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Screening of lentil genotype against collar rot of lentil caused by *Sclerotium rolfsii* Sacc. Under field conditions

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Abstract
Total 271 lentil entries received from AICRP-MuLLaRP (All India Coordinated Research Project) on lentil, were evaluated at Raipur under field condition during the year 2018-19 and 2019-20 to identify sources of genetic resistant against collar rot disease incited by the fungus *Sclerotium rolfsii*. The fungus was isolated from diseased lentil plants collected from research farm at seedling and vegetative stage of the crop, purified and maintained on PDA for further screening process. Total 271 lentil entries were screened against collar rot disease, out of which 139 entries were screened during 2018-2019 while, 132 entries were screened in 2019-2020. During first *rabi* season 2019-2020, all lentil entries were susceptible to highly susceptible to collar rot**.** While, during second *rabi* season 2018-19, Only 3 germplasm, DPL-62, VL-1 and VL-4**,** were found highly resistant to this disease, whereas, 10 germplasm DPL-15, ASHA, NDL-1, PL-5, Ranjan, PL-406, PL-234, VL-103 Kirsey fokar and Dehati Masoor were identified as resistant. Rest of germplasm lines were found from susceptible to highly susceptible to the "disease.

Keywords: lentil, Collar rot, *Sclerotium rolfsii,* Screening

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) affect lentil and cause severe yield loss.
Several diseases are known to infect lentil (*Lens culinaris* Medik) during its growth stages. Among them, collar rot caused by *Sclerotium rolfsii* Sacc., is very common in all the major lentil growing areas (Butler and Bisby, 1931) [5]. The disease causes appreciable loss in yield

due to which, area under this crop is consistently decreasing. For restoring the area production and productivity of lentil, it is necessary to reduce the loss caused by this disease. Therefore, some new seed dressing fungicides along with existing ones were tested in the present study to the manage the above disease in-vitro and results are reported in this investigation. *S. rolfsii* is a polyphagous soil borne pathogen infecting over 500 plant species worldwide causing huge losses. Though the fungus is seed and soil borne, soil borne inoculum is more important in causing infection and disease development. The fungus *S. rolfsii* produces abundant white fluffy, branched, septate mycelium with clamp connections only on the main hyphae, which spread like a fan. Small white tufts were formed on mycelium which later give rise to smooth, hard and dark brown sclerotia. Sclerotia may be spherical or irregular in shape and at maturity resemble the mustard seed

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) [4]. 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 collar rot resistance has not been reported. The released varieties exhibit variation for resistance. Stable sources are required for breeding collar rot resistant varieties. Hence this study was carried out with specific objective of identifying lentil genotypes resistant to *Schlerotium rolfsii* lentis through field screening.

Material and method

Isolation of pathogen *Sclerotium rolfsii* from diseased samples

Isolation was made from the fresh diseased plant samples collected from research farm at seedling and vegetative stage of the crop. The roots of diseased plant showing symptoms were washed thoroughly with water, small pieces of infected roots were cut with the help of sterilized blade. These pieces were surface sterilized with 1:1000 mercuric chloride ($HgCl_2$) solution for one minute followed by three washings with sterilized distilled water to remove traces of $HgCl_2$. The pieces were then transferred aseptically to Petri plates containing sterilized PDA and incubated at 25±2 ^{0}C for three to five days and examined at frequent intervals to see the growth of the fungus developing from different pieces. As and when fungal colony appears they were transferred to PDA slant for purification of culture.

Mass multiplication of *Sclerotium rolfsii*

The *S. rolfsii* was mass multiplied in wheat grain media. Wheat grains were soaked in water for 6 hrs then little boiled, drained excess water, air dried and supplemented with 50 g calcium carbonate in 1 kg wheat grains. Two hundred gram wheat grains were filled in 6 x 11 inches polythene bags and plugged with non-absorbent cotton with the support of one inch diameter PVC ring (length 1.5 inch).

These bags were sterilized in autoclave with 1.02 kg/cm^2 pressure for 25-30 minutes. The sterilized bags were inoculated with 2-3 mycelial discs (5 mm) taken from the periphery of the with 5 days old culture of *S. rolfsii* previously grown on PDA. The inoculated bags were incubated in BOD incubator at 25±2 ^{0}C for 15 days. Multiplied culture of *S. rolfsii* inoculated in collar zone of lentil plant, 15 days after sowing.

Screening of lentil entries

Seeds were procured from AICRP on MULLaRP ("All India Coordinated Research Project) on Lentil, Raipur. The field experiment was laid out during *rabi* season 2018-19 and 2019-2020 at the research farm, IGKV Raipur. Total 271 entries were screened against *Sclerotium rolfsii*, out of which 139 entries during 2018-2019 were screened while, 132 entries were screened in 2019-2020. Each test entries was sown in a plot of two rows of 5 meter length 30 cm apart with one row of susceptible check variety JL3 after every two test entry and replicated twice in randomized block Design. Observations on emergence were recorded at ten and twenty DAS. Light irrigation was given just to activate the growth of fungus. Observations on per cent mortality were started from ten days and recorded at five day intervals upto marurity, finally computed as follows.

