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KP Manju
Department of Entomology,
Krishi Vigyan Kendra, Kannur,
Kerala Agricultural University,
Kerala, India

K Vijaya Lakshmi
Department of Entomology,
PJTSAU, Hyderabad,
Telangana, India

B Sarath Babu
NBPGR, Regional Station,
Rajendranagar, Telangana, India

K Anitha
NBPGR, Regional Station,
Rajendranagar, Telangana, India

Corresponding Author:
KP Manju
Department of Entomology,
Krishi Vigyan Kendra, Kannur,
Kerala Agricultural University,
Kerala, India

Morphological and biochemical basis of resistance in okra to whitefly, *Bemisia tabaci* and okra yellow vein mosaic virus (OYVMV)

KP Manju, K Vijaya Lakshmi, B Sarath Babu and K Anitha

Abstract
Field screening studies were conducted with 25 okra germplasm accessions during the summer season of 2015 and 2016 at the National Bureau of Plant Genetic Resources (NBPGR) Regional Station, Rajendranagar, Hyderabad. The morphological and biochemical parameters analyzed in the selected okra germplasm lines indicated that among the morphological parameters the germplasm lines possessing high trichome density, less leaf area and dark green leaf colour and biochemical parameters like low nitrogen, protein, less total and reducing sugars and high phenol content offered resistance mechanism against the vector as well as the virus. The three immune okra accessions *viz.,* RJR-124, PSRJ-12952 and IC344598 possessing the above characters were completely free from OYVMV, while the highly susceptible accessions PSRJ-13040 and RJR-193 and the susceptible check Pusa Sawani possessing less trichome density, more leaf area, high sugars, nitrogen content and low phenol content were highly preferred by the vector and were highly susceptible to OYVMV. Total sugars, reducing sugars, nitrogen and protein content had a positive correlation with the whitefly population and OYVMV incidence. The multiple linear regression analysis revealed that the morphological and biochemical parameters together explained the variation in whitefly population and subsequent OYVMV incidence to an extent of 99.00 per cent.

Keywords: okra germplasm, whitefly, OYVMV, morphological parameters, biochemical parameters

Introduction

Okra (*Abelmoschus esculentus* L.), commonly known as bhendi, belongs to the family Malvaceae, is one of the important vegetable crops cultivated throughout India. The production and quality of okra fruits are affected by an array of sucking and fruit boring pests from sowing until harvest. The key sucking pests of okra are whiteflies, aphids, jassids, thrips and mites. Among the sucking pests, whitefly, *Bemisia tabaci* Gennadius causes economic damage to okra by feeding on phloem sap, there by contaminating leaves and fruits with honey dew that causes sooty mould formation [23]. Besides causing direct damage, it also transmits an economically important viral disease caused by *Okra yellow vein mosaic virus* (OYVMV), resulting in significant yield losses especially when it occurs in the early stages of crop growth. As compared to healthy plants, diseased plants showed a reduction of 24.9% in plant height, 15.5% decrease in root length, and 32.1% in number of fruits per plant, whereas stem girth was reduced by 16.3% [34].

OYVMV belongs to the genus *Begomovirus* of the family Geminiviridae. Geminiviruses make up a large diverse family of plant viruses and cause heavy crop losses worldwide. A roving survey conducted in the okra growing areas of Karnataka, Andhra Pradesh, Tamil Nadu, Kerala, Maharashtra, Haryana, Uttar Pradesh, Delhi, Chandigarh and Rajasthan revealed that the disease incidence caused by OYVMV ranged from 23.00 to 67.78 per cent. [43]. The disease is characterized by different degrees of chlorosis and yellowing of veins and veinlets, smaller leaves, fewer and smaller fruits and stunting [44].

Efforts were made by number of scientists for identification of resistant sources against OYVMV [12, 31]. Several OYVMV resistant okra varieties have been released, but none of them had retained resistance for long**.** Besides this, the rainy season crop in the subtropical regions and spring summer crop in tropical regions of India is highly vulnerable to the attack of OYVMV disease. The disease cannot be controlled satisfactorily by anti-viral chemicals and insecticide applications. Therefore, the ideal way of controlling this viral disease in okra would be to develop resistant cultivars against the virus as well as the vector. Therefore, the present study was undertaken to screen and analyze okra accessions to identify resistant sources against whitefly vector and YVMV disease incidence.

Methodology
For assessing the various factors responsible for resistance or susceptibility of okra germplasm against whitefly and OYVMV, okra germplasm lines were selected based on the disease score, and the following morphological and biochemical parameters were analyzed.

Morphological Parameters of the Okra Germplasm
The following morphological parameters were recorded in the selected okra germplasm lines.

Estimation of Trichome Density
Light Microscopy
The trichome density of the 25 okra germplasm lines was assessed by counting the number of trichomes in a 1 cm^2 round disc cut from the distal part of the lower side of the fully opened leaf of the plant. Three discs from each of five plants (standardized stage) from each replication of the treatment were examined. The total number of trichomes were counted under a binocular stereomicroscope at 40x magnification and expressed as trichomes per mm^2 area [13].

