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Screening of Lentil Genotypes against Highly Aggressive Strain of *Fusarium oxysporum* f. sp. *lentis*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Lentil is one of the most nutritious pulse crops known as masur and grown as a rainfed crop throughout the world in winter season. It is rich in protein, starch, micronutrients and dietary fiber. In the global scenario, India ranked first in the area and second in the production with Bihar being one the major lentil growing state. The majority of the lentil crop is grown in Tal area of the state. As it is cultivated as a rainfed crop, it gets severely affected by several biotic and abiotic stresses. Among the biotic stresses, Fusaium wilt, caused by *Fusarium oxysporum* f. sp. *lentis (Fol)* is one of the major fungal diseases and remarkably causing severe crop damage from vegetative to reproductive stage producing significant yield reduction. *Fol* isolate exhibit great variability and aggressiveness based on agro- climatic conditions. AGLF-11 isolate of *Fol* collected from Tal area of Bihar was

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found to be highly aggressive based on previous studies. For this, 50 diverse genotypes were screened against this isolate under greenhouse condition, out of which 14 genotypes showed high susceptibility, 29 genotypes showed moderate susceptibility, 5 genotypes exhibited moderately resistance and only 2 genotypes (L 7920 and DPL 58) exhibited resistance reaction.

Keywords: Fusarium oxysporum; lentil; fungal diseases.

1. INTRODUCTION

Lentil (*Lens culinaris* Medikus subsp*. culinaris*), also known as masur are grown as a rainfed crop throughout the world in winter season. It is herbaceous, annual, self -pollinated pulse crop of Fabaceae family with erect / sub-erect growth habit. It is diploid (2x=2n=14) with genome size 4063 Mbp [1,2] It is rich in protein (24-32 %), starch (63 %), micronutrients and dietary fiber (11 %) [3]. It is one of the oldest domesticated pulse crops grown for over 8,000 years. This crop is believed to be originated in Mediterranean region (Turkey-Syria-Iraq region) having the position of 6th major pulse crop grown in more than 50 countries of the World, with 39.79 per cent and 22.79 per cent of the world area and production respectively [4]. India ranked first in terms of area and second in volume with Bihar being one the major lentil growing state with 0.15 m hectare area with production of 0.12 m tones having productivity of 793 kg/hectare [5]. The majority of the lentil crop is grown in Tal area of the state. Lentil can withstand harsh environmental conditions including drought and high temperature and can be cultivated in semi-arid areas without irrigation [6]. It may grow in a variety of soil types. Moreover, the crop can be cultivated in rotation with cereal crops to help reduce soil erosion, weed management, and nitrogen fertiliser requirements. Cultivation of lentil is more profitable than other crops under rain-fed conditions [7].

Production of lentil crop is constrained by a number of abiotic and biotic stresses. Abiotic factors including drought, terminal heat, and salt susceptibility cause considerable yield loss. Among biotic stresses fungal diseases are most common which greatly reduces the crop production and productivity. Among fungal diseases, ascochyta blight and fusarium wilt are major diseases which reduces its yield remarkably [8,9,10]. Wilt in lentil, caused by *Fusarium oxysporum* f.sp *lentis (Fol)*, is a one of the major cause of crop loss on every continent except Australia where lentils are produced [11,6,12]. It infects its host by entering through the root and blocking the vascular system (xylem and phloem), preventing water and nutrient transfer to the plant, causing wilting, discoloration, and eventually causing death [8]. Wilt disease occurs at both pre- emergence as well as post emergence stages. In case of post emergence infection, plants are affected at seedling stage [13,14]. If it infects during seedling to pod filling stage it can cause yield loss up to 50-78% and infection during seedling stage can cause yield loss up to 100 % [7]. The disease is primarily transmitted through contaminated soil and plant debris, where it infects the plant through the root system [15]. Disease management is mandatory to ensure the stable lentil production. Development of resistant cultivars is the most long-term and cost-effective solution to this challenge [16,17]. *Fol* isolate exhibit great variability and aggressiveness based on agro-climatic conditions. Studies for genetic diversity have been done by researcher across several countries. According to experiment conducted in lab of department of Molecular Biology and Genetic Engineering, Bihar Agricultural University Sabour, Bhagalpur in which 12 *Fol* isolates, collected from major lentil growing areas in Bihar were evaluated for its aggressiveness on two identified wilt resistant (PL-6) and susceptible (JL-3) checks. Among the 12 isolates, AGLF-11 isolate was found to be highly aggressive [18]. The released varieties exhibit variation for resistance, there hasn't been any evidence of a high level of wilt resistance. Therefore, stable sources are essential for breeding wilt resistant varieties. Keeping that in view, the present experiment was carried out to identify lentil genotypes resistant against this highly aggressive strain of *Fol* under greenhouse screening.

