

Association analysis and evaluation of sources for resistance to phyllody and foliar diseases in sesame (*Sesamum indicum* L.)

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ABSTRACT

In the present investigation, a set of advanced mutant and breeding lines (133) including checks from different geographical regions were evaluated against phyllody and *Alternaria* leaf spot during *kharif*, 2017 and against powdery mildew during *summer*, 2018 under natural epiphytotic field conditions. Among the entries, two genotypes (LW-2 and SDSN-15-98) were found to be promising for phyllody during *kharif*, 2017 whereas, the genotype LW-2 was also found to be moderately resistant against powdery mildew during *summer*, 2018. None of the genotypes were promising against *Alternaria* leaf spot. The association of agronomic traits namely plant height, thousand seed weight, number of branches/plant, number of capsules/plant was correlated negatively significant with diseases. The genotype LW-2 identified promising against phyllody and powdery mildew will be useful in hybridization programme as donor parent for development of multiple disease resistant sesame varieties.

Key words: Phyllody, Powdery mildew, *Alternaria* leaf spot, Field screening, Correlation

Introduction

The sesame (*Sesamum indicum* L.) seed is known as the "Queen of Oilseeds". It is commonly known as gingelly, til and vernacular name is *nuvvulu*. It is a major and ancient oil seed crop known to man due to its simple extraction procedure, high stability, and drought resistance. India, China, Sudan, Mexico, Turkey, Burma, and Pakistan are some of the major sesame growing countries. Sesame seed contains 50-

60% oil and 19-25% protein, along with two lignans, sesamin and sesamolin. These lignans prevent rancidity, prolonging the shelf life of sesame oil and have physiological effects that seem to be advantageous to both human and animal health (Ashakumary *et al.*, 1999). Sesame oil contains important unsaturated fatty acids like oleic acid and linoleic acid, each about 40%. Farmers have given sesame less attention, as it is showing poor yield due to a lack of high yielding cultivars which suit the

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diverse agro-climatic conditions, and also its susceptibility to biotic and abiotic stresses. Diseases such as phyllody, powdery mildew, and *Alternaria* leaf spot were indeed important biotic stresses since they cause significant yield losses in farmer's fields. Sesame yield losses due to major diseases have been reported to be approximately 53.5 % in total (Kumar and Mishra, 1992; Gupta *et al.*, 2018).

Use of host plant resistance is the most feasible and economical means of managing the phyllody, powdery mildew, *Alternaria* leaf spot in sesame, as the crop is grown by resource poor and marginal farmers, who cannot afford the expensive chemicals and it is a second alternative crop during summer season in Telangana state. Several sources of resistance to phyllody like *Sesamum Mulayanum*, *Sesamum prostratum* and SVPRI (Mehetre *et al.*, 1993); TH-6, JLT-408, YLM-66, RT-223, GT-10, AKT-101, Chandana, TNAU-11, 22, Naveli-5, Cultivar PhauleTil No.1, B67, HT-12, HT-16, Jabalpur local (Kaushik *et al.*, 1986); KAU-05-2-12, PC-14-2 and Kanakapura local (Mahadevaprasad *et al.*, 2017), for powdery mildew *S. malabaricum* and *S. mulayanum*, VRI-1, Co-1, T-12, N-32, SSD-4, SSD-7, SSD-19, SSD-20 (Mallaiah *et al.*, 2016), RT 54 (Reddy and Haripriya, 1990); TKG-22, NSKMS-260 and G-55 (Rao *et al.*, 2013) and for *Alternaria* No.1, JT-7, No.2 and E-8 (Jayaramaiah *et al.*, 1981); RT-273, *Sesamum radiatum*, *Sesamum prostratum*, *Sesamum mulayanum* (Naik *et al.*, 2003); Navile-1, TC-28, Madhavi, Tarikere local (Basavaraj *et al.*, 2007); S-122 (Marri *et al.*, 2012); IVT-14-10, IVT-14-11 (Pawar *et al.*, 2013) were reported. Besides the available resistant sources, inducement of wider range of genetic variation through mutations is a logical or appropriate approach as it causes memorized altered gene expression by epigenetic modifications, creating an opportunity of obtaining novel traits enriching the crop germplasm base and also helps in conserving the biodiversity by stopping gene erosion (Suprasanna *et al.*, 2015). The mutants with high yield and disease resistance can be directly used as a variety or can be used as a donor in the hybridization programme (Sarwar and Akhtar, 2009) like Uma (for yield), Usha (for yield), Kalika (for yield and disease resistance) which are the mutant sesame varieties released in India (Bhatia *et al.*, 1999). However, non-availability of multiple resistant sources or donors against key diseases of sesame could be one of the major hinderances to sesame breeders as well as farmers. With this objective in view, the current

