

## Evaluation of Lentil Genotypes for Resistance of *Fusarium oxysporum* f.sp. *lentis*

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### Abstract

*Lentil (Lens culinaris Medikus Sub sp. Culinaris) is a highly nutritious pulse crop grown globally as a rainfed crop in the winter season and also a cheap source of protein for rural people. Fusarium wilt, caused by Fusarium oxysporum f.sp. lentis (Fol), is a major fungal disease that affects lentil crops, leading to a significant yield reduction. In this study, 50 lentil genotypes were screened against a highly aggressive Fol isolate (AGLF-11) under greenhouse conditions. Out of the tested genotypes, 14 showed high susceptibility, 29 showed moderate susceptibility, 5 exhibited moderate resistance and only 2 genotypes (L 7920 and DPL 58) showed resistance to the Fol isolate. Thus, the two genotypes (L 7920 and DPL 58) hold promise for breeding programmes aimed at developing lentil varieties with improved resistance to this devastating fungal disease, which could help mitigate yield reduction and ensure the continued cultivation of this highly nutritious pulse crop for ensuring nutritional security among rural people.*

**Keywords:** Lentil; Fusarium wilt, *Lens culinaris*, disease resistance

### Introduction

Lentil (*Lens culinaris* Medikus subsp. *culinaris*), also known as masur, is an annual, self-pollinated pulse crop grown worldwide as a rainfed crop in the winter season. It is highly nutritious, rich in protein, starch, micronutrients, and dietary fiber. Lentils are an excellent protein

source, containing 192% more protein than mushrooms (Nayak et al., 2021; Nayak et al., 2022) but 2-3 times less protein per calorie than meat/ beef. Lentil protein bridges the nutritional gap in the diets of rural communities, providing a sustainable and affordable source of plant-based protein essential for their health and well-being. Lentil cultivation is prevalent in

India, with Bihar being a major lentil-growing state. However, lentil crops face various biotic and abiotic stresses, including Fusarium wilt caused by Fol, which results in significant yield loss (Vasudeva and Srinivasan, 1952). Fusarium wilt is a vascular disease that blocks nutrient and water transfer in plants, causing wilting and eventual death. Developing resistant cultivars is crucial for stable lentil production (Infantino et al., 2006). Due to limitations such as the confounding effects of drought and other root rot pathogens, field screening alone is insufficient. Therefore, it is necessary to conduct screening in controlled conditions in a glasshouse (Meena et al., 2017). Hence this study was carried out with specific objective of identifying lentil genotypes resistant to *Fusarium oxysporum* f.sp. *lentis* through greenhouse screening.

## Materials and Methods

Fifty lentil genotypes from various sources were collected and screened against the highly aggressive Fol isolate AGLF-11 and the experiment were re-conducted for validation. The screening was conducted under controlled greenhouse conditions using sick soil micropots. The genotypes were evaluated for pre-germination and post-germination mortality. The experiment followed a completely randomized design with three replications per genotype. Disease rating scales were used to categorize genotypes based on their mortality percentages.

### Detail of 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.

### 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). Sub culturing of isolate was done time to time. The isolate was mass multiplied as per the protocol of Kamdi et al. (2012) 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. 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 15 lbs. psi pressure. The 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°C. 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 (Bayaa and Erskine, 1990; Bayaa et al., 1995; Kamdi et al., 2012). 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:

The genotypes on the basis of mortality percentage recorded were categorized into different categories, viz., immune resistant, moderately resistant, moderately susceptible and highly susceptible, on a scale of 1 to 9 (Stoilova and Chavdarov, 2006) (Table 1).

$$\text{Pre germination mortality (\%)} = \frac{\text{Total germination of healthy seeds} - \text{Total germination of treated seeds}}{\text{Total germination of healthy seeds}} \times 100$$

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

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

**Table 1: Disease rating scale for Fusarium wilt**

Sl. 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)

### Results and Discussion

The screening results revealed a wide range of pre-germination and post-germination mortality percentages among the tested lentil genotypes. The genotypes exhibited

varying levels of susceptibility or resistance to the Fol isolate. Among the tested genotypes, 14 were highly susceptible, 29 were moderately susceptible, 5 exhibited moderate resistance, and only 2 genotypes (L 7920 and DPL 58) showed resistance to

the AGLF-11 isolate. These resistant genotypes hold promise for developing wilt-resistant lentil varieties.

**Under controlled conditions, the reactions of several lentil genotypes to the AGLF-11 isolate of *Fusarium oxysporium* f. sp. lentis**

After the experiment was completed, genotypes of lentil plants were tested based on their reactivity to the AGLF-11 isolate. The screening resulted in the lentil genotypes being classified into four separate groups: resistant, moderately resistant, moderately susceptible, and extremely susceptible. L 7920 and DPL-58 were resistant among the fifty genotypes tested, whereas HUL-57, PL-639, L 4717,

L 4147, and L 4771 were somewhat resistant. The 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, 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, and PL-05 indicated moderate susceptibility. LKH-I, Titua, VL-138, Pusa Vaibhav, DBGL-62, P-3236, DKL-37, DPL-62, IPL-316, IPL-526, BRL-3, Shivalik, IPL-81, and PL-04, on the other hand, showed high susceptibility reactivity to the AGLF-11 isolate. There was no immunological response in any of the genotypes.

**Table 2. 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-81, PL-04

The effective screening of genotypes was dependent on the accumulation of critical knowledge about the biology and

interaction of the host and pathogen (Beniwal et al., 1993; Bhat et al., 2006; Chandra et al., 2019; Choudhary et al.,

2013). The use of known tests in both field and greenhouse conditions aided in the identification and selection of lentil plants with wilt resistance (Meena et al., 2017). The genotypes identified as resistant through screening might be useful sources of resistance for developing wilt-resistant lentil varieties in future studies. Furthermore, the cloning of resistance genes using differential display expression analysis shows promise for future advances in this field.

### Conclusion

Fusarium wilt caused by Fol is a significant threat to lentil production worldwide. Screening lentil genotypes for resistance to highly aggressive Fol isolates is essential for developing wilt-resistant cultivars. In this study, two lentil genotypes (L 7920 and DPL 58) exhibited resistance to the AGLF-11 isolate, indicating their potential for breeding wilt-resistant varieties, which could help mitigate yield reduction and ensure the continued cultivation of this highly nutritious pulse crop for ensuring nutritional security among rural people. Further research is needed to understand the genetic basis of resistance and develop effective breeding strategies to combat Fusarium wilt in lentil crops.

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