2. MATERIALS AND METHODS

Fifty lentil genotypes procured from various parts of country and ICARDA, Lebanon and screened against highly aggressive *F. oxysporium* f.sp*. lentis* AGLF-11 / *Fol-11* isolate collected from Mokama (Tal) area in Bihar, India during 2020-2021 crop season under controlled condition using sick soil micro pots. Screening was carried out in greenhouse of Bihar Agricultural University, Sabour (Bhagalpur), Bihar, India. The

effect of this isolate on pre- and post-germination mortality of these 50 genotypes was studied. The genotypes used in this experiment along with their origin are enlisted in Table 1. Screening for lentil wilt resistance must account two factors first is the varying time of symptom development among genotypes and other is uneven and patchy distribution of disease in the field. [19]. Simulation of natural soil and ambient conditions, as well as consistent inoculum load across all samples, are required for successful and efficient screening for resistance to soil borne pathogens such as *Fusarium* spp.

2.1 *Fol* Isolate Used

Highly aggressive *Fol* isolate, AGLF-11/*Fol-11* was collected from major lentil growing region mainly collected from Tal area of Barh, Patna, Bihar, India in year 2019. Different *Fol* isolates have different morphological characteristics viz. white, light pink, pink and dark pink colony colour and sparse, fluffy, centrally fluffy and crystal growth patterns. The colony colour of this isolate is mainly pink with sparse growth pattern (Fig. 1).