study was aimed to identify the sources of multiple disease resistance in a set of genotypes consisting of mutants, advanced breeding lines along with checks against key diseases of sesame that could be utilized in disease resistant breeding programmes as donors.

Material and Methods

A total of 133 genotypes were evaluated at the Regional Agricultural Research Station in Polasa, Jagtial, Telangana State, including checks, TKG-22 (Natioanl level), Pragathi (Zonal level) and Swetha til (Local level). The seed material comprising of 37 advanced mutant breeding lines, 30 germplasm lines, 15 local cultivar lines and 4 RILs obtained from University of Agriculture Sciences, Raichur and remaining material containing germplasm lines (20), genotypes from IVT *kharif*-2016 (10), AVT *kharif*-2016 (2), IVT *summer*-2017 (2), MLT *summer*-2017 (4), popular varieties (5), local cultivar line (1) and checks (3) were received from AICRP on Sesame, RARS, Polasa, Jagtial, Telangana State. During *kharif*, 2017 the material was screened for phyllody and *Alternaria* leaf spot under natural epiphytotic conditions whereas, the same set of entries were again screened for powdery mildew during *summer*, 2018. This is due to the weather conditions are highly conducive for phyllody and *Alternaria* leaf spot during *kharif* season whereas, powdery mildew during *summer* season at RARS, Jagtial.

Field screening

The standard infector row technique was used for material screening. The experimental study was conducted in Randomized Complete Block Design (RCBD) with three replications following 30 cm inter-row and 10 cm intra-row spacing in 2 rows of 2 m length in both seasons (*kharif*, 2017 and *summer*, 2018). The evaluating material was sown in such a way that two rows of susceptible check (Swetha til) is repeated for every 10 test entries following infector row method. Sufficient disease pressure is obtained without any artificial inoculation due to highly favourable weather conditions during both the seasons. Screening done in field situations at adult stage is most widely practiced as it reflects the resistance shown by plants under actual field conditions (Kumar and Banga, 2017). For seven quantitative characters (days to 50% flowering, days to maturity, plant height, number of branches/plant, number of capsules/plant, and seed yield/plant),

data were collected from five randomly selected plants in each genotype in each replication. Diseases were scored based on the scale on the entire row. The same set of material was also evaluated during *summer*, 2018 against powdery mildew. Analysis of the data was done using WINDOSTAT statistical package.

Scale used for foliar diseases (Powdery mildew and *Alternaria* leaf spot)

The severity of foliar diseases was estimated based on relative portion of the plant tissue infected according to the 0-5 rating scale (Sherwood and Hagedorn, 1958; Kushwaha and Kaushal, 1970) and per cent disease index (PDI) where, 0 = 0% infected leaf area as immune, 1=1-10% infected leaf area as resistant, 2=10-25% infected leaf area as moderately resistant, 3=25-50% infected leaf area as moderately susceptible, 4=50-75% infected leaf area as susceptible, and 5=75-100% infected leaf area as highly susceptible. The genotypes were categorised by calculating the percent disease index using the formula below (Wheeler, 1969).

