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RESEARCH ARTICLE

Inheritance pattern of okra enation leaf curl disease among cultivated species and its relationship with biochemical parameters

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Abstract. Okra production in eastern India at present is severely threatened by whitefly-mediated okra enation leaf curl disease (OELCuD). Identification of resistant genotype and understanding the genetic control and biochemical relationship of OELCuD resistance are prerequisite for developing an effective breeding strategy. This study was conducted employing six populations (P_1, P_2, F_1, F_2, BC_1 and BC_2) of two selected (resistant × susceptible (R×S)) crosses. Associationship between severity of OELCuD and biochemical parameters of parents and hybrids at preflowering and flowering stages was studied. Segregation pattern of the genotypes in F_2 generation showing OELCuD reaction of two crosses suggested that two duplicate recessive genes was operative for resistance to OELCuD. Generation mean analysis revealed involvement of both additive and nonadditive effects in the inheritance of disease resistance. Hence, postponement of selection in later generations or intermating among the selected segregates followed by one or two generations of selfing to break the undesirable linkage and allow the accumulation of favourable alleles could be suggested for the development of stable resistant genotype against this disease. Higher peroxidase activity and total phenol content in leaf emerged as reliable biochemical markers for early selection of genotype resistant to OELCuD.

Keywords. biochemical parameters; okra enation leaf curl disease; genetics; okra.

Introduction

India is the largest producer of okra (*Abelmoschus esculentus* L. Moench) in the world (Anonymous 2017). The most important constraint in okra production is susceptibility of the crop to various insect pests (Acharya *et al*. 2018) and viral diseases (Das *et al*. 2013; Seth *et al*. 2017). The gangetic plains of eastern India share a coveted position in okra production. Over the years, this particular region serves as one of the hot spots of okra yellow vein mosaic disease (OYVMD) which occurs at all growth stages in okra (Das *et al*. 2013). At present, okra enation leaf curl disease (OELCuD) is an emerging viral disease of Indian subcontinent as a whole and in eastern parts of India in particular (Yadav *et al*. 2018; Jamir *et al*. 2020). It was first reported in Karnataka, India (Singh and Dutta 1986; Singh 1996). Later, report on incidence of this disease came from different parts of the world, namely Nigeria (Atiri 1984), Pakistan (Nadeem *et al*. 1997), Saudi Arabia (Ghanem 2003), China (Lubin *et al*. 2005) and Iran (Bananej *et al*. 2016). This disease is caused by viruses which belong to the group begomovirus, family Geminiviridae and characterized by the formation of small pin-head enations on the under surface of leaves followed by a warty and rough texture of the leaves which later curl upwards, showing a twisting of the stem and petioles with leaves becoming thick and leathery. This disease results stunted growth of okra plants with small and deformed fruits, which are unfit for marketing and consumption (Singh 1996; Sanwal *et al*. 2014). It is not seed transmitted disease but it is reported to be transmitted by whitefly (Venkataravanappa *et al*. 2015).

The wide diversity among begomoviruses associated with mixed infections results in recombination and pseudorecombination events leading to evolution of geminiviruses

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(Seal *et al*. 2006) including the origin of OELCuD as the sequences making up OELCuD have originated from other malvaceous begomoviruses; cotton leaf curl Bangaluru virus (CLCuBaV), mesta yellow vein mosaic virus (MeYVMV), and OYVMD (Venkataravanappa *et al*. 2015). They also reported that OELCuD may be produced in association with at least two distinct betasatellites: bhendi yellow vein betasatellite (BYVB) and okra leaf curl betasatellite (OLCuB). An alpha satellite DNA associated with OELCuD has also been characterized (Chandran *et al*. 2013).

From southern parts of India, it has also been reported that okra plant showed either OYVMD or OELCuD symptoms (Sohrab *et al*. 2013). However, under northern Indian conditions, both OYVMD and OELCuD symptoms together on the same plants have been noticed which could be due to lack of genetic purity of a particular genotype or due to the emergence of new viral strains or due to the recombination or pseudorecombination (Mishra *et al*. 2017). In the Gangetic plains of eastern India, the infection of YVMV and OELCV did not occur simultaneously in the same plant (Devi *et al*. 2018; Yadav *et al*. 2018).

Lack of resistance source to this disease has forced breeders to look into the wild species (*A. crinitus*, *A. ficulneus*, *A. angulosus* and *A. manihot*) in okra which have stable and reliable sources of resistance to OELCuD (Singh *et al*. 2007, 2009). However, the transfer of resistance from wild relatives is hampered by sterility problems and it is difficult to produce subsequent generations or even carryout backcrosses (Sanwal *et al*. 2014). Hence, continuous search for new sources of resistance from indigenous germplasm or prebreeding lines, and development of varieties/hybrids with higher level of resistance against OELCuD should be the prime objective.

The role of proximate compositions and enzyme activities for imparting resistance against OELCuD has not yet been documented, although several reports are available on biochemical basis of resistance to OYVMD (Prakasha 2009; Prabu and Warade 2009; Seth *et al*. 2017). Plant phenolics are secondary metabolites involved in the defense mechanisms of plants against pathogens and insect herbivores (Saini *et al*. 1988; Lattanzio *et al*. 2006). Plants respond to wide array of environmental enemies with a varying degree of responses, which use constitutive and induced phenolic substances affecting the susceptibility/resistance reactions of the host plant (Lattanzio *et al*. 2006). Phenolic substances provide mechanical strength to the host cell wall and also check the entry of invading pathogens (Saini *et al*. 1988). The first step of the defense mechanism in plants responding to infection is the rapid accumulation of phenols at the infection site, which restricts or slows down the pathogen growth (Matern and Kneusel 1988). The enzymes, phenylalanine ammonia lyase (PAL), polyphenoloxidase (PPO), and peroxidase (POD) are involved in phenol oxidation and exert their increased activities during hypersensitive reactions to infection (Barberan and Espin 2001). Our previous study revealed that higher activities of POD, PPO, phenol and contents of ascorbic acid in okra leaves exhibited negative relationships with severity of OYVMD over the crop growth stages (Seth *et al*. 2017). Activities of defense related factors varied with genotypes and crop growth stages (Jabeen *et al*. 2009). The main drawback of the conventional resistant breeding is the time required for the identification of resistant or tolerant lines which cannot be identified at early plant growth stage. Selection at early growth stage can reduce the time required for population screening. Different marker systems are available which can help to select disease resistant genotypes at early growth stages. The synthesis of different biochemical compositions including enzyme activities at various growth stages can be used as biochemical markers that could help for early identification of resistant genotype during population screening.

