



Source of resistant against *Fusarium* wilt and *Stemphylium* blight in lentil (*Lens culinaris* Medikus)

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Abstract

The crop yield of lentil is below attainable levels which are mainly attributed to pathological factors especially lentil wilt caused by *Fusarium oxysporum* f.sp. *lentis* and *Stemphylium* blight caused by *Stemphylium botryosum*. *Fusarium* wilt is a potential threat to lentil production not only in Nepal but worldwide that can cause complete crop failure. While *stemphylium* blight is recently being serious problem to Bangladesh, Nepal and India. In Nepal, multiple disease resistance work was not reported in lentil before the present study. Two trials separately using 185 genotypes were conducted to study the wilt and stem phylium blight were conducted. For wilt screening one hundred eighty five lentil lines including resistant check ILL7164 and susceptible check sindur, were sown in a previously developed wilt sick bed, while for stem phylium blight serarate trial was conducted in the research area of Regional Agricultural Research Station Nepalgunj using augmented design. Natural inoculum were also relied upon. Disease severity data were recorded. Results revealed that incase of *Fusarium* wilt sixteen genotypes were found resistant and twenty-three moderately resistant while in case of *Stemphylium* blight 87 resistant and 36 moderately resistant in field condition. It also indicated that 9 genotypes i.e RL-13,RL-21,ILL6468,ILL9996,ILL6024,ILL6811,ILL7164,Arun,Maheswar bharti showed combined resistant to both the diseases.

Keyword: Lentil; *Fusarium* wilt; *Stemphylium* blight; Source of resistant; Combine resistant.

Introduction

Lentil (*Lens culinaris* fsp. *culinaris* Medikus) is affordable source of dietary proteins (22–35%), minerals, fiber, and carbohydrates to poor people and plays a vital role in alleviating malnutrition and micronutrient deficiencies in developing countries. It is highly recommended by physicians for the people suffering from diabetes, obesity, and cardiovascular diseases (Srivastava and Vasishtha, 2012). In fact, vegetable protein is gaining preference over the animal protein for consumption by the health conscious people in the present day. This could be one of the reasons for increased per capita consumption (Vandenberg, 2009) and fivefold increase in global lentil production (from 0.85 to 4.43 Mt) during the last five decades, through a 155% increase in cultivated area and the doubling of average yields from 528 to 1068 kg ha⁻¹ (FAOSTAT, 2014)

Lentil ranks first among pulse crops in Nepal. Its area and production is 2,05,939 ha and 2,26,830 metric ton respectively with productivity of 1,101 kg per hectare

(MoAD, 2014). Its production seems adequate but current status of lentil is prone to a number of pathological threats including lentil wilt, *stemphylium* blight, collar rot and root rot in Nepal (Yadav, 2004) Among these diseases, lentil wilt caused by *Fusarium oxysporum* f.sp. *lentis* (*Fol*) is believed to be the most important constraint in lentil production worldwide (Taylor et al., 2007).

Lentil wilt is characterized by a sudden drooping of the leaves (more like wilting and damping off), followed by the leaves drying and the eventual death of the seedling. Apparently, the root system seems healthy but with a reduced proliferation and nodulation rate. Other symptoms at the seedling stage include seed rot (Kraft et al., 1981.) It is soil born disease causing 5-10% yield losses lentil worldwide but sometimes severe damage may result complete crop failure under favorable conditions of disease development (Chaudhary and Kaur, 2002), especially in a warm spring and dry / hot summer. Temperature and soil moisture are the main factors in determining fungal growth rates and symptom expression (Falahati et al., 2010).

