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RESEARCH ARTICLE

Evolving phyllody resistant mutant(s) in sesame (*Sesamum indicum* L.) through marker validation and phytochemical quantification

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Abstract

Sesame (*Sesamum indicum* L.) is one of the most important oilseed crops in India. Among the various factors affecting the productivity of sesame, phyllody caused by *phytoplasma* and transmitted by *Orosius albisinctus* is a major disease which will reduce the yield up to 80 %. As sesame phyllody is a vector bone disease it is very difficult to control this disease and evolving resistant/tolerant variety may be one of the low-cost solutions. With the objective of evolving a high yielding phyllody resistant sesame variety through mutation, this research was executed. About 100 handpicked good quality seeds of sesame varieties viz. TMV 7, CO 1 and VRI 3 were subjected to irradiation at Atomic Power Station, Kalpakkam (IGCAR) on 18th September 2020. Three doses of gamma irradiation *viz.*, 30 kR, 40 kR and 50 kR were fixed for our mutation studies. The M_1 generation was raised at Agricultural Research Station, Vaigai Dam on 21.09.2020 by following Randomized Block Design (RBD) with three replications. At the time of flowering, selfing was carried out to ensure self-pollination in the mutants and the selfed seeds of the M_1 generation were used to raise the M_2 generation. In the M_2 generation 132 phyllody-free sesame plants were identified at the time of maturity and forwarded as families to M_3 generation. In the M_3 generation a total number of eighteen families were identified as phyllody free families and forwarded to M_4 generation. Among the eighteen families of the M_4 generation, one family i.e. PR 375 is recorded as the phyllody tolerant species with the score of 16.06^{a}. While screening for phyllody resistance none of the plant protective measures were carried out. From these families, seven phyllody resistant mutants (PR 375-1, PR 375-2, PR 375-3, PR 375-4, PR 375-5, PR 375-6 and PR 375-7 were identified. The resistance for phyllody in the PR 375 family was confirmed by the nested polymerase chain reaction (PCR) assay by using the universal primer pairs P1/P7 and R16F2n / R16 R2 as marker and quantification of secondary metabolites viz. phenols, tannin, alkaloids and flavonoids. The progeny of PR 375 will be forwarded to subsequent generations for further evaluation.

Keywords: mutants; phyllody resistance; sesame

Introduction

Sesame is an important oilseed crop which is having the oil content 40 to 52 % and it is termed as queen of oilseeds because of its oil quality. The global area of the sesame cultivation is 117.43 lakh hectares with the productivity of 502 kg/ha. In India the sesame is cultivated in 17.30 lakh hectares with the productivity of 431 kg/ha (1). Main reasons for the low productivity of sesame in India are biotic and abiotic stresses experienced during cropping season. Among the biotic stresses, root rot and phyllody are the two destructive diseases causing yield reduction up to 90 % (2). The sesame phyllody is caused by prokaryotic phytoplasma that are wall less obligate pathogen transmitted by the vector *Orosius albicinctus*. As this disease is spread by a vector, availability of alternate hosts ensures the presence of phyllody causing phytoplasma throughout the year. Sesame phyllody symptoms are deformation of capsules and the flowers lost its fertility. Finally, the seeds will not be produced in the phyllody affected plants (3). Since controlling this disease will be a very difficult task for the sesame farmers, raising phyllody resistant sesame cultivars is the only viable option to mitigate this disease. Several breeding methodologies were attempted to evolve a phyllody

resistant sesame variety, but it was ended up with no fruitful results because of lack of proper resistance source and availability of various biotypes of phyllody causing phytoplasma. Inheritance studies of sesame phyllody resistance revealed that the phyllody resistance is governed by a recessive gene (4). Hence, mutation breeding is one of the viable options to create phyllody resistant sesame cultivar (5). Among the several mutagens the gamma ray is an important mutagen which is being employed for the creation of variation almost in every crop species. In this present investigation, the gamma rays were used as a mutagen to create variability in sesame to get phyllody resistant sesame cultivar. Screening for phyllody is a critical procedure as there are chances for escaping of host plants from disease causing vectors. A fool proof method should be identified to confirm the genetic nature of the resistance. As the phyllody causing phytoplasma can be detected through the presence of bands in the PCR products for the primer pairs P1/P7 and R16F2n/ R16 R2 of asymptomatic plants in phytoplasma infested field which ensures the genetic nature of the resistance (6). Diseased plants also having the elevated level of phytochemicals such as phenols, tannins, flavonoids, alkaloids and quantification of these phytochemicals will also give secondary confirmation for the presence of disease causing phytoplasma in asymptomatic plants. With these principles the present investigation was executed to evolve phyllody resistant sesame mutants through mutation, marker validation and phytochemical quantification.