Under greenhouse condition, agro-inoculation is an useful and efficient technique to screen okra germplasm against OELCuD (Venkataravanappa *et al*. 2014). However, evaluation under field condition in hotspot can be equally an effective method for screening large number of germplasm lines for OELCuD resistance and susceptibility (Devi *et al*. 2019; Jamir *et al*. 2020). A single report is available on the inheritance pattern of the disease till date (Devi *et al*. 2019). For developing the high-yielding OELCuD resistant varieties of okra, it is worthwhile to identify resistant source among cultivated species of okra for the study of the inheritance of host resistance. The present investigation was carried out to understand the genetic basis of the host resistance to OELCuD with emphasis on biochemical parameters related to resistance with a view to frame the breeding strategy for development of OELCuD resistant variety.

Material and methods

Field evaluation of okra germplasm

The field screening of 35 okra genotypes were obtained from ICAR-IIVR, Varanasi and ICAR-IIHR, Bengaluru, India, which was carried out for the identification of OYVMD/OELCuD resistant genotypes under climatic conditions (humidity (65 to 95%) and mean day temperature 24°C to 39°C) during June–September 2015. During this time the activity of whitefly and incidence of viral diseases reaches its peak as observed in our previous studies (Das *et al*. 2012; Acharya *et al*. 2018). Each line was sown in augmented block design keeping spacing at 60 cm × 30 cm between rows and plants in raised plots that were 3.0-m long and 1.8-m wide, accommodating 30 plants per plot. To allow the growth of vector population throughout the growing season, no protection measures were taken against whitefly infestation. Every plant in the population was observed visually at 20 days interval for susceptibility against both YVMD and OELCuD starting from 20 days after sowing (DAS) till 80 DAS. The evaluated lines either expressed OYVMD or OELCuD symptom in a

population at a time which supported the previous observations (Sohrab *et al*. 2013; Yadav *et al*. 2018; Devi *et al*. 2018). For the purpose of disease reaction classification, a genotype found resistant to any of the disease at any stage was considered resistant and *vice versa*.

Development of F_1 generations

Based on the severity of symptoms development of OELCuD, two most resistant genotypes 285-1-3A-1-17-1 and VRO-178, and one susceptible genotype BCO-1 were selected by confirming artificial feeding with viruliferous whitefly (Yadav *et al*. 2018) for the hybridization programme during February–May of 2016.

Development of F_2 and backcross generations

Hybrids of two selected contrasting crosses involving most resistant genotypes (285-1-3A-1-17-1 and VRO-178) and susceptible genotype (BCO-1) were sown during June–September 2016 (humidity (60 to 92%) and mean day temperature 27°C to 38.5°C) in the Gangetic plains of West Bengal at the research field of All India Coordinated Research Project on Vegetable Crops, Bidhan Chandra Krishi Viswavidyalaya, Nadia, India, situated at 23.5°N latitude and 89°E longitude at an elevation of 9.75 m above the mean sea level. Hybrids were selfed to obtain F_2 progenies as well as backcrossed to their respective parents (P_1 and P_2) in the following fashion to obtain backcross progenies of BC_1 (cross with resistant parent) and BC_2 (cross with susceptible parent). (i) Cross I: resistant × susceptible [285-1-3A-1-17-1 × BCO-1]; (ii) cross II: resistant × susceptible [VRO-178 × BCO-1].

Experimental layout

Seeds of parents (P_1 and P_2) and their generations (F_1, F_2, BC_1, and BC_2) of two contrasting crosses were sown following compact family block design replicated thrice during June–September 2017. A total of 25 plants, each for P_1, P_2 and F_1; 250 plants for F_2; and 35 plants each for BC_1 and BC_2 keeping five plants in a row were kept per replication for evaluation. Standard agronomic practices for raising good crop were followed (Chattopadhyay *et al*. 2007). No spraying of systemic insecticide against whitefly infestation was taken to allow the growth of vector population throughout the growing season.

Data recording

Different biochemical parameters of total chlorophyll and phenol contents, enzyme activities of POD, PPO, and PAL were estimated from the top third leaf of two resistant (285-1-3A-1-17-1, VRO-178) and one susceptible (BCO-1) parents, and their cross combinations (285-1-3A-1-17-1 × BCO-1 and VRO-178 × BCO-1) at preflowering (30 DAS) and flowering (45 DAS) stages. Measurement of total chlorophyll content (mg/100 g tissue) and estimation of total phenol content (mg/100 g tissue) in leaf was performed (Sadasivam and Manickam 1996). The changes in the activities of PPO (Malik and Singh 1980), POD and PAL were done spectrophotometrically (Sadasivam and Manickam 1996).

Days to first appearance of OELCuD was noted in each generation. The total number of plants infected with OELCuD was counted starting from 20 to 80 DAS in the population and per cent disease index (PDI) was calculated (McKinney and Davis 1925) by using disease severity scale (0, no disease; 1, top leaves curled; 3, top leaves curled and slight stunting of plant; 5, all leaves curled, twisting of petiole and slight stunting of plant; 7, severe curling of leaves, twisting of petiole, stunting of plant and proliferation of auxiliary branches) as per Alegbejo (1997). Relative spread of OELCuD was also computed among different generations of two contrasting crosses using the area under the disease progress-curve (AUDPC) following the standard method (Campbell and Madden 1990).