$$\text{PDI} = \frac{\text{Sum of numerical ratings}}{\text{No. of leaves/ plants observed} \times \text{Maximum disease grade}} \times 100$$

Scale used for phyllody

The phyllody incidence was calculated by counting the number of infected plants out of the total number of plants in a row for every genotype. The genotypes were categorised into various reaction groups based on disease incidence (Sherwood and Hagedorn, 1958; Kushwaha and Kaushal, 1970), where immune = 0% infected plants, resistant = 1 - 10% infected plants, moderately resistant = 10 - 25% infected plants, moderately susceptible = 25 - 50% infected plants, susceptible = 75 - 100% infected plants. The reaction shown by each genotype was estimated using a formula, and the material was grouped into various reaction categories.

$$\text{Phyllody incidence (\%)} = \frac{\text{Number of phyllody infected plants}}{\text{Total number of plants}} \times 100$$

Chi-square and correlation

The segregation for resistance and susceptible reaction in genotypes was calculated using the chi-square test: $\chi^2 = (O - E)^2 / E$, where 'E' is the expected value and 'O' is the observed value (Zuki *et al.*, 2020), with the significance set at 5% ($P < 0.05$).

al., 2020), with the significance set at 5% ($P < 0.05$).

Correlation was conducted in order to determine the degree of relationship between yield attributes and diseases at both, phenotypic and genotypic levels employing Falconer's formulae (1981). Test of significance for the correlation coefficients were compared with the estimated values at 5 per cent and 1 per cent level of significance with (n-2) degrees of freedom.

Results and Discussion

The experiment location could be assumed a "hot spot" for evaluating sesame genotypes against phyllody, powdery mildew, and *Alternaria* leaf spot as the weather patterns favour disease havoc, limiting artificial inoculation in both seasons. This allowed the screening purely under natural epiphytotic conditions and majority of the genotypes were found susceptible to phyllody during *kharif*, 2017. The severity of disease is determined not only by prevailing climatic conditions, but also by local non-climatic epidemiologic and ecological factors (Cagirgan *et al.*, 2013). The severe incidence of phyllody during late *kharif*, 2017 may be due to presence of wild host as well as weeds in neighbouring fields under high rainfall situations, as the pathogen was capable of transmitting to healthy plants despite the low vector population (Chakraborty *et al.*, 2000). Therefore, the vector (leafhopper) population has no significant effect or influence on disease severity (Murugesan *et al.*, 1973; Abraham *et al.*, 1977), as evidenced by the fact that a single leafhopper from the inoculum outside the field could have inoculated a number of plants (Tan, 2010).

During first screening season, *kharif* 2017, two genotypes (SDSN-15-98 and LW-2) were observed showing resistance reaction to phyllody with a per cent incidence of 6.33 and 9.83, respectively, and seven genotypes managed to show moderately resistant reaction with an incidence range of 11-25 % for phyllody. The findings from the present study were in line with the results of previous researchers using limited number of entries (Rajeswari *et al.*, 2010) while our study was done in a comprehensive manner representing large number of entries (Akthar *et al.*, 2013).

During *kharif*-2017, none of the entries were found to be resistant to *Alternaria* leaf spot, and there is sight of combined resistance for all diseases among the genotypes, as well as the appearance of a

combination of symptoms (Min and Toyota, 2019). So, there is a large scope to explore the wild relatives for identification of resistance to *Alternaria* leaf spot. The incidence of powdery mildew was negligible during *kharif*, 2017 due to high rainfall prevailed during that period. The same set was evaluated against powdery mildew during *summer*, 2018 which is highly favourable for occurrence of powdery mildew. During *summer*-2018, ten genotypes (V-72, IISL-4, 10KRE8-2, 30KRDS-1-14, TKG-22, SDSN-15-70, SDSN-15-99, RT-376, 30KRDS-1-13, 30KRDS-1-7) were found to be resistant to powdery mildew with PDI ranging from 0-10 per cent, whereas 29 genotypes were moderately resistant with PDI ranging from 10.1-25 per cent. On the basis of results obtained from screening, the genotype LW-2 was found to be resistant to phyllody and moderately resistant to powdery mildew under natural field conditions.