Materials and Methods

Mutation studies

About 100 numbers of hand-picked seeds of sesame varieties *viz.*, CO 1, TMV 7 and VRI 3 were subjected to irradiation at IGCAR, Kalpakkam on 18th September 2020.The selected sesame varieties are popular varieties but susceptible to sesame phyllody. The seeds were irradiated with Gamma rays @ 30 kR, 40 kR and 50 kR from the source Co 60. The treated seeds were sown in the field at Agricultural Research Station, Vaigai Dam within 24 hr by following Randomised Block Design (RBD) with three replications as M_1 generation (Fig. 1). To assess the irradiation effect the observations on survival percentage on 30 DAS, days to 50 % flowering, plant height at maturity, number of primary branches per plant, number of capsules per plant, single plant yield per plant were recorded in 10 plants per replication per treatment. The phyllody infestation was also recorded at the time of maturity in natural condition and the percentage of infestation was calculated by following the standard procedure (7). The data were analysed using analysis of variance and SAS statistica computer package ($P < 0.1$ %). The M_2 generation was raised in the field from the selfed seeds of M_1 generation. As the segregation is more in the M_2 generation, we raised as many as number of plants in each treatment to screen the segregants for phyllody resistance. An alternate host for phyllody causing phytoplasma i.e., *Vinca rosea* was also planted in each plot to increase the phyllody infestation (8). None of the control measures was followed to assure the presence of vector *Orosius albicinctus*. During the early stages, the M_2 segregants were perceived for the presence of chlorophyll mutants, plant type mutants and at the time of maturity the plants completely free from phyllody symptoms were selected, counted and tagged. Capsules were harvested from each healthy plant separately and advanced to M_3 generation. The seeds collected from 132 phyllody free mutants in the M_2 generation were sown in the field as M_3 generation. The progenies of a single plant were raised in a separate plot as family and hence 132 phyllody free mutant families were raised. The plot size was 4 X 3 m. In M_3 generation also, the control measures on leaf hopper were not carried out. On the 70th day the observation on phyllody infestation was recorded and expressed in percentage of phyllody infestation. The assessment scale was followed based on earlier scientific studies (7) as furnished below.

% of incidence	Disease reaction
0	Highly resistant
Up to 10 %	Resistant
11 to 20 %	Tolerant
to 30 %	Susceptible
>31 %	Highly susceptible

A total number of 18 families recorded as resistant for sesame phyllody in M_3 generation were forwarded to M_4 generation. The progenies of 18 M_3 mutant families were sown in 4 X 3 m sized plot with the spacing of 30 X 30 cm and replicated twice. In M_4 generation also the control measures on leaf hopper were not practised to increase the probability of phyllody incidence. The observation on final plant stand per plot and number of plants infested with phyllody per plot were recorded.

Fig. 1. Field view of M_1 generation.

Molecular confirmation for phytoplasma in phyllody resistant mutants

In M_4 generation, the families were screened for phyllody resistance based on the presence of symptoms in the plants at later stage. The non symptomatic plants are being considered as resistant or tolerant whereas the plants producing symptoms are termed as susceptible. However, escaping from the vector attack may also produce symptom less plants. To screen the plants with real resistance, asymptomatic plants can be subjected to molecular confirmation for the presence of phyllody causing phytoplasma. The phyllody causing phytoplasma is successfully detected by nested polymerase chain reaction (PCR) assays using primer pairs P1/P7 and R16F2n/ R16 R2 (5) as marker in the DNA samples of asymptomatic plants. Leaf samples were collected from the 7 asymptomatic plants, 2 symptomatic plants of PR 375 families and the five asymptomatic healthy plants of control (TMV 7).