Statistical analysis

The means and variances of means for two disease related traits (days to first appearance of OELCuD and PDI of OELCuD) were computed for each generation (Gomez and Gomez 1984). Observations recorded in the segregating generations were subjected to χ^2 test for goodness of fit. The three quantities of scaling tests, namely A, B and C were calculated (Mather 1949) to detect the presence or absence of epistasis. The generation means were analysed (Hayman 1958) to provide information on the inheritance of various traits. The generation means were used to estimate the six genetic parameters, namely (m), (d), (h), (i), (j) and (l) of digenic interaction model representing F_2 mean, additive gene action, dominance genetic effect, additive × additive gene interaction effect, additive × dominance interaction effect and dominance × dominance gene effects, respectively assuming that no linkage and no higher order gene interaction exists. The significance of each estimate was judged by *t*-test against its standard error of estimate. Pearson's correlation coefficients were worked out among two tolerant and one susceptible parents, and their cross combinations by taking PDI of OELCuD as dependent variable, and different biochemical parameters as independent variables, at preflowering (30 DAS) and flowering (45 DAS) stages. All the analyses were computed using computer software programme INDOSTAT (ver. 8.1, Indostat, Hyderabad, India).

Results and discussion

Mean performances of two OELCuD related traits

The expressions of parents (P_1 and P_2) of two contrasting crosses differed significantly for two OELCuD related traits, namely days to first appearance of OELCuD and PDI of OELCuD (table 1). The degree of disease severity varied greatly among different generations of two contrasting cross combinations. Comparatively, a high disease severity (44.06%) was observed in F_2 population of VRO-178 × BCO-1 cross. Significant heterosis in negative direction for days to 1st appearance of OELCuD and positive direction for PDI of OELCuD over mid-parent in F_1 generations of two crosses was observed (table 1). Significant heterosis was noted in both negative and positive directions over mid-parent in F_2 generation for both the traits.

The appearance of OELCuD in the Gangetic plains of West Bengal, India commences within 15–20 days after sowing, which resulted in high infection rate of disease (Jamir *et al*. 2020). The crop could produce significant number of marketable fruits if infected at post flowering stage as compared to the plant infected before the flowering stage. Therefore, days to first appearance of OELCuD would be a useful indicator governing susceptibility/resistance of okra germplasm for breeders to develop resistant varieties. The spread of OELCuD depends on several factors, such as presence of virulent strains of the viruses, population of viruliferous cryptic whitely-species complex, environmental conditions and inoculum load (Sanwal *et al*. 2014; Vyskocilová *et al*. 2018). In the present study, significant heterosis in negative and positive directions over mid-parent in F_1 generation indicates bidirectional dominance of two disease related traits. Transgressions in segregating generation in the present study may be due to wide genetic distance of parents and recombination of additive alleles. This recombination results in new pairs of alleles at two or more loci. Both bidirectional dominance and transgressive segregates were also noticed in two YVMD related traits (Seth *et al*. 2017). Moreover, the data of two disease related traits in backcross generations (BC_1 and BC_2) were superior to their matching generations (F_1 and F_2) which might also indicate an accumulation of some favourable alleles. The data in F_2 generation for PDI of OELCuD was inferior to its matching progeny generations (F_1, BC_1 and BC_2), which might be due to the maximum segregation of desirable alleles that may result in higher frequency of inferior segregates in two crosses. The superiority of F_1 could be due to an accumulation of favourable dominant alleles, while the superiority in performance of segregating generations (F_2, BC_1 and BC_2) might suggest a higher frequency of their transgressive segregates (Marame *et al*. 2009). The least difference in days to 1st appearance of OELCuD and PDI of OELCuD between backcross generations might be due to dispersion of increasing and

Table 1. Mean values for OELCuD related traits in six generations.

Trait	Cross combination	P_1	P_2	F_1	F_2	BC_1	BC_2	MP heterosis in F_1	MP heterosis in F_2	P_1–P_2
Days to first appearance of OELCuD	285-1-3A-1-17-1 × BCO-1 (R × S)	45.33	25.33	28.67	29.67	35.67	31.33	−18.80**	−16.02**	20.00**
	VRO-178 × BCO-1 (R × S)	46.67	25.67	26.33	27.67	36.67	30.67	−27.20**	−23.50**	21.00**
PDI of OELCuD (%)	285-1-3A-1-17-1 × BCO-1 (R × S)	11.01	48.93	40.86	43.67	30.26	36.91	36.33**	45.71**	−37.92**
	VRO-178 × BCO-1 (R × S)	14.23	51.63	41.23	44.06	29.31	37.85	25.20**	33.79**	−37.40**

**Significant at 0.01 level of probability.

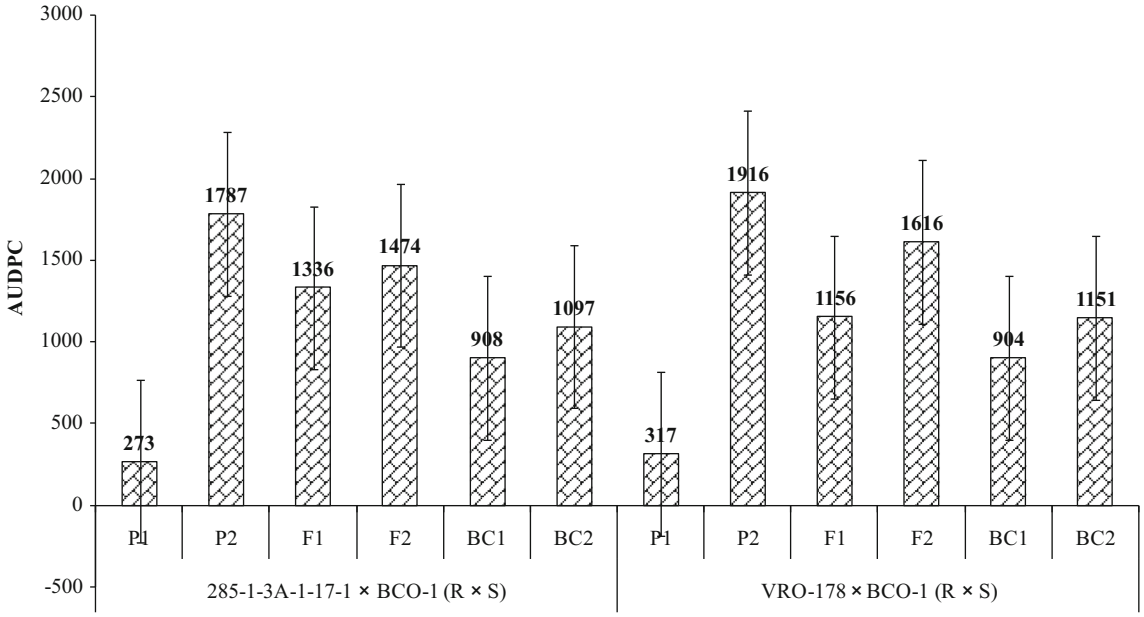

Figure 1. Comparative study on OELCuD progression in six generations of two different cross combinations.

decreasing alleles among the parents (Mather and Jinks 1982).

