

Original Research Article

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Screening of Lentil (*Lens culinaris* Medikus sub sp. *culinaris*) Germplasm against Fusarium Wilt (*Fusarium oxysporum* f. sp. *lentis*)

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ABSTRACT

Fusarium wilt is major disease in Central India, the major lentil growing region of country. The disease is soil borne causing huge loss and development of wilt resistant varieties is most effective means of controlling this disease. Highly resistant sources of wilt in lentil have not been reported from the Indian lentil breeding programme. Ninety three lentil accessions including twelve varieties, six ICARDA germplasm lines and seventy five advanced breeding lines were evaluated in field and controlled conditions against wilt. Field screening was carried out at RKVV, Sehore (hot spot) using infector row technique. Based on field and controlled condition screening- IG 69549 and IG 70238 have been identified as highly resistant genotypes. These can be used in hybridization programme for wilt resistance breeding and studying the inheritance of wilt resistance in lentil.

Keywords

Lentil, Screening, Fusarium wilt and donars.

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Introduction

Lentil (*Lens culinaris* Medik) ranks third in the world after chickpea and pea (FAO 2015). It is considered as one of the oldest domesticated crop in the Near East based on the archaeological evidence (Cubero, 1981; Zohary and Hopf, 1973) and is grown as an important food source over the last 8,000 years (Dhuppar *et al.*, 2012; Oplinger *et al.*, 1990). Lentil is an annual, autogamous, diploid crop (2n=14) with genome size of approximately 4 Gbp in its haploid component (Arumuganathan and Earle, 1991). Lentil is planted as rotational crop for deriving ecological and environment benefits

by improving rhizosphere diversity through biological nitrogen fixation increase in fertility of soil, carbon sequestration, and by management of diseases, weeds and insect pests (Kumar *et al.*, 2013). It is an economical source of proteins, carbohydrates, minerals and fiber for resource poor. The major lentil producing countries are Australia, North America, Western Asia, the Middle East, Nepal, China, Ethiopia, Syria, Bangladesh and India (FAOSTAT, 2014). In India, main lentil growing states are Madhya Pradesh, Bundelkhand region of Uttar Pradesh and Bihar. The global cultivated area of lentil is

around 4.34 million hectares producing 4.95 million tons of production with an average production of 1140 kg/ha (FAOSTAT, 2014). In India lentil was grown in 1.89 mha with production of 1.13mt with an average production of 598 kg/ha during 2013-14. However, yield of lentil remains low due to biotic and abiotic stresses. Biotic stresses such as fusarium wilt (*Fusarium oxysporum* f.sp. *lentis*), ascochyta blight (*Ascochyta lentis*), stemphylium blight (*Stemphylium botryosum*), anthracnose (*Colletotrichum truncatum*), root rot (*Rhizoctonia solani*), rust (*Uromyces viciae-fabae*), white mold (*Sclerotinia sclerotiorum*) and collar rot (*Sclerotium rolfsii*), (Kumar *et al.*, 2013; Sharpe *et al.*, 2013) affect lentil and cause severe yield loss.

Among them Fusarium wilt caused by *Fusarium oxysporum* f.sp. *lentis* is one of the major disease affecting lentil all over the world (Bayaa *et al.*, 1998; Khare 1981). It was first reported from Hungary (Fleischmann, 1937) for the first time, and later on from many countries including India (Padwick, 1941), USA (Wilson and Brandsberg, 1965), USSR (Kotava *et al.*, 1965), Syria (Bayya *et al.*, 1986) and Turkey (Bayya *et al.*, 1998). Globally wilt is considered as the most harmful soil borne disease of lentil (Khare, 1981; Bayya *et al.*, 1998). *Fusarium oxysporum* f. sp. *lentis* Vasudeva and Srinivasan affect lentil at every growth stage like seed, seedling, flowering and at crop maturity in stem and root which causes seed rot, stem rots, damping off, wilt and root (Khare *et al.*, 1979; Vasudeva and Srinivasan, 1952). Warm and dry conditions are the most ideal condition for the proliferation of the disease (Bayaa and Erskine 1990). In India, fusarium wilt is the major factor limiting lentil production in the states of Uttar Pradesh, Madhya Pradesh, Himachal Pradesh, Bihar, West Bengal, Assam, Rajasthan, Haryana and Punjab

(Agrawal *et al.*, 1993; Chaudhary *et al.*, 2009; 2010). In India, the incidence of this disease has been reported at seedling, flowering and pod stages at temperature 25°C or above (Kannaiyan and Nene, 1976).