DNA extraction method

The total genomic DNA of leaf samples were separated using CTAB based phenol chloroform method. The quality of genomic DNA was assessed using 0.7 % agarose gel and the quantification of genomic DNA was done through UV- spectrometer (9)

PCR testing and gene amplification

Amplification of phyllody specific gene was executed for all the samples. PCR generated amplicon was confirmed and purified using GeneJET PCR purification Kit (Rhermoscientific, Eu-Luthinana) to remove the primer dimer and carryover contaminations. The quality of the product was evaluated using 1.8 % agarose gel along with 100 bp DNA ladder. The universal primer pair sequences for detecting phytoplasma are furnished below.

Primer sequences

Primer	Sequence
P1	AAGAGTTTGATCCTGGCTCAGGATT
P7	CGTCCTTCATCGGCTCTT
R16 F2n	GAAACGACTGCTAAGACTGG
R16R2	TGACGGGCGGTGTGTACAACCCCG

Phytochemical quantification in the phyllody resistant mutants

The phytochemicals *viz.,* total phenol, tannin, alkaloid and flavonoid contents were quantified in the 7 asymptomatic plants and 2 symptomatic plants from resistant family (PR 375) and five asymptomatic healthy plants of control. Phenolic compounds of sesame leaves were extracted and estimated following Folin- Ciocalteus spectrophotometric method (10). The results were expressed in mg of gallic acid equivalents (GAEs) per gram DW (mg GAEs g^{-1} DW). The tannin content was assessed using Folin-Ciocalteu and sodium carbonate solution following the spectrophotometric method (11) and values were expressed as mg of tannic acid equivalents (TAEs) per g DW (mg TAEs g^{-1} DW). The alkaloid was quantified following the gravimetric method (12) and indicated in mg g^{-1} DW. The flavonoid compounds of dry sesame leaves were quantified by the aluminium chloride spectrophotometric method (13). The results were expressed as mg of quercetin equivalents (QUEs) per g DW of extract (mg QUEs g^{-1} DW).

Results and Discussion

Effect of irradiation

The repercussions of gamma rays on phenotypic expression in M_1 generation of sesame were analysed and expressed in percentage reduction over control. (Table 1). For survival (%) on 30 DAS, VRI 3 variety is highly sensitive to gamma rays and mortality rate is higher than other varieties in all doses and the maximum value of (%) reduction (78.81) for survival was recorded in 50 kR of VRI 3 sesame variety and for the trait plant height on maturity 40 kR of CO 1 recorded highest value (16.86). For the traits number of primary branches per plant and single plant yield the highest values for percentage reduction over control were observed in 50 kR of VRI 3 as 30.0 % and 41.53 % respectively. 40 kR of TMV 7 recorded highest percentage reduction over control for the trait number of capsules per plant. Inhibitory effect of irradiation on germination (%) is due to alteration in cytochrome oxidase content, destruction of cell organelles at molecular level and altered enzyme activity. Reduced shoot length which might have been due to

Table 1. Effect of different dosages of gamma irradiation on M_1 generation in sesame

Characters	TMV 7				CO 1				VRI 3				CD @ 1%
	Control	30 kR	40 kR	50 kR	Control	30 kR	40 kR	50 kR	Control	30 kR	40 kR	50 kR	
Survival (%) on 30 DAS	85	48.6** (42.82)	41.3** (51.41)	39** (54.11)	89	65** (26.96)	53.6** (39.77)	37.6** (57.7)	90	21* (76.6)	20** (76.6)	19** (78.8)	5.95
Days to 50 % flowering	37.6	41** (9.04)	42** (11.70)	41** (9.04)	40	43** (7.5)	44** (10)	44** (10)	38	39.5** (3.9)	41** (7.8)	42** (10.5)	0.81
Plant height on maturity (cm)	127	121 (4.7)	121 (4.7)	115** (9.44)	166	139** (16.26)	138** (16.86)	153** (7.83)	105	104 (0.09)	104 (0.04)	103 (1.9)	11.76
Number of primary branches per plant	4	4	3.5** (12.5)	3.5** (12.5)	5	4.5** (10)	4.5 (10)	4.5 (10)	5	4** (20)	4** (20)	3.5* (30)	0.81
Number of capsules per plant	69.6	64.3 (7.6)	46** (33.9)	60.6 (12.93)	79.5	63** (20.7)	67 (15.72)	68 (14.46)	51	46 (9.8)	48 (5.8)	54 (13.72)	13.4
SPY (g)	10.1	8.9 (11.88)	7.8 (22.77)	6.5 (35.64)	12.5	10.5 (10)	10.2 (18.4)	9.5 (24)	6.5	4.5 (30.7)	4.2 (35.3)	3.8** (41.53)	2.5
Phyllody infestation (%)	45.6	38.5 (15.57)	34.9** (23.46)	44.2 (3.07)	44.5	35.06** (21.21)	37.4 (15.95)	36.6 (43.6)	58.8	46.3** (21.2)	48.5** (17.51)	48.2** (18.02)	7.52