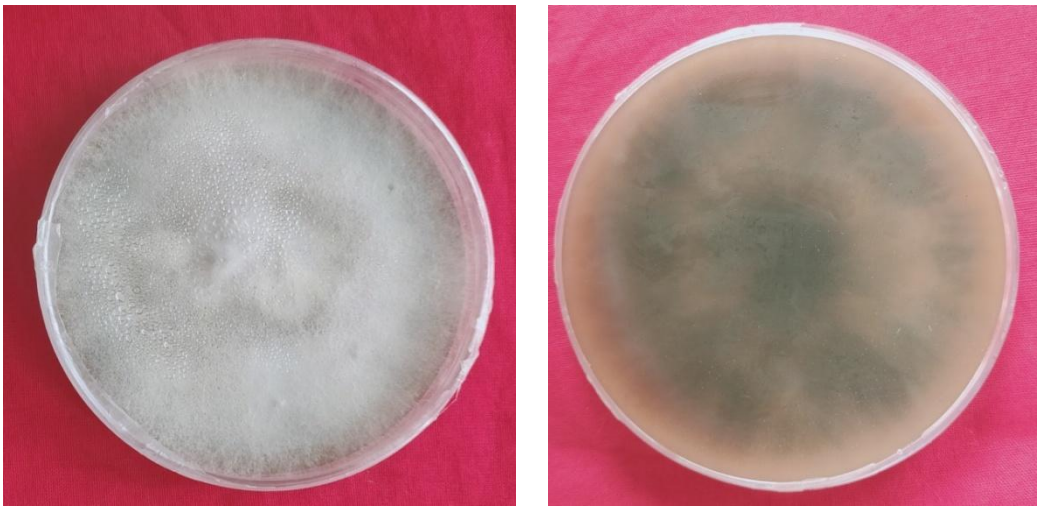

Fig. 1. Plates of AGLF-11 Isolate on Potato dextrose agar medium

Table 1. The list of materials used in the study along with its source

S.No	Genotypes	Source
1.	JL 3	JNKV, Jabalpur
2.	P-13108	Procured from IARI, New Delhi
3.	Noori	IIPR, Kanpur
4.	GP-3221	IIPR, Kanpur
5.	LKH-I	Collected from Lakhisarai, Bihar
6.	Moitree	BCKV, West Bengal
7.	Titua	Local collection
8.	BRL-2	Selection from landrace of Rajoun block, Banka
9.	VL 138	VPKAS, Almora
10.	DBGL 105	ICAR-Research complex for Eastern Region, Patna
11.	Pusa Vaibhav	IARI, New Delhi
12.	DBGL-62	ICAR-Research complex for Eastern Region, Patna
13.	DBGL-135	ICAR-Research complex for Eastern Region, Patna
14.	IG -195	Procured from IARI, New Delhi
15.	IG- 122133	Procured from IARI, New Delhi
16.	P-15115	Procured from IARI, New Delhi
17.	JL-1	JNKV, Jabalpur
18.	JL-7	JNKV, Jabalpur
19.	P 3236	Procured from IARI, New Delhi
20.	P 43120	Procured from IARI, New Delhi
21.	DKL 37	Procured from IARI, New Delhi

S.No	Genotypes	Source
22.	IG 559831	Procured from IARI, New Delhi
23.	NDL-I	NDUAT, Faizabad
24.	MC 6	Procured from IARI, New Delhi
25.	Flip 96-51	ICARDA, Lebnon
26.	PL-6	GBPUAT, Pantnagar
27.	PL-8	GBPUAT, Pantnagar
28.	PL-27	GBPUAT, Pantnagar
29.	DPL-62	IIPR, Kanpur
30.	DPL-316	IIPR, Kanpur
31.	IPL 526	IIPR, Kanpur
32.	KLS-218	CSAU, Kanpur
33.	HUL-57	BHU, Varanasi
34.	Pusa Ageti	IARI, New Delhi
35.	BRL-3	Selection from SL-2-24
36.	Shivalik	IARI, New Delhi
37.	IPL 406	IIPR, Kanpur
38.	IPL 315	IIPR, Kanpur
39.	DPL 58	IIPR, Kanpur
40.	DPL 15	IIPR, Kanpur
41.	PL-4	GBPUAT, Pantnagar
42.	PL- 639	GBPUAT, Pantnagar
43.	PL- 406	GBPUAT, Pantnagar
44.	IPL-321	IIPR, Kanpur
45.	K-75	CSAU, Kanpur
46.	PL-05	GBPUAT, Pantnagar
47.	L-4717	IARI, New Delhi
48.	L-4147	IARI, New Delhi
49.	L-4771	IARI, New Delhi
50.	L-7920	IARI, New Delhi

2.2 Greenhouse Screening

AGLF-11 isolate was maintained in laboratory using potato dextrose agar (PDA) medium supplemented with streptomycin sulphate and stored in a refrigerator (4°C) [20]. Sub culturing of isolate was done time to time. The isolate was mass multiplicated as per the protocol [21] with necessary modifications. Initially, sorghum grains were soaked in water overnight. Excess water was drained out and seeds were soaked in dextrose water @ 100g in 1 litre water (1-1.5 hrs). It is then spread and air dried on the clean blotting paper. Moistened grains (about 150 g) were filled in each autoclavable poly bags and autoclaved for 30 minutes at 121 ℃temperature and 15 lbs. psi pressure. The fungal mycelium bit of pure culture of AGLF-11 isolate was inoculated under aseptic condition in the poly bags containing grains and incubated for 12-14 days at 25±2 ºC. Meanwhile, polybags were shaken regularly to facilitate early growth of the fungus or to avoid clumping of grains. Due to mycelial growth of the test fungus, the grains turned whitish.

To prepare sick soil micropots, the grains colonized by isolate were mixed in the soil [21]. Fusarium wilt susceptibility of each genotype was tested in infected soil. For this, sterilized sandy-loam soil was mixed with mass multiplied culture of AGLF-11 @ 100g / kg soil. Seeds of 50 lentil genotypes were rinsed with distilled water. 10 Seeds of each lentil genotypes were sown in each well of the infected soil following Completely Randomized Design (CRD). The experiment was carried out in three replications for each genotype. Controlled soil micropot was also used without AGLF-11 isolate infection with each genotype. Observations on pre-germination mortality and post-germination mortality percentage were recorded up to 15 days. The post emergence mortality was recorded time to time and the final data was recorded at the end of the experiment on 15th day.

