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Ashwini R
Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Raichur, Karnataka, India

Amaresh YS
Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Raichur, Karnataka, India

Yenjerappa ST
Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Raichur, Karnataka, India

RV Beladhadi
Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Raichur, Karnataka, India

Corresponding Author:
Ashwini R
Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Raichur, Karnataka, India

Influence of biochemical composition on resistance and susceptibility of okra genotypes screened against powdery mildew disease

Ashwini R, Amaresh YS, Yenjerappa ST and RV Beladhadi

Abstract
Bhendi powdery mildew caused by *Erysiphe cichoracearum* DC. is important foliar disease affecting all the stages of the plant growth by causing premature defoliation and resulting 17.0 to 86.6 per cent yield loss in bhendi. Biochemical studies were carried out to know the biochemical changes due to powdery mildew using different okra genotypes. Results revealed that, *i.e*., lower amount of reducing, non-reducing and total sugar of 10.16, 9.24, 19.40 mg g^{-1} was recorded in moderately resistant genotype (EC329404), while highly susceptible genotypes *viz*., Arka Anamika and IC42531 recorded higher amount of reducing, non-reducing and total sugar of 16.65 and 15.47 mg g^{-1}, 20.12 and 19.10 mg g^{-1}, 36.77 and 34.57 mg g^{-1} respectively in diseased leaf and were found on par with each other. Healthy leaves of highly susceptible genotypes (Arka Anamika and IC42531) have recorded 6.4, 6.1 mg g^{-1} of total phenol which increased to 9.95, 8.63 mg g^{-1} after infection by *E. cichoracearum*. The increase in phenol content was at higher rate in moderately resistant genotype *i.e*., EC329404; before infection phenol content recorded was 9.7 mg g^{-1} and it increased to 16.29 mg g^{-1} after infection by *E. cichoracearum*. It was noticed that sugar content and resistance to disease were negatively correlated while phenol content and resistance to disease were positively correlated.

Keywords: Powdery mildew, *Erysiphe cichoracearum*, biochemical, reducing, non-reducing, total sugars, phenol, resistance

Introduction
Bhendi (*Abelmoschus esculentus* (L.) Moench) is globally important annual vegetable belongs to a family malvaceae, it is most broadly distributed vegetable all over the world. Many factors responsible for yield loss of the crop, one of them are the diseases which are the major constraints for low yield of bhendi (Sastry and Singh, 1974) [13]. A number of fungal, bacterial and viral diseases have been reported in India. Among the fungal diseases affecting bhendi crop, powdery mildew caused by *Erysiphe cichoracearum* is the most important disease causing considerable yield losses. The disease initiates as white minute powdery patches first on the upper surface of leaf and lower older leaves and then spreads to younger ones. Grayish white powdery coating is visible on severely affected leaves. Leaves finally show necrosis resulting in withering, drying and defoliation. Powdery mildew affects plants at all the growth stages and may result yield losses up to 17 to 86.6 per cent (Sridhar and Sinha, 1989) [16]. Powdery mildew occurs in severe form at a particular stage of the crop covering large host surface. Sometime, crop gets infected at an early stage, if the inoculum potential coincides with favorable environmental conditions. Host plant resistance is one of the most realistic, economical and feasible method of management of plant diseases. Use of resistant varieties is inexpensive and reliable approach than other methods. Therefore, it is vital to carry out screening of genotypes to become aware of resistant lines which play an important role in the management of diseases. Further, the studies on biochemical parameters would be helpful to find out their relationship, if any with the phenomenon of resistance against the disease.

Material and methods
Biochemical studies and enzymatic analysis
Biochemical studies were carried out to know the biochemical changes due to powdery mildew disease caused by *E. cichoracearum* using different okra genotypes screened under pot conditions. Fresh healthy and infected leaf samples were collected from okra genotypes for assessing the biochemical factors responsible for imparting resistance to powdery mildew disease.

Sampling of leaves to estimate phenols and total sugars
At 60 DAS, the leaf samples were collected during early morning hours from the different germplasm lines. For sampling, healthy and diseased plants were chosen for the purpose of biochemical analysis. The leaf samples were collected in polythene bags separately, labelled and carried to the laboratory.