The range of AUDPC of different generations in two cross combinations varied widely between resistant and susceptible populations (figure 1). The disease severity of OELCuD among populations of two contrasting crosses progressed slowly at the initial stage but accelerated as the plants were maturing. In the present study, the AUDPC values were found to be the minimum (less than 400) within resistant group and the maximum (904 to 1916) within the susceptible group under naturally infected field conditions. Okra generations among two crosses showed that susceptible and highly susceptible reactions had extremely high AUDPCs.

It is difficult to judge OELCuD severity of the same line at later growth stages on the basis of single observation. Plant-disease epidemiologists rely on temporal increase of disease data for the calculation of the OELCuD (Madden *et al*. 2000) which uses multiple evaluations and do not rely on transformations, thus lead to more accurate phenotypic evaluation (Campbell and Madden 1990) and represents disease progress throughout the entire growth period (Roy *et al*. 2009). In this investigation, the AUDPC values varied greatly between resistant and susceptible groups, indicating low cumulative disease progress in resistant genotype and vice versa.

Table 2. Chi-square test for different genetic ratios in crosses involving OELCuD resistant and susceptible parents of okra.

Cross combinations	Generation	Number of resistant plant	Number of susceptible plant	Total	Genetic ratio T : S	χ^2	*P*
285-1-3A-1-17-1 × BCO-1 (R × S)	P_1	63	12	75	–	–	–
	P_2	9	66	75	–	–	–
	F_1	12	63	75	–	–	–
	F_2	294	456	750	7:9	2.10	0.1–0.2
	BC_1^*	90	15	105	1:0	∞	∞
	BC_2^{**}	57	48	105	1:1	0.26	0.5–0.7
VRO-178 × BCO-1 (R × S)	P_1	57	18	75	–	–	–
	P_2	12	63	75	–	–	–
	F_1	15	60	75	–	–	–
	F_2	282	468	750	7:9	3.83	0.05–0.10
	BC_1^{***}	84	21	105	1:0	∞	∞
	BC_2^{****}	60	45	105	1:1	0.72	0.3–0.5

R, tolerant; S, susceptible; *BC_1, backcross with 285-1-3A-1-17-1; **BC_2, backcross with BCO-1; ***BC_1, backcross with VRO-178; ****BC_2, backcross with BCO-1.

Inheritance pattern of OELCuD resistance

Two parental lines (P_1, P_2) and their generations (F_1, F_2, BC_1, and BC_2) of two contrasting cross combinations of resistant × susceptible type were taken to carry out this experiment during June–September 2015. The number of resistant and susceptible genotypes against OELCuD were recorded from parental lines and their generations. The qualitative analysis interpreted in terms of ratios and frequencies was carried out by using χ^2 test on the segregation of resistant and susceptible plants in F_2 and backcross generations of two crosses, presented in table 2. In cross I (285-1-3A-1-17-1 × BCO-1), of the total population of 75 in each P_1, P_2 and F_1 in three replications, 12, 66, and 63 plants showed OELCuD symptoms, respectively suggesting resistance to OELCuD in the parent 285-1-3A-1-17-1 and susceptibility in other parent BCO-1. In case of cross II (VRO-178 × BCO-1), of the 75 plants in three replications, 18, 63, and 60 plants showed OELCuD symptoms in P_1, P_2 and F_1, respectively revealing resistance to OELCuD in the parent VRO-178 and susceptibility in BCO-1. Appearance of a few resistant plants in F_1 population in two crosses suggested the possibility of more than single gene controlling the resistance to OELCuD in 285-1-3A-1-17-1 and VRO-178. Segregation pattern for OELCuD in F_2 generation of these two crosses showed a good fit for seven (resistant): nine (susceptible) and their acceptable homogeneity chi-square (χ^2) confirmed existence of two genes with duplicate recessive epistasis (table 2). The nonsignificant values of χ^2 suggested that the observed ratio did not deviate significantly from the expected 7:9 ratio. The dominant allele of one gene was epistatic to the recessive allele of the other. Of the 105 plants in three replications, 15 and 48 plants in cross I, and 21 and 45 plants in cross II in each BC_1 and BC_2 generations, respectively produced OELCuD symptoms which supported the expected ratio of almost 1:0 in BC_1 (backcross with resistant parent) and 1:1 in BC_2 (backcross with susceptible parent).

The inheritance study of OELCuD through χ^2 test amply suggested that resistance to OELCuD was controlled by two duplicate recessive genes in resistant × susceptible (R × S) crosses. In our previous study with different genetic background of parental lines, the inheritance pattern of ELCuD of okra also revealed that two duplicate recessive genes were operative in tolerant × susceptible cross (Devi *et al*. 2019). The joint actions of two or multiple loci conferring disease resistance in plant systems have been reported (Kang *et al*. 2005). When two loci, *pvr2* and *pvr6* in pepper, are in a recessive-homozygous condition, they can confer resistance to pepper veinal mottle virus (PVMV) (Ruffel *et al*. 2006). In the present study, appearance of few numbers of susceptible plants in BC_1 generations (backcross with tolerant parent) of two crosses may be due to presence of additional factors in the resistance system of 285-1-3A-1-17-1 and VRO-178 or minor resistance factor contributed by the susceptible parent BCO-1. Hence, the inheritance of resistance to OELCuD appears to be more complicated, quantitative in nature, and dependent on gene-dosage resistance mechanism. Fraser (1990) stated that such mechanism allows virus to infect the plant, but simultaneously inhibit virus multiplication within the plant or showing no phenotypic symptom, which support the findings of Nariani and Seth (1958) who stated the situation as symptomless carriers. Therefore, one could transfer major resistant genes to other varieties, but the resistant breaking virus strains might obstruct to get resistant plants in stable condition. Thus, accumulation of more resistant genes may be required for getting a stable OELCuD resistant phenotype in okra. In such cases some breeding lines 2014/OKYV RES-5, 2014/OKYV RES-10, 2014/OKYV RES-11, IC433532 and IC411692 which were identified as promising donors could be utilized in OELCuD resistant breeding programme (Devi *et al*. 2018).

