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IDENTIFICATION OF MULTIPLE SOURCES OF RESISTANCE IN LENTIL AGAINST SOME POTENTIAL FUNGAL DISEASES

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Four hundred-sixty-six lentil genotypes were evaluated to identify the potential sources of resistance against 4 major fungal diseases namely; Fusarium wilt (FW), collar rot (CR), botrytis grey mould (BGM) and Lentil rust (LR). Screening of germplasm was performed at hotspots under high inoculum pressure at Sialkot and Faisalabad regions during 2012-14. Most of the genotypes were susceptible to highly susceptible against all diseases. One genotype LPP 12089 was found highly resistant against FW disease while LPP 11002 was found highly resistant against BGM and LR diseases. However, 23 genotypes were found having resistance against more than one diseases. One genotype LPP 12012 was found to be resistant against all four diseases; 3 genotypes (LPP 11001, LPP 11002 and LPP 12040) were identified as best sources of multiple disease resistance for traditional lentil growing regions of Pakistan whereas 12 genotypes showing resistance against FW and CR were identified as best sources of multiple disease resistance for the regions other than traditional areas of Pakistan. These identified sources of resistance can either be used directly as resistant varieties after verifying their agronomic traits or can be used in lentil breeding programs for the evolution of multiple disease resistant varieties for commercial cultivation.
Keywords: Lentil wilt, collar rot, botrytis grey mold, rust, multiple resistance

INTRODUCTION

Lentil (*Lens culinaris* Medik.) yield is being affected by all morphological, physiological and biochemical processes occurring in plants at different stages. It also suffers from various diseases caused by fungi, viruses, bacteria, phytoplasma and nematodes (Sarwar *et al*., 2014; Akhtar *et al*., 2016a,b). Among these, diseases caused by fungi are of prime importance which reduces plant vigor, threatening its life and ultimately yields (Chen *et al*., 2011).

Fusarium wilt, Collar rot, Rust and Botrytis grey mold are four common and major diseases of lentil. Lentil wilt caused by *Fusarium oxysporum* is a serious soil-borne disease in almost every continent of its cultivation. It was found to cause severe losses in Bangladesh, Morocco, India, Turkey, Nepal, Ethiopia, Syria, Pakistan, Algeria, Argentina, Bulgaria, Chile, Colombia, Hungary, Jordan, Brazil, Canada, Egypt, France, Myanmar, former Soviet Union, Sudan, Tunisia, US and Uruguay (Chen *et al*., 2011; Akhtar *et al*. 2016b). *F. oxysporum* is a diverse and quite adaptive ascomycete fungus whose pathogenic strains are divided into special forms or *formae speciales* based on host specificity (Muhammad *et al.*, 2017). It causes 5-10% yield loss but sometimes severe damage may occur depending upon the crop stage and severity of the pathogen. Seed rottening, seedling death, wilting and death of adult plants may occur and spread in patches but in severe attack, whole field may get affected (Khare, 1981; Chaudhary and Amarjit, 2002). CR of lentil caused by *Sclerotium rolfsii* is another major reason of low yield. It can cause up to 50% yield losses at farmers field. CR occurs in almost every region where lentil is grown especially in areas with high soil moisture and high temperature at seedling stage. This disease is of economic importance in Bangladesh, Ethiopia, India and Pakistan (Chen *et al*., 2011). Major symptoms of CR include rottening and death of seedlings at ground level in small patches (Khare *et al*., 1979). LR caused by *Uromyces viciae-fabae* is the most important foliar disease of lentil in Bangladesh, Morocco, India, Turkey, Nepal, Ethiopia, Syria, Pakistan, Algeria, Iran, Argetina, Bulgaria, Chile, Colombia, Cyprus, Hungary, Israel, Italy, Jordan, Palestine, Peru, Portugal and Sicily. In Pakistan LR is widely occurring disease in Sialkot district, the major growing area of lentil. Under favorable weather conditions, complete crop failure may also occur (Khare and Agrawal, 1978; Beniwal *et al*., 1993). Epiphytotic of LR are common in many countries including Pakistan (Erskine and Saxena, 1993). This disease is common in South Asia with symptoms like lesions on the leaves and stem, which drops and lead to premature plant death (Ahmad and Morrall, 1996). BGM also known as botrytis stem and blossom blight is another important fungal disease infecting all above ground parts of lentil plant. This disease has also worldwide distribution and has been found to cause severe epidemics in Australia, Bangladesh, Canada, Colombia, Nepal, New Zealand, Pakistan, India, Morocco, Syria and the United States (Chen *et al*., 2011). In Pakistan its major niche is Sialkot region. *Botrytis fabae* and *B. cinerea* are the major causes of BGM but in Pakistan only *B. cinerea* was found to be the chief cause of this malady. Its symptoms appear when crop reaches canopy closure under humid and warm microclimate. The disease first starts to appear on the lower foliage of plant by forming discrete lesions on leaves, which then enlarges and coalesces to infect whole plant. BGM is widespread problem damaging plants from 30% to complete failure in some fields depending upon the climatic conditions (Bayaa *et al*., 1997).