Genotypes used for biochemical and enzymatic analysis

Sl. No.	Genotype/variety
1.	EC329404
2.	EC329405
3.	EC329399
4.	IC42524
5.	IC42535
6.	IC42531
7.	Arka Anamika

Extraction of plant tissues in alcohol
One gram of tissue was weighed and made into small pieces and plunged immediately in boiling alcohol. Then it was cooled and passed through double-layered muslin cloth. The pieces of the tissue were ground thoroughly in a mortar and pestle with 10 ml alcohol, later it was passed through muslin cloth. The above step was repeated once again. The filtrates were pooled and filtered through Whatman No. 41 filter paper and made up to 10 ml with alcohol. The extract was stored in a refrigerator at 4 ^{0}C. This alcoholic extract was used further for analysis of reducing sugar, non-reducing sugar, total sugar and phenols.

Clarification of alcoholic extracts
Dark colored alcoholic extracts of the tissues create a great problem in analytical procedure. Saturated solution of neutral lead acetate and saturated solution of disodium hydrogen phosphate were used for clarification of alcoholic extracts.

Procedure
Two ml of saturated lead acetate solution was added drop wise to 10 ml of the colored alcoholic extract with three ml of saturated solution of di-sodium hydrogen phosphate till the precipitation is completed. The above solutions were mixed thoroughly and kept overnight and filtered through Whatman No. 1 filter paper and volume was made up to 15 ml with 80 per cent alcohol and stored in a refrigerator at 4 ^{0}C.

Estimation of total sugars
Estimation of reducing sugars
Reducing sugars in the leaf samples were estimated by Nelson's modification of Somogyi's method (Nelson, 1944) [11].

Reagents
A. Alkaline copper reagent
Solution A: Twenty-five gram of anhydrous sodium carbonate, 25 g of sodium potassium tartrate, 20 g of sodium bicarbonate and 200 g of sodium sulphate were dissolved in 800 ml of distilled water and volume was made up to one liter.
Solution B: Fifteen gram of copper sulphate was dissolved in distilled water to which one or two drops of concentrated sulphuric acid was added and made up to 100 ml with distilled water.
Solution A and B were mixed in 24:1 (v/v) proportion just before use.

B. Arsenomolybdate reagent
1. Twenty-five gram of ammonium molybdate was dissolved in 450 ml of distilled water. Twenty-one ml of concentrated sulphuric acid was added and mixed with above solution.
2. Three gram of sodium orthoarsenate was dissolved in 25 ml of distilled water.

Both the solutions (1 and 2) were mixed with stirring and placed in an incubator at 37 ^{0}C for 24-48 hrs. The reagent was stored in brown colored bottle.

Procedure
One ml of each sample (alcohol extract) was pipetted out into a test tube. To each one ml of extract, one ml of mixture of solution A and B was added. The test tubes were heated on a hot water bath for 20 min. The tubes were then cooled under running tap water. After cooling one ml of arsenomolybdate reagent was added. After 15 min the solution was diluted to 15 ml. The absorbance of the solution was measured in spectrophotometer at 620 nm. The amount of reducing sugars was determined by using standard curve prepared with glucose.

Acid hydrolysis of non-reducing sugar and its estimation as reducing sugar
Non-reducing sugar was first hydrolyzed with the help of diluted hydrochloric acid. The hydrolysate was neutralized and the reducing sugar was estimated by Nelson Simonyi's method (Nelson, 1944) [11].

Reagents
1. 0.1 and 1 N hydrochloric acid and 1 N sodium hydroxide
2. Phenolphthalein indicator solution in alcohol

Procedure
One ml of alcohol extract was added to one ml of 1 N HCl in a test tube, the test tubes were then kept on hot water bath at 50 ^{0}C for 20 min. After cooling, one drop of indicator was added and mixed well. To the solution, 1 N sodium hydroxide was added drop wise till the colour turned pink due to excess alkali. The excess alkali was reneutralized with 0.1 N hydrochloric acid till the solution became colourless, then the volume was made up to 5 ml. From this, 1 ml was taken and reducing sugar present in hydrolysate was estimated by Nelson Simonyi's method. The reducing sugar in the hydrolysate was a measure of total sugar. To get the quantity of non-reducing sugar, the quantity of reducing sugar was subtracted from total sugar and it was multiplied by a conversion factor of 0.95.

