Chapter 8

Fusarium Wilt: A Killer Disease of Lentil

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Additional information is available at the end of the chapter

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Abstract

Lentil (*Lens culinaris* Medikus subsp. *culinaris*) is an important dietary source of protein and other essential nutrients in South and West Asia, North and East Africa. Lentil crops are vulnerable to a number of diseases caused by fungi, viruses, nematodes, insect pests, parasitic plants and abiotic stresses. Among them, the most significant and serious soil-borne disease is Fusarium wilt (*Fusarium oxysporum* f.sp. *lentis*: *Fol*). Fusarium wilt causes yield loss up to 50% in farmers' fields. The pathogen showed high levels of phenotypic and genotypic diversity in India, Algeria, Syria and Iran. The disease thrives at 22–25°C temperature and affect lentil either at seedling and vegetative or the reproductive stages of the crop. To minimize yield losses, an integrated management strategy comprising resistant/partial resistant cultivars, adjusting sowing time, bio-control and chemical seed treatments is the best approach to reduce the incidence of the Fusarium wilt of lentil. This review covers past achievements in managing the disease, pathogen diversity and identify gaps in managing Fusarium wilt to improve productivity and production of the crop.

Keywords: lentil, *Fusarium oxysporum* f.sp. *lentis*, Fusarium wilt, disease management

1. Introduction

Lentil (*Lens culinaris* Medikus subsp. *culinaris*) is a cool season, diploid (2n = 2X = 14) self-pollinating grain legume with genome size of approximately 4 Gbp [1]. It is an ancient crop originated in the Near East and after that rapidly spread all through the Mediterranean Basin, Central Asia and later to the New World including Latin America. It is one of the oldest grain legumes domesticated about 10,000 years ago [2–4]. Production and consumption of lentil involve more than 100 countries. The total world lentil production is about 4.8 million tons from an estimated area of 4.5 million ha with an average yield of 0.11 t/ha [5]. The cultivated *L. culinaris* sub spp. *culinaris* includes two physio-morphological cultivated lentil types: small

seeded (microsperma) and large seeded (macrosperma) [6]. Lentil is recognized as one of the most nutritious pulse crops ranking next to chickpea among cool-season food legumes. It contains 57–60% carbohydrate, 24–26% protein, 3.2% and 1.3% fiber. It is also a rich source of minerals containing calcium (69 mg per 100 g), phosphorus (300 mg per 100 g) and Iron (7 mg per 100 g) of seed [7, 8]. Lentil seed contains lysine, an essential amino acid, found only at low levels in cereal protein. Lentil is a valuable human food, mostly consumed as dry seeds as well as used as fodder, and generally grown as a crop rotation after cereals to enrich the soil by their nitrogen fixing ability [9]. In South East Asia, lentil mostly grows on residual soil moisture after post rainy season under rainfed conditions. The inclusion of lentil as a crop rotation can benefits the succeeding crops by improving the soil health through biological nitrogen fixation and carbon sequestration. The amount of nitrogen fixed by plants varies from 0 to 192 kg total N/ha around a mean of 80 kg total N/ha [10]. This estimate of N fixation is similar to the quantities fixed by chickpea and dry bean.

Since 1970s there have been significant achievements in national and international lentil programs in developing phenologically adapted, stress resistant and high-yielding cultivars [11]. During the past three decades, different national agricultural systems released more than 90 improved cultivars from germplasm developed by the International Centre for Agricultural Research in the Dry Areas (ICARDA) [11]. Therefore, the current review covers past achievements in managing the disease, pathogen diversity and identify gaps in managing Fusarium wilt to improve productivity and production of the crop.

2. Production and constraints

In the global lentil scenario, India ranked first in the area and second in the production with 39% and 22% of world area and production respectively. Canada ranking first in production (41.2%). The highest yield is recorded in Croatia (0.3 tons per ha) followed by New Zealand (0.25 tons per ha) [5] (**Table 1**).

