



Identification of Lentil Genotypes for Resistance to Ascochyta Blight (*Ascochyta Lentis*)

Tolesa Bedasa

Department of Agricultural Research, Debre Zeit Agricultural Research Center, Ethiopia

ABSTRACT

Ascochyta blight, caused by *Ascochyta lentis*, is one of the most globally important diseases of lentil. The disease is seed and air borne causing huge loss and development of ascochyta blight resistant varieties is most effective means of controlling this disease. The diseases of lentil not only reduce yield but also deteriorate seed quality. To date, no highly resistant sources of ascochyta blight in lentil have not been reported from the Ethiopian lentil breeding programme. Past efforts have been directed towards developing improved varieties with resistance to one or the other biotic stress, improving the seed size, color of seed cotyledon, market quality and shortening the crop duration to fit lentil in various cropping systems. And also breeding for host resistance has been suggested as an efficient means and sustainable to control this disease. In present study, total of sixty five lentil entries received from Austria, is one of our partners, were evaluated at Alemtena and Minjar naturally hot spot field condition during the year 2018-19 and 2019-20 to identify sources of genetic resistant against ascochyta blight disease incited by the fungus *Ascochyta lentis*. These entries were assigned in augmented design with two replications that of checks were replicated after every eight test entries for the comparison purpose. The spacing was 20 cm between rows with 4m row length. The disease severity was recorded three times at different growth stage every 21 days intervals using (1-9) point disease ratings scale. High variations were observed in resistance level among the tested genotypes ranged from resistant to highly susceptible. Based on the reactions, 7 genotypes were resistant, 15 were moderately resistant and other become susceptible to highly susceptible which is 10 and 30 lines, respectively at Alem Tena. In another hand, 1 was resistant, eight were moderately resistant, twelve were susceptible and forty one genotypes were highly susceptible at Minjar. The promising genotypes would be used as a source of parental materials in the next breeding stages. Ethiopia still has different opportunities for enhancing the productivity of lentil including varied agro ecology, diversity of grain legumes, population and urbanization trends, and increased demand for animal feed and processed foods. Identification of more sources of resistance genes, good characterization of the host-pathogen system, and identification of molecular markers tightly linked to resistance genes are suggested as the key areas for future study.

Key words: Ascochyta blight; *Ascochyta lentis*; Alemtena; Minjar; Diversity of grain legumes

INTRODUCTION

Lentil is one of the most important legume crop in Ethiopia. It provides important economic advantages to the small scale farm households in providing food, income as a high-value crop, soil improvement and foreign currency earnings. Lentil straw is used as livestock feed, nodulated lentil fixes atmospheric nitrogen

(2018) and lentil provides a disease break in cereal cropping systems (2017). Lentil requires a cold climate and is grown in areas with temperatures ranging from 18°C to 30°C and an annual rainfall as little as 250 mm to a maximum of >1000 mm. Lentil is cultivated on a wide range of soil types; however, higher yields are realized when grown on sandy loam to clay loam soils that are fertile, have good drainage, water holding capacity and

*Correspondence to: Tolesa Bedasa Department of Agricultural Research, Debre Zeit Agricultural Research Center, Ethiopia; E-mail: naafsijira@gmail.com

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neutral to alkaline pH (6-7) (2009). Lentil do not grow well in soils with high boron (B) content, sodicity or salinity, which cause plant death and substantially limits yields (Pulse Australia, 2016). In Ethiopia, the private peasant holders grow lentils primarily for the seed which has relatively higher contents of protein, fast cooking characteristics, economic benefits, carbohydrate and calories as compared to other legumes (2013).

The major lentil producing countries are Australia, North America, Western Asia, the Middle East, Nepal, China, Ethiopia, Syria, Bangladesh and India (FAOSTAT, 2014). In Ethiopia, the major lentil growing regions are Oromia, Amhara and Tigray (CSA, 2019/20). The area coverage of lentil is about 87,443.89 hectare (ha) of the total cultivated land which is 1.193, 288.93 Quintal of total production and providing 13.65Qt/ha of the total productivity in Ethiopia (CSA, 2019/20). However, the crop is often challenged by biotic and abiotic stresses that reduce the full yield potential. Among these, *Ascochyta* blight (AB) caused by *Ascochyta lentis* affects gross profits and yield stability in Ethiopia. *Ascochyta lentis* (teleomorph *Didymella lentis*) is the causal agent of *ascochyta* blight of lentil (*Lens culinaris*), is a highly destructive disease in most lentil-growing areas of Ethiopia. Several workers have studied on the conventional breeding of lentil for resistance to *Ascochyta* blight, however, the susceptibility of previously released resistant cultivars was happened in its potential areas and compressive work on the source of resistant to *Ascochyta* blight will be focused. The pathogen grows asexually on the host plant, while its perfect stage can be recovered from previous lentil debris. *A. lentis* is specific to cultivated and wild species of lentil. *Ascochyta* blight is caused by fungus *Ascochyta lentis* which is able to attack all aboveground plant parts at any growth stage under favorable conditions. This disease favored by cool, moist weather and occurs on shoots, stems, pods and seeds. In severe conditions, *Ascochyta* blight infected crops may lose up to 70% grain yield, leading, and the disease causes reduction in yield and seed quality (1983). The seed harvested from *ascochyta* blight -infected plants are poor in quality, yield and appear small, discolored and shriveled, which makes them unmarketable. Additionally, the subsequent use of infected seed serves as primary inoculum for the spread of the disease (1997). *A. lentis* populations are highly variable in terms of aggressiveness on different lentil cultivars and wild accessions. Movement of the host germplasm has disseminated the pathogen worldwide where it is primarily introduced to new sites through infected seed [1-10].