Screening from 37 advanced mutant lines revealed that only one genotype (30KRDS-1-8, 14%) was observed showing moderately resistant reaction to phyllody with PDI less than 20%. Similar screening reactions in mutant lines such as moderate resistance reaction was observed by Mahalaxmi (2018), while Sarwar and Haq (2006) observed immune to moderately resistant reaction and resistant to moderately resistant reaction was reported by Akthar *et al.* (2013). During the *kharif* season of 2017, all the advanced mutant breeding lines were found to be susceptible to *Alternaria* leaf spot due to highly favourable weather conditions for leaf spot, which could result in a susceptible reaction in all of the tested genotypes.

When the same set of advanced mutant lines were screened against powdery mildew during the *summer* of 2018, ten genotypes (50KRE8-3, 30KRDS-1, 30KRDS-1-2, 30KRDS-1-3, 30KRDS-1-6, 30KRDS-1-18, 30KRDS-1-20, 30KRDS-1-28, 30KRDS-1-28, 30KRDS-1-29, and 30KRDS-1-31) evinced moderate resistance response with PDI ranging between 12-24 per cent. The majority of these genotypes also have

desirable agronomic traits and thus have the potential to be used in sesame improvement programmes aimed at developing high yielding and disease resistant varieties.

Genetic ratio of goodness of fit for advanced mutant lines

Visual symptoms determine the resistance reaction, which can be supported by knowledge of resistance gene inheritance or the role of each gene contributing to resistance or susceptible reaction (Rajput and Raghuvanshi, 2017).

The advanced mutant lines in the present study were fit into a ratio of 3:1 (susceptible: resistance) for powdery mildew ($p < 0.05$), signifying that susceptibility was considered dominant over resistance reaction giving an idea that resistance reaction was governed by a single recessive gene which is according to the view of Buschges *et al.* (1997). Sravani *et al.* (2012) and Ramana Rao *et al.* (2012) reported similar reports of resistance governed by recessive alleles, demonstrating complementary gene action (9:7), *i.e.*, resistance was governed by two recessive genes. For the purposes of calculating the ratio, immune, resistant, and moderately resistant reactions were considered as resistance group, whilst moderately susceptible and highly susceptible reactions were considered as susceptible group (Table 1).

The goodness of mutant lines for phyllody didn't quite fit into any genetic ratio or gene action. The experiment results contradict previous findings by Shinde *et al.* (2011), Rajput and Raghuvanshi (2017), who reported 9:7 ratio indicating complementary gene action or a single recessive gene resistance by Parani *et al.* (1996). This could be due to differences in disease rating methodology, screening methodology, and phyllody severity. In the present investigation natural infestation was adopted as the location of experimentation is considered as hotspot location against artificial infestation which may be the reason to cause the difference in disease rating (Rao *et al.*, 2013).

Table 1. Chi square test for goodness of fit with standard ratios against powdery mildew screening conducted during *Summer*, 2018.

Disease	Generation	Segregation						Expected ratio S:R	χ^2 value
		O		S	E				
		T	R		T	R	S		
Powdery mildew	M ₀	31	13	18	31	7.75	23.25	3:1	1.81