Fusarium oxysporum f.sp *lentis* (*Fol*) is considered as warm-weather pathogen generally found in sandy and acidic soil. It can remain in the soil for up to 10 years. *Fusarium oxysporum* attacks plants and is most severe at air and soil temperatures of 24°C to 32° or 35°C (75° to 90° or 95°F). The optimal soil and air temperature for the pathogen is about 28°C (Eagling, 2009). The management of pathogen is difficult, because of its wide host range and ability to grow saprophytically or survive for extended periods in the form of thick walled chlamydospores in absence of a susceptible crop. Excessive use of chemical fungicides may apparently lower the density of pathogens in soil for short period of time but might give rise to mutant strains of pathogens with altered pathogenicity; making the previously resistant varieties of crops susceptible. Selection of durable resistant germplasm and effective breeding programs are needed to employ. Use of bio-control methods, crop rotations and genetically modified crops are some of the methods of disease management to curb down extreme use of chemicals on field to control pathogens

Stemphylium blight cause by *Stemphylium botryosum* is a serious problem in some parts of the world especially in West Asia and South Asia, North Africa and widely distributed in Saskatchewan, Canada where it was considered to be a minor disease of lentil. The disease has been first time reported in Bangladesh by Erskine and Sarker (1997) and it was mentioned that it can cause 70% yield loss up or even total crop failure in epidemic years. It is reportedly spread by air borne conidia in the field. It overwinters on seed and as mycelium on dead stems and leaves in many crops left in the soil. Limited information is available on whether the pathogen is seed-borne in lentil or not (Bayaa and Erskine, 1998). In alfalfa, it spreads by airborne and water-borne conidia (conidia and ascospore) and by sowing infected seed. Airborne conidia of *S. botryosum* land on host tissue and germinate when conditions are favorable. Generally penetration occurs through stomata but *Stemphylium* spp. also produce the toxin stemphol that may aid host infection (Solfrizzo *et al.*, 1994). Environment plays a major role in stemphylium blight disease development and that is why understanding the environmental role in disease development is important for effectively controlling the disease. The diverse host range *S. botryosum* that includes leguminous and non leguminous crops in different parts of the world indicates the adaptability of the pathogen to different environmental conditions. Most of the research on infection by *Stemphylium* spp. of different hosts has confirmed that temperature and moisture are the two most important environmental factors. In South Asia, temperatures of 18°-20°C and relative humidity over 85% have been reported to favor the development of disease (Erskine and Sarker, 1997). The pathogen requires at least 8 hr of wetness at low temperatures (10°C) for successful infection and infection

increases with increased leaf wetness for 24h (Mwakutuya, 2006)

The pathogen causes a leaf blight, plant defoliation and death. The disease is poorly understood but apparently there is some resistance available in the germplasm.

Use of resistant varieties is the only practical measure for controlling the disease in the field (Tamiatti and Valentino, 2006). The search for sources of resistance to diseases is a primary and most eminent research for most of the work carried out in the past and also is continuing presently (Shankar *et al.*, 2013).

Materials and Methods

The present investigation was carried out during winter growing season in 2012/13 at the crop field of Nepalgunj, The Latitude, Longitude and altitude of Nepalgunj is 28° 05' N, 81° 61' E and 181 masl respectively with 1111mm annual rain fall. Two trials using 185 genotypes were conducted to study the wilt and Stemphylium blight separately. The experimental materials used in the experiment were collected from International Centre for Agricultural Research in the Dry Areas (ICARDA) and National Grain Legumes Research Program, Rampur (NGLRP). Each germplasm line as one treatment was planted in road row design with 4 block.

Fusarium Wilt Screening Nursery

One hundred eighty five lentil genotypes/varieties including resistant check ILL7715 and susceptible check Sindur, were sown in a previously developed wilt sick plot, Debris of previous crops were incorporated to the soil to develop sick plot. Sindur (a highly susceptible line) was repeatedly planted after every two-test entries to increase the inoculum pressure of *Fol* under natural conditions. Plot size was of 1 row of 2 meter length. Crop was planted at 25 cm row to row and 5cm plant to plant spacing. Fertilizer was applied at the rate of 20:40:20 kg NPK /ha. . No insecticide and fungicide were applied. The weather conditions were highly favorable for disease development. Disease severity data were recorded. Disease reaction, on individual seedlings basis, was estimated using a 1-9 scale. 1=no symptoms (Highly resistant); 3=yellowing of the basal leaves only (resistant); 5=yellowing on 50% of the foliage (moderately susceptible); 7=complete yellowing of the foliage; 9=flaccidity of the top leaves, & partial drying (susceptible); 9=the whole plant or a unilateral shoot is wilted and/or dry (highly susceptible).