Scanning Electron Microscopy
Trichome density of the 6 selected okra germplasm lines based on their reaction to BYVMV was estimated using Scanning Electron Microscope (SEM) at Ruska laboratories, Rajendranagar, Hyderabad. Fresh leaf pieces (10×10 mm^2) were immersed in a fixation solution of 2.5 % glutaraldehyde in 0.1 m phosphate buffer for 24 h at 4 ºC and post fixed in 2 % aqueous osmium tetroxide for 4 h. Samples were washed for 15–30 min with the buffer and dehydrated using the following ethanol concentrations: 25 and 50 % for 5 min; 70, 80, 95 and twice 99 % for 10 min and dried to critical point drying with critical point dryer (CPD) unit. The processed samples were mounted over the aluminium stubs with double sided carbon conductivity tape and a thin layer of the gold coating was done over the samples by using an automated sputter coater (Model- JEOL JFC-1600) for 3 min and scanned under SEM (Model: JOEL-JSM 5600) at 75x,150x, and 200x magnification [42].

Plant Height
The plant height was taken at 50% flowering stage in five plants of each replication and expressed as cm.

Leaf Area
The leaf area was measured by recording the length and width at 50% flowering stage in five plants of each replication and expressed as cm^2.

Leaf Colour
Leaf colour of the germplasm lines was recorded based on visual observations at 50 % flowering stage in five plants of each replication using the leaf colour chart.

Biochemical Parameters
To find out the role of biochemical parameters in offering resistance/susceptibility against the vector and the virus, the following biochemical parameters were analyzed in selected okra germplasm lines at vegetative stage of the crop.

Estimation of Total Sugars, Reducing Sugars and Non-Reducing Sugars
The total sugars, reducing sugars and non- reducing sugars were estimated by following Somogyi's modification of Nelson's method [30].

Estimation of Total Phenols
The phenol content was estimated in the okra leaves by following the method described by [30].

Estimation of Nitrogen and Total Proteins
The nitrogen content in the okra leaves was determined by using the Kjeldahl method [30]. The total protein content was estimated by multiplying the nitrogen content in the okra leaves obtained through the Kjeldahl method with the nitrogen to protein conversion factor (6.25) as suggested by Sadashivan and Manickam, (1992). The crude protein (CP) content was calculated by using the following formula

CP (DM basis) = % N (DM basis) × F, Where F = 6.25

Statistical Analysis
Data was collected from different okra germplasm on the population counts of whitefly, percent disease incidence (PDI) of OYVMV, morphological parameters, and biochemical parameters. Later it was analyzed statistically by using the factorial RBD concept. To find out the influence of morphological and biochemical parameters on pest and disease occurrence in different germplasm lines, simple correlation coefficients were worked out between the incidence of whitefly and OYVMV with morphological and biochemical parameters. Step down multiple linear regression analysis was carried out for whitefly population and OYVMV with various morphological and biochemical parameters to find out the combined effect of these parameters on whitefly and OYVMV incidence [25, 22].

Results and Discussion
Morphological Parameters of the Okra Germplasm
To find out the role of morphological factors of okra germplasm in offering resistance/susceptibility against the vector and the virus, the following morphological parameters were assessed in okra crop. The morphological parameters assessed in the study include trichome density, leaf area, leaf colour and plant height.

Estimation of Trichome Density
Trichome density of 25 okra germplasm lines was observed under stereo binocular microscope whereas that of selected germplasm lines based on their resistance or susceptibility reaction to the vector and virus was observed under the scanning electron microscope (SEM) and expressed as trichomes per mm^2 area.

The data about the leaf trichome density of 25 germplasm lines observed under stereo binocular microscope are presented in Table 1. The observations revealed that the mean trichome density varied significantly among all the accessions and ranged from 10.33 per mm^2 (RJR-193) to 57.00 per mm^2 (RJR-124). Significantly highest trichome density of 57.00 per mm^2 was observed in the immune germplasm line RJR-124 whereas the significantly lowest trichome density was observed in highly susceptible germplasm lines RJR-193 (10.33/mm^2) and PSRJ-13040 (13.00/mm^2). In general the immune and highly resistant germplasm lines recorded high density of trichomes than the susceptible and highly susceptible germplasm lines indicating that, greater the trichome density or leaf pubescence, lower the whitefly population and BYVMV incidence.

The trichome density of selected 6 okra germplasm lines *viz.*, RJR-124, PSRJ-12952 (Immune), 1C-433597 (Highly resistant), NSJ-401 (Tolerant), RJR-193 and PSRJ 13040 (Highly susceptible) were observed under the scanning electron microscope (Figure 1 and 2). The results indicated that, among the six okra germplasm lines, the immune okra accession RJR-124 (80/mm^2) recorded the highest trichome density. The other immune accession PSRJ-12952 and tolerant accession NSJ-401 recorded a trichome density of 30 per mm^2. The highly susceptible germplasm lines *viz.*, RJR-193 (20/mm^2) and PSRJ-13040 (10/mm^2) and highly resistant accession 1C-433597 (20/mm^2) recorded the lowest trichome density.

The correlation studies (Table 2) indicated that trichome density had a highly significant negative correlation (r= -0.76) with the population of whiteflies and the subsequent OYVMV incidence (r= -0.62). The population of whiteflies decreased with an increase in the trichome density resulted in less transmission and subsequent OYVMV disease incidence in low proportion.