Gene action based on generation mean analysis

Mean data for six generations along with the estimates on the scales 'A', 'B', 'C' and 'D' from the crosses are summarized (table 3). The scaling tests were applied in the present study to test the presence of nonallelic gene interactions (Mather and Jinks 1982). The estimates of the gene effects by using a six-parameter model (Kearsey and Pooni 1996) are presented in table 4.

Table 3. Estimates of gene effects based on scaling test for OELCuD related traits in okra.

Cross combination	Scale			
	A	B	C	D
Days to first appearance of OELCuD				
285-1-3A-1-17-1 × BCO-1 (R×S)	−2.66** ± 1.50	8.66** ± 1.57	−9.32** ± 3.17	−7.66** ± 1.47
VRO-178 × BCO-1 (R×S)	0.34 ± 2.60	9.34** ± 2.27	−6.32** ± 4.46	−8.00** ± 2.15
PDI of OELCuD (%)				
285-1-3A-1-17-1 × BCO-1 (R×S)	5.88** ± 1.90	−18.74** ± 1.59	24.24** ± 2.99	18.55** ± 1.67
VRO-178 × BCO-1 (R×S)	0.33 ± 2.10	−19.99** ± 2.05	10.94** ± 3.52	15.30** ± 2.06

**Significant at 0.01 level of probability.

Table 4. Estimates of gene effects and narrow sense heritability based on joint scaling test for a six-parameter model in intervarietal crosses of OELCuD related traits.

Genetic components/traits	Days to first appearance of OELCuD		PDI of OELCuD (%)	
	285-1-3A-1-17-1 × BCO-1 (R × S)	VRO-178 × BCO-1 (R × S)	285-1-3A-1-17-1 × BCO-1 (R × S)	VRO-178 × BCO-1 (R × S)
M (mean)	29.67** ± 0.62	29.67** ± 0.87	42.86** ± 6.33	41.23** ± 0.79
d (additive effect)	4.34** ± 0.64	6.00* ± 4.53	−6.65** ± 0.73	−8.74** ± 0.98
h (dominance effect)	8.66** ± 3.06	6.16* ± 4.45	−23.44** ± 3.43	−19.47** ± 4.27
i (additive × additive type gene interaction)	15.32** ± 2.94	16.00** ± 4.31	−37.10** ± 3.34	−30.60** ± 4.11
j (additive × dominance type gene interaction)	−11.32** ± 1.27	−9.00** ± 2.41	24.67** ± 2.01	20.32** ± 2.22
l (dominance × dominance type gene interaction)	−21.32** ± 4.43	−25.68** ± 6.75	49.96** ± 5.29	50.26** ± 6.31
Epistatic gene action	Duplicate	Duplicate	Duplicate	Duplicate
Narrow sense heritability (%)	48.72		56.27	

*,**Significant at 0.05 and 0.01 level of probability, respectively.

Days to first appearance of OELCuD: In cross I (285-1-3A-1-17-1 × BCO-1), all the four scales and gene effects were found significant revealing dominance, additive, additive × additive, additive × dominance and dominance × dominance type gene interactions controlled days to first appearance of OELCuD. The opposite sign of [h] and [l] was the indicative of duplicate type (gene effect) of epistasis.

For the second cross (VRO-178 × BCO-1), the significant estimates of four scales indicated the presence of additive × additive [i], additive × dominance [j], and dominance × dominance [l] types of non-allelic gene interactions. The values of [d] and [h] gene effects were significant which revealed the presence of additive and dominance types of gene interactions, respectively. The values of [h] and [l] attained different signs which suggested duplicate type of epistasis.

Percent disease index (PDI) of OELCuD

For cross I, the significant estimates of four scales indicated the presence of all three types of nonallelic gene interactions. Additive, dominance, additive × additive and dominance × dominance type of gene interactions controlled percent disease index of OELCuD. The values of [h] and [l] were of the opposite sign which indicated the presence of duplicate type of epistasis.

In second cross, presence of all types of non-allelic gene interactions were also found due to significant estimates of scales 'B','C' and 'D' except 'A'. In this cross, percent disease index of OELCuD was controlled by additive, dominance, additive × additive and dominance × dominance gene actions. The values of [h] and [l] had opposite signs which suggested duplicate type of epistasis for this trait.

From the study, it revealed that days to first appearance of OELCuD and PDI of OELCuD were governed by both additive and nonadditive gene actions. Medium range of narrow sense heritability (48.72% to 56.27%) for both the disease related traits was recorded (table 4).

An additive–dominance model was inadequate to express the gene effects due to significance of all four scales in two crosses. Both additive and dominance components of genetic variations were significant in two crosses. However, positive additive × additive (i) type gene action and duplicate epistasis found in days to 1st appearance of OELCuD suggested the possibility of obtaining transgressive segregates in later generation from two crosses. A number of researchers reported the gene action of different quantitative traits of okra but information regarding gene effects of OELCuD related traits is lacking. All the three types of gene action, i.e. additive, dominance and interaction components were found to play a profound role in the inheritance of days to first appearance of OELCuD and PDI of OELCuD. However, their degree differed with crosses. The presence of