Moreover, these diseases are very difficult to control using chemicals or cultural practices. Chemical control is also costly and even the recovery of heavily infected, shriveled and damaged plants is impossible, leaving only the cultivation of resistant varieties, the safe and economical method. There are few reports available on sources of resistance against individual fungal diseases in lentil but no reports are available on multiple disease resistance in lentil germplasm in Pakistan. Keeping in view the above facts, present study was planned for the evaluation of newly developed (through hybridization and induced mutations in local germplasm) lentil germplasm for multiple disease resistance against severely threatening four major fungal diseases (Fusarium wilt, collar rot, botrytis grey mould and rust).

MATERIALS AND METHODS

Four hundred and sixty six genotypes developed through hybridization and induced mutations of exotic and indigenous germplasm at Nuclear Institute for Agriculture and Biology (NIAB) and Pulses Research Institute (PRI), Ayub Agricultural Research institute (AARI), Faisalabad were evaluated in this study. Screening of germplasm was performed under high inoculum pressure at two different locations. BGM and LR screening was performed at Adaptive Research Station, Sahowali/Kot Nainan in Sialkot region. For FW and CR, it was done at Plant Pathology Research Institute, AARI, Faisalabad. The study was carried out during Rabi seasons of 2012-13 and 2013-14.

FW and CR sick plots were prepared by adding locally available *F. oxysporum* f. sp. *lentis* and *S. rolfsii* isolates in separate fields, respectively. Before screening, FW and CR susceptible genotypes were grown in these fields for mass culturing and equal distribution of inoculums for two consecutive years (2010-2011). For BGM and LR, areas having previous history of severe epidemic with highly favorable weather conditions for the disease were selected in Sialkot district. Plants were also inoculated artificially by spraying the spore suspension of *B. cinerea* and *U. viciae fabae* in separate experimental fields to ensure high inoculums pressure when there were favorable conditions for disease development. All genotypes were planted in triplicates in a single row of 4 m length keeping inter and intra row spacing of 30 cm and 10 cm, respectively, following randomized complete block design. Uniform agronomic practices were followed for the maintenance of crop at both locations. Data was recorded for the response of lentil genotypes against FW, CR, BGM and LR by following scale given in Table 1.

RESULTS AND DISCUSSION

Host plant resistance is the major component in the management of fungal diseases in lentil. A Set of 466 lentil genotypes was evaluated for disease response against four economically important fungal diseases (FW, CR, BGM and LR) and several new resistant sources were identified (Table 2). A wide range of variation was observed among genotypes for reaction against all diseases during multiyear evaluations. These genotypes were categorized and grouped based on their reaction to each disease under field conditions.

Table 1. Disease scale for the rating of lentil germplasm against *Fusarium* wilt, collar rot, botrytis grey mould and lentil rust diseases.

Rating	Fusarium wilt and Collar rot	Botrytis grey mold	Rust	Disease response
1	0% plants infested	No lesions visible	No pustule visible	Highly Resistant
3	0.1-10% plants infested	Few scattered lesions seen after careful searching	Few scattered pustules usually seen after careful searching	Resistant
5	10.1-20% plants infested	Lesions common and some lesions coalesced, little defoliation	Pustules common on leaves and easily observed but causing no apparent damage	Moderately Resistant
7	20.1-50% plants infested	Large lesions, very common and damaging, some defoliation	Pustules very common and damaging, few pustules on petiole and stem	Susceptible
9	50.1-100% plants infested	Lesions very large, very extensive defoliation	Pustules very extensive on all plant parts, some death of leaves and other plant parts	Highly Susceptible