detrimental effect on physiological system and growth hormones. Similar results were obtained in an earlier research investigation (14) in M_1 generation of sesame due to sodium azide. As for the phyllody infestation is concerned all the treatments recorded lower incidence than the respective controls.

Screening for phyllody resistance in M_2 and M_3 generation

A spectrum of mutants such as chlorophyll mutants (Albina, Chlorina, Xantha) and plant type mutants observed in M_2 generation are given in the Table 2. Thirteen chlorophyll mutants (1 albina, 8 chlorina, 4 Xantha) four plant type mutants were observed in M_2 generation (Fig. 2 a-b, 3). The frequency of total number of induced mutants was maximum in 40 kR of CO 1 (3.06×10^{-3}). The frequency of macro mutations did not necessarily increase with increase in doses of radiation. The results on spectrum of mutants are in harmony with the results of previous research studies (14, 15). The observations recorded on phyllody infestation in M_2 generation are furnished in the Table 3. A total number of 132 phyllody free plants were selected and tagged as phyllody resistant. Similarly, based on the visual scoring (16) identified seven genotypes with phyllody resistance after evaluating 150 genotypes, 32 cultivars in natural condition. For all the three varieties, the percentage of phyllody infestation was high in control when comparing the segregants. The highest and lowest percentage of phyllody infestation was recorded in VRI 3 control (98.43 %) and VRI 3 - 50 kR (90.69 %) respectively. In M_3 generation based on the phyllody infestation 18 families were recorded as resistant (< 10 % phyllody infestation), 32 families were recorded as tolerant (11 to 20 % phyllody infestation), 57 families were recorded as susceptible (21 to 30 % phyllody infestation), 25 families were recorded as highly susceptible (31 to 40 % phyllody infestation (Table 4).

Validation through markers in M_4 generation

In M_4 generation, based on the phyllody infestation (%) on 70th day, only one family *ie*. PR 375 recorded as tolerant family with the score of 16.06^{a} (23.62) and four families *viz*., PR 124 (20.01^{ab}), PR 159 (21.65 ^{abc}) PR 197 (28.13^{cd}) and PR 211 (27.865^{bcd}) recorded as moderately tolerant against phyllody (Table 5). To ensure that the plants did not show phyllody symptoms are due to genotypic resistance only, the presence of phyllody causing phytoplasma in molecular level is also assessed. The phyllody causing phytoplasma is successfully detected by the nested polymerase chain reaction (PCR) assay by using the universal primer pairs P1/P7 and R16 F2/R16R2 as marker. The presence of phyllody causing phytoplasma in resistant plants was confirmed by the appearance of amplified bands in the gene specific (P1/P7) amplification (Fig. 4). The gene specific P1/ P7, R16 F 2n) R16 R2) bands were documented for the samples of plant number PR 375-1 to PR 375- 7 (asymptomatic plants) and plants PR 375-8 & PR 375-9 (symptomatic plants). Presence of bands for the plants PR 375-8 and PR 375-9 is due to the presence of phyllody causing phytoplasma in the symptomatic plants, whereas the plants of TMV 7 control (lane 11, 13 to lane 17) showed no amplification for P1/P7 gene which confirms the escapism of the host plant from the vector. Phytoplasmas may be present in plant system without producing any symptoms (17) and this condition is due to tolerant/resistant reaction of plants (18).