The pre germination mortality and post germination mortality percentage were recorded using following formula:

$$Pre\ germination\ mortality\ (\%) = \frac{Total\ germination\ of\ healthy\ seeds - Total\ germination\ of\ treated\ seeds}{Total\ germination\ of\ healthy\ seeds} x100$$

$$Post\ germination\ mortality\ (\%) = \frac{Total\ germination\ of\ treated\ seeds - Plant\ survived\ in\ treated\ seeds}{Total\ germination\ of\ treated\ seeds} x\ 100$$

Where, complete yellowing and dropdown of the plants were considered complete mortality of the plants.

Table 2. Disease rating scale for Fusarium wilt

S.No.	Rating	Mortality (%)	Disease reaction
1.	**1**	≤ 1 %	Immune (I)
2.	**3**	2-10 %	Resistant (R)
3.	**5**	11-20 %	Moderately resistant (MR)
4.	**7**	21-50 %	Moderately susceptible (MS)
5.	**9**	>50 %	Highly susceptible (HS)

The genotypes on the basis of mortality percentage recorded were categorized in to different categories viz. immune resistant, moderately resistant, moderately susceptible and highly susceptible on a scale of 1 to 9 (Table 2) [1].

3. RESULTS AND DISCUSSION

In this study, fifty lentil genotypes were screened against fusarium wilt in control condition using sick soil micropot technique under controlled condition. The recorded pre- germination mortality and post-germination mortality percentage data are enlisted in Table 3. A perusal of data in the table-3 reveals that, a significant wide range of pre and post germination mortality was observed among all the genotypes of lentil. The pre-germination mortality among all the genotypes ranged between 9.09 to 80.23 %. The highest pre-germination mortality was observed in Titua genotype treatment which was about 80.23 % and the lowest pre-germination mortality was observed in the treatment of L7920 genotype having only 9.09 %. Other genotypes also exhibited significant pre-germination mortality percentage that ranges from 9.62 to 73.58 %. This highly aggressive isolate of *Fol* not only affected the germination of seed but also caused significant post-germination mortality in some genotypes. Post-germination mortality was ranged between 12.01 to 30.56 %. The highest post germination mortality was observed in treatment of Titua genotype which was 30.56% while, genotypes L7920 showed lowest post germination mortality percentage which was 12.01 %. Several genotypes with high level of resistance under controlled condition were identified.

3.1 Reactions of Lentil Genotypes against AGLF-11 Isolate of *Fusarium oxysporium* f. sp. *lentis*

At the end of the experiment, the screening of genotypes was done based on reaction of genotypes against AGLF-11 isolate. A perusal of data in Table 4 reveals the same. The result classified the studied lentil genotypes in four groups viz. resistant, moderately resistant, moderately susceptible and highly susceptible reaction. Out of fifty screened genotypes, L 7920, DPL-58 genotypes exhibited resistance reaction, HUL -57, PL-639, L 4717, L 4147, L 4771 genotypes expressed moderate resistance reaction, genotypes JL-3, P-13108, Noori, GP 3221, Moitree, BRL-2, DBGL-105, DBGL-135, IG-195, IG 122133, P-15115, JL-1, JL-7, P 43120, IG-55983, NDL-I, MC-6, Flip-96-51, PL-6, PL-8, PL-27, KLS-218, Pusa Ageti, IPL-406, DPL-15, PL-406, IPL-321, K-75, PL-05 exhibited moderate susceptibility, while LKH-I, Titua, VL-138, Pusa Vaibhav, DBGL-62, P-3236, DKL-37, DPL-62, IPL-316, IPL-526, BRL-3, Shivalik, IPL-315, PL-04 genotypes expressed high

susceptibility reaction against the isolate. None of the genotypes exhibited immune reaction. Similar approaches for screening wilt resistance have been reported by [22,23,9,24]. Twelve accessions were identified as resistant [25]. [26] [27] and [28] have also identified fusarium wilt resistant germplasm lines in lentil under wilt sick plot and controlled condition.