Estimation of total phenols
Total phenol content was determined by following Folin - Ciocalteau reagent (FCR) method (Bray and Thrope, 1954) [1].

Reagents
1. Folin- Ciocalteau reagent (FCR 1 %)
2. Sodium carbonate (2 %)
3. Stock catechol solution
4. Working standard solution

Procedure
One ml each of alcoholic extract was taken in a test tube, to which 1 ml of FCR reagent was added followed by 2 ml of

sodium carbonate solution (2 %). The tubes were shaken well and heated in a hot water bath for exactly one minute and then cooled under running tap water. The blue coloured solution was diluted to 20 ml with distilled water and its absorbance was recorded at 650 nm in spectrophotometer. The amount of phenols present in the sample was calculated from a standard curve prepared from catechol.

Results and discussion

Sugar

Healthy and powdery mildew infected leaf samples were collected separately from different genotypes as mentioned in ''Material and Methods'' and the levels of sugars were analysed and results obtained are presented in Table 1.

Reducing sugar

Reducing sugar content varied from 7.8 to 12.89 mg g^{-1} in healthy leaf among various genotypes. There was significant increase in reducing sugar content in all the genotypes after infection by the pathogen and was inversely proportion to disease resistance. *i.e*., lower amount of reducing sugar of 10.16 mg g^{-1} was recorded in moderately resistant genotype (EC329404), while highly susceptible genotypes *viz*., IC42531 and Arka Anamika recorded higher amount of reducing sugar of 15.47 and 16.65 mg g^{-1}, respectively in diseased leaf and were found on par with each other. Moderately susceptible genotypes *i.e*., EC329405, EC329399 recorded reducing sugar content of 12.28, 12.90 mg g^{-1}, respectively, in diseased leaf and were found on par with each other. While, in susceptible genotypes *i.e*., IC42524, IC42535 reducing sugar content of 14.45,15.23 mg g^{-1} respectively was recorded in diseased leaf and were on par with each other (Fig. 1).

The results obtained are in accordance with the earlier workers Mandahar and Garg (1975) [10] that, the okra leaves infected with powdery mildew (*E. cichoracearum*) had higher reducing sugar content than healthy leaves. However, Shete and Munjal (2002) [14] reported that, there was an increase in level of reducing sugars in leaves infected with powdery mildew pathogen in mung bean cultivars. Such increase was more pronounced in susceptible cultivars than in resistant ones. Dhanumjayarao *et al.* (2007) [4] screened sixty genotypes of grapes among them they selected three varieties in each category *i.e*., highly susceptible, susceptible and resistant for biochemical study against powdery mildew and found that highly susceptible variety Parlette recorded lowest amount of acidity and higher amount of reducing sugar and total sugar. Whereas, resistant variety Pearl of Casaba recorded higher amount of acidity and lowest amount of reducing sugar and total sugar.

Non Reducing sugar

Non-reducing sugar in leaves before or after infection were lower in resistant genotypes than in moderately resistant, susceptible and highly susceptible genotypes. The highest content (9.62, 9.02 mg g^{-1}) of non-reducing sugar was observed in highly susceptible genotypes *i.e*., Arka Anamika and IC42531 that were on par with each other, whereas lowest content (4.65 mg g^{-1}) was recorded in moderately resistant genotype EC329404 before infection by the pathogen. After infection, the highest content of non-reducing sugar (20.12, 19.10 mg g^{-1}) was observed in highly susceptible genotypes *i.e*., Arka Anamika and IC42531 that were on par with each other, while lowest content (9.24 mg g^{-1}) was recorded in moderately resistant genotype EC329404. Moderately susceptible genotypes *i.e*., EC329405, EC329399 recorded non-reducing sugar content of 6.71, 7.47 mg g^{-1} before infection by the pathogen and were on par with each other and susceptible genotypes *i.e*., IC42524, IC42535 recorded non-reducing sugar content of 8.52, 8.43 mg g^{-1} before infection by the pathogen and were on par with each other. Whereas, after infection by the pathogen moderately susceptible genotypes *i.e*., EC329405, EC329399 recorded non-reducing sugar content of 11.62, 12.49 mg g^{-1}, respectively and were on par with each other and susceptible genotypes *i.e*., IC42524, IC42535 recorded non-reducing sugar content of to 13.87, 14.16 mg g^{-1}, respectively and were on par with each other (Fig. 1). However, non-reducing sugar content in leaves increased in all the genotypes after infection by the pathogen.