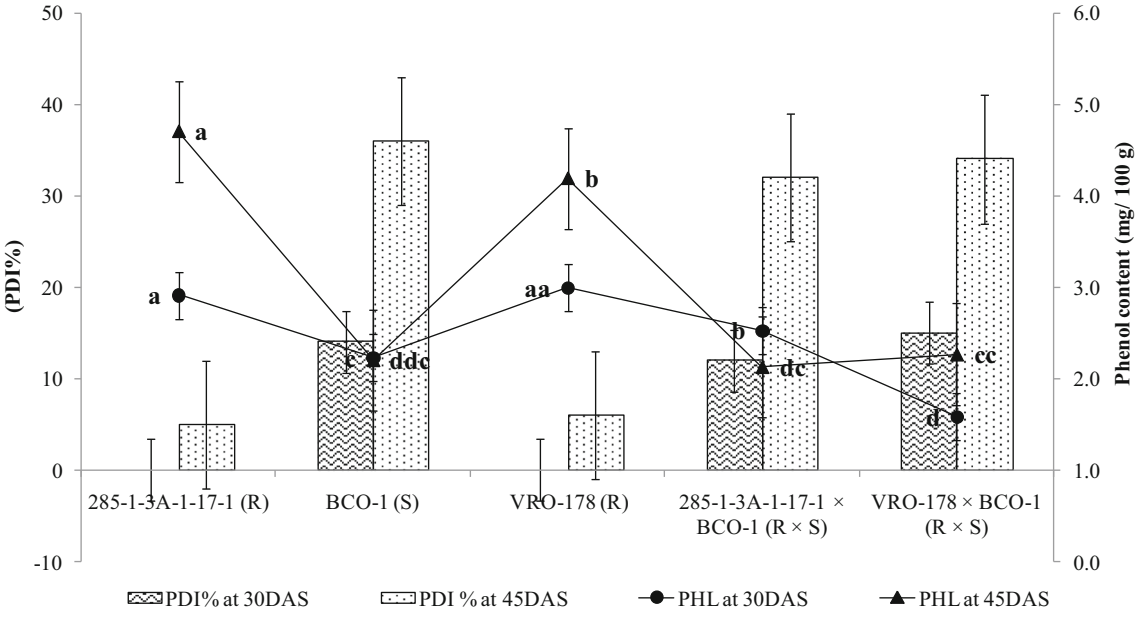

Figure 2. Relationship between PDI of OELCuD and phenol (PHL) content at different growth stages of okra.

nonadditive gene effects for days to 1st appearance of OELCuD and PDI of OELCuD was also reported (Devi *et al*. 2019). This could be due to differences in magnitude of the gene effects and genetic background of the parents (Murthy and Deshpande 1997). Presence of dominance and additive components suggested that the selection for OELCuD related traits would be delayed till dominance and epistatic components are reduced through selfings.

Presence of duplicate type epistasis for days to 1st appearance of OELCuD and PDI of OELCuD in two crosses would like to obstruct the selection procedure in early generations. Additive × additive type interaction was found significant for PDI of OELCuD but with negative sign which indicated little scope of improvement through simple selection. Selection in later generations (F_4 or F_5) or crossing among the selected segregates followed by one or two generation(s) of selfing to break the undesirable linkage and allow the accumulation of favourable alleles for improvement of this trait.

Relationship between OELCuD severity and biochemical parameters

The biochemical substances responsible for the expression of different traits related to OELCuD resistance at different growth stages varied significantly among parents and their crosses. Two resistant parents (285-1-3A-1-17-1, VRO-178) and resistant × susceptible crosses (285-1-3A-1-17-1 × BCO-1, VRO-178 × BCO-1) had the maximum contents/activities of total chlorophyll, phenol, POD, PPO and PAL, while susceptible parent (BCO-1) had minimum of these contents/activities at 30 DAS (figures 2–6). All the biochemical traits increased significantly irrespective of genotypes both in parental lines as well as in the crosses but the tolerant parents had higher contents/activities of total chlorophyll, phenol, POD, PPO and PAL than susceptible parent at 45 DAS.

The OELCuD resistance in okra is complex, and a number of biochemical parameters and defense related enzymes might be associated with OELCuD resistance responses. The knowledge of relationship between OELCuD severity and biochemical traits is crucial through which selection program could be sensibly formulated in disease resistance breeding programme. Hence, a straightforward correlation between PDI of OELCuD and five biochemical parameters was computed at different growth stages, i.e. preflowering (30 DAS) and flowering (45 DAS) involving two tolerant and one susceptible parents, including their cross combinations (resistant × susceptible), depicted in table 5. Negative significant relationship between PDI of OELCuD and biochemical traits is desirable to have better understanding of correlated response. Two biochemical parameters POD activity and phenol content were found negatively and significantly correlated with PDI of OELCuD at 30 and 45 DAS, whereas, other traits (PPO, PAL and total chlorophyll) expressed nonsignificant negative correlations with ELCuD severity.

To screen desirable OELCuD resistant genotypes in okra breeding programme, it is necessary to understand the role of biochemical parameters conferring resistance to this disease by limiting the multiplication of the viral infection. Hence, quantification of biochemical parameters and their variation at progressive crop growth stages become imperative in recognizing response of the host plant against OELCuD. No reports indicate a correlation between the biochemical