From the results it was evident that among the two OYVMV Immune germplasm lines, one accession RJR-124 recorded the highest trichome density of 80 per mm^2 whereas the other immune germplasm PSRJ-12952 recorded only 30 trichomes per mm^2 while the tolerant NSJ-401 also recorded a trichome density of 30 per mm^2. The two highly susceptible accessions RJR-193 (20/mm^2) and PSRJ 13040 (10/mm^2) supported with very less trichome density. Hence it can be inferred from the results that in general the trichome density adversely affects whitefly population density and subsequent OYVMV incidence - except for PSRJ-12952. PSRJ-12952 supported low whitefly population and showed immune reaction to OYVMV despite having low trichome density. Other morphological and biochemical parameters might have played a significant role in the immune reaction of PSRJ-12952.

It was evident from the results that the okra germplasm lines with high trichome density harboured fewer number of whiteflies thus less OYVMV incidence. The present findings are in close agreement with the findings of [39] who reported that trichome density was negatively correlated with whitefly, *B. tabaci* eggs, nymphs and adults in blackgram. Similarly, [22] also evaluated the attractiveness and ovipositional preference of *B. tabaci* for 17 tomato genotypes. The results indicated that the glandular trichome density was negatively correlated with whiteflies attractiveness and oviposition. Studies conducted on brinjal also revealed a negative correlation between leaf trichome density and resistance to *B. tabaci* [37, 36, 8]. Leaf trichome density is a defensive character that prevents the infestation of whiteflies by deterring or limiting their establishment [29] and thus making the locomotion, feeding and oviposition difficult [21, 16] also reported the non-occurrence of oviposition by *B. tabaci* on the apical leaves of eggplant having abundant trichomes density.

However, the results of the present investigation are in contrary with the results of [38] who reported a linear relationship between trichome density and oviposition preference by whitefly, *B. tabaci* in tomato. [17] also noted that cultivars with low trichome density sustained less whitefly oviposition than cultivars with high trichome densities. The trichome density in cotton was found to be positively correlated with whitefly population by several authors [10, 20, 6, 7]. They revealed that the whitefly population on cotton had a positive and significant correlation with the trichome density.

The differential preference of whitefly to trichome density could be due to variation in the chemical constituents of trichomes or the host plant.

Plant Height

The plant height recorded in the different germplasm lines indicated that it ranged from 38.33 (IC-90402) cm to 92.67cm (RJR-279). It was observed that in general the wild accessions exhibited lesser plant height ranging from 38.33 to 60 cm compared to the cultivated accessions which recorded a plant height ranging from 59.33 to 92.67 cm (Table 1).

Out of 25 germplasm lines, the maximum plant height of 92.67cm cm was recorded by RJR-279 followed by RJR-405 (84.00 cm) and RJR-193 (82.60 cm). These accessions *viz.*, RJR-279, RJR-405 and RJR-193 recorded a high whitefly population and exhibited moderately susceptible to highly susceptible reaction to OYVMV. However, the susceptible check Pusa Sawani and moderately susceptible germplasm RJR-110 in spite of possessing less plant height of 56.67cm and 59.67 cm, respectively showed a susceptibility reaction. Similarly some of the wild accessions *viz.*, IC-344598 and IC141020 which possessed plant height of 60 cm were either immune or highly resistant to pest and disease incidence.

From the correlation data, (Table 2) it was observed that the plant height had a non-significant positive influence on whitefly population (r=0.35) and OYVMV (r=0.47) incidence.

From the present study it was evident that plant height did not have any significant influence on the whitefly population build up and OYVMV incidence. The results are also in agreement with the findings of (28) who advocated that plant height did not affect adult and nymph populations of whitefly in cotton. The results of the present investigation are contradictory with the findings of (14) who reported that plant height had a negative correlation with whitefly adult and nymph populations in cotton.

Leaf Area

The leaf area of 25 germplasm lines was measured at 50% flowering stage (Table 1). From the data it was observed that the highest leaf area was recorded in highly susceptible germplasm PSRJ-13040 (86.19 cm^2) followed by RJR-193 (82.18 cm^2) and significantly less leaf area was recorded from the immune germplasm IC-344598 (44.76 cm^2) followed by IC-433667 (48.15 cm^2), RJR-124 (48.17 cm^2) and IC-433597 (49.51 cm^2). The resistant check (Parbhani Kranti) recorded 67.50 cm^2 leaf area while the susceptible check (Pusa Sawani) recorded a leaf area of 82.50 cm^2.

The correlation studies (Table 2) revealed that leaf area had significant positive correlation with whitefly population (r= -0.64) and OYVMV (r= -0.78) incidence. The okra germplasm with more leaf area recorded a high whitefly population and OYVMV incidence.

From the results it can be inferred that the leaf area had a significant role in influencing the resistance or susceptibility of okra germplasm against whitefly population and subsequent OYVMV infestation among the different okra germplasm lines. It was observed that the germplasm lines, IC-344598, IC-433667, RJR-124 and IC-433597 which recorded less leaf area ranging from 44.76 to 49.51 cm^2 recorded less whitefly population and low OYVMV incidence than the highly susceptible germplasm lines, Pusa Sawani, PSRJ-13040 and RJR-193 which recorded the highest leaf area of 82.50 cm^2, 82.18 cm^2 and 86.19 cm^2, respectively. The reason for high susceptibility of germplasm lines possessing more leaf area may be due to the availability of a larger leaf area for oviposition and feeding by the whitefly. The results of the present investigation are in agreement with the finding of [39] who reported that leaf area influences the egg laying and subsequent population build up of whitefly nymps and adults.