Observations of infected/wilted plant on plot basis were recorded at vegetative, and maturity stages following 1-9 scoring scale (Bayaa *et al.*, 1997) 1 = No infection (Highly Resistant), 3 = 1-10% plants affected, (Resistant), 5 = 10-20% of plants affected (Moderately Resistant), 7 = 20-50% of plants affected (Susceptible), 9 = More than 50% of plants affected (Highly Susceptible)

Stemphylium blight screening nursery

One hundred eighty five lentil genotypes/varieties including Bari masur-4 as a resistant check and Shital as a susceptible checks were planted .Plot size was of 1 row of 2 meter length. Crop was planted at 25 cm row to row and 5cm plant to plant spacing. Fertilizer was applied at the rate of 20:40:20 kg NPK/ha. Debris of previous crops were incorporated to the soil to develop sick plot, Shital (susceptible check of SB) were repeatedly planted after two test entries. The lentil crop was raised by following recommended agronomic practice No insecticide and fungicide were applied. The weather conditions were highly favorable for disease development, particularly for Stemphylium blight.

Horsfall-Barrat's logarithmic scale had unequal intervals in disease scores and is difficult to use for quantitatively inherited traits. To overcome this problem Hashemi (2005) modified this scale to a 0-10 linear semi-quantitative scale. This scale considered disease development pattern consisting of the appearance of chlorotic spots followed by gradual defoliation of plants. 0=free of disease, 1=a few tiny tan spots, 2=few small to large chlorotic spots, 3=expanding lesions on leaves to defoliation started, 4=20% nodes on main stem showing necrotic symptoms and defoliation,

5=40% nodes on main stem showing necrotic symptoms and defoliation, 6= 60% nodes on main stem showing necrotic symptoms and defoliation, 7=80% nodes on main stem showing necrotic symptoms and defoliation, 8=100% leaves defoliate but small green tip recovering, 9=100% leaves defoliate but stem still green, 10= Completely dead). Kumar (2007) also used this scale (0-10) for stemphylium blight screening. Observations of infected plant on plot basis were recorded at vegetative, and maturity stages following 1-9 scoring scale suggested by Bayaa et al. 1997 for foliar diseases. 1 = No infection (Highly Resistant), 3 = 1-10% plants affected ,(Resistant),5 = 10-20% of plants affected (Moderately Resistant),7 = 20-50% of plants affected (Susceptible),9 = More than 50% of plants affected (Highly Susceptible)

Results and Discussions

This study was conducted under natural infection conditions in the field of RARS, Nepalgunj during 2012/13 lentil growing season, The climatic conditions during the experiment were favorable for the development of wilt and Stemphylium blight. Results of disease reaction of germplasm accessions have been summarized in Table 1. A wide range of variation in disease reaction was observed among lentil genotypes.