These findings are in agreement with the reports of Sanjay Guleria *et al*. (1997) [12] who worked with pea powdery mildew indicated that concentration of non-reducing sugars was negatively correlated with degree of resistance. Similarly, Devendra *et al*. (2011) [3] observed that the quantified estimates of reducing, non-reducing and total sugars were low in resistant genotypes as compared to susceptible genotypes. Divya (2012) [6] found that, the non-reducing sugar in leaves before or after infection were lower in resistant genotypes than moderately resistant, susceptible or highly susceptible genotypes. The non-reducing sugar content increased in leaves after powdery mildew infection (*E. polygoni*) in all the genotypes. The non-reducing sugar content of 1.41 and 2.02 mg/g was noticed with resistant genotypes TARM-18 and Kannerimath Local respectively before infection and increased to 5.72 and 12.63 mg/g after infection by the pathogen. Highly susceptible genotypes Shining Mung and Meha recorded highest non-reducing sugar content (24.73, 19.24 mg/g) after infection and was 5.37, 7.7 mg/g before infection by pathogen.

Total Sugar

Sugars are precursors and basic molecules for the synthesis of phenols, phytoalexines and farm a skeleton for the synthesis of nucleic acids (Vidaysekaran, 1974) [19]. Sugars play important role in inhibition of pectiolytic and cellulolytic enzymes which are essential for pathogenicity (Vidaysekaran, 1987) [18]. In general, infection by pathogen bring about changes in respiratory pathway and photosynthesis, which are vital processes taking place inside the plant leading to wide fluctuations in sugar content in plant system (Farkar and Kiraly, 1962, Klement and Goodman, 1967) [7, 8].

The total sugar content was increased in leaves after infection in all the genotypes. The highest content of total sugar (22.33, 21.91 mg g^{-1}) was observed in highly susceptible genotypes *i.e*., Arka Anamika and IC42531 that were on par with each other whereas lowest content (12.45 mg g^{-1}) was recorded in moderately resistant genotype *i.e*., EC329404 before infection by the pathogen. After infection by the pathogen the highest content (36.77, 34.57 mg g^{-1}) of total sugar was observed in highly susceptible genotypes *i.e*., Arka Anamika and IC42531 and were on par with each other and lowest content (19.40 mg g^{-1}) was recorded in moderately resistant genotype *i.e*., EC329404. Before infection by the pathogen, moderately susceptible genotypes *i.e*., EC329405, EC329399 recorded total sugar content of 16.39, 16.67 mg g^{-1}, respectively and were on par with each other and susceptible genotypes *i.e*., IC42524, IC42535 recorded total sugar content of to 20.10, 18.63 mg g^{-1}, respectively and were on par with each other.

Whereas, after infection by the pathogen, moderately susceptible genotypes *i.e*., EC329405, EC329399 recorded total sugar content of 23.90, 25.39 mg g^{-1}, respectively and were on par with each other but susceptible genotypes *i.e*., IC42524, IC42535 recorded total sugar content of 28.32, 29.39 mg g^{-1}, respectively that were on par with each other (Fig. 1).

The total sugar content in moderately resistant genotype was lower before or after infection than in highly susceptible, moderately susceptible and susceptible genotypes. The reason may be that the powdery mildews are high sugar diseases and susceptibility of plant to powdery mildew increased with the increase in level of sugars (Yarwood, 1957) [21]. Further, the higher level of sugars in the pathogen infected plants compared to the healthy plants may be because of post-infectional induction of hydrolytic enzymes that helped in the breakdown of sugars as reported by Shukla and Bhattacharya (1992) [15]. Further, diseases would also impair the translocation of sugars from source to sink and hence contribute to the higher sugars accumulation in infected plants.