Leaf Colour

The leaf colour of the 25 germplasm lines was recorded based on visual observations by using a leaf colour chart at 50 % flowering stage. The leaf colour of the 25 germplasm lines ranged between slightly light green and dark green (Table 1).

Among all the germplasm lines, three immune accessions *viz.*, IC-344598, PSRJ-12952 and RJR-124 which recorded the lowest whitefly population and OYVMV incidence exhibited dark green (DG) leaf colour. Whereas the two highly susceptible germplasm lines *viz.*, RJR-193 and PSRJ-13040 which recorded the highest population of whitefly and OYVMV incidence exhibited a slightly light green leaf colour. In the rest of the germplasm lines, the leaf colour ranged from slightly light green (SLG) to light green (LG). The results indicated that significantly highest whitefly population and OYVMV incidence was recorded in the germplasm lines possessing slightly light green to light green.

The results of the present investigation revealed that dark green leaf colour had a negative influence on whitefly population and subsequent OYVMV incidence. Moreover, green color intensity was negatively correlated with the whitefly adults indicating that higher the bright green color, the lower the number of adult whiteflies and less OYVMV incidence. The results obtained from the present investigation are in agreement with findings of (1) who observed a significant and negative correlation between the green leaf colour with the whitefly adult population and oviposition in brinjal.

Based on the results obtained from the study, it was clear that morphological parameters significantly influenced the resistance or susceptibility against the vector and virus. Among the four leaf characters studied, leaf area, leaf colour and trichome density played a significant influence on vector and virus incidence. The leaf area had a positive effect on population build up of whiteflies and OYVMV incidence while the okra germplasm lines possessing dark green colour and high trichome density had a negative effect on the vector as well as virus incidence. Therefore the germplasm lines RJR-124 and PSRJ-12952 possessing the dark green leaves with less leaf area and more trichome density can be used in plant resistance breeding programme.

Estimation of Biochemical Parameters of Okra Germplasm

The biochemical components such as total phenols, total sugars, reducing sugars, non- reducing sugars, nitrogen and crude protein content were estimated in the selected germplasm lines by using standard protocols at vegetative stage of the crop. A total of eight okra germplasm lines *viz.*, three immunes (IC-344598, PSRJ-12952, RJR-124), three highly resistant (IC-433597, IC-141020, NIC-9402) and two highly susceptible accessions (PSRJ 13040 and RJR-193) were selected to find out the relation of these parameters with whitefly and OYVMV resistance or susceptibility.

Estimation of Total Sugars, Reducing Sugars and Non-Reducing sugars

The total sugars, reducing sugars and non- reducing sugars were estimated in eight selected okra germplasm lines and expressed in mg g^{-1} leaf (Table 3).

Among the eight germplasm lines, RJR-193 and PSRJ-13040, which were highly susceptible to whitefly and OYVMV recorded more total sugars of 2.93 and 2.48 mg g^{-1}, respectively. The germplasm lines *viz.*, IC-344598, PSRJ-12952 and RJR-124 which were proved to be immune to the disease incidence recorded low sugars of 1.68, 0.58, 1.47 mg g^{-1}, respectively. The remaining three highly resistant germplasms *viz.*, IC-141020 (2.05 mg/g), IC-433597 (2.09 mg/g) and NIC-9402 (0.55 mg/g) also recorded low sugars.

Out of eight selected okra germplasm lines, highest reducing and non-reducing sugars were recorded from the highly susceptible germplasm lines, RJR-193 (2.93, 0.69) and PSRJ 13040 (2.48, 0.62). The three immune germplasm lines *viz.*, IC-344598, PSRJ-12952 and RJR-124, recorded low reducing sugars of 1.43, 0.96 and 0.97 mg g^{-1} and non- reducing sugars of 0.25, 0.38 and 0.50 mg g^{-1}, respectively. The remaining three highly resistant germplasm lines *viz.*, IC-141020 (1.53, 0.52 mg/g), IC-433597 (2.07, 0.02 mg/g) and NIC-9402 (0.24, 0.31 mg/g) also contained low reducing and non-reducing sugars when compared to the highly susceptible germplasm lines.

The correlation studies (Table 4) revealed that highly significant and positive correlation was observed between the total and reducing sugars with whitefly population (r= 0.82, 0.79) and OYVMV (r= 0.84, 0.78) incidence in different genotypes. No significant correlation was obtained between non-reducing sugars and whitefly population and OYVMV incidence. The okra germplasm lines with high sugars recorded high whitefly population and more OYVMV incidence. It was observed that there was an increase in the whitefly population and OYVMV incidence with the increase in sugars in the leaves of the germplasm lines.

The present results clearly indicated that the population of whiteflies was high in germplasm lines containing high total and reducing sugars and *vice versa*. Since sugars are considered as one of the vital nutrients in plants, the difference in the relative amount of sugars between different germplasm lines with differential susceptibilities to whitefly indicated that these compounds might act as phago-stimulants to whitefly feeding on okra. The present findings are in agreement with findings of [9] who reported that leaves of susceptible and highly susceptible cultivars of okra to OYVMV showed higher content of reducing, non-reducing and total sugars than resistant ones. [40] reported that the leaves, stem tips, squares and boll rind of cotton susceptible genotypes to whitefly invariably have higher levels of sugars, where as the resistant genotypes have comparatively lower levels of sugars. [41] reported low content of polysaccharides in all the tissues of resistant genotypes to whiteflies when compared to the susceptible genotypes in cotton.