Table 1: IIPR rating scale

S. No.	Reaction	Per cent mortality
1	R- Resistant	0-10
2	MR- Moderately Resistant	11-20
3	MS- Moderately Susceptible	21-30
4	S- Susceptible	31-50
5	HS- Highly Susceptible	51-100

Total 271 entries were screened against *S. rolfsii*. Each test entry was sown in a plot of two rows of 5 meter length 30 cm apart alternating with one row of susceptible check variety JL-3 after every two test entry and replicated twice.

Observations on emergence were recorded at ten and twenty DAS. Observations on per cent mortality were started from ten days after inoculation and recorded at five day intervals upto marurity, finally computed as follows.

$$\text{Per cent incidence} = \frac{\text{Total infected plant}}{\text{Total emergence of plant}} \times 100$$

Result and discussion

Screening of lentil entries

To find out the sources of resistance, lentil entries were evaluated for their reaction against *Sclerotium rolfsii* under natural field condition as per standard evaluation system. The reactions of the entries are depicted in Table 2.

During first *rabi* season 2018- 2019, total 139 lentil entries were screened against collar rot pathogen under field conditions. All the entries tested under Lentil LS AVT-1(7), Lentil LS AVT-2(6), Lentil EE IVT(21), Lentil EE IVT(10), Lentil MMLT(13), Lentil Germplasm(52), Lentil Released Variety(30) were susceptible to highly susceptible to collar rot. None entry was resistant or moderately resistant to collar rot.

While during second *rabi* season 2019-20, total 132 lentil entries were screened against collar rot pathogen under field conditions. All the entries tested under lentil AVT 2 Large seed CV(5), Lentil IVT Rice Fallow NEPZ(13), Lentil IVT Large Seed(22), lentil germplasm(34), lentil germplasm(52)

132 lentil germplasm accessions were screened against collar rot pathogen under natural conditions. Only 3 germplasm, DPL-62, VL-1 and VL-4**,** were found highly resistant to this

disease, whereas, 10 germplasm DPL-15, ASHA, NDL-1, PL-5, Ranjan, PL-406, PL-234, VL-103 Kirsey fokar and Dehati Masoor were identified as resistant (Table 2). Fourteen germplasm lines were found tolerant while 02 were moderately susceptible and 05 were highly susceptible to the disease. These resistant sources can further be exploited inbreeding program for the development of disease resistant commercial cultivars.

Gaurkhede *et al.* (2015) [7] reported that in a field screening of 284 lentil germplasm accessions against collar rot, 9 were found free from disease and 29 exhibited < 10 per cent mortality due to collar rot. Gupta and Mishra, (2009) [8] screened among 120 lines of lentil in disease sick fields for 3 consecutive years and 32 entries performed consistent resistant reaction to collar rot. Twelve accessions were found free from collar rot during the testing years under high disease pressure. Hussain *et al*., (2005) [9], screened 57 cultivars and found only one genotype highly resistant. Sugha *et al*., (1991) [17] evaluated 210 lentil lines/cultivars from different sources. None of these were resistant or even moderately resistant.

Table 2: Reaction of lentil genotypes to *Sclerocium rolfsii* under natural conditions 2018-19