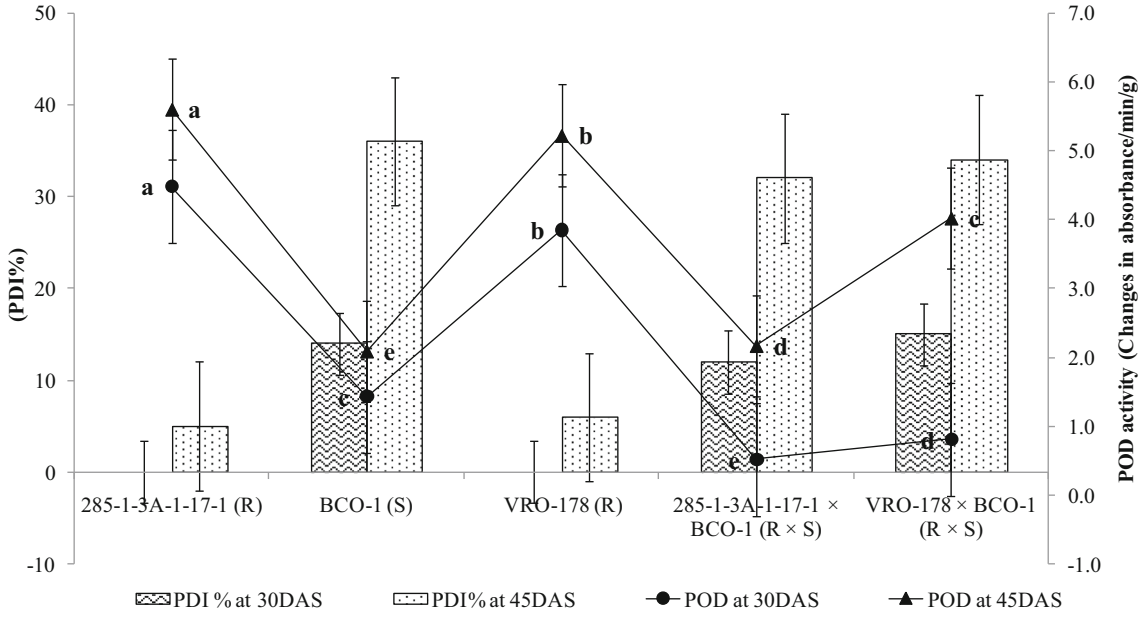

Figure 3. Relationship between PDI of OELCuD and peroxidase (POD) activity at different growth stages of okra.

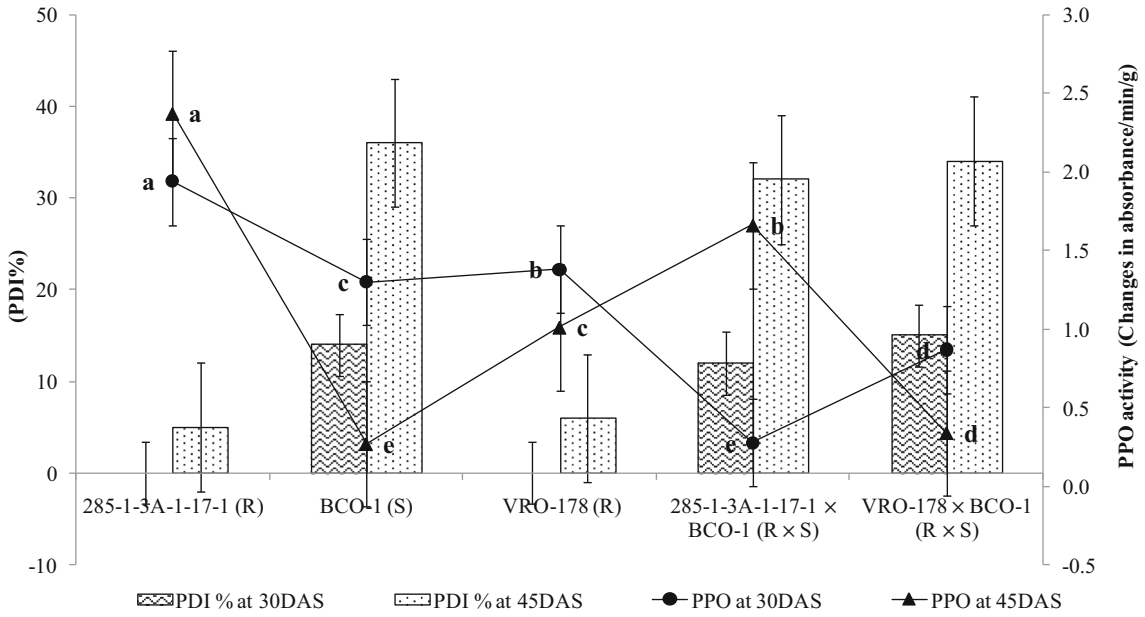

Figure 4. Relationship between PDI of OELCuD and PPO activity at different growth stages of okra.

parameters and resistant or susceptibility to OELCuD. The leaves of okra exhibited a progressive increase in content/activities of chlorophyll, total phenol, POD, PPO and PAL over the growth stages. Activities/contents of chlorophyll, total phenol, POD, PPO and PAL over the growth stages differ varyingly among the susceptible and tolerant okra genotypes. Genotypes showing OELCuD resistance activity contained more biochemical substances than those showing susceptibility. This phenomenon has tremendous prospect in selecting resistant genotype at early growth stages through quantifying the biochemical molecules. More accumulation of phenolic compounds and defense related enzymes following viral infection convert them to more toxic forms that inhibit the multiplication of virus inside the resistant host plant up to 45 DAS. Plants with acquired resistance having high levels of peroxidase catalyze the polymerization of monolignols during lignification of cells, which is an early response to hypersensitive cell death, and also oxidizes

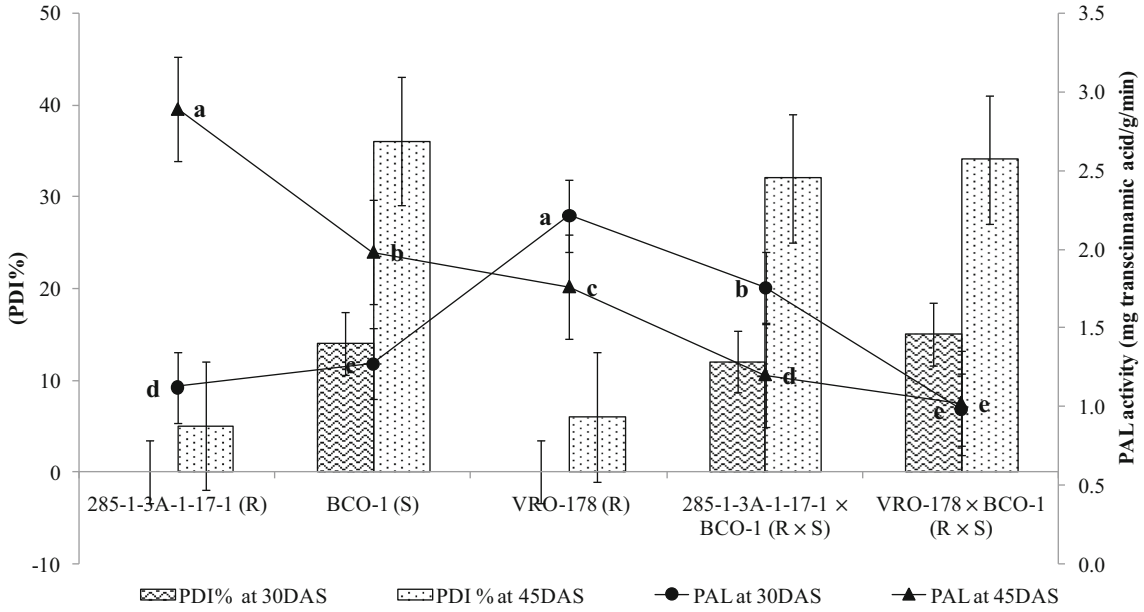

Figure 5. Relationship between PDI of OELCuD and PAL activity at different growth stages of okra.