Table 1: Reaction of *Fusarium* wilts resistant in 185 lentil genotypes

D score	Disease reaction	Infection%	No of genotypes	Genotypes
1	Highly resistant	0	0	N0
2	Resistant	1-10	16	RL-13,ILL1672, ILL6468, ILL6260, ILL8191, ILL9996, ILL7164, RL-85, Arun, ILL6811, ILL6024, RL-77, DPL-62, M-Bharatai, RL-21, Sagun
3	MR	10-20	23	ILL1920, ILL9932, ILL7980, RL-51, RL-44, ILL6021, ILL7157, RL-6, ILL8187, ILL3490, ILL2526, FLIP2009-60L, ILL6256, ILL9949, LN-0111, ILL8132, LN-0137, FLIP05-24L(ILL10045), 39-s-66L, ILL6025, LN-0135, , ILL9993, , ILL7715
4	S	20-50	67	ILL7162, RL-74, RL-83, PL-4402, ILL6408, ILL3496, ILL4139, LN-0136, ILL7163, RL-55, ILL6465, ILL8188, Khajura-1, ILL2573, ILL2527, Cumara, ILL7664, RL-20, ILL7978, WBL-77, Bari masuri -4, FLIP04-60L(ILL10013), RL-81, ILL3236, FLIP2008-7L, ILL7537R, FLIP 95-1L, X49s-48, ILL7220, sindur, RL-35, shisir, F2003-49L, ILL9926, RL-9, RL-76, FLIP2009-59L(ILL), ILL6458 ,HUL-57, FLIP2009-54L, RL-94, RL-4, RL-70, RL-84, Aarial, RL-45, ILL9885, ILL9976, ILL1970, X94s-48, Baitadi6A, RL-47, Simal, PL-639,PL-4, NRx2001-71-3, ILL3768, ILL10068, RL-75, LN-3885, ILL3280, PL-406, Digger, ILL7979, RL-26, ILL10071,ILL7616
	HS	50%and above	79	RL-11, ILL7723, ILL9927, ILL-6458, RL-58, ILL6467, ILL7990, RL-25, ILL2716, RL-78, FLIP05-44L(ILL100), ILL590, simrik, RL-38, ILL9881, ILL1704, Flip2006-55L(ILL1), MangalBazar, RL-68, RNx99S-95-1-12, LG-12, ILL8186, ILL9992, RL-80, ILL3111, RL-73, RL-39, ILL6447, RL-60, ILL10134, RL-43, RL-8, khajura-2, NRx2001-72-3, shital, RL-95, ILL8605, FLIP05-24L(ILL10065), ILL7986, ILL280, ILL6818, RL-15, shikhar, ILL2712, ILL27001-1, ILL9943, NRx9901-1, NRx2001-71-4, FLIP-2006-99L, ILL2373, RL-23, RL-12, RL-42, RL-56, NRx995-95-1, RL-22, ILL9924, FLIP05-52(ILL10073), ILL7538, RL-28, IL-1, ILL3338, RL-67, ILL9990, ILL3490, RL-71, Jutpani, RL-69, X95s83, ILL6821, RL-49, RL-62, ILL2501, X39S-66L, ILL6829, ILL6256, RL-79, NRX9801-1, RL-41

Screening for Wilt

Out of 185 genotypes tested, fifteen genotypes i.e. RL-13, ILL1672, ILL6468, ILL6260, ILL8191, ILL9996, ILL7164, RL-85, Arun, ILL6811, ILL6024, RL-77, DPL-62, M-Bharati, RL-21, and Sagun were found resistant to Fusarium wilt. Similarly 23 genotypes, i.e ILL1920, ILL9932, ILL7980, RL-51, RL-44, ILL6021, ILL7157, RL-6, ILL8187, ILL3490, ILL2526, FLIP2009-60L, ILL6256, ILL9949, LN-0111, ILL8132, LN-0137, FLIP05-24L (ILL10045), 39-s-66L, ILL6025, LN-0135 were found moderately resistant to Fusarium wilt on the basis of plant infection (Table-1). Sixty-seven genotypes showed susceptible while 79 genotypes showed highly susceptible reaction to Fusarium wilt.

Bayaa et al. (1997) reported that genotypes. ILL590, ILL632, ILL2313, ILL2692, ILL4829, ILL4954, ILL6120, ILL6404, ILL6797, ILL6811, ILL6994, ILL7193, ILL7199, ILL7363, ILL7502, ILL7553, ILL7617, ILL7668, ILL7683, ILL7698, ILL7713, ILL7803, ILL7880, ILL8076, ILL8077, ILL8174, ILL9918, ILL9981, and ILL10124 complete resistance to Fusarium wilt over years in ICARDA, Aleppo, Syria.

Joshi (2006) observed that varieties i.e Simal, Simrik, Khajura-1, Shital, Bari Masur-4, and Maheswor Bharati possess field resistance to vascular wilt at Khumaltar, Nepal, while Gharti et al. (2011) found that genotypes ILL

9993 and ILL 7715 were resistant and ILL 590, PL 406, ILL 7164 and F 2003-49L were moderately resistant to wilt/ root rot complex in Nepalgunj condition.