In many countries, lentil is cultivated as a rainfed crop and affected by several biotic (fungi, viruses, nematodes, insect pests and parasitic plants) and abiotic stresses (terminal drought, heat stress, cold, waterlogging and low soil fertility). Biotic stresses caused by pathogenic fungi include Fusarium wilt (*Fusarium oxysporum* f.sp. *lentis: Fol*), Ascochyta blight (*Ascochyta lentis*), Anthracnose (*Colletotrichum truncatum*), Stemphylium blight (*Stemphylium botryosum*), Rust (*Uromyces viciae-fabae*), Collar rot (*Sclerotiun rolfsii*), Root rot (*Rhizoctonia solani*), and Botrytis gray mold (*Botrytis cinerea*) [12, 13]. It is also known that lentil is susceptible to several species of *Orobanche* and *Phelipanche* prevalent in the Mediterranean region [14]. Till now, only *F. oxysporum* f.sp. *lentis* has been reported as the cause of Fusarium wilt of lentil but recently *F. redolens* was found associated with lentil wilt in Italy [15].

Among the biotic stresses, Fusarium wilt is a serious disease in reducing lentil yield in India, West Asia, North Africa and East Africa [16]. Fusarium wilt can cause yield losses up to 50% of the production to complete yield loss if severely affected. The disease appears at seedling stage (early wilt) or during the reproductive stage (late wilt) [17, 18]. The pathogen can survive in the soil as chlamydospores which can remain viable for many years [19] making crop rotation as a control option ineffective.

Rank	Area			Production			Yield	
	Country	Area	% to world	Country	Prod	% to world	Country	Yield
1	India	1.80	39.8%	Canada	1.99	41.2%	Croatia	0.286
2	Canada	1.22	26.9%	India	1.10	22.8%	New Zealand	0.247
3	Turkey	0.24	5.4%	Turkey	0.35	7.1%	Armenia	0.226
4	Nepal	0.21	4.6%	Australia	0.24	4.9%	China	0.208
5	Iran	0.17	3.7%	Nepal	0.23	4.7%	Egypt	0.206
6	Australia	0.16	3.6%	Bangladesh	0.16	3.3%	Canada	0.163
7	Bangladesh	0.12	2.8%	USA	0.16	3.2%	Iraq	0.157
8	Syria	0.11	2.5%	Ethiopia	0.14	2.8%	USA	0.149
9	U.S.A	0.10	2.3%	China	0.13	2.6%	Australia	0.147
10	Ethiopia	0.10	2.2%	Iran	0.08	1.8%	Lebanon	0.146
	World	4.52		World	4.83		World	0.107

Source: FAOSTAT 2015
http://faostat3.fao.org/home/index.html

Table 1. Global ranking in area, production and yield: (area—million hectare, production—million tons, yield—tons/hectare).

Fusarium wilt epidemics depend on crop stages (seedling or adult flowering), environment and crop variety [20, 21]. Fusarium wilt is part of a disease complex under field conditions. In India, 12 fungal pathogens were identified where *Fol* is the dominant pathogen (30.8%), followed by *Rhizoctonia bataticola* (17.5%) and *Sclerotium rolfsii* (15.7%) [22]. The prevalence of wilt-root rot complex and their associated pathogens were reported by Chaudhary et al. (2010) from India and the main pathogens associated with plant mortality were *Fusarium oxysporum* f.sp. *lentis* (62.0%), *Rhizoctonia bataticola* (25.2%) and *Sclerotium rolfsii* (9.8%) [23]. In India, under natural conditions wilt incidence can reach 50–78% [21, 24] and cause up to 100% yield loss if the crop is affected at the seedling stage [17]. In Czechoslovakia yield losses can reach as high as 70% [25]. In South and Northwest Syria disease incidence can reach up to 29% [26–29]. Moreover, field experiments indicated that the percentage seed yield loss per unit change in wilt incidence was 0.89 [30]. The disease incidence due to lentil wilt in Pakistan was recorded as 5–10% and may result in 100% crop loss under favorable conditions [31, 32]. Recently, the first report of *Fusarium nygamai* causing wilt disease on lentil in Pakistan is also reported [33]. Presence of the nematode significantly increased Fusarium wilt incidence. It causes significant reduction in shoot length, root length and nodulation in both susceptible and resistant cultivars [34].

3. Fusarium wilt pathogen

Fusarium oxysporum is a pathogenic fungus commonly found around the world. It is a soil borne ascomycete causing Fusarium wilt, on many economically important crops. The pathogen comprises of over 120 known strains and each of which is specific to unique host plant in which it causes disease. *F. oxysporum* strains infect and kills many commercially harvested crops and

legumes. The spores of *F. oxysporum* survive in a dormant stage in the soil for several years and are easily spread in water, it can infect vegetative cuttings, and can transmit to other individuals. Scientists around the world proposed developing *F. oxysporum* as a universal model for the understanding of fungal virulence [35]. *Fusarium oxysporum* infects its host by entering through the root and grows in the plant xylem. It blocks the vascular system and prevents transport of water and nutrients to the plant that causes wilting, discoloration, and eventually death of the plant.