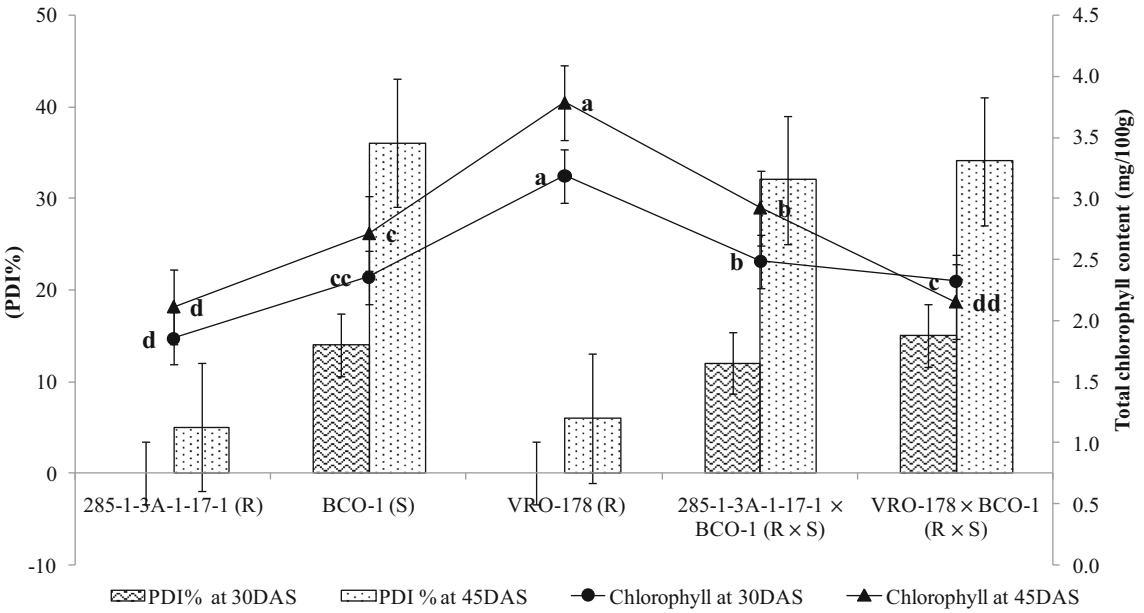

Figure 6. Relationship between PDI of OELCuD and total chlorophyll content at different growth stages of okra.

phenols to quinones which are toxic to the pathogen (Brisson *et al*. 1994). Previous studies also suggested that plants resistant to virus diseases show more accumulation of phenols (Dasgupta 1988; Seth *et al*. 2017) and increased activity of oxidative enzyme like peroxidase (Seth *et al*. 2017) during hypersensitive reactions to infection. Therefore, more number of marketable fruits would be produced by okra plant showing tolerance against OELCuD up to 45 days as compared to the plant infected before flowering stage.

In the present study, the higher content of phenols and POD activity with decreased spread of OELCuD in the host plant over the growth stages might be due to the fact that phenolic compounds or after conversion to their oxidation products responsible for the resistance mechanism in tolerant host plant either by inhibiting the virus activity or by reducing the virus multiplication rate inside the host plant (Seth *et al*. 2017). POD has a specific role in lignifications strengthening the plant cell wall that is resistant to biodegradation (Jaiti *et al*. 2009). POD together with PPO consumes oxygen and producing quinones, which may reduce plant digestibility for the insect vector (whitefly) (Ranger *et al*. 2009). Negative and significant correlated

Table 5. Correlation coefficients between biochemical parameters and PDI of OELCuD at different growth stages of okra.

	PDI of OELCuD (%)	
Biochemical parameter	30 DAS	45 DAS
POD activity (changes in absorbance/min/g leaf tissue)	−0.904**	−0.793*
PPO activity (changes in absorbance/min/g leaf tissue)	−0.370	−0.339
PAL activity (mg transcinnamic acid/g leaf tissue/min)	−0.379	−0.347
Phenol content (mg/100 g tissue)	−0.731*	−0.913**
Total chlorophyll content (mg/100 g tissue)	−0.540	−0.662

*,**Significant at 0.05 and 0.01 level of probability, respectively.

response of POD activity and phenol content with PDI of OELCuD at 30 and 45 DAS revealed that increase activity/content of these biochemical parameters leading to disease resistance in okra. Hence, on the basis of this study, it can be suggested that phenol content and POD activity in plant body have the potential to be used as biochemical marker to identify OELCuD resistant okra genotype, both early as well at later plant growth stage. To the best our knowledge, no previous studies have been documented for associate between OELC uD severity and biochemical traits to support our findings.

In conclusion, the study concluded that resistance to OELCuD was controlled predominantly by two duplicate recessive genes in resistant × susceptible crosses with the involvement of duplicate epistasis suggesting somewhat complicated inheritance pattern. Peroxidase activity and total phenol content in okra leaf exhibited consistent and significant negative correlation with the severity of OELCuD over the growth stages suggesting their implication as biochemical 'markers' for identification of okra genotype resistant to this disease. Two OELCuD related traits examined exhibit complex genetic behaviour. Simple selection in early segregating generations may not be effective for improvement of these traits. Selection for improvement of OELCuD related traits should be delayed to later segregating generations in okra. Medium range narrow sense heritability for OELCuD related traits also recommend modified bulk method of selection for improvement of such traits.

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