Present findings are in close proximity with reports of Divya (2012) [6] that, the total sugar content in resistant genotypes was lower before or after infection than moderately resistant, highly susceptible and susceptible green gram genotypes. The total sugar content was increased in leaves after powdery mildew infection (*E. polygoni*) in all the genotypes. The total sugar content in resistant genotypes TARM-18 and Kannerimath Local were 7.71 and 10.77 mg/g before infection and it increased to 16.86 and 12.1 mg/g after infection by the pathogen. The highest total sugar content (36.83 mg/g) was noticed with highly susceptible genotypes Shining Mung and Meha (33.4 mg/g) after infection by pathogen while it was 24.57 and 23.84 mg/g before infection. Korra and Kumar (2020) [9] found that significantly lowest total sugar content was observed in highly resistant genotypes KUP-34 (4.48 mg/100 mg) KUP-40 (4.62 mg/100 mg) and were on par in their total sugar content with moderately resistant genotypes *viz*., KUP-12 (4.63 mg /100 mg), KUP-6 (4.65 mg/100 mg), KUP-11 (4.66 mg/100 mg) and KUP-31 (4.74 mg/ 100 mg). While, highest total sugar content was observed in highly susceptible genotype LBG-623 (7.39 mg/100 mg). They concluded that resistance of genotypes was inverse to the total sugar content.

Phenol

Plant tissue responds to injury with the production of chemical substances which includes mostly phenolics, phytoalexins and products on their oxidation. One of the major biological properties of phenolic compounds is their antimicrobial activity and their main role in plants is to act as protective compounds against disease causing agents such as fungi, bacteria and viruses. High concentration of phenols (toxic level) causes an instant lethal action by general tanning effect (Dasgupta, 1988) [2]. Study was undertaken to analyse the levels of total phenols in different okra genotypes before and after powdery mildew infection in the leaves. Results obtained are presented in Table 1 and Figure 1. In the present study there was significant increase in total phenol content from the onset of infection up to the disease development. The total phenol content increased in leaves after infection in all the genotypes. It was noticed that, moderately resistant genotype had high phenol content compared to moderately susceptible, susceptible and highly susceptible genotypes.

Healthy leaves of highly susceptible genotypes (Arka Anamika and IC42531) have recorded 6.4, 6.1 mg g^{-1} of total phenol which increased to 9.95, 8.63 mg g^{-1} after infection by *E. cichoracearum*. Whereas, susceptible genotypes *i.e*., IC42524 and IC42535 have recorded 6.9 and 7.1 mg g^{-1} of total phenols respectively, before infection that increased to 10.63 and 10.95 mg g^{-1}, respectively in the diseased leaves. Moderately susceptible genotypes *i.e*., EC329405, EC329399 have recorded 8.1 and 8.3 mg $g^{-1}$of total phenol before infection and it increased to 13.33 and 12.95 mg g^{-1} after infection by the pathogen (Fig. 1). The increase in phenol content was at higher rate in moderately resistant genotype *i.e*., EC329404; before infection phenol content recorded was 9.7 mg g^{-1} and it increased to 16.29 mg g^{-1} after infection by *E. cichoracearum*. It was noticed that phenol content was directly proportional to resistance to the disease.

The present findings are in accordance with the observations of Dinesh (2009) [5], who found that, initially healthy leaves of susceptible variety of sunflower (Morden) had 1.204 mg g^{-1} of total phenols and it increased to 3.980 mg g^{-1} after infection by *E. cichoracearum*. Tirupathiswamy *et al.* (2017) [17] conducted biochemical analysis using black gram powdery mildew infected leaves of LBG 17 (resistant inbred), Nethiminumu and Chikkuduminumu (Susceptible parents). The total phenol content was estimated by Folin-ciocalteau reagent method and it was noticed that the highest phenol content was observed in LBG 17 (3.68 mg/g) followed by Chikkuduminumu (2.77 mg/g) and Nethiminumu (2.68 mg/g). Waghmare and Nasreen (2018) based on field screening results selected one each of powdery mildew resistant (BDU-1, LGG and JP-71) and susceptible cultivars (TAU-1, K-851 and Arkel) of black gram, green gram and pea and subjected for biochemical analysis. Both healthy and infected leaves of respective genotypes were analysed and found that, irrespective of genotypes analysed, phenol content was higher in infected leaves than in the healthy leaves. The phenol content in the susceptible cultivars *i.e*., TAU-1, K-851 and Arkel before infection was 4.97, 4.25 and 5.69 mg/g while it was increased to 7.42, 6.68 and 7.56 mg/g in the powdery mildew infected leaves, whereas in resistant cultivars *i.e*., BDU-1, LGG and JP-71 phenol content was 9.23, 9.15 and 7.57 mg/g in healthy leaves that increased to 15.59, 17.02 and 15.26 mg/g in the infected leaves.