Table 3. Effect of AGLF-11 isolate on pre- and post-germination mortality on various lentil genotypes

S.No.	Genotypes	Pre-germination mortality (%)	Post-germination mortality (%)
1	JL-3	48.04	23.15
2	P-13108	39.96	18.28
3	Noori	32.65	16.24
4	GP 3221	48.04	23.15
5	LKH-I	61.27	15.87
6	Moitree	42.77	21.48
7	Titua	80.23	30.56
8	BRL-2	37.69	18.28
9	VL-138	58.39	13.69
10	DBGL-105	41.50	19.39
11	P. Vaibhav	52.64	22.41
12	DBGL-62	72.61	20.56
13	DBGL-135	47.92	23.15
14	IG-195	28.56	15.02
15	IG 122133	30.96	15.02
16	P-15115	33.89	24.36
17	JL-1	30.17	16.24
18	JL-7	49.02	23.15
19	P3236	58.19	13.10
20	P43120	30.17	16.24
21	DKL 37	53.52	23.15
22	IG 559831	40.74	18.79
23	NDL I	24.02	23.72
24	MC-6	22.66	14.65
25	Flip-96-51	30.31	15.45
26	PL-6	43.36	20.13
27	PL-8	26.36	15.45
28	PL-27	26.71	14.65
29	DPL-62	73.09	21.67
30	IPL-316	73.41	23.33
31	IPL-526	73.53	21.67
32	KLS-218	37.04	17.68
33	HUL-57	17.22	15.81
34	Pusa Ageti	46.94	23.15
35	BRL-3	62.54	15.87
36	Shivalik	50.87	12.04
37	IPL-406	44.23	20.74
38	IPL-315	62.48	14.48
39	DPL-58	9.31	12.78
40	DPL-15	46.08	10.74
41	PL-04	53.52	23.15
42	PL-639	19.15	13.06
43	PL-406	42.59	19.39
44	IPL-321	25.87	15.02
45	K-75	28.70	16.24
46	PL-05	47.27	20.74
47	L4717	18.85	13.97
48	L4147	19.69	13.37

S.No.	Genotypes	Pre-germination mortality (%)	Post-germination mortality (%)
49	L4771	16.28	13.06
50	L7920	9.06	12.01
	SE(m)	**±2.35**	**±0.923**
	CD	**6.60**	**2.63**
	CV	**9.85**	**8.9**

Table 4. Reactions of lentil genotypes against Fusarium wilt (AGLF-11 isolate) in controlled conditions

Rating scale	Reaction	Mortality (%)	Genotypes
1	Immune (I)	≤ 1 %	Nil
3	Resistant (R)	2-10 %	L 7920, DPL-58
5	Moderately resistant (MR)	11-20 %	HUL -57, PL-639, L 4717, L 4147, L 4771
7	Moderately susceptible (MS)	21-50 %	JL-3, P-13108, Noori, GP 3221, Moitree, BRL-2, DBGL-105, DBGL-135, IG-195, IG 122133, P-15115, JL-1, JL-7, P43120, IG-55983, NDL-I, MC-6, Flip-96-51, PL-6, PL-8, PL-27, KLS-218, Pusa Ageti, IPL-406, DPL-15, PL-406, IPL-321, K-75, PL-05
9	Highly susceptible (HS)	>50 %	LKH-I, Titua, VL-138, Pusa Vaibhav, DBGL-62, P-3236, DKL-37, DPL-62, IPL-316, IPL-526, BRL-3, Shivalik, IPL-315, PL-04

4. CONCLUSION

For successful screening, important information about host-pathogen biology and interaction is required. The availability and accessibility of diverse germplasm collections, as well as the accuracy of the screening methodologies used in the study, are essential for successful disease resistance screening [29]. It's also crucial for identifying susceptible and resistant genotypes, as well as finding suitable resistance parents for use in hybridization. The use of well-established assays in the field and in the greenhouse aids in the identification and selection of wilt resistant donors. In this present research, two genotypes (L 7920, DPL-58) exhibited resistance reaction, along with five genotypes (HUL -57, PL-639, L 4717, L 4147, L 4771) which exhibited moderate resistance against the highly aggressive isolate. These screened resistance genotypes could be used as resistance sources for breeding wilt resistant lentil varieties and cloning of the resistant genes via differential display expression analysis in future research programmes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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