Screening for Stemphylium Blight

One hundred eighty five genotypes including resistant (Barimasuro-4) and susceptible (Shital) checks. Out of that 87 genotypes, i.e ILL 7980, RL- 9, RL- 12, PL 406, ILL 3490, ILL 6821, ILL 6447, RL- 11, LN-0135, Khajura-1, ILL 7990, ILL 9949, LN-0137, ILL 8187, ILL 7986, ILL 9992, Cumara, ILL 8188, Simal, ILL7978, ILL7349, ILL 7163, Jutpani, Mangal Bazar, ILL2712, ILL1970, ILL10071, ILL6465, ILL9926, ILL6458, ILL1920, ILL6811, M.Bharati, ILL7723, ILL3768, ILL7537R, WBL-77, ILL6467, ILL6256, RL-60, FLIP2009-60L, RL-73, RL-75, RL-35, RL-43, RL-69, RL-44, RL-39, RL-58, RL-21, FLIP05-52L (ILL10073, ILL6260, RL-94, RL-74, RL-20, RL-5, ILL 7664, Bari Masuro-4 (Res check), Aarial, ILL 6458, FLIP 2009 - 59L (ILL 10716), RL- 28, RL- 78, ILL 6256, ILL 6408, ILL 10134, ILL 10065, ILL 2716, ILL 8186, PL 639, F 2003-49L, ILL 9990, RL- 77, ILL 9993, ILL 6818, ILL 3236, PL 4, LN 0111, NRx 99S-95-1-12, Arun, 94-S-38, ILL 1672, ILL 7715, X 94s43, RL-70, Digger, X49S-48 showed resistant reactions. While 36 genotypes were found moderately resistant to Stemphylium blight are shown in Table-2). Forty-four genotypes were found susceptible while 17 were recorded as highly susceptible.

Table 2: Reaction of Stemphylium blight in 185 lentil genotypes

D score	Disease reaction	Infection%	No of genotypes	Genotypes
1	Highly resistant	0		N0
3	Resistant	1-10	87	ILL 7980, RL- 9, RL- 12, PL 406, ILL 3490, ILL 6821, ILL 6447, RL- 11, LN-0135, Khajura-1, ILL 7990, ILL 9949, LN-0137, ILL 8187, ILL 7986, ILL 9992, Cumara, ILL 8188, Simal, ILL7978, ILL7349, ILL 7163, Jutpani, Mangal Bazar, ILL2712, ILL1970, ILL10071, ILL6465, ILL9926, ILL6458, ILL1920, ILL6811, M.Bharati, ILL7723, ILL3768, ILL7537R, WBL-77, ILL6467, ILL6256, RL-60, FLIP2009-60L, RL-73, RL-75, RL-35, RL-43, RL-69, RL-44, RL-39, RL-58, RL-21, FLIP05-52L (ILL10073, ILL6260, RL-94, RL-74, RL-20, RL-5, ILL 7664, Bari Masuro-4 (Res check), Aarial, ILL 6458, FLIP 2009 - 59L (ILL 10716), RL- 28, RL- 78, ILL 6256, ILL 6408, ILL 10134, ILL 10065, ILL 2716, ILL 8186, PL 639, F 2003-49L, ILL 9990, RL- 77, ILL 9993, ILL 6818, ILL 3236, PL 4, LN 0111, NRx 99S-95-1-12, Arun, 94-S-38, ILL 1672, ILL 7715, X 94s43, RL-70, Digger, X49S-48
5	MR	10-20	36	RL-79, RL-8, X94S-48, ILL9924, HUL-57, Sagun, DPL-62, LG-12, ILL3111, FLIP-2006-99L, FLIP04-60L (ILL10013), ILL6021, RL-76, RL-26, RL-23, X 95 s83, Khajura - 2, ILL7220, NRX9801-1, 39-5-66L, ILL 6468, ILL 9943, PL 4402, ILL 6024, ILL 3496, ILL 8191, RL- 85, RL-83, IL-1, ILL7164 , ILL 1704, FLIP 05-24L (ILL10045), RL-47, RL-62, ILL 9976, ILL7980
7	S	20-50	45	RL-45, RL-67, RL-49, ILL3338, RL-56, RL-68, ILL7162, LN-0136, ILL7979, RL-4, ILL2527, FLIP95-1L, RL-71, NRx2001-72-3, FLIP2008-7L, FLIP2009-54L, RL-42, RL-41, RL-80, X39S-66L, NR2001-71-4, RL-25, RL-95, ILL10068, RL-22, RL-38, RL- 13, ILL 6025, ILL 3280, ILL 9996, RL-55, ILL 6829, ILL8132, ILL8605, RL-84, Simrik, ILL 9927, RL-51, ILL590, ILL2501, ILL27001-1, ILL4139, ILL 2573, Shikhar, ILL280
9	HS	50% and above	17	Sindur, NRx-99s-95-1-1, Shisir, ILL9932, LN 3885, ILL9885, RL- 6, ILL7157, ILL7538, Baitadi6A, ILL 2526, ILL9881, RL-81, ILL7616, Shital (sus.check) NRx9901-1, ILL2373