Disease management is required to ensure the stable lentil production. Application of fungicide is one of the solutions to overcome this problem but field applications is not feasible due to the expense required and technical difficulty in infusing chemicals into the soil (Taylor *et al.*, 2007). The most sustainable and effective solution to this problem is the development of resistant cultivars (Bayaa *et al.*, 1995; Kraft *et al.*, 2000).

Lentil germplasm can be screened in fields with high levels of natural inoculum of *Fusarium oxysporium* f.sp. *lentis* (Kraft *et al.*, 1994, Bayaa *et al.*, 1994). Field screening has limitations such as, confounding effect of drought and other root rot pathogens. Hence screening under controlled conditions in glasshouse is required. High level of wilt resistance has not been reported. The released varieties exhibit variation for resistance. Stable sources are required for breeding wilt resistant varieties. Hence this study was carried out with specific objective of identifying lentil genotypes resistant to *Fusarium oxysporum* f.sp. *lentis* through field and green house screening.

Materials and Methods

Ninety three genotypes of lentil from various parts of India and Mediterranean region were screened against fusarium wilt in wilt sick plot during 2015/16 crop season at hot spot. The field screening was carried out RAK, Sehore, Madhya Pradesh (Central Zone; 23° 11' N 77°04'E 457masl) and screening under controlled condition was carried out at Indian Agricultural Research Institute, New Delhi in

greenhouse condition. The genotypes used in this study along with their origin are listed in Table 1. Screening for resistance to lentil wilt must take into account two factors: the varied timing of symptom expression among genotypes and the uneven and patchy distribution of the disease in the field. For effective and efficient screening for resistance to soil borne pathogens such as *Fusarium* spp. simulation of natural soil and environmental conditions and uniform inoculum load across all the plants of test genotypes to discriminate between resistant and susceptible genotypes (Porta-Puglia and Aragona, 1997) is necessary.

Screening under wilt sick plot

The method for screening in wilt sick plot has been described by Bayaa and Erskine (1990), Bayaa *et al.*, (1995, 1997), and Eujayl *et al.*, (1998). The experimental material for the present study comprised of 93 lentil genotypes. The field experiment was laid out in Randomized Complete Block Design (RCBD) with three replications per entry (3 rows per replication) with plant distance of 5 cm × 25 cm and row length of 4 metre. Susceptible cultivar 'L 9-12' was planted between every two rows of genotypes screened as infector / spreader row. Observations on wilt incidence were recorded at fortnightly interval just after appearance of the disease.

Greenhouse screening

Laboratory and glasshouse screening techniques for resistance to wilt of lentil have been described in previous reviews (Bayaa *et al.*, 1994; Khare *et al.*, 1993; Kraft *et al.*, 1994). The seeds were superficially sterilized in 3% sodium hypochlorite for 3 min, then washed in sterile water and then germinated in towel paper for 10 days. Inoculum of pure culture of *Fusarium oxysporium*, isolated

from naturally wilt infected lentil plants was used for multiplication. Single spore culture of *F. oxysporium* was multiplied on 100 g of 9:1 sand: lentil meal medium for 15 days at 28-30 °C . Two hundred gram of these inoculums was mixed well with 2 kg autoclaved soil and placed in one 15 cm plastic pots. The 10-day-old uprooted seedlings were washed under water to remove soil particles. Root tips approximately 0.6 cm long were cut to facilitate the entry of pathogen in roots. The roots of the seedlings were then immersed in the spore suspension (5×10^6 conidia ml⁻¹) for 5 min to enable conidia to stick to the roots. Inoculated seedlings were transplanted into a mixture of equal parts of sterile soil, sand, peat and perlite which had been potted and pre-irrigated 2 days previously. Seedlings were irrigated after their planting in pots, and incubated at $25 \pm 3^\circ\text{C}$.

The appearance of disease symptoms, the percentage of dead plants was recorded following the method proposed by Bayaa and Erskine (1990).The following formula was used to calculate wilt disease incidence

Disease *incidence* (%) = Total number of plants examined/ No. of plants infected × 100

Results and Discussion

Wilt disease of lentil is caused by *Fusarium oxysporium* f. sp. *lentis*. In this study lentil genotypes were screened for resistance to fusarium wilt under controlled and field conditions. Several accessions with high level of resistance under both controlled and field conditions were identified.