Estimation of Total Phenols

The total phenols estimated in the leaves of selected germplasm lines of okra showed significant differences among the germplasm lines (Table 3). The total phenols in the immune gremplasm lines *viz.*, RJR-124, RJR-12952 and IC-344598 ranged from 221.4 mg/100g to 262.4 mg/100g, while it ranged between 146.4 mg/100g and 164.5 mg /100g, in highly susceptible okra germplasm lines, PSRJ-13040 and RJR- 193. Out of 8 okra germplasm lines, significantly highest phenol content of 262.4 mg/100g sample was recorded in immune wild accession IC-344598 followed by immune cultivated accession PSRJ-12952 (243.2 mg/100g) and RJR-124 (221.4 mg/100g). The germplasm lines *viz.*, IC-141020, IC-433597 and NIC-9402 which were highly resistant to OYVMV possessed the phenol contents of 201.5, 183.5 and 221.0 mg $100g^{-1}$, respectively. The susceptible genotypes RJR-193 (164.5 mg/100g) and PSRJ-13040 (146.4 mg/100g) recorded less phenol content.

The correlation coefficients computed for total phenols with whitefly population and OYVMV incidence of selected germplasm lines revealed that there was a significant negative correlation between total phenol content with whitefly population (r= -0.81) and OYVMV (-0.90) incidence in different okra germplasm lines (Table 4). The okra germplasm lines with high phenol content recorded low whitefly population and OYVMV incidence. It was observed that there was a decrease in the whitefly population and OYVMV incidence with the increase of phenol content in different germplasm lines.

From the present study it was evident that the immune germplasm lines *viz.*, IC-344598, PSRJ-12952 and RJR-124 had higher phenol content than the other accessions. 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 [11]. Similar results were observed by [5] and [32] opined that the levels of total phenols were higher in resistant cultivars than susceptible cultivars before and after the appearance of OYVMV. The present results are in close agreement with [27] who reported that phenolics in a large concentration could ward off insect pests because of direct toxicity. [24] also noted that whitefly resistant variety showed 1 to 1.2 mg more phenolics than susceptible varieties at all growth stages in cotton. Similarly, [26] also reported that total phenols, ortho dihydroxy phenols, tannins and gossypol showed significant negative relation with egg, nymph and adult whitefly population in upland cotton. The present findings are in confirmity with the results of [3, 4] who revealed the occurrence of more phenols coupled with high peroxidase and polyphenol oxidase in the whitefly resistant lines of cotton. It was also observed that total phenols, polyphenol oxidase and peroxidase activity in the healthy plants were significantly higher in the resistant wild species and their inter-specific hybrids as compared to susceptible cultivated *A. esculentus* cultivars. Similar results were also obtained by [33, 15, 35, 2] whose findings revealed that the amount of total phenols was negatively correlated with the population density of whiteflies. [25] also revealed that wild parents resistant to OYVMV had maximum phenolics, peroxidase, and polyphenol oxidase activity. [18] found that the okra germplasms which were highly resistant in their reaction to OYVMV had higher content of phenols, ortho-dihydroxyphenols and flavonols.

Estimation of Nitrogen Content

The results obtained from the estimation of nitrogen content from the leaves of 8 selected okra germplasm lines indicated that susceptible okra germplasm lines possessed high nitrogen content compared to resistant gemplasm lines (Table 3). The total nitrogen content ranged from 1.39 mg/ g fresh wt of leaf (RJR-124) to 2.42 mg/ g fresh wt of leaf (RJR-193) in selected okra germplasm.

Among all the selected germplasm lines, immune germplasm lines which supported lowest whitefly and OYVMV incidence *viz.* IC-344598 (1.64 mg/g), PSRJ-12952 (1.47 mg/g) and RJR-124 (1.39 mg/g) recorded the lowest amount of total nitrogen. Whereas the highly susceptible germplasm lines *viz.*, RJR-193 and PSRJ 13040 which recorded the highest amount of total nitrogen of 2.42 and 2.24 mg/g sample, respectively had highest incidence of whitefly and OYVMV. The germplasm lines *viz.*, IC-141020, IC-43359 and NIC-9402 which were proved as highly resistant to OYVMV also recorded low nitrogen contents of 1.65, 1.93, 1.90 mg g^{-1}, respectively

The correlation studies (Table 4) revealed a highly significant and positive correlation between the amount of total nitrogen with whitefly population (r= 0.74) and OYVMV (r= 0.77) incidence in different germplasm lines. The okra germplasm lines with high nitrogen content recorded high whitefly population and OYVMV incidence.

The results revealed that the nitrogen content was relatively lower in immune germplasm lines *viz.*, IC-344598, PSRJ-12952 and RJR-124 compared to the highly susceptible germplasm lines, RJR-193 and PSRJ 13040. Similar to the present findings low nitrogen content in the host plant has been associated with resitance in *Gossypium* sp to *B. tabaci* [45, 19] explained that under high nitrogen supply to plant, phenolic compounds and lignin contents are reduced, which play a key role in resistance to pest attack. An excess of N and K deficiency have been reported to lead to higher accumulation of amino acids in plants and a higher degree of insect attack, such as whitefly. The present results are also in agreement with the findings of [25] who revealed that the total nitrogen content was found to be in a minimum concentration in resistant wild parents resistant to OYVMV and maximum in cultivated okra and intermediate in case of inter-specific hybrids.