The pathogen Fol affecting lentil crop was first reported from Hungary [36] and later from many countries including India [37], USA [38], Czechoslovakia [39], USSR [40], Brazil [41], France [42], Argentina [43], Bangladesh [44], Turkey [45], Syria [26, 46], Myanmar and Pakistan [47], Nepal [48], Ethiopia [49], Egypt [50], Italy [51]. In India, Fusarium wilt is known to limit the production of lentil in the states of Uttar Pradesh, Madhya Pradesh, Himachal Pradesh, Bihar, West Bengal, Assam, Rajasthan, Haryana and Punjab [22].

Wilt appears in the field as patches at both seedling i.e. early wilting and adult stages i.e. late wilting. Early wilting is characterized by sudden drooping and drying of leaves and seedling death (**Figure 1**). The roots are healthy but having reduced proliferation and nodulation and no internal discoloration of the vascular system. Late wilting appears from flowering to late pod-filling stage and sudden drooping of top leaflets of the affected plant and dull green foliage followed by wilting of the whole plant or in the individual branches [45]. The pod filling stage of the plant is severely affected and eventually a huge yield loss occurs. The disease thrives at 22–25°C temperature, with warm and dry soil conditions [52].

A culture of *Fol* display hyaline, septate and much branched mycelium. On media the growth pattern varies from fluffy to appressed and also vary in color from no color to pink. The pathogen is known to produce three kinds of asexual spores; micro conidia, macro conidia and chlamydospores [53]. Microconidia are usually single celled, ovoid or kidney-shaped and hyaline. Macroconidia are usually two to seven celled, long with pointed apical cell and notched basal cell. Chlamydospores are single celled, oval or spherical shaped and thick walled, formed singly in macroconidia or apical or intercalary in the hyphae [53]. In laboratory, the culturing of infected plant tissue should be done with caution because other saprophytic *Fusarium* spp. may be present that appears similar to *Fol*.

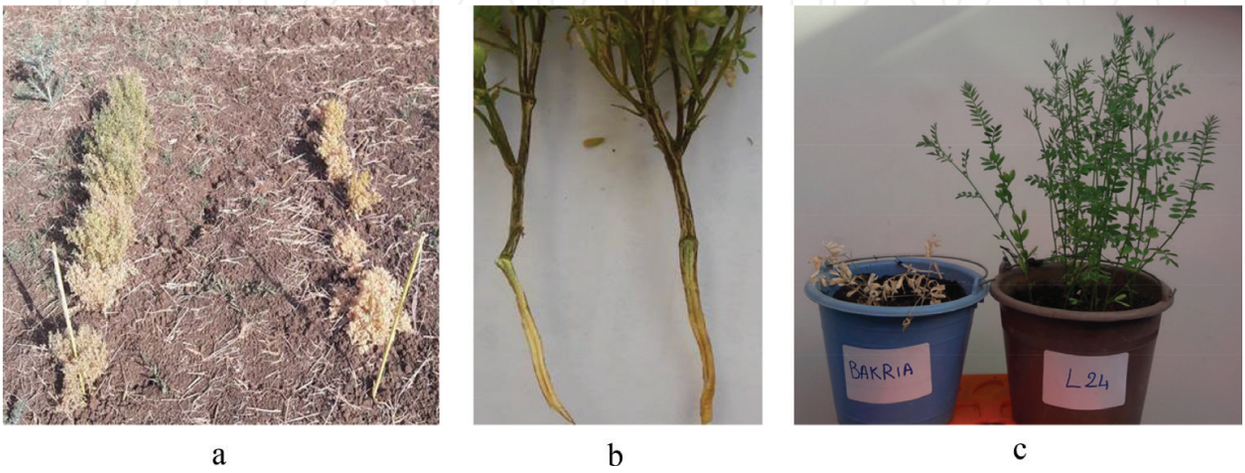

Figure 1. Lentil wilt disease: (a) lentil plants infected by wilt disease in field; (b) cross section showing internal discoloration of tap root in wilted lentil plant; (c) Fusarium wilt symptoms on artificial inoculated lentil plants.

