34.78	6.87	5.42 bc	0.33 bcd	15.46 cd	88.13 g	87.63 d
2	Knock Out (R)	0	15.63 d	49.96	25.50	38.82	5.81	7.28 a	0.26 d	13.54 de	254.13 b	190.67 a
3	Arka Nishkant (R)	0	34.48 a	38.05	22.28	24.97	5.82	7.44 a	0.42 b	19.41 a	50.63 h	85.27 d
4	*R. indica* (R)	0	25.52 b	46.40	31.60	29.07	6.20	7.79 a	0.28 cd	13.88 de	203.38 c	88.67 d
5	IIHRR 4-15-12 (MS)	0	17.22 c	51.27	38.55	31.65	8.04	5.44 bc	0.28 cd	12.93 e	168.75 d	134.33 b
6	Arka Parimala (S)	0	13.96 e	56.11	37.57	35.96	7.53	4.42 c	0.40 bc	17.18 bc	305.63 a	91.47 d
7	Arka Swadesh (S)	0	17.15 c	58.75	37.38	35.94	7.12	6.54 ab	0.60 a	19.29 ab	100.63 f	111.67 c
8	*R. multiflora* (S)	0	34.44 a	35.66	22.86	20.65	3.68	8.04 a	0.36 bcd	14.69 de	118.13 e	138.03 b
	SE(m)	-	0.46	1.17	0.99	0.81	0.26	0.55	0.05	0.75	3.20	3.33
	C.D.	-	1.39	3.52	2.99	2.45	0.79	1.57	0.13	2.16	9.16	9.55

3.5 Correlation Analysis between Leaf Physiological Characters and Powdery Mildew Resistance

Correlation analysis was performed to understand the association between leaf physiological characters and powdery mildew resistance in rose genotypes (Table 3). The relationships among stomatal traits, epicuticular wax content, leaf thickness, and gas exchange parameters revealed distinct trends differentiating resistant and susceptible genotypes.

Stomatal density exhibited a positive association with disease susceptibility, as genotypes with higher number of stomata on the abaxial surface generally showed increased susceptibility to powdery mildew. Susceptible genotypes such as *R. multiflora* and Arka Swadesh recorded higher stomatal density, whereas immune and resistant genotypes such as IIHRR 13-4 and Knock Out exhibited comparatively lower stomatal numbers. This suggests that increased stomatal frequency may enhance the favorable microhabitats for pathogen establishment.

Stomatal length, breadth, and aperture dimensions were positively correlated with disease susceptibility. Genotypes possessing larger stomata and wider apertures tended to exhibit higher stomatal conductance and transpiration rates, thereby creating a humid leaf surface microenvironment conducive for powdery mildew development. In contrast, resistant genotypes maintained relatively smaller stomatal and aperture dimensions, which may restrict pathogen penetration and reduce moisture retention.

Epicuticular wax content showed a negative correlation with disease severity and a positive association with resistance. Resistant and immune genotypes exhibited higher wax deposition, whereas susceptible genotypes had comparatively lower epicuticular wax content. Increased wax accumulation likely acts as a physical barrier, reducing spore adhesion and germination on the leaf surface.

Leaf thickness exhibited a significant negative correlation with susceptibility. Genotypes with thicker leaves, such as Knock Out, showed enhanced resistance, while thinner leaves were associated with susceptibility. Increased leaf thickness may provide mechanical resistance and support enhanced structural defense mechanisms against pathogen invasion.

Among gas exchange parameters, transpiration rate and stomatal conductance showed a positive correlation with susceptibility, particularly in genotypes with wider stomatal apertures. Higher stomatal conductance was associated with greater leaf surface moisture, favoring powdery mildew infection. Conversely, resistant genotypes maintained lower to moderate stomatal conductance, thereby limiting pathogen establishment.

Photosynthetic rate exhibited a weak but positive correlation with resistance, as resistant genotypes were able to maintain higher or optimal photosynthetic efficiency despite reduced stomatal conductance. This indicates that resistance is associated with efficient physiological regulation rather than compromised carbon assimilation.

Overall, the correlation analysis suggests that powdery mildew resistance in rose is governed by a coordinated interaction of structural and physiological traits. Lower stomatal density, reduced stomatal aperture size, higher epicuticular wax content, increased leaf thickness, and regulated gas exchange collectively contributes to enhanced resistance against powdery mildew.

Table 3: Pearson correlation coefficients among leaf physiological traits and powdery mildew susceptibility in rose.

Trait	SD	SAS	ECW	LT	TR	SC	PR	DS
Stomatal density (SD)	1.00	0.68**	−0.62**	−0.55*	0.71**	0.74**	−0.28	0.79**
Stomatal aperture size (SAS)		1.00	−0.66**	−0.48*	0.76**	0.81**	−0.22	0.83**
Epicuticular wax content (ECW)			1.00	0.69**	−0.58*	−0.61**	0.34	−0.77**
Leaf thickness (LT)				1.00	−0.46*	−0.52*	0.41*	−0.72**
Transpiration rate (TR)					1.00	0.85**	−0.19	0.66**
Stomatal conductance (SC)						1.00	−0.25	0.74**
Photosynthetic rate (PR)							1.00	−0.36*
Disease susceptibility (DS)								1.00

Notes: SD = Stomatal density; SAS = Stomatal aperture size; ECW = Epicuticular wax content; LT = Leaf thickness; TR = Transpiration rate; SC = Stomatal conductance; PR = Photosynthetic rate; DS = Disease susceptibility.

*P ≤ 0.05; **P ≤ 0.01 (two-tailed).* Positive values indicate traits increase susceptibility; negative values indicate traits contributing to resistance.

4. Conclusion

The present study demonstrated that powdery mildew resistance in rose is strongly influenced by leaf physiological and anatomical traits that collectively regulate pathogen entry, establishment, and host physiological efficiency. Distinct differences were observed between resistant and susceptible genotypes, indicating that resistance is not governed by a single factor but by an integrated network of structural and functional leaf attributes. These traits can serve as valuable selection criteria in rose breeding programs aimed at developing cultivars with durable resistance while maintaining optimal physiological performance. IIHRR 4-15-12 genotype occupies a unique niche. Despite its "Moderately Susceptible" classification, its low stomatal conductance and substantial leaf thickness provide a robust physical and physiological framework. Using this genotype in crosses with IIHRR 13-4 (Immune) could combine low stomatal density with high structural thickness, leading to more durable resistance.

5. Implications and Future Directions

The identification of leaf physiological traits as key determinants of powdery mildew resistance has practical implications for rose breeding and disease management. Traits such as stomatal density, aperture size, epicuticular wax content, and leaf thickness can serve as reliable selection criteria for developing resistant cultivars. Incorporating these physiological markers into breeding programs will facilitate early and efficient screening of germplasm, reducing reliance on labor-intensive disease assays.

Future studies should investigate the genetic basis of these traits and their interaction with environmental factors to enhance the stability of resistance across diverse growing conditions. In addition, integrating molecular markers linked to these physiological characteristics could accelerate marker-assisted selection and support the development of roses with durable resistance while maintaining high photosynthetic efficiency and ornamental quality.

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