Estimation of Total Proteins

Among the 8 germplasm lines, RJR-193 and PSRJ-13040 which were highly susceptible to whitefly and OYVMV recorded more protein content of 15.12 and 14.00 mg /g sample, respectively and the immune germplasm lines *viz.*, IC-344598 (10.25 mg /g), PSRJ-12952 (9.19 mg /g) and RJR-124 (8.68 mg /g) recorded low protein content (Table 3). The remaining three germplasm IC-141020, IC-433597 and NIC-9402 which were proved as highly resistant to OYVMV and whitefly possessed low protein content of 10.27, 12.07 and 11.87 mg g^{-1}, respectively.

The correlation studies (Table 4) revealed that there was a highly significant and positive correlation between the amount of total protein and whitefly (r= 0.74) and OYVMV (r= 0.78) incidence in different germplasm lines. It was observed that there is an increase in the whitefly population and OYVMV incidence with the increase of protein content in the different germplasm lines.

The present results revealed that okra germplasm lines, RJR-193 and PSRJ 13040 possessing high protein content supported high whitefly population and subsequent high incidence of OYVMV whereas the immune okra gemplasm accessions *viz.*, IC-344598, PSRJ-12952 and RJR-124 recorded low protein content. These findings are in agreement with findings of [25] who found that wild parents resistant to OYVMV had minimum nitrogen and protein contents.

From the present investigation, it can be concluded that the immune okra gemplasm accessions *viz.*, IC-344598, PSRJ-12952 and RJR-124 recorded highest phenol content and low total sugars, reducing sugars, non-reducing sugars, nitrogen and protein contents while in highly susceptible accessions, RJR-193 and PSRJ-13040 the composition of chemical components showed the reverse trend. This information could be useful in developing the resistant varieties against the whitefly vector and OYVMV either by following conventional breeding or by biotechnological tools.

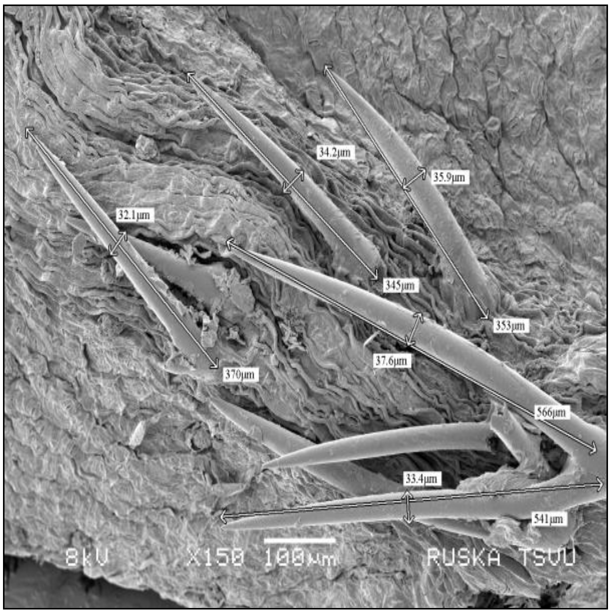
Relation of Morphological and Biochemical Factors on Whitefly and OYVMV Incidence

The step down multiple linear regression analysis studies done with morphological and biochemical parameters (Table 5) could explain the combined influence of both the parameters to an extent of 99.00 per cent for both the whitefly vector and OYVMV incidence. Thus the results showed that both morphological as well as biochemical parameters significantly influenced the build up of whitefly population as well as subsequent OYVMV incidence on okra crop.

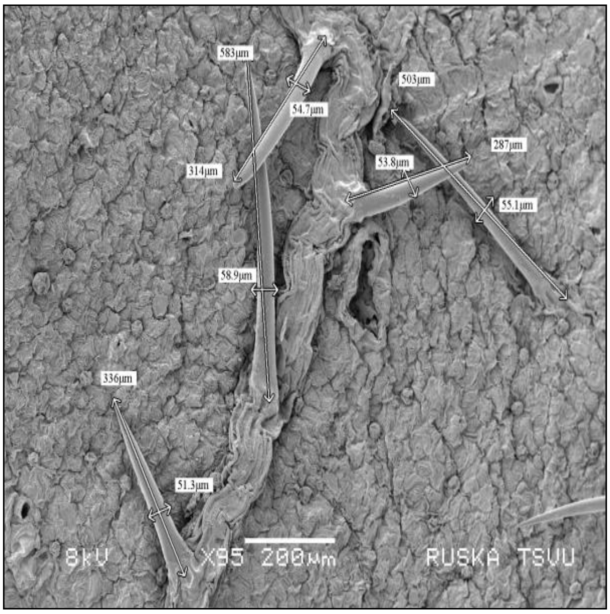
In India okra crop is highly susceptible to OYVMV disease probably due to warm tropical climate and intensive crop cultivation which supports the survival of whitefly population round the year. Host plant resistance to virus is one of the most practical, economical and environmental friendly strategies for reducing yield loss in okra. In the present study two of the cultivated accessions *viz.*, RJR-124 and PSRJ-12952 showed immune reaction to OYVMV during both the seasons and they possessed good yield attributes and recorded significantly high yield. The morphological and biochemical bases of resistance studies undertaken in the germplasm lines demonstrated the role of trichomes, leaf area, phenols, total sugars, nitrogen and protein in offering resistance or susceptibility of okra germplasm against the vector and virus. Since it is very difficult to transfer the genes of desirable traits of wild accessions in the high yielding varieties, the promising cultivated germplasm lines identified in the present study can be efficiently utilized in the breeding programme. Moreover the occurrence of OYVMV is severe in certain locations in certain seasons, like in summer. So the screening studies need to be done in these hot spot areas. Similarly attempts should be made to incorporate broad spectrum resistance through gene pyramiding and develop okra varieties with desirable resistance or tolerance to OYVMV followed by maintenance breeding. To confirm the immune reaction, the two immune cultivated germplasm lines identified in the present study need to be screened in the hot spot areas and greenhouse conditions by subjecting them to artificial infestation with vector population to confirm the immune reaction of this germplasm before using in the breeding programme.