4. Pathogen diversity

The pathogen can interact with specific host which results in breakdown of plant resistance within very short duration of time [54]. Therefore, it is important to determine the pathogen genetic diversity in *Fol* population, which can be used by plant breeders for disease resistance and can also help in studying its epidemiology, taxonomy, and detection [55]. The amount of genetic variation can be evaluated by molecular markers techniques like Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR) and Inter-Simple Sequence Repeat (ISSR).

The genetic diversity of *Fol* was studied by different researchers in many countries. Pouralibaba (2005) has studied the pathogenic diversity based on growth media (Czapecs Agar, PDA and Lentil Extract Agar) and a set of host differentials (ILL590, Gachsaran and Moghan lentil genotypes) using 13 isolates collected from Iran and Syria. The results showed that the difference among pathogen population was not related to different aggressiveness properties with no virulence patterns [56]. In other studies, Iranian isolates of *Fol* were grouped into 10 using RAPD markers and to six groups using ISSR markers [57]. In a recent study, 101 *Fol* isolates from five countries in Ilam provinces of western showed low level of genetic variability using Simple Sequence Repeat (SSR) markers [58]. In Algeria, all isolates were in one Vegetative Compatibility Group [59].

In India, 43 cultural and morphological groups were grouped into three clusters based on their aggressiveness of lentil genotypes [60]. Datta et al. (2011) showed varying degree of genetic diversity ranging from 54% in case of RAPD to up to 35% with ITS markers of *Fol* collected from different agro-ecologies in India where isolates from north region fall in same cluster, whereas isolates from north east regions and eastern region fall in different group [61]. In Syria, three major groups of *Fol* were identified using RAPD, SSR and ISSR markers [62].

5. Race concept in *Fusarium oxysporum* f.sp. *lentis*

In order to devise strategies for conferring resistance against disease, it is important to have knowledge of pathogen variability and prevalence of particular races in the target environment. The pathogen populations are primarily characterized by its virulence analysis on cultivars carrying differential resistance genes. Many researchers have studied the pathogen variability based on their grown on different solid media and on the basis of their pathogenicity [63, 64]. Later, Pandya et al. (1980) has evaluated the line (Pant-406) against seven races proposed by Kannaiyan and Nene [64], and found it immune to race 5, resistant against races 3 and 6, and partially resistant against race 4 [65].

Belabid et al. (2004) has reported that all the 32 Algerian isolates of *Fol* under study represent a single race but differ in their aggressiveness on the susceptible line on the basis of virulence and vegetative compatibility [66]. In India, on the basis of disease reactions against seven lentil differentials, the isolates were grouped into three clusters [60]. In an another study based on genetic variability, the *Fol* isolates collected from north eastern Indo-Gangetic plains revealed two sub-populations groups [61]. Sallam and Abdel-Monaim (2012) have collected 10 isolates

of *Fol* from different locations at Minia, Assuit and New Valley governorates, which were varied in their virulence [67].

Altaf et al. (2014) have characterized 15 *Fol* isolates (Fol-1 to Fol-15) collected from nine district of Pakistan on the basis of their pathogenicity and morphology [68]. Recently, Pouralibaba et al. (2016, 2017) have identify seven pathotypes (1–7) of *Fol* on the basis of their different pattern of virulence on lentil genotypes. Fifty-two *Fol* isolates originated from Iran, Syria and Algeria were used in the study. The results suggest that the pathogen 7 was virulent on all the accessions under study and there was no correlation found between the pathotype and the geographical origin of the isolates. The study was further confirmed by analyzing histopathology pattern of infection on resistant/susceptible varieties by pathotypes 1 and 7, which suggests that lower disease index was measured with plants inoculated with pathotypes 1 but not with pathotype 2 [69, 70]. Further studies are required to identify region specific pathogenic races using differential lines for conferring resistance against them in the respective agro-climatic regions.

6. Host ranges

Due to the presence of high mutations and variations among the pathogen populations limit the effectiveness of natural resistance in the host plants against the pathogens [71]. Therefore, it is important to access the variability among the pathogen and regarding its host resistance for a successful breeding program. It is also important to replace the low yielding genotypes and disease susceptible varieties with those of high yielding and disease resistance ones.