Table 1: Influence of biochemical composition on resistance and susceptibility of okra genotypes against powdery mildew disease

Sl. No.	Genotype/variety	PDI (%)	Reducing sugar (mg g of fresh wt^{-1})		Non-reducing sugar (mg g of fresh wt^{-1})		Total sugar (mg g of fresh wt^{-1})		Total Phenol (mg g of fresh wt^{-1})	
			H	I	H	I	H	I	H	I
1	EC329404	24.62	7.80	10.16	4.65	9.24	12.45	19.40	9.7	16.29
2	EC329405	45.94	9.68	12.28	6.71	11.62	16.39	23.90	8.1	13.33
3	EC329399	35.95	9.20	12.90	7.47	12.49	16.67	25.39	8.3	12.95
4	IC42524	65.94	11.58	14.45	8.52	13.87	20.10	28.32	6.9	10.63
5	IC42535	61.98	10.20	15.23	8.43	14.16	18.63	29.39	7.1	10.95

6	IC42531	75.93	12.89	15.47	9.02	19.10	21.91	34.57	6.1	8.63
7	Arka anamika	76.60	12.71	16.65	9.62	20.12	22.33	36.77	6.4	9.95
	Mean	-	9.71	14.02	7.16	14.34	16.87	28.36	7.51	11.53
	S. Em. (±)		0.53	0.40	0.37	0.34	0.64	0.58	0.33	0.52
	C.D. at 1%		1.85	1.40	1.32	1.22	2.30	2.14	1.12	1.92

*H – Healthy, I – Infected, PDI - Per cent disease index

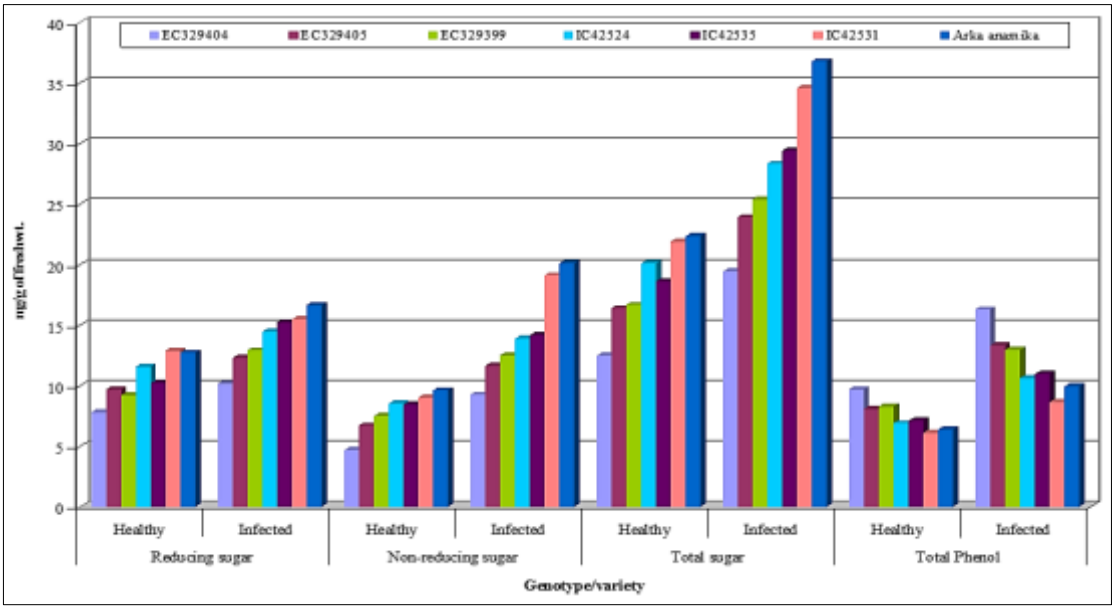

Fig 1: Influence of biochemical composition on resistance and susceptibility of okra genotypes against powdery mildew disease

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