Wilt disease caused by *F. oxysporum* f. sp. lentis, is believed to be a serious threat to lentil worldwide. Most of the commercial varieties in field were found to be susceptible to FW in Pakistan (Sarwar *et al*., 2014). The only solution to this problem is breeding for host resistance which is the most effective, efficient and environment friendly method (Sarwar *et al*., 2014). Successful breeding program for disease resistance must be based on the availability of large and diverse germplasm collections (Pouralibaba *et al*., 2015; Akbar *et al*., 2017). In present study several genotypes with different levels of resistance were identified, providing new sources of FW-resistance to breeders. Out of 466 diverse lentil genotypes tested, one genotype, i.e. LPP 12089 was found to be highly resistant, 32 were resistant, 275 were moderately resistant and 158 were susceptible to FW (Table 2, 3). A series of disease symptoms were recorded depending upon the resistance/ susceptibility levels. Disease symptoms were started as a slight yellowing of the leaves, later as the disease progressed, the leaves got completely yellow and finally showed desiccation and defoliation. Ultimately, in the susceptible lines, the whole plant wilted and

Table 2. Response of all lentil genotypes to Fusarium wilt, Collar rot, Botrytis grey mould and lentil rust.

Diseases	**Number of genotypes**					
	Highly Resistant	**Resistant**	**Moderately Resistant**	**Susceptible**	**Highly Susceptible**	**Total**
Fusarium wilt	01	32	275	158	0	466
Collar rot	0	60	163	243	0	466
Botrytis grey mould	01	20	186	187	72	466
Rust	01	32	141	176	116	466

Table 3. Lentil genotypes showing resistance to fungal diseases as identified from their disease reaction.

Disease	**Reaction of genotype**	**Name of Genotypes**	**Number**
Fusarium wilt	Highly resistant	LPP 12089	01
	Resistant	LPP 11002, LPP 11030, LPP 11035, LPP 11045, LPP 11058, LPP 11122, LPP 12005, LPP 12011, LPP 12012, LPP 12017, LPP 12025, LPP 12031, LPP 12034, LPP 12051, LPP 12053, LPP 12068, LPP 12072, LPP 12091, LPP 12099, LPP 12120, LPP 12127, LPP 12137, LPP 12142, LPP 12143, LPP 12161, LPP 12176, LPP 12181, LPP 12198, LPP 12201, LPP 12205, LPP 12216, LPP 12222	32
	Total		**33**
Collar rot	Highly resistant	-	0
	Resistant	LPP 12004, LPP 12005, LPP 12009, LPP 12011, LPP 12012, LPP 12016, LPP 12020, LPP 12022, LPP 12030, LPP 12031, LPP 12038, LPP 12043, LPP 12045, LPP 12051, LPP 12053, LPP 12061, LPP 12062, LPP 12068, LPP 12071, LPP 12076, LPP 12090, LPP 12099, LPP 12100, LPP 12104, LPP 12105, LPP 12106, LPP 12107, LPP 12110, LPP 12120, LPP 12126, LPP 12127, LPP 12129, LPP 12130, LPP 12136, LPP 12137, LPP 12143, LPP 12144, LPP 12145, LPP 12152, LPP 12153, LPP 12161, LPP 12167, LPP 12168, LPP 12169, LPP 12182, LPP 12183, LPP 12187, LPP 12188, LPP 12190, LPP 12191, LPP 12198, LPP 12201, LPP 12205, LPP 12208, LPP 12213, LPP 12215, LPP 12219, LPP 12221, LPP 12223, LPP 12225	60
	Total		**60**
Botrytis grey mould	Highly resistant	LPP 11002	01
	Resistant	LPP 11001, LPP 12011, LPP 12012, LPP 12018, LPP 12019, LPP 12026, LPP 12030, LPP 12036, LPP 12040, LPP 12044, LPP 12051, LPP 12052, LPP 12059, LPP 12075, LPP 12083, LPP 12085, LPP 12094, LPP 12104, LPP 12164, LPP 12188	20
	Total		**21**
Lentil rust	Highly resistant	LPP 11002	01
	Resistant	LPP 11001, LPP 11014, LPP 11021, LPP 11022, LPP 11023, LPP 11027, LPP 11059, LPP 11061, LPP 11063, LPP 11072, LPP 11084, LPP 11085, LPP 11096, LPP 11103, LPP 11108, LPP 11146, LPP 11147, LPP 11148, LPP 11169, LPP 11170, LPP 11171, LPP 11191, LPP 11212, LPP 11213, LPP 11214, LPP 12012, LPP 12025, LPP 12040, LPP 12074, LPP 12099, LPP 12108, LPP 12163	32
	Total		**33**

died as it has been previously reported by Pouralibaba *et al.* (2015).