O-Observed, E-Expected, T-Total, R-Resistant, S-Susceptible

Correlation between diseases and agronomic traits

Alternaria leaf spot has positive correlation with phyllody ($r=0.04$, $p<0.05$) showing, both the diseases together having larger effect on yield (Table 2). Under favourable conditions, powdery mildew has appeared at the active vegetative to flowering stage but later was negligible during first season (*kharif*, 2017) due to unfavourable weather conditions and weak inoculum (Divya, 2018). Keeping the results in view, the same set of genotypes were again evaluated during *summer*, 2018 for identification of resistance sources against powdery mildew. The genotypes exhibited wide range of diversity for different agronomic traits. Especially genotypes have shown significant negative correlation between diseases (phyllody and *Alternaria* leaf spot) and yield ($r= -0.18^{**}$ and $r= -0.26^{**}$) indicating that with the severity of diseases there will be a decrease in the yield of the crop. When compared to *Alternaria* leaf spot, the phyllody disease has a very low significant negative relationship with yield. This can be explained by the fact that the disease surfaced at the mid-growth stage, by that time interval plants had attained plant height and capsules which have already reached actual genetic potentiality of genotype. Later, the incidence of leaf spot has significant impact on seed development. *Alternaria* ($r= -0.43^{**}$) has shown nega-

tively significant (Ojiambo *et al.*, 2000) and phyllody ($r=-0.03$) has shown negative relation with plant height also. This negative influence will affect the source-sink relation (Rangaswami and Mahadevan, 2001; Laxmi, 2004) by decreasing the number of internodes thereby branches and ultimately the capsules.

Days to 50% flowering has shown negative correlation with *Alternaria* leaf spot ($r=-0.03$, $p<0.05$) and days to maturity has shown positive correlation with both phyllody ($r=0.00$) and *Alternaria* leaf spot ($r=0.03$) which might be due to the prior flowering before the incidence of diseases being the main cause for the lesser influence on the maturity to some extent. However, there were contrasting responses revealing negative correlation between diseases and days to maturity (Shobharani, 1999).

Performance of genotypes which have shown resistance reaction for diseases

The promising lines from both seasons were pooled, along with their mean performances for agronomic traits and diseases, as well as yield in Table 3. The genotypes LW-2 and SDSN-15-98 have shown resistance reaction for phyllody, indicating that there was additive gene action where the trait was less controlled by the environment and the genotypes

Table 2. Correlation between yield, agronomic traits and diseases in sesame genotypes conducted under field conditions during *kharif*, 2017.

Traits		DFF (days)	DM (days)	PH (cm)	BPP	P/CPP	TSW (g)	Phyllody (% incidence)	ALSPDI (%)	SYP (g)
DFF (days)	G	1.00	0.88**	0.21**	0.15**	0.04	0.01	0.00	-0.03	-0.09
	P	1.00	0.87**	0.19**	0.14**	0.04	0.01	0.02	-0.02	-0.09
DM (days)	G		1.00	0.29**	0.24**	-0.02	-0.05	0.00	0.03	-0.01
	P		1.00	0.26**	0.21**	-0.01	-0.04	0.00	0.02	-0.09
PH (cm)	G			1.00	0.39**	0.11*	0.33**	-0.03	-0.43**	0.28**
	P			1.00	0.30**	0.08	0.25**	-0.02	-0.29**	0.26**
BPP	G				1.00	0.82**	0.25**	0.03	-0.15**	0.18**
	P				1.00	0.10*	0.17**	0.02	-0.21**	0.14**
P/CPP	G					1.00	0.29**	-0.03	-0.19**	0.69**
	P					1.00	0.22**	-0.06	-0.17**	0.66**
TSW (g)	G						1.00	-0.18**	-0.25**	0.59**
	P						1.00	-0.16**	-0.15**	0.47**
Phyllody (% incidence)	G							1.00	0.04	-0.18**
	P							1.00	0.03	-0.17**
ALS PDI (%)	G								1.00	-0.26**
	P								1.00	-0.21**

*- significance at 5% level **- significance at 1% level

DFF- Days to 50% flowering; DM- Days to maturity; PH- Plant height (cm); BPP- Number of branches/plant; P/CPP- Number of capsules/plant; TSW- Thousand seed weight (g); ALS- *Alternaria* leaf spot; SYP- Seed yield/plant (g)

Table 3. Mean performance of promising genotypes showing agronomic traits and diseases during *kharif*, 2017 and *summer*, 2018

