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 donors.

**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 remais 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 (Sclerotiun 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 oxysporum 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 ± 3°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

\[
\text{Disease incidence (\%) = Total number of plants examined/ No. of plants infected \times 100}
\]

**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     |
| No. | Code   | Origin       | Description                           |
|-----|--------|--------------|---------------------------------------|
| 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, Kota   | 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       | DPL15 × DPL 15                        |
| 62  | DKL37  | Dhaulakuan   | 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  | RKL112-11E-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                         |
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, ILL 10965 | 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, IPL315, IPL325, IPL335, PL406, IPL533, DPL62, DKL14-20, RKL14-26, RKL24C-59, RKL603-1, RLG195, KLB13-6, VL524, PL175, L1374, L4708, L4593 |
| **9**        | Highly susceptible (>50%) | L9-12, 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, L9-12, 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                  | Reaction                              |
|-------------------------------|---------------------------------------|
| Wilt incidence percent        |                                       |
| 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 L9-12 (infector 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, PL 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, IPL315, IPL325, IPL335, PL406, IPL533, 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, PL165, 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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**How to cite this article:**

Jitendra Kumar Meena, Akanksha Singh, H.K. Dikshit, G.P. Mishra, M. Aski, N. Srinivasa, Soma Gupta, Deepa Singh and Aparna Tripathi. 2017. Screening of Lentil (*Lens culinaris* Medikus sub sp. *culinaris*) Germplasm against Fusarium Wilt (*Fusarium oxysporum* f. sp. *lentis*). *Int.J.Curr.Microbiol.App.Sci.* 6(11): 2533-2541.

doi: [https://doi.org/10.20546/ijcmas.2017.611.298](https://doi.org/10.20546/ijcmas.2017.611.298)