Reactions of lentil promising lines against *Fusarium* wilt under field conditions

Field screening was carried out against fusarium wilt in sick plot.

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

S. No.	Genotype	Source	Pedigree
1	L4721	IARI, New Delhi	-
2	L4712	IARI, New Delhi	-
3	L4717	IARI, New Delhi	ILL 7617 × 91516
4	L4076	IARI, New Delhi	PL234 × PL 639
5	L4715	IARI, New Delhi	-
6	L4590	IARI, New Delhi	-
7	L4716	IARI, New Delhi	-
8	L4718	IARI, New Delhi	-
9	L4719	IARI, New Delhi	-
10	L4147	IARI, New Delhi	(L 3875 × P4)PKVL1
11	L4720	IARI, New Delhi	-
12	L4714	IARI, New Delhi	-
13	L4713	IARI, New Delhi	-
14	L4709	IARI, New Delhi	-
15	L4710	IARI, New Delhi	L4603 × PL406
16	L4593	IARI, New Delhi	-
17	L4711	IARI, New Delhi	-
18	L4592	IARI, New Delhi	-
19	L4708	IARI, New Delhi	-
20	L 9-12	IARI, New Delhi	-
21	L1373	IARI, New Delhi	-
22	L4739	IARI, New Delhi	-
23	L4737	IARI, New Delhi	-
24	L4730	IARI, New Delhi	-
25	L4726	IARI, New Delhi	-
26	L4727	IARI, New Delhi	-
27	L4117	IARI, New Delhi	-
28	LL1320	PAU, Ludhiana	LL158 × DPL15
29	LL1316	PAU, Ludhiana	DPL15 × L967
30	L1318	IARI, New Delhi	-
31	IG 69549	ICARDA, Aleppo, Syria	-
32	IG 70238	ICARDA, Aleppo, Syria	-
33	IG 71487	ICARDA, Aleppo, Syria	-
34	ILL 10916	ICARDA, Aleppo, Syria	-
35	ILL 10921	ICARDA, Aleppo, Syria	-
36	ILL 10965	ICARDA, Aleppo, Syria	-
37	PL6-9	Pantnagar	-
38	DPL15	IIPR, Kanpur	PL406 × L4076
39	SLC101	RARS, Sahillongani	Pure line selection from 'Chirarg Local'
40	PL178	Pantnagar	PL 5 × DPL 15
41	IPL332	IIPR, Kanpur	IPL517 × DPL62) DPL62

42	HUL57	Varanasi	Mutant of HUL-11
43	PL175	Pantnagar	PL02×DPL58
44	PL157	Pantnagar	PL02 × DPL58
45	KLS13-3	-	-
46	KLB13-6	CSA, Kanpur	KLB08-4 × KLB 303
47	IPL334	IIPR, Kanpur	(ILL 6002 × DPL 62) × JL1
48	IPL222	IIPR, Kanpur	-
49	IPL227	IIPR, Kanpur	98/155 × Pant L 5
50	IPL335	IIPR, Kanpur	-
51	KLB14-12	CSAUT, Kanpur	KLB345 × KLB303
52	IPL331	IIPR, Kanpur	-
53	RKL1003-21C	ARS, Kota	Mutant of DPL 62
54	IPL81	IIPR, Kanpur	PL639 × K-75
55	PL194	Pantnagar	PL02 × DPL15
56	VL524	Almora	VL 501 × VL 502
57	RVL13-5	Sehore	JL3 × DPL 62
58	RKL14-26	ARS, Kota	RKL1001 × KLB339
59	RVL13-7	Sehore	JL 1 × Black Masara
60	VL148	Almora	DPL15 × L4076
61	VL525	Almora	VL120 × DPL 15
62	DKL37	Dhaulakaun	DPL-6 × PL-5
63	RLG195	RARI, Durgapura	IPL 313 × PL5
64	IPL315	IIPR, Kanpur	-
65	PL-165	Pantnagar	DPL 15 × PL639
66	RKL24C-59	ARS, Kota	Mutant of DPL62
67	DPL62	IIPR, Kanpur	JL1 × LG171
68	IPL329	IIPR, Kanpur	KL178 × DPL62
69	IPL220	IIPR, Kanpur	(DPL44 × DPL 62) × DPL58
70	KLS14-1	CSAU, Kanpur	KLS9-3 × KLS133
71	IPL576	IIPR, Kanpur	-
72	NDL14-22	Faizabad	NDL 1 × PusaVabhav
73	LL1374	PAU, Ludhiana	IPL406 × FLIP2004-7L
74	IPL406	IIPR, Kanpur	DPL 35 × EC 157634/382
75	RKL12-11E-119	ARS, Kota	Mutant of DPL62
76	IPL228	IIPR, Kanpur	VKS16/21 × DPL62
77	PL191	Pantnagar	DPL 15 × LL992
78	IPL321	IIPR, Kanpur	K75 × DPL62
79	NDL14-21	Faizabad	NDL 1 × PANT L 4
80	IPL325	IIPR, Kanpur	-
81	RVL11-6	Sehore	JL3 × DPL 62