Table 1: Morphological attributes of okra germplasm recorded during summer, 2015 and 2016 (Pooled data)

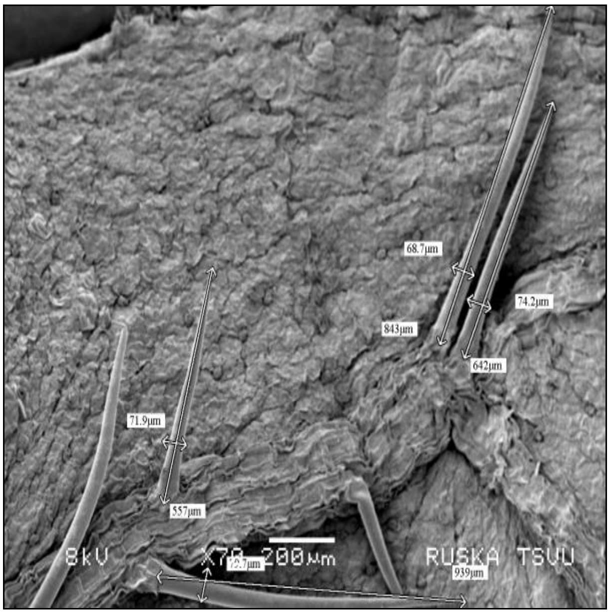
Okra germplasm		Trichome density/mm^2	Plant height (cm)	Leaf area (cm^2)	Leaf colour
Wild accessions	IC-141020	39.33	60.00	52.40	LG*
	IC-433597	31.33	56.00	49.51	LG
	EC-305672	33.67	57.00	60.16	LG
	IC-433438	34.67	42.00	50.14	LG
	IC-433667	40.00	40.00	48.15	SLG**
	EC-305619	32.00	51.00	70.14	SLG
	EC-305736	26.33	54.00	64.19	DG***
	NIC-9402	28.67	39.00	52.17	DG
	IC-344598	42.33	60.00	44.76	DG
	IC-90402	40.33	38.33	55.18	LG
Cultivated accessions	PSRJ-13040	13.00	79.00	86.19	SLG
	PSRJ-12952	47.67	67.33	50.17	DG
	RJR-265	29.00	79.67	76.53	SLG
	RJR-193	10.33	82.60	82.18	SLG
	RJR-124	57.00	62.00	48.17	DG
	RJR-479	35.33	81.33	70.52	LG
	RJR-279	28.00	92.67	81.57	LG
	RJR-405	24.00	84.00	79.53	SLG
	RJR-587	24.00	71.33	80.14	SLG
	RJR-670	20.00	70.00	64.17	SLG
	RJR-110	41.33	59.33	70.15	LG
	RJR-45	42.67	71.33	65.34	LG
	NSJ-401	40.00	72.67	61.81	LG
Susceptible check	Pusa Sawani	36.00	56.67	82.50	LG
Resistant check	Parbhani Kranti	37.67	77.33	67.50	DG
	S. Em.±	1.25	1.15	1.32	-
	C.D. at 5%	3.55	3.27	3.86	-



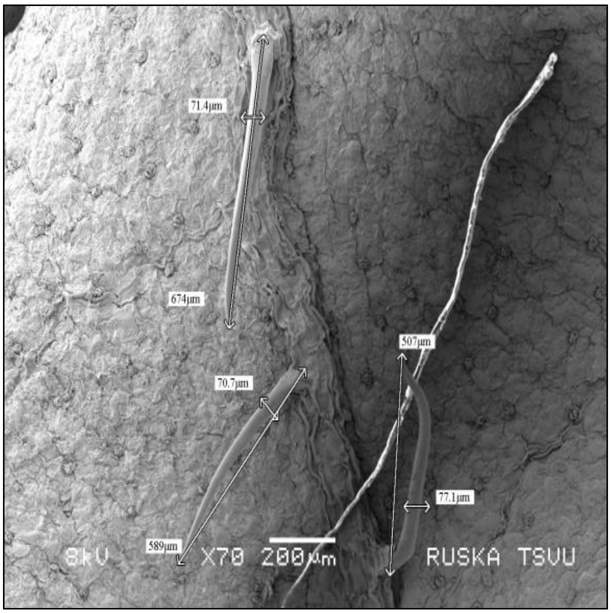
RJR-124 - Immune (80 trichomes/mm^2)



PSRJ-12952 - Immune (30 trichomes/mm^2)