The forma specialis of lentil has a very limited host range and can induce disease of lentil only under natural conditions. Khare (1980) and Taheri et al. (2010) studied the host range of *Fol* by inoculating it on plants such as cowpea, french bean, bengal gram, lathyrus, mungbean, urdbean, pea, soybean, tomato and eggplant (Solanaceae), melon (Cucurbitaceae) or red gram which results in no infection [53, 72]. Recent host range studies on soybean, chickpea and tomato did not result to infection [73].

7. Fusarium wilt management options

Different Fusarium wilt management are used by lentil growers in different countries. These include cultural, biological, chemical, host plant resistance and an integration of two or more control options.

7.1. Chemical control

Several fungicides have been tested against the *Fol* in different parts of the world. The study reveals that the systemic fungicides found to be superior to non-systemic fungicides in inhibiting the fungal mycelial growth in plates as well as in pot seed treatment. Benomyl (76.6%) showed the most positive results against the pathogen followed by thiophanate methyl (73.0%) whereas non-systemic fungicides viz. captan (67.8%) and dithane M-45 (62.3%) were the least efficient in reducing the fungal growth compared to the systemic fungicides [74]. On the other hand, Kasyap et al. (2008) has found much reduced fungal growth with captan (88.3%) [75].

Maheshwari et al. (2008) tested the effect of seven fungitoxicants against *Fol*. The results suggest that carbendazim was the most effective (5.6 mm) followed by captan (9.9 mm) and hexaconazole and diniconazole for reducing the fungal growth [76]. Several studies were carried out for determining the concentration of the fungicides to control the growth. The results suggest that the best fungus control was observed at highest fungicidal concentration (100 ppm) with benomyl followed by thiophanate methyl, second most effective at 100 ppm concentration [74, 77]. In Syria, seed treatment with benomyl-thiram did not affect Fusarium wilt incidence [78].

7.2. Biological control

Biological control is known to be the best and effective method, against soil-borne pathogens. This method has many advantages such as environment friendly, cost effective and extended plant protection. Many fungal and bacterial species like *Pseudomonas*, *Trichoderma* and *Streptomyces* have antagonistic effect on Fusarium wilt of lentil. Among them *Trichoderma* species are been extensively used as bio-control agent against soil and seed-borne diseases [74]. A study revealed that the seed treatment with *Gliocladium virens* + *P. fluorescens* + carboxin and *Bacillus subtilis* + carboxin + *T. harzianum*/*T. viride*/*G. virens* have been found more effective in controlling Fusarium wilt incidence in lentil [79, 80]. In the recent study, two species of *Trichoderma* were employed against highly virulent isolate of *Fusarium* responsible for lentil wilt. The results revealed that *T. harzianum* was highly effective in controlling wilt disease in comparison to other isolate, when applied as a soil drench [74].

In an experiment conducted by Garkoti et al. (2013) observed significant reduction in disease incidence and maximum grain yield in field trials using 'Pant L-639' a popular cultivar against lentil wilt with *T. harzianum* + *Pseudomonas fluorescence* [81]. Similarly, in another report the result suggest that the disease severity was reduced with increased plant height with the combination of *T. harzianum* + *S. vermifera* [82]. Likewise, El-Hassan and Gowen (2006) has evaluated the formulation and delivery of the bacterial antagonist *Bacillus subtilis* against Fusarium wilt of lentil. The result reveal that the seed treatments with formulations of *B. subtilis* on glucose, talc and peat significantly enhanced its biocontrol activity against *Fusarium* compared with a treatment in which spores were applied directly to seed [83]. Additionally, several studies have also proved the importance of the organic material in reducing the disease incidence caused by plant pathogen like bacteria [84], fungi [85] and nematode [86] species.

7.3. Cultural practices

The cultural control generally depends on date and depth of sowing and manipulation of agronomic practices [68, 87]. It is reported that delay sowing usually lowers the wilt incidence whereas compared with early sowing (end of July), late sowing resulted in low yield [88]. The most suitable dates vary according to the different production regions. Use of clean seed for sowing and use of fungicidal seed treatment can reduce contaminating inoculum sources. To prevent the crop from various diseases a proper depth (10–12 cm) of seed planting should be used [89]. Intercropping/mixed cropping is being suggested for reduced wilt incidence and increased crop yield. Haware (1982) suggested that deep ploughing and removal of infected trash can reduce inoculum levels of Fusarium wilt of chickpea [90]. Soil solarization is another way to minimize the disease incidence [91]. In order to control the lentil wilt pathogen, chemical amendments (Mn and Zn) and foliar application on lentil wilt is also recommended. The

study suggests that the application of Zn and Mn salts at 80 ppm concentration on presoaked seeds of lentil has shown promising results on the control of wilt disease [92].