Phytochemical quantification in asymptomatic mutants

The estimation of secondary metabolites (phytochemicals) in the M_4 generation reveals that all the 7 asymptomatic plants which are all confirmed for the presence of phytoplasma through DNA amplification having elevated level of secondary metabolites (phytochemicals). The mutant PR 375-3 recorded the highest (1.82) of total flavonoids whereas the mutant PR 375-7 recorded the highest level of total phenols (11.85) (Table 6). The mutant PR 375-4 and PR 375-7 recorded highest level of total alkaloids (5.29) and total tannins (0.29) respectively. The mutants PR 375-8 and PR 375-9 recorded lower levels of secondary metabolites which are on par with the control. Polyphenols are well-known antimicrobial metabolites involved in defence mechanisms of plant system, which will give signals to activate plant defence genes (19). A high level of polyphenols in plants after infection reflects the host's response to phytoplasma infection and their increased accumulation could be related to the defence mechanisms of the host (20). The increased levels of phenols and other phytochemicals in phytoplasma-infected mutants in our study may be related to the phytoplasma-induced biosynthesis of L-phenylalanine and hydroxycinnamic acid (21).

Table 2. Spectrum and frequency of induced macro mutants in M_2 generation of sesame

Varieties	Treatment dosages	Total number of plants	Number of mutants identified				Total number of mutants and its frequency	
			Chlorophyll mutants			Plant type mutants	Mutants	Frequency
			Albino	Chlorina	Xantha			
TMV 7	**30 kR**	1481	-	1	-	1	2	1.35×10^{-3}
	40 kR	1774	-	2	-	1	3	1.69×10^{-3}
	50 kR	1522	-	2	-	-	2	1.31×10^{-3}
CO 1	**30 kR**	1612	-	-	-	-	-	-
	40 kR	1307	1	1	2	-	4	3.06×10^{-3}
	50 kR	1859	-	-	-	1	1	5.37×10^{-4}
VRI 3	**30 kR**	1174	-	-	1		1	8.51×10^{-4}
	40 kR	2226	-	2	1	1	4	1.79×10^{-4}
	50 kR	1221	-	-	-	-	-	-

Fig. 2a. Chlorophyll mutant 1.

Fig. 2b. Chlorophyll mutant 2.

Table 3. Observations recorded on phyllody infestation in M_2 generation

Variety	Treatment	Total number of infected plants	Total number of plants	% of phyllody infestation	% of plants free from phyllody
CO 1	Control	1319	1356	97.27	2.72
	30 kR	1481	1561	94.87	5.12
	40 kR	1774	1865	95.12	4.8
	50 kR	1522	1603	94.94	5.05
TMV 7	Control	1902	1952	97.43	2.56
	30 kR	1612	1700	94.82	5.17
	40 kR	1307	1427	91.59	8.40
	50 kR	1859	1963	94.70	5.29
VRI 3	Control	1378	1400	98.42	1.57
	30 kR	1174	1198	97.99	2.0
	40 kR	1226	1344	91.22	8.77
	50 kR	1121	1236	90.69	9.3

Fig. 3. Plant type mutant.

Table 4. Screening for phyllody resistance in the mutant families of M_3 generation

Sl No	Pedigree	Total number of plants	Number of plants infested with phyllody	% of phyllody infestation	Disease reaction
1	**CO 1 Control**	**42**	**24**	**57.14**	**HS**
2	CO 1 30 kR -17	65	14	21.54	S
3	CO 1 30 kR -34	24	2	8.33	R
4	CO 1 30 kR -35	83	15	18.07	T
5	CO 1 30 kR -50	20	6	30.0	S
6	CO 1 30 kR -52	52	13	25.0	S
7	CO 1 30 kR -61	31	10	32.26	HS
8	CO 1 30 kR -62	38	8	21.05	S
9	CO 1 30 kR -63	40	12	30.0	S
10	CO 1 30 kR -64	47	10	21.28	S
11	CO 1 30 kR -65	44	8	18.18	T
12	CO 1 30 kR -66	75	19	25.73	S
13	CO 1 30 kR -67	62	7	11.29	T
14	CO 1 30 kR -68	28	15	53.57	HS
15	CO 1 30 kR -69	71	6	8.45	R
16	CO 1 30 kR -70	48	9	18.75	T
17	CO 1 30 kR -71	71	9	12.68	T
18	CO 1 30 kR -72	55	8	14.55	T
19	CO 1 30 kR -73	41	4	9.75	R
20	CO 1 30 kR -74	68	19	27.94	S
21	CO 1 30 kR -75	38	3	7.89	R
22	CO 1 30 kR -76	68	16	23.53	S
23	CO 1 30 kR -79	24	6	25.0	S
24	**TMV 7 Control**	**58**	**39**	**67.24**	**HS**
25	TMV 7 30 kR -101	32	08	25.0	S
26	TMV 7 30 kR -102	28	9	32.14	HS
27	TMV 7 30 kR -103	40	6	15.0	T
28	TMV 7 30 kR -105	70	21	30.0	S
29	TMV 7 30 kR -108	30	12	40.0	HS
30	TMV 7 30 kR -109	36	6	16.67	T
31	TMV 7 30 kR -110	21	9	42.86	HS
32	TMV 7 30 kR -113	28	7	25.0	S
33	TMV 7 30 kR -117	46	13	28.26	S
34	TMV 7 30 kR -118	41	16	39.02	HS
35	TMV 7 30 kR -119	34	12	35.29	HS
36	TMV 7 30 kR -120	16	4	25.0	S
37	TMV 7 30 kR -121	47	14	29.79	S
38	TMV 7 30 kR -122	32	8	25.0	S
39	TMV 7 30 kR -123	45	17	37.78	HS