FW disease can also occur both at seedling and flowering stages. In early sown crop, wilting may occur in November but the disease ceases during the cool months of December and January due to severe cold. In February with the increase in temperature, FW reappear and showed its maximum incidence in March (Bashir and Malik, 1988; Sarwar *et al*., 2014). The final symptoms that a plant shows depend on the level of host resistance which determines the final damage caused by the disease (Pouralibaba *et al*., 2015). In present study one highly resistant genotype, LPP 12089 showed slight yellowing of the leaves with slow development of disease showing no mortality of plants at both stages. However, 32 genotypes responded as resistant had more advance symptoms than the highly resistant ones and were less affected by the disease than the susceptible lines. In these genotypes, the pace of disease development was also slower than susceptible ones with limited wilting as only less than 10% mortality (Table 2, 3) was observed till flowering. Identification of source of resistance to FW in lentil germplasm is not rare and many other workers have also reported the occurrence of high level of resistance to FW (Erskine *et al*., 1994: Bayaa *et al*., 1997; Pouralibaba *et al.*, 2015).

Lentil germplasm evaluated under present study exhibited various levels of resistance/ susceptibility to CR disease caused by *S. rolfsii*. None of the tested genotypes were found highly resistant against CR disease. However, a low frequency of genotypes comprising of 60 out of 466 were found resistant. Whereas, remaining 163 were found to be moderately resistant and 243 were susceptible (Table 2, 3). This low frequency of resistance may be due to the high level of aggressiveness of the pathogen or relatively narrow diversification of genetic material under study. The soil of sick plot infested with *S. rolfsii* exhibited mycelial growth on the soil surface and around some of the seedlings after 20th day of germination. A high level of CR infection was observed at seedling stage. In severely infected seedlings, soft watery rot symptoms and brown lesions advanced up to 3 cm above the soil level appeared at the collar region. On the lesions, white mycelial growth having white and brown sclerotia depending on the maturity was observed. The seedlings of susceptible genotypes were killed within 10-15 days after the initiation of disease symptom. However, in case of resistant genotypes pace of disease development was slower than susceptible ones and less than 10% mortality was observed (Table 2, 3). CR of lentil is a serious disease inflicting heavy losses every year however, a literature survey revealed the scarcity of information regarding the identification of resistance sources in Pakistan prior to this study. So, present findings are helpful to manage the disease using resistant sources in areas having high incidence of CR to avoid future losses.

BGM is also an economically important disease of lentil, especially in areas where cool, cloudy, and humid weather persists. Screening lentil germplasm in the field for BGM resistance is necessary to enable sources of resistance to be identified that are effective in Pakistani field conditions. Considerable variation was observed among 466 tested genotypes in their response to BGM. One genotype, LPP 11002 was found to be highly resistant; 20 were resistant; 186 were moderately resistant; 187 were susceptible and 72 were found highly susceptible. Disease was observed throughout the growing period of crop but it becomes severe after canopy closer. Infection was first developed on flowers and pods or on the lower foliage of established plants as dark green lesion that later become pale tan spots and caused severe defoliation followed by the death of the plants on susceptible genotypes. A furry layer of grey mould was also found on infected flowers, pods, branches and stems on these genotypes during favorable conditions. However, in case of one highly resistant genotype, there were no visible lesions on any plant throughout the crop growing period during all seasons. But in case of 20 resistant genotypes, few scattered lesions were seen after carful observation and most of these genotypes showed recovery after few days. There are several reports available on sources of resistance against individual fungal diseases in lentil. Lentil germplasm with resistance to BGM has previously been reported in Canada, Nepal, Pakistan and Australia (Lindbeck *et al*., 2008).

Out of 466 genotypes tested against LR disease only one genotype LPP 11002 was observed to be resistant as no rust pustules were observed on any plant throughout the cropping seasons during multiyears. However, 32 genotypes were found resistant, 141 were moderately resistant, 176 were susceptible and 116 were highly susceptible to LR. Initial symptoms on susceptible genotypes started as yellowish white pycnidia and aceial cups on leaf lets and pods in circular pattern which later developed into brown uredial pustules. On these genotypes pustules were very extensive on all plant parts causing some death of leaves and other plant parts. However, in case of 32 resistant genotypes, few scattered pustules were usually seen after careful searching. Previously few LR resistant lines were observed in India and Ethiopia but there was no evidence of resistance in lentil against LR disease in Pakistan. Currently, the conditions are favorable for LR in Sialkot region, a major lentil growing area in Punjab province of Pakistan. So, growing highly resistant and resistant genotypes identified in present study will be helpful to manage the LR disease in these areas to avoid future losses.