7.4. Fusarium wilt resistant cultivars

The initial step in managing the disease is to develop a reliable and reproducible disease screening techniques, so that a large number of germplasm (cultivated and wild relatives) can be evaluated in wilt sick plot and in greenhouse. The varietal resistance is a major goal of lentil improvement programme currently running at the International Centre for Agricultural Research in the Dry Areas (ICARDA). In order to identify the resistant variety of Fusarium wilt, screening under field and controlled conditions (green house and laboratory conditions) has been suggested [93, 94]. The systematic utilization of resistant source for wilt from cultivated accessions such as 'ILL 5883', 'ILL 5588', 'ILL 4400' and 'ILL 590' has resulted in the development of a wide spectrum of Fusarium wilt resistant varieties at ICARDA. Some of the prominent wilt resistant varieties in Syria ('Idleb 2', 'Idleb 3', 'Idleb 4' and 'Ebla 1'), Lebanon ('Talya 2', 'Rachayya' and 'Hala'), Turkey ('Firat 87' and 'Syran 96'), Ethiopia ('Adaa', 'Alemaya', 'Assano', 'Alemtena' and 'Teshale'), Iran ('Kimiya') and Iraq ('IPA 98') [95]. In India, several wilt resistant varieties are released such as 'L 4147', 'Pant L 406', 'Pant L 4', 'Pant L 639', 'Priya', 'Seri', 'JL 3', 'Noori', and 'VL 507' under national program [65, 96, 97].

The lentil germplasm can be screened under natural condition with natural inoculum of *Fol* in field. Wilt sick plot (WSP) is the most common method used to screen disease resistant plants under natural conditions. The WSPs have been developed by ICARDA, and NARS. The advantage of this method is that, large number of genotypes can be screened. Bayaa et al. (1997) has screened a core collection of 577 lentil germplasm accessions from 33 countries. The result reveals that the most resistant accessions came from Chile, Egypt, India, Iran and Romania and also emphasize the relative uniformity of disease pressure in WSPs [19].

Different inoculation methods have been used to for the infectivity of wilt in chickpea but in lentil very limited work has been conducted [12, 98, 99]. The inoculum density of about 10^6 conidia ml^{-1} is generally been used to establish the pathogen [100]. Wild species are an invaluable source for disease resistance. The wild germplasm of lentil was evaluated for resistance against biotic and abiotic stresses was done at ICARDA [101]. The crosses were made between the wild lentil (*L. culinaris* ssp. *orientalis*) and the cultigen has resulted in high-yielding selections under dryland conditions. Similarly, another study was done to screen the 221 accessions representing five species/subspecies, showed resistance in ILWL 113 (*L. culinaris* ssp. *orientalis*) from Turkey and ILWL 138 (*L. ervoides*) from Syria [102]. In India, seventy accessions representing four wild species/sub-species were evaluated and the donors for *Fol* resistance were identified in all species. The wild accessions of lentil (one of *L. culinaris* ssp. *orientalis* (ILWL76), five of *L. odemensis* (ILWLs 35, 39, 153, 237, 300), eleven of *L. ervoides* (ILWLs 40, 41, 42, 133, 204, 251, 258, 261, 271, 280 and 299) and six of *L. nigricans* (ILWLs 22, 26, 31, 37, 38, 430) can provide an important source of alien genes for disease resistance [103].

7.5. Genetic of Fusarium wilt resistance

The most economical means to control the Fusarium wilt of lentil is through the development of resistant varieties [12]. Due to the evolution of new races and co-existence of more than one

pathotypes, it is difficult to develop the resistant cultivars. Hence, the knowledge of about the inheritance and genetics of wilt resistance is important to develop resistant or moderately resistant cultivars. The studies in genetics of resistance to Fusarium wilt will eventually help to produce more resistant lentil cultivars [104]. Resistant or moderately resistant lentil cultivars (OPL 58, DPL 61 and DPL 62) significantly reduced wilt incidence and severity of root rot, and increase grain yield [31]. Very limited studies are available on the genetics and inheritance pattern of wilt resistance in lentil. Kamboj et al. (1990) has reported five independent genes to confer resistance to Fusarium wilt in lentil [105]. Eujayl et al. (1998) has also recorded the monogenic inheritance in 'ILL 5588' for wilt resistance and designated the gene as Fw. A study based on allelism test, identified 2 genes each of duplicate genes and complementary genes imparting resistance in the variety PL 234, JL 446 and PL 286, respectively [104]. However, Abbas (1995) has reported that only one dominant gene is controlling the wilt resistance found in the crosses made at ICARDA [106].