82	RKL603-1	ARS, Kota	L 4682 × DPL 62
83	VL149	Almora	(VL 4 × VL 105) × VL126
84	DKL14-20	Dhaulakuan	-
85	IPL316	IIPR, Kanpur	Sheore 74-3 × DPL 58
86	PL172	Pantnagar	PL 5 × DPL 15
87	KLS218	CSAU, Kanpur	KLS133 × LG362
88	IPL533	IIPR, Kanpur	-
89	RLG192	RARI, Durgapura	RLG32 × L4076
90	IPL330	IIPR, Kanpur	KL 178 × DPL 62
91	RL3-5	IGKV, Raipur	Selection from germplasm
92	PL192	Pantnagar	-
93	Sehore 74-3	JNKVV, Jabalpur	-

Table.2 Reactions of lentil genotypes against *Fusarium* wilt

Rating scale	Reaction	Field screening	Glasshouse screening
1	Resistance ((≤1%)	IG 69549, IG 70238, IG 71487, ILL 10916, ILL 10921, ILL10965	IG 69549, IG 70238
3	Moderately resistant (2-10%)	L4712, L4713, L4714, L4717, L4719, L4720, LL1374, IPL334, PL175, DPL15	IG 71487, ILL 10916, ILL 10921, ILL 10965
5	Moderately susceptible (11-50 %)	L4592, L4593, L4709, L4710, L4711, L4715, IPL321, IPL332, IPL576, PL178, PL192, HUL57	L4713, L4714, L4719, DPL15, IPL334, PL175, L4720, LL1374, L4708, L4593
7	Susceptible (21-50 %)	L1318, L1373, L4076, L4117, L4590, L4708, L4716, L4718, L4721, L4726, L4727, L4737, L4739, LL1320, IPL220, IPL222, IPL227, IPL228, IPL315, IPL325, IPL335, IPL406, IPL533, PL157, PL172, PL191, PL194, RKL24C-59, RKL603-1, RKL14-26, RLG195, VL524, NDL14-21, NDL14-22, KLB13-6, DKL14-20, DPL62	L4709, L4710, PL178, PL192, HUL57, IPL321, IPL332, IPL576, L1318, L1373, L4076, L4117, L4590, L4592, L4711, L4712, L4715, L4716, L4717, L4721, L4726, L4727, L4737, L4739, LL1320, IPL220, IPL222, IPL227, IPL228, IPL315, IPL325, IPL335, PL406, IPL533, DPL62, DKL14-20, RKL14-26, RKL24C-59, RKL603-1, RLG195, KLB13-6, VL524, PL157, PL172, PL191, PL194, NDL14-21, NDL14-22
9	Highly susceptible (>50%)	L 9-12, L4147, L4730, LL1316, Sehore 74-3, IPL81, IPL316, IPL329, IPL330, IPL331, PL6-9, PL-165, KLS14-1, KLS13-3, KLS218, RLG192, RL3-5, KLB14-12, RKL1003-21C, RKL12-11E-119, RVL11-6, RVL13-5, RVL13-7, VL148, VL149, VL525, DKL137, SLC101	L4718, L 9-12, LL1316, L4147, L4730, Sehore 74-3, RLG192, IPL81, IPL316, IPL329, IPL330, IPL331, RL3-5, PL-165, PL6-9, KLS13-3, KLS14-1, KLS218, KLB14-12, RKL1003-21C, RKL12-11E-119, RVL11-6, RVL13-5, RVL13-7, VL148, VL149, VL525, DKL37, SLC101