Sl No	Pedigree	Total number of plants	Number of plants infested with phyllody	% of phyllody infestation	Disease reaction
40	TMV 7 30 kR-124	39	3	7.69	R
41	TMV 7 30 kR-125	68	19	27.94	S
42	TMV 7 30 kR-126	35	8	22.86	S
43	TMV 7 30 kR-127	11	3	27.27	S
44	TMV 7 30 kR-131	68	21	30.88	HS
45	TMV 7 30 kR-133	45	16	35.56	HS
46	TMV 7 30 kR-138	20	5	25.0	S
47	TMV 7 30 kR-141	51	12	23.53	S
48	TMV 7 30 kR-143	40	10	25.0	S
49	TMV 7 30 kR-144	35	5	14.29	T
50	TMV 7 30 kR-145	44	14	31.82	HS
51	TMV 7 30 kR-147	32	4	12.5	T
52	TMV 7 30 kR-148	35	12	34.29	HS
53	TMV 7 30 kR-149	39	10	25.64	S
54	TMV 7 30 kR-150	25	9	36.0	HS
55	TMV 7 30 kR-151	43	13	30.23	HS
56	TMV 7 30 kR-153	57	10	17.54	T
57	TMV 7 30 kR-156	47	7	14.89	T
58	TMV 7 30 kR-158	13	3	23.08	S
59	TMV 7 30 kR-159	32	3	9.37	R
60	TMV 7 30 kR-161	47	15	31.91	HS
61	TMV 7 30 kR-165	34	9	26.47	S
62	TMV 7 30 kR - 183	27	6	22.22	S
63	TMV 7 30 kR -184	39	10	25.64	S
64	TMV 7 30 kR -187	45	6	13.33	T
65	TMV 7 30 kR -188	67	14	20.90	S
66	TMV 7 30 kR -191	50	5	10	R
67	TMV 7 30 kR -192	35	11	31.43	HS
68	TMV 7 40 kR -196	35	3	8.57	R
69	TMV 7 40 kR -197	17	5	29.41	S
70	TMV 7 40 kR -198	61	16	26.23	S
71	TMV 7 40 kR -199	65	15	23.08	S
72	TMV 7 40 kR -200	30	12	40	HS
73	TMV 7 40 kR -203	50	14	28	S
74	TMV 7 40 kR -205	7	7	100	HS
75	TMV 7 40 kR -207	29	4	13.79	T
76	TMV 7 40 kR -209	40	8	20.0	T
77	TMV 7 40 kR -211	42	4	9.52	R
78	TMV 7 40 kR -214	60	20	33.33	HS