S. No	Grade	Disease reaction	Name of the genotypes	Total entries
1	0	R-Resistant (<10%)	DPL-62, VL-1 and VL-4,	03
2	1	MR-Moderately Resistant (10-20%)	DPL-15, ASHA, NDL-1, PL-5, Ranjan, DPL-15, ASHA, NDL-1, PL-5, Ranjan, PL-406, PL-234, VL-103 Kirsey fokar and Dehati Masoor	10
3	3	MR-Moderately Resistant (21-30%)	VKG-15/367, IC-201665 RL-9, IC-4197, IC-208353, Masoor Dal, Deshi Lal, Chhoti Sunhari, Chhoti Masoor, Adlika, Chootki Masoor K, Local, Desi Safed, Deshi Masoor Gol, VK-15/362	15
4	4	S-Susceptible (31-40%)	LLS 18- 82, LLS 18- 83, LLS 18- 85, LLS-18-68, LLS-18-69, LLS-18-70, LLS-18-71, LLS-18-72, LLS 18- 131, LLS 18- 132, LLS 18- 133, LLS 18- 134, LLS 18- 135, LLS 18-137, LLS 18- 138, LLS 18- 139, LLS 18- 140, LLS 18- 141, LLS 18- 142, LLS 18- 145, LLS 18- 146, LLS 18- 148	22
5	5	HS-Highly Susceptible (>40%)	JL-1, JL-3, K-75, PL-4, Deshi Masoor-1, Masoor-1, Masoor- 2, Moti Masoor, Baban Masoor, Subrata, Masuri, Kashor Masur, LLS 18- 84, LLS 18- 86 , LLS 18- 129, LLS 18- 130, LLS 18- 136, LLS 18- 143, LLS 18- 144, LLS 18- 147, LLS 18- 149, LEE18-165, LEE18-166, LEE18-167, LEE18-168, LEE18-169, LEE18-170, LEE18-171, LEE18-172, LEE18-173, RL-6-1, RL-8, RL9, RL10, RL-11, RL-12, JL-3, RVL-13-5, L- 4796, RVL-6071-1, IPL-316, RVL-14-4, RL-3-5-1, RL-4, RL-8, IPL-532, IPL-522, L-4676, JL-3, RL-3, IC-29983, SKY/AC-1420, IC-496773-1, EC-299645, IC-408019, IC-201689, PRCOI-EE-758, EC-78451-A, IC-558821, JL-29, IC-201714, IC-271999, IC-201101, IC-2220168, IC-267114, IC-2016IC-299647, IC-299647, EC-28514, RI-5, RL-10, PL-10, IPL-521, L-4049, RL-6, RL-7, VKG-15/275, IC-21268, IC-211609, IC-268271, IC-267121, IC-201714, IC-212276, IC-267657, IC-277444, IC-271999, EC-299644, VKG-15/151, IC-271332.	88
			Total Entries	139

Table 3: Reaction of lentil genotypes to *Sclerocium rolfsii* under natural conditions during Rabi 2019-20

S. No	Grade	Disease reaction	Name of the genotypes	Total entries
1	0	R-Resistant	NIL	-
2	1	MR-Moderately Resistant	NIL	-
3	3	MR-Moderately Resistant (21-30)	LRF19-159, LLS19-136, LLS19-144, RLV-31, L-4717, DPL-62, IPL-406, Pant L-7, IC-267363, IC-261720, SKY-IC-1420-2, IC-20168, VK-15/362, IC-201688, IC-560127, IC-560212, IC-560299, IGL-1014-5, L-4735	19
4	4	S-Susceptible (30-40%)	LRF19-150, LRF19-154, LRF19-157, LLS19-130, LLS19-140, JL-3, RLG-5, L-4076, LH-84-8, LL-1373, DPL-15, IPL-316, WBL-77, Pant L -8, IC-201697, IC-267113, EC-78540, EC-267601, VKG-LE-1, L-4727, L-4762, L-4147, RLV13-5, LLS19-146, LLS19-137, LLS19-133, LLS19-141	27
5	5	HS-Highly Susceptible (>40%)	LLS 19-73, LLS 19- 74, LLS 19-75, LLS 19- 76, LLS 19-77, LRF19-148, LRF19-149, LRF19-151, LRF19-152, LRF19-153, LRF19-155, LRF19-156, LRF19-158, LRF19-160, LLS 19-126, LLS 19-127, LLS 19-128, LLS 19-129, LLS 19-131, LLS 19-132, LLS 19-134, LLS 19-135, LLS 19-138, LLS 19-139, LLS 19-142, LLS 19-143, LLS 19-145, LLS 19-147, RLV11-6, RLV13-7, HUL-57, Kota Mashoor-1, KotaMashoor-2, L-4727, LH-89-48, LH-82-6, LL-699, LL-931, IPL-81, IPL-220, Narendra Mashoor-1 , Narendr Mashoor 2, IC-267198, IC-421795, IC-429195, IC-201694, IC-208343, IC-207029, IC-4967732, VKG-15/362, VKG-15/319, IC-371632, VKG-15/227, VKG-15/336, EC-303712, IC-201740, IC-55944, IC-559776, C-S-CL-4595, EC-78415, EC-78461, CE-267554, EC-267604, EC-267627, EC-267638, EC-223222, EC-267537, EC-267538, EC-267626, EC-267628-B, IC-396758, RVL-13-7, RVL-13-5, L-4769, RVL-14-4, RVL-14-5.	86
			Total entries	132

Conclusion

Total 271 lentil entries were screened against collar rot disease, Only 3 germplasm, DPL-62, VL-1 and VL-4**,** were found highly resistant to this disease, whereas, 10 germplasm DPL-15, ASHA, NDL-1, PL-5, Ranjan, PL-406, PL-234, VL-103 Kirsey fokar and Dehati Masoor were identified as

resistant. Screening of lentil genotype against the collar rot of lentil could identify the resistance to moderately resistant lines. These lines can be used for future breeding programme.

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