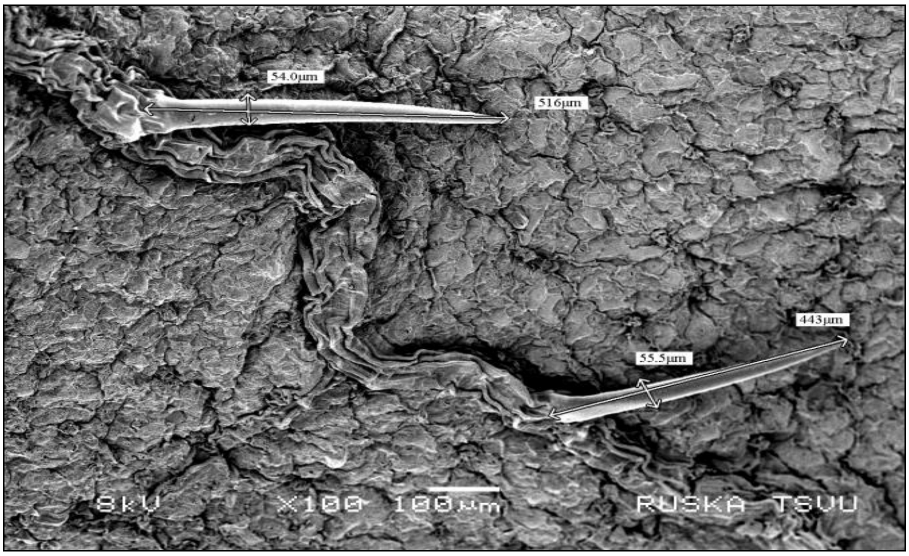
NSJ-401 - Tolerant (30 trichomes/mm^2)



IC-433597- Highly resistant (15 trichomes/mm^2)

Fig 1: Scanning electron microscope (SEM) images depicting trichome density in immune, tolerant and highly resistant okra germplasm lines

PSRJ-13040- Highly susceptible (10 trichomes/mm^2)



RJR-193- Highly susceptible (20 trichomes/mm^2)

Fig 2: Scanning electron microscope (SEM) images depicting trichome density in highly susceptible okra germplasm lines

Table 2: Correlation coefficients of *B. tabaci* population and OYVMV incidence with morphological and yield parameters in okra

Morphological and yield parameters	Correlation coefficients (r)	
	B. tabaci	**OYVMV**
Trichome density	-0.76**	-0.62**
Plant height	0.35	0.47
Leaf area	0.64**	0.78**
Yield	-0.85**	-0.89**

* Significant at 5 % level ** Significant at 1 % level

Table 3: Biochemical parameters of selected okra germplasm screened against *B. tabaci* and OYVMV

Okra germplasm	Total sugars (mg/g)	Reducing sugars (mg/g)	Non- reducing sugars (mg/g)	Total phenols (mg/100g)	Total nitrogen (mg/g)	Total proteins (mg/g)
IC-41020 (HR)	2.05	1.53	0.52	201.5	1.65	10.31
IC-433597 (HR)	2.09	2.07	0.02	183.5	1.93	12.07
NIC-9402 (HR)	0.55	0.24	0.31	221.0	1.90	11.87
IC-344598 (I)	1.68	1.43	0.25	262.4	1.64	10.25
PSRJ-12952 (I)	0.58	0.96	0.38	243.2	1.47	9.19
RJR-124 (I)	1.47	0.97	0.50	221.4	1.39	8.68
PSRJ-13040(HS)	3.10	2.48	0.62	146.4	2.24	14.00
RJR-193 (HS)	3.62	2.93	0.69	164.5	2.42	15.12
S. Em.±	0.12	0.09	0.01	0.11	0.06	0.17
C.D. at 5%	0.28	0.30	0.04	0.34	0.17	0.50

HR- Highly resistant I- Immune HS- Highly susceptible

Table 4: Correlation coefficients of *B. tabaci* population and OYVMV incidence with biochemical parameters of selected okra germplasm

Biochemical parameters	Correlation coefficients (r)	
	B. tabaci	**OYVMV**
Total sugars	0.82*	0.83*
Total reducing sugars	0.79*	0.78*
Total non- reducing sugars	0.47	0.40
Total phenols	-0.81*	-0.90**
Total nitrogen	0.74*	0.77*
Total proteins	0.74*	0.78*

* Significant at 5 % level ** Significant at 1 % level

Table 5: Multiple Regression equations developed for *B. tabaci* and OYVMV based on morphological and biochemical parameters in okra germplasm during summer, 2015 and 2016 (pooled data)

B. tabaci		**OYVMV**	
Multiple Regression equation	**R^2**	**Multiple Regression equation**	**R^2**
Y= -109.66 -2.16 X1 + 1.12 X2 +2.95 X3 + 2.47 X4 - 1.48 X5 +1.01 X6	0.99	Y= -119.72 -2.92 X1+ 1.98 X2 +3.45 X3 + 3.32 X4 - 1.86 X5 +1.29 X6	0.99

4. Conclusion

Based on the outcome of the present investigation entitled "Morphological and Biochemical basis of resistance in Okra to whitefly, *Bemisia tabaci* and Okra yellow vein mosaic virus (OYVMV)" it was evident that, the immune cultivated accessions *viz.*, RJR-124 and PSRJ- 12952 with good yield possessed high trichome density, high phenol content, low sugars, low nitrogen and protein content were found to be promising lines against the vector and virus. Hence, these genotypes can be utilized for developing new varieties with pest and disease resistance through breeding programmes.

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