Table 3: Reaction of combined disease resistant to Fusarium wilt and Stemphylium blight in lentil genotypes

S.N.	Particular	No	Genotypes
1	Combined resistant to Wilt and Stemphylium blight	9	RL-13, RL-21, ILL6468, ILL9996, ILL6024, ILL6811, ILL7164, Arun, Maheswar bharti.
2	Resistant to wilt and MR to SB	2	DPL-62, Sagun
3	Resistant to SB and MR to FW	6	ILL6021, ILL6256, ILL9949, ILL8132, LN0137, ILL10045
4	MR to F.W and SB	2	ILL3490, ILL7980

Gharti *et al.* (2011) reported that genotypes ILL 7164, ILL 6458, ILL 1704, ILL 9927, ILL 8006(BM-4), ILL 1672, X94s43, ILL 2573, ILL 9992, ILL 6025, Aarial, ILL 8093, ILL 9976, ILL 6256, IL-1, ILL 6818, ILL 2700-1, X94s29, ILL 9931, ILL 9996, ILL 5787 and ILL 8191 were found moderately resistant to Stemphylium blight

Khadka *et al.* (2014) reported that genotypes X94-s-38, RL-75 were found to be resistant both the year while other genotypes ILL 1672, ILL 7537, ILL 7164, NRx 99595-2-4, ILL 8603, ILL 7715, Cumara, Arun, RL-47, ILL 10638, ILL 10134, FLIP 2008-7L and ILL 8009 were found to be resistant only in 2013 to SB at Nepalgunj condition.

Screening for combine resistant

Combined observation of both the diseases showed that 9 genotypes *i.e.* RL-13, RL-21, ILL6468, ILL9996, ILL6024, ILL6811, ILL7164, Arun, Maheswarbharti were found combine resistant to both the diseases. While DPL-62, Sagun resistant to wilt and moderately resistant to SB. Genotypes ILL6021, ILL6256, ILL9949, ILL8132, LN0137, ILL10045 were found resistant S.B and moderately resistant to FW while genotype ILL3490 and ILL7980 exhibits moderately resistant to both diseases (Table-3). Hussain *et al.*, 2008, studied multiple resistant in lentil and found two lines 66013-3 and 66013-4 were resistant to blight and rust

Conclusion

Based on findings of the present study, it is concluded that 9 genotypes *i.e.* RL-13, RL-21, ILL6468, ILL9996, ILL6024, ILL6811, ILL7164, Arun, Maheswarbharti were found combine resistant to both the diseases. While DPL-62, Sagun resistant to wilt and moderately resistant to SB. Genotypes ILL6021, ILL6256, ILL9949, ILL8132, LN0137, ILL10045 were found resistant to S.B and moderately resistant to FW, while genotype ILL3490 and ILL7980 exhibits moderately resistant to both the diseases. These genotypes could be used in future breeding program to get stable and higher yield of lentil in the country.

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