Stevenson et al. (1995) has explained that the plant root exudates and the difference in resistance in genotypes depend on the amount of root exudates and their antifungal compound [107]. Another study reveals that the root exudates release considerable amounts of organic substance in soil including the amino acids and sugars and the amino acid (Glycine and phenylalanine) were found to have an inhibitory effect upon the spore germination of pathogen [108]. Iftikhar et al. (2005) analyzed the presence and involvement of antifungal compounds in wilt resistance. The result suggests that the phenolics have an important role in imparting resistance against wilt disease because only wilt-resistant lines produced this compound [109]. Similarly, in another study the potential of the lines to produce phytoalexins influences their resistance to fungal infections [73, 110]. Similarly, a peptide with a molecular mass of 11 kDa, was isolated from dry seeds of red lentil has exhibited antifungal activity against *Fusarium oxysporum* [111]. These selected lines serve as a reliable source of disease resistance and can be used in Fusarium wilt resistance breeding programs.

7.6. Modern breeding approach

The classical plant breeding is based on recombination breeding approach by selecting the desirable plants on the basis of their phenotypic characters. However, this approach is less precise and time consuming when dealing with quantitative traits which are highly influenced by environment and genotype-environment (GE) interaction [112]. Therefore, it is important to integrate modern biotechnological tools such as genetic engineering and marker assisted selection (MAS) in lentil breeding program to mainstream new genetic variability in the cultivated gene pool.

In early 1980s, the first genetic linkage map of lentil was constructed using morphological and isozyme markers [113, 114]. Later, Eujayl et al. (1998) has reported first comprehensive linkage map with 177 RAPD, AFLP, RFLP, and morphological markers was developed using inter specific recombinant inbred lines (RIL) population of a single cross of *L. culinaris* × *L. orientalis* [104, 106]. Hamwieh et al. (2005) added 39 SSR and 50 AFLP markers to the comprehensive Lens map constructed by Eujayl et al. (1998), comprising 283 genetic markers covering 715 cM. They have constructed first genomic library from a cultivated accession, ILL5588 using the restriction enzyme Sau3AI (*Staphylococcus aureus* 3A) and screened with (GT)10, (GA)10, (GC)10, (GAA)8, (TA)10, and (TAA) probes. This study reveals that only SSR59-2B

was closely linked with Fw at 19.7 cM [115]. In an another study, a set of 122 functional SSR markers have been developed using a genomic library enriched for GA/CT motifs for utilization in the lentil breeding program [116].

As lentil has a narrow genetic base an inter-varietal linkage maps were developed by utilizing diverge parents from the wild and cultivated species but these maps have low recombination rate and the map size is also small. QTLs responsible for many traits can be identifying by intra specific mapping population and desirable gene of interest can be tagged. First intra specific lentil map was developed by Ford et al. (2003) through RAPD and ISSR markers [117]. Bi-parental mapping populations derived from the most divergent parents are always better for developing recombinant inbred line and through that a dense mapping or fine mapping can be done from the population developed through the cross of resistant and susceptible parents. These maps are useful to identify genes and major QTLs responsible for the variation of the trait of interest.

Gene cloning can help to characterize the function of the gene or QTLs responsible for the wilt and the knowledge of the genes cloned in lentil can facilitate the development of functional markers for the marker assisted selection. Resistant genes for different functions have cloned in lentil [118]. Using functional genomics approaches, genes expressing differentially in contrasting lentil genotypes can be identified.