Sl No	Pedigree	Total number of plants	Number of plants infested with phyllody	% of phyllody infestation	Disease reaction
79	TMV 7 40 kR -215	45	12	26.67	S
80	TMV 7 40 kR -217	50	13	26.0	S
81	TMV 7 40 kR -222	38	9	23.68	S
82	TMV 7 40 kR -223	27	6	22.22	S
83	TMV 7 40 kR -224	36	3	8.33	R
84	TMV 7 40 kR -229	27	6	22.22	S
85	TMV 7 40 kR -234	60	6	10.0	R
86	TMV 7 50 kR-296	36	10	27.78	S
87	TMV 7 50 kR-299	38	7	18.42	T
88	TMV 7 50 kR-302	30	3	10	R
89	TMV 7 50 kR-303	40	8	20	T
90	TMV 7 50 kR-304	17	5	29.41	S
91	TMV 7 50 kR-310	43	6	13.95	T
92	TMV 7 50 kR-312	8	2	25	S
93	TMV 7 50 kR-313	74	13	17.57	T
94	TMV 7 50 kR-314	63	8	12.7	T
95	TMV 7 50 kR-315	50	18	36.	HS
96	TMV 7 50 kR-318	82	24	29.27	S
97	TMV 7 50 kR-320	50	6	12.0	T
98	TMV 7 50 kR-322	17	6	35.29	HS
99	TMV 7 50 kR-324	60	17	28.33	S
100	TMV 7 50 kR-325	29	2	6.89	R
101	TMV 7 50 kR-327	51	8	15.69	T
102	TMV 7 50 kR-330	31	7	22.58	S
103	TMV 7 50 kR-331	32	3	8.37	R
104	TMV 7 50 kR-333	55	19	34.55	HS
105	TMV 7 50 kR-335	49	8	16.33	T
106	TMV 7 50 kR-337	50	9	18.0	T
107	TMV 7 50 kR-342	25	7	28.0	S
108	TMV 7 50 kR-344	48	10	20.83	S
109	TMV 7 50 kR-346	30	6	20	T
110	TMV 7 50 kR-347	16	3	18.75	T
111	TMV 7 50 kR-348	10	2	20	T
112	TMV 7 50 kR-349	38	3	7.89	R
113	TMV 7 50 kR-350	41	10	24.39	S
114	TMV 7 50 kR-352	8	2	25.0	S
115	TMV 7 50 kR-353	22	5	22.73	S
116	TMV 7 50 kR-358	55	16	29.29	S
117	TMV 7 50 kR-360	25	9	36.0	HS
118	TMV 7 50 kR-363	43	7	16.28	T
119	TMV 7 50 kR- 365	33	6	18.18	T
120	TMV 7 50 kR-366	47	3	6.38	R
121	TMV 7 50 kR-368	11	6	54.55	HS
122	TMV 7 50 kR-372	28	6	21.43	S
123	TMV 7 50 kR-374	63	11	17.46	T
124	TMV 7 50 kR-375	34	2	5.88	R
125	TMV 7 50 kR-377	40	3	7.5	R
126	TMV 7 50 kR-378	36	4	11.11	T
127	TMV 7 50 kR-380	42	9	21.43	S
128	TMV 7 50 kR-383	52	11	21.15	S
129	TMV 7 50 kR-387	36	8	22.22	S
130	TMV 7 50 kR-390	25	8	32.0	HS
131	TMV 7 50 kR-391	45	3	6.67	R
132	TMV 7 50 kR-394	52	14	26.92	S
133	TMV 7 50 kR-395	33	7	21.21	S
134	TMV 7 50 kR-398	45	6	13.33	T

Table 5. Screening for phyllody resistance in the mutant families of M_4 generation

Sl No	Mutant family	Pedigree	Phyllody infestation (%)
1	PR 75	CO 1 50 kR	32.23 ^{de} (34.76)
2	PR 95	TMV 7 - 30 kR	34.69 ^{def} (36.03)
3	PR 124	TMV 7 – 30 kR	20.01 ^{ab} (26.53)
4	PR 158	TMV 7 – 40 kR	38.05 ^{efg} (38.06)
5	PR 159	TMV 7 – 30 kR	21.69 ^{abc} (27.67)
6	PR 196	TMV 7 – 40 kR	48.75 ^{hij} (45.02)
7	PR 197	TMV 7 – 40 kR	28.13 ^{cd} (31.97)
8	PR 211	TMV 7 – 40 kR	27.86 ^{bcd} (31.86)
9	PR 216	TMV 7 – 40 kR	48.73 ^{hij} (44.27)
10	PR 302	TMV 7 – 50 kR	41.40 ^{efgh} (40.03)
11	PR 312	TMV 7 – 50 kR	46.62 ^{ghi} (43.05)
12	PR 325	TMV 7 – 50 kR	54.90 ^{ij} (47.82)
13	PR 352	TMV 7 – 50 kR	47.22 ^{ghij} (43.38)
14	PR 353	TMV 7 – 50 kR	66.6 ^{k} (54.71)
15	PR 375	TMV 7 – 50 kR	16.06 ^{a} (23.62)
16	PR 377	TMV 7 – 50 kR	53.44 ^{ij} (47.0)
17	PR 378	TMV 7 – 50 kR	47.81 ^{hij} (43.74)
18	PR 391	TMV 7 – 50 kR	42.64 ^{fgh} (40.76)
19	Check	TMV 7	56.42 ^{j} (48.69)
20	Check	CO 1	55.815 ^{ij} (48.34)
		C.D @ 0.05 % P	**5.38**
		CV %	**6.45**