S. No.	Promising lines	Kharif, 2017									Summer, 2018
		DFP (days)	DM (days)	PH (cm)	BPP	P/CPP	TSW (g)	SYP (g)	ALSPDI (%)	Phyllody (% incidence)	PMPDI (%)
1.	10KRE8-2	57	87	98.3	7.5	14.0	2.93	3.95	96.0	63.3	0
2.	30KRDS-1-14	44	76	47.2	4.5	22.0	2.23	3.08	93.0	85.0	0
3.	30KRDS-1-7	63	96	130.3	5.5	12.0	2.25	1.21	86.5	95.3	0
4.	LW-2	44	74	85.9	4.0	21.5	2.90	4.38	94.0	9.8	20
5.	V-72	50	83	113.9	3.0	19.0	2.81	3.12	74.0	85.6	0
6.	IISL-4	46	73	76.175	4.0	27.0	2.64	2.86	90.5	53.3	0
7.	TKG-22 (NC)	59	90	77.9	3.0	6.0	1.96	0.67	91.5	74.0	0
8.	SDRN-15-70	54	87	88.6	4.5	17.5	2.39	1.76	94.0	98.6	0
9.	SDRN-15-98	56	87	92.0	4.0	15.0	2.34	2.51	91.0	6.33	60
10.	SDRN-15-99	55	86	99.7	3.5	12.0	1.68	1.04	88.0	17.0	0
11.	RT-376	54	85	122.8	3.0	10.0	1.62	1.69	84.5	96.6	0
12.	Pragathi (ZC)	54	84	111.7	5.5	19.0	2.98	5.32	82.0	80.6	—
13.	Swetha til (LC)	57	112	119.5	6.5	11.0	1.08	0.56	100	88.3	—

DFP- Days to 50% flowering; DM- Days to maturity; PH- Plant height (cm); BPP- Number of branches/plant; P/CPP- Number of capsules/plant; TSW- Thousand seed weight (g); SYP- Seed yield/plant (g); ALS-*Alternaria* leaf spot; PM- Powdery mildew, (NC- national Check, ZC- Zonal Check, LC- Local Check)

showing resistance were purely innate (Divya *et al.*, 2018). LW-2 performed the best among the genotypes tested for diseases and other traits.

Days to 50% flowering of the genotype LW-2 were 44 days, which influenced days to maturity and yield directly by avoiding disease incidence at the actual reproductive stage as meteorological conditions, morphological host characters, or physiological host activities may aid in disease escape (Wingard, 1941). Even after the incidence of diseases, the number of capsules per plant was high (21) next to IISL-4 (27) with high seed yield for LW-2 as 4.38 g more than the check TKG-22 (0.22 g). The findings could be interpreted as adult plant resistance or as the plant's ability to withstand disease. This concept of disease endurance is sometimes deduced from a plant's capacity to grow in spite of the attack, either through exceptional vigour or a harder structure, like in the case of some melons, which thrive leaf-blight attacks better than ordinary melons because their leaves do not dry out as quickly (Orton, 1908). The genotype is also resistant to powdery mildew, with a PDI of 20%. Based on the screening data and agronomic traits, LW-2 is a promising genotype that needs to be evaluated further for its resistance stability at multilocation for use as a donor in breeding programmes.

Conclusion

In the present study, LW-2 was identified as a promising genetic resource for phyllody and powdery mildew as it has shown the ability to obstruct the major damage caused by disease pressure on its own. The genetic ratios also show that, in addition to physiological escape or endurance, the innate recessive genes conferred disease resistance. Furthermore, the LW-2 resistant reaction must be confirmed in a range of geographical locations across India. Once phenotypically confirmed, this line can be used as a donor parent in hybridization programmes to develop multiple disease resistant lines, which is the most efficient and cost-effective way of increasing yield. Along with phenotypic screening, molecular techniques should be used to identify QTLs and selection methods such as MAS helps aids in early detection of resistance. This effort to identify promising resistant sources in sesame ensures that breeding programmes will have a better chance of improving cultivars with a broad genetic base.

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