Table 4. Lentil genotypes having multiple resistance against potential fungal diseases.

Disease Reaction	Name of Genotypes	Number
Resistant to FW, CR, BGM and LR	LPP 12012	01
Resistant to FW, CR and BGM	LPP 12011, LPP 12051	02
Resistant to FW, CR and LR	LPP 12099	01
Resistant to FW, BGM and LR	LPP 11002	01
Resistant to CR, BGM and LR	-	0
Resistant to FW and CR	LPP 12005, LPP 12031, LPP 12053, LPP 12068, LPP 12120, LPP 12127, LPP 12137, LPP 12143, LPP 12161, LPP 12198, LPP 12201, LPP 12205	12
Resistant to FW and BGM	-	0
Resistant to FW and LR	LPP 12025	01
Resistant to CR and BGM	LPP 12030, LPP 12104, LPP 12188	03
Resistant to CR and LR	-	0
Resistant to BGM and LR	LPP 11001, LPP 12040	02
Total		23

Exploitation of host plant resistance is the best option for managing above mentioned fungal complex consisting of four economically important diseases in Pakistan. Because of the complex association of fungal diseases of lentil, successful production of lentil often requires cultivars resistant to multiple diseases. There are few reports available on sources of resistance against individual fungal diseases in lentil but no reports are available on multiple disease resistance in local lentil germplasm. In present study, twenty-three genotypes with multiple disease resistance were identified (Table 4). One genotype, LPP 12012 was found to be resistant against all four diseases whereas two genotypes, viz; LPP 12011 and LPP 12051 showed resistance to FR, CR and BGM. Similarly, LPP 12099 was also found as a multiple disease resistant source against FW, CR and LR disease. Resistance to FR, BGM and LR was also identified in genotype, LPP 11002. This genotype and two others (LPP 11001 and LPP 12040) also showed high level of resistance against BGM and LR and were identified as best sources of multiple resistance for Sialkot region where these diseases are causing heavy losses to lentil crop. Twelve genotypes, viz; LPP 12005, LPP 12031, LPP 12053, LPP 12068, LPP 12120, LPP 12127, LPP 12137, LPP 12143, LPP 12161, LPP 12198, LPP 12201, LPP 12205 were found as sources of resistance against FW and CR. These sources are important for lentil growing areas other than Sialkot region where FW and CR are causing damage to lentil crop due to favorable weather conditions like high temperature and less humidity. Only one genotype, LPP 12025 was found resistant against FW and LR whereas three genotypes (LPP 12030, LPP 12104, LPP 12188) were identified as effective resistant sources against CR and BGM. In general, multiple disease resistant sources of lentil developed through hybridization and induced mutations in local germplasm were identified against FW, CR, BGM and LR. Earlier, Mansehra-89 (ILL 4605) was released as a multiple resistant source against lentil blight and LR in Pakistan. However, such unique multiple disease resistant sources against major fungal diseases have not been previously identified in the country. Local germplasm has the potential and it carries the genes to be included in breeding programs for developing high yielding and multiple disease resistant varieties for commercial cultivation.

***Conclusion*:** From the above discussion, it may be concluded that in present study many genotypes were screened against major fungal diseases that yielded 147 resistant and 23 multiple resistant genotypes. Presently the conditions are favorable for LR and BGM in Sialkot region, a major lentil growing area in Punjab province of Pakistan but there was no evidence of resistance in lentil against LR disease in Pakistan. Under present study one genotype LPP 12012 was found to be resistant against all four diseases; 3 genotypes (LPP 11001, LPP 11002 and LPP 12040) were identified as best sources of multiple disease resistance for traditional lentil growing regions (like Sialkot) of Pakistan whereas 12 genotypes showing resistance against FW and CR were identified as best sources of multiple disease resistance for the regions other than traditional areas of Pakistan. So, these genotypes can be either used directly as resistant varieties for these areas after verifying their agronomic traits or can be used in lentil breeding programs for the evolution of multiple disease resistant varieties for commercial cultivation.

These resistant genotypes can also be useful as genetic source for lentil breeding as well as for genetic mapping to identify and characterize putative resistance gene**.** Such type of resistant breeding material can not only minimize the impact of these diseases on lentil production but would also be an aid to the lentil breeders in developing high yielding and disease resistant varieties.

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