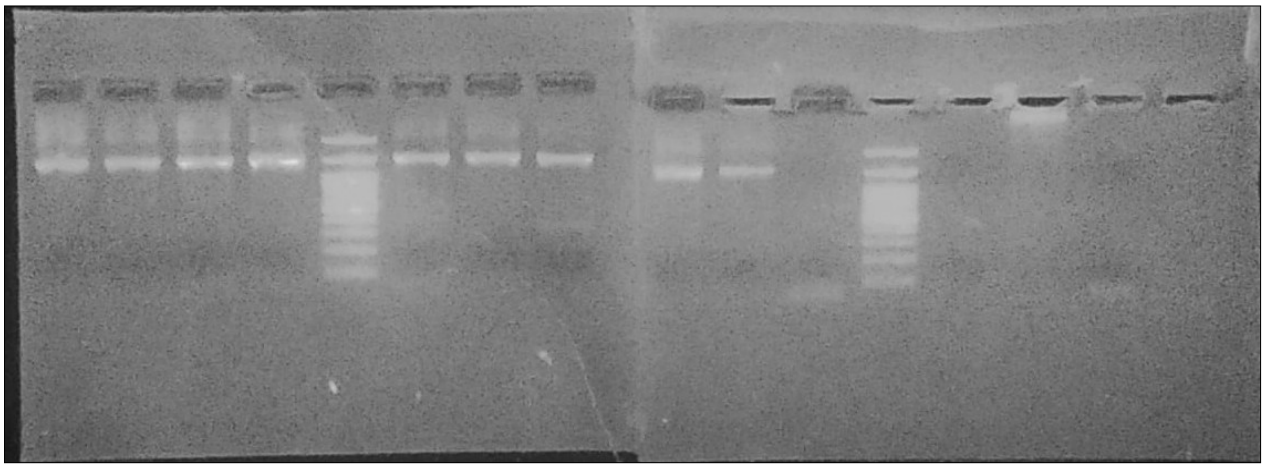

Fig. 4. Marker validation.

Lane 1:PR 375-1, 2:PR 375-2, 3:PR 375-3, 4: PR 375-4, 6: PR 375-5, 7: PR 375-6, 8: PR 375-7, 9: PR 375-8, 10: PR 375-9, 11: TMV 7 control-1. 13: TMV 7 control-2, 14: TMV 7 control 3, 15: TMV 7 control -4, 16: TMV 7 Control -5. Lanes 5 & 12 100 bp ladder

Conclusion

Through the present study on mutation in sesame seven phyllody resistant sesame mutants (PR 375 -1, PR 375-2, PR 375 -3, PR 375-4, PR 375-5, PR 375-6 and PR 375-7) were developed. The presence of resistant gene(s) in the mutants was confirmed through Polymerase Chain Reaction (PCR) validation for the universal primer pairs P1/P7 and R16 F2/R16R2. The phytochemical analysis in the mutants also confirmed the presence of the elevated levels of phenols, alkaloids, flavonoids and tannins which is evident for signalling the activation of the resistant mechanism against phyllody causing phytoplasma. The mutants evolved in M_4 generation will be forwarded to subsequent generations for further genetic utilisation.

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Authors' contributions

MJ carried out the mutational studies in all the generations and collected data. MT did the screening for phyllody resistance in M_2 and M_3 generations. JH and CP carried out the data analysis work in mutation studies. KM, JR and SK carried out the screening for phyllody resistance in M_4 generation and PCR validation. SG carried out critical and intellectual reviews. All the authors read and agreed with the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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