Focusing towards the natural defense of host plant may reduce the impact of the pathogen on productivity. However, our poor knowledge about the molecular interaction between the crop and the pathogen limits support for breeding disease-resistant varieties. Due to the development of sequencing technologies, several genes coding transcription factors (TFs) and candidate defense genes (CDGs) from lentil are identified [112]. The full sequence of candidate defense genes like a β-1,3-glucanase (GLU1) (CV793598), a pathogenesis-relate (PR) protein from the Bet v I superfamily (AY792956), a disease resistance response protein 230 (DRR230-A) from pea (AJ308155), another disease resistance response protein (DRRG49-C) from pea (J03680), a pathogenesis-related 4 (PR4) type gene (DY396388) and a gene encoding an antimicrobial SNAKIN2 protein from tomato (HQ008860) are available in NCBI Genbank [112]. A partial sequence of translation elongation factor-1α (TEF-1α) (KR061303 and KR061304) from *Fusarium nygamai* infecting lentil were also deposited in Genbank [33]. These candidate genes and TFs should be further biologically characterized and can help us in decoding the defense pathways and pathogen recognition.

7.7. Integrated management of Fusarium wilt of lentil

Integration of two or more disease management option can reduce the impact of any disease affecting crops. The expected benefit in opting this strategy is improved and sustainable control of disease. The use of biocontrol agents in combination with chemical control can act as one of the strategies in controlling some soil-borne diseases. Therefore, some researchers have used the combination of *Bacillus megaterium* with carbendazim, which provided an effective control of Fusarium crown and root rot of tomato [119]. Similarly, the combination of soil amendments and biological control agents such as *Trichoderma* spp., have been shown to increase disease control and horticultural productivity [120]. Nowadays the use of organic amendments to improve soil properties, plant health and yield has expanded [121].

In Syria, the effect of different control options on disease parameters and yields were conducted as field experiment. The control options are changes in sowing dates, host plant resistance and fungicide seed treatment and the disease parameters like wilt onset, duration, per cent terminal wilt and areas under the disease progress curve were considered. The results revealed that the lentil genotypes had a greater effect on the onset and duration of Fusarium wilt than planting date or fungicide seed treatment. The percent terminal wilt and areas under the disease progress curve was observed lowest during November plantings for all lentil genotypes [78]. Therefore, different individual control options should be recommended to mitigate the effect of Fusarium wilt on lentil yield include manipulation of sowing date, fungicide seed treatment, biological control agents and host resistance [52].

8. Future directions

Knowledge about the pathogen has improved since it was observed, but still few challenges remain. A region specific race of the pathogen is needed. Since there are potential differences in the reaction of lentil cultivars to different races of the pathogen, so information about the distribution of races will be of great importance for breeding programs and the development of resistant genotypes. Along with this, a standardized set of host differentials is required to correlate pathogenicity with DNA techniques. A robust screening techniques for resistant to the pathogen is also required. With the lack of host-pathogen interaction studies, management remains elusive and additional research is needed in this area. Marker assisted selection (MAS) offers great opportunity for improved efficiency and effectiveness in the selection of plant genotypes with a desired combination of traits. Through marker assisted selection, disease resistance can be evaluated in the absence of the disease and in early stages of plant development. Implementation of markers for routine use in lentil breeding programs is currently very limited, integration of the markers within the breeding program to ensure that cost effective utilization of the technology is achieved. Establishment of a tight linkage between a molecular marker and the chromosome allocation of the gene(s) governing the trait to be selected in a particular environment is required. The information from multiple population-specific genetic maps can be integrated to produce high-density consensus structures utilizing the sequence-linked genetic markers which enables the identification of bridging loci between maps. This will further assist in the identification of more closely linked markers for Fusarium wilt resistance in lentil that can be effectively used in breeding and there is a need to develop and map more functional markers like EST-SSRs and SNPs on such maps to enhance their relevance in lentil genetics and breeding. The study based on SNP markers is still limited in lentil due to the lack of available sequence data. For effective variety development marker assisted selection is very imprint that requires much attention in lentil breeding program. Comparative genomics and synteny analyses with closely related legumes can play an important role in enhancing the knowledge of the lentil genome and can provide the genes and selectable markers for use in MAS. Transgenic and non-transgenic approaches including RNAi technology and virus-induced gene silencing (VIGS) can be explore to understand the molecular mechanisms of host resistance in lentil. Additional refined genetic materials are required in order to apply advanced genomic tools such as transcriptome profiling and

map-based gene cloning of lentil. Germplasm with wilt resistance and drought tolerance are key areas of emphases since the later pre-dispose the crop to *Fusarium* infection. In addition to host plant resistance, integrated management of Fusarium wilt is very important to narrow the yield gap due to Fusarium wilt in many countries.

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