The studied genotypes were rated on rating scale (1-9)

Rating Scale [Wilt incidence percent]	Reaction
1 1% or less plants wilted	Resistant
3 2-10% plants wilted	Moderately Resistant
5 11-20% plants wilted	Moderately Susceptible
7 21-50% plants wilted	Susceptible
9 Above 50% plants wilted	Highly Susceptible

The uniform distribution of inoculum in sick plot was evident from 100% mortality of L 9-12 (infecter row). Out of 93 genotypes screened only six genotypes IG 69549, IG 70238, IG 71487, ILL 10916, ILL 10921, ILL 10965 showed the resistance expression while ten genotypes 4712, L 4713, L 4714, L 4717, L 4719, L 4720, LL 1374, IPL 334, PL 175, DPL 15 expressed the moderate resistant reaction in field condition. ICARDA genotypes expressed relatively higher resistance in comparison to Indian genotypes. Whereas L 4592, L 4593, L 4709, L 4710, L 4711, L 4715, IPL 321, IPL 332, IPL 576, PL 178, PL 192, HUL 57 exhibited moderate susceptibility, while genotypes L 1318, L 1373, L 4076, L 4117, L 4590, L 4708, L 4716, L 4718, L 4721, L 4726, L 4727, L 4737, L 4739, LL 1320, IPL 220, IPL 222, IPL 227, IPL 228, IPL 315, IPL 325, IPL 335, IPL 406, IPL 533, PL 157, PL 172, PL 191, PL 194, RKL 24C-59, RKL 603-1, RKL 14-26, RLG 195, VL 524, NDL 14-21, NDL 14-22, KLB 13-6, DKL 14-20, DPL 62, were revealed susceptibility to wilt.

The genotypes L 9-12, L 4147, L 4730, LL 1316, Sehore 74-3, IPL 81, IPL 316, IPL 329, IPL 330, IPL 331, PL 6-9, PL 165, KLS 14-1, KLS 13-3, KLS 218, RLG 192, RL3-5, KLB14-12, RKL1003-21C, RKL12-11E-119, RVL11-6, RVL13-5, RVL13-7, VL148, VL149, VL525, DKL137, SLC101 expressed moderate to very high susceptibility reaction against the fusarium wilt. Bhat *et al.*, (2003) and De *et al.*, (2003) have also identified

fusarium wilt resistant germplasm line in lentil based on field screening.

Reactions of lentil genotypes against Fusarium wilt under greenhouse condition

Under the controlled greenhouse condition two genotypes IG 69549 and IG 70238 exhibited resistance reaction while, genotypes IG 71487, ILL 10916, ILL 10921, ILL 10965 exhibited moderate resistance reaction. Genotypes L4713, L4714, L4719, DPL15, IPL334, PL175, L4720, LL1374, L4708, and L4593 revealed moderate susceptibility. The genotypes L4709, L4710, PL178, PL192, HUL57, IPL321, IPL332, IPL576, L1318, L1373, L4076, L4117, L4590, L4592, L4711, L4712, L4715, L4716, L4717, L4721, L4726, L4727, L4737, L4739, LL1320, IPL220, IPL222, IPL227, IPL228, IPL315, IPL325, IPL335, PL406, IPL533, DPL62, DKL14-20, RKL14-26, RKL24C-59, RKL603-1, RLG195, KLB13-6, VL524, PL157, PL172, PL191, PL194, NDL14-21, NDL14-22 expressed susceptible reaction. The genotypes L4718, L 9-12, LL1316, L4147, L4730, Sehore 74-3, RLG192, IPL81, IPL316, IPL329, IPL330, IPL331, RL3-5, PL-165, PL6-9, KLS13-3, KLS14-1, KLS218, KLB14-12, RKL1003-21C, RKL12-11E-119, RVL11-6, RVL13-5, RVL13-7, VL148, VL149, VL525, DKL37, SLC101 revealed high susceptibility against the fusarium wilt (Table 2). The significant information about host-pathogen biology and interaction is necessary for successful screening. The use of

well-established tests for both field and greenhouse screening help in identification and selection of donors for wilt resistance. The screened resistance genotypes would have immense potential for use as resistance sources for breeding wilt resistant lentil varieties and cloning of the resistant genes through differential display expression analysis in future research programs.

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