Ascochyta blight infection on lentil seed and pods can affect grain yield and quality through seed abortion and seed staining. While infection on the foliage influences severity of seed and pod infection via rain-splash of conidia, cultivar responses to *ascochyta* blight on seed and foliage appear to differ (2012). *Ascochyta* blight causes varying degree of yield losses which can be as high as eighty percent depending on the level of resistance of the genotype. *Ascochyta* blight resistance is one of the traits of interest in improving lentil crop. Other traits such as yield, plant height and maturity are important for the success of a new cultivar. Kaiser and Hellier (1993) found the sexual stage of *A. lentis* on lentil straw in the USA in 1992. They showed that the fungus is heterothallic with two mating types, and that it is

probably a species of *Didymella*. confirmed the presence of two mating types of *A. lentis* by controlled crossing in the laboratory. The roles of the two mating types in the field leading to increased genetic diversity and adaptive potential or promoting variability in the pathogen population and in the disease cycle are not known. (2013) [11-15].

Infection and disease development and spread are favoured by cool, wet weather (Nene et al., 1988). The fungus may complete both sexual (teleomorph) and asexual (anamorph) stages in its life cycle (1997). Upon favorable climatic conditions, either of the stage can infect lentil and produce symptoms. The asexual stage produces a fruiting body known as a pycnidium within the lesions on the lentil plant, crop debris or seeds and releases pycnidiospores upon rain splash/heavy winds (1997). The dispersed spores settle and germinate on healthy lentil plants under favorable conditions (temperatures within a range 20°C-24°C and availability of moisture) and spread the disease (1994)[16-20].

The disease can be managed through the application of fungicides but host plant resistance (resistant genotypes) is the most reliable, environmental friendly, economically affordable to small scale farmers and durable method of management of *Ascochyta* blight and other diseases of lentil (2017). Many resistant cultivars/lines have been identified in both cultivated and wild lentil. The genetics of resistance to *Ascochyta* blight coming from *Lens orientalis* was first reported by Ahmad Resistance to *Ascochyta* blight in lentil is mainly under the control of major genes, but minor genes also play a role (2002). To breed for *Ascochyta* blight resistance, in lentils, it is necessary to identify resistant genetic sources and to have a good understanding of the genetics of the resistance in these resources. Resistances identified so far in lentil crops against the *ascochyta* blights provide only incomplete protection (2011). Resistance breeding in lentil crops has been slow due to the complex nature of resistance and the relatively low investment on genetics, genomics, and biotechnology of legume crops. However, continuous cultivation of relatively few resistant cultivars with narrow genetic base has likely led to episodes of resistance breakdown through selection of adapted and aggressive isolates [21].

Tripathi also reported as the disease may be effectively controlled by chemical methods such as seed treatment and foliar sprays. However, an indiscriminate use of fungicides contaminates the crop products and results in a negative impact on the environment. Additionally, the efficacy of fungicides has been affected in some situations due to the development of resistant strains within the target pathogen populations as evident a Carbendazim-resistant strain within the Australian *A. lentis* population (Lopez and Kay). Therefore, instead of a complete reliance on chemical control, resistant cultivars integrated with other management practices such as crop rotation, burial of infested residue, use of disease-free seed, and weather based sowing dates are more environmentally friendly and sustainable to combat AB. Breeding for resistance has been suggested as an efficient means to reduce the economic loss caused by *ascochyta* blight in lentil (2000). These resistances are mostly polygenic traits controlled by quantitative trait loci

(QTLs) (2012). Quick shifts in aggressiveness of the population of the causal agent *A. lentis* mandates developing germplasm with novel and durable resistance (2018). Moreover, the efficiency of resistant in lentil cultivars in *Ascochyta* blight is limited by pathogenic variability in the natural populations, location-specific occurrence of races which causes resistant cultivar to lose resistance over a period of time, due to this problem breakdown of genetic in lentil cultivars happened [22].

Control of the disease currently consists of the integrated selection of the most resistant varieties and best cultural practices, plus applications of fungicides on seed and foliage (2012). Fungicide applications are a considerable cost, both financially and environmentally, and can be difficult to apply in a timely fashion due to adverse weather and soil conditions therefore the development of highly resistant lentil varieties continues to be a primary breeding goal. Management of *ascochyta* blight requires an integrated approach including the use of certified disease-free seeds, sowing depth, crop rotation, burial of plant debris from previous planting season to prevent overwintering of the inoculum, fungicide seed treatment, use of resistant cultivars and foliar fungicides (2011) [23].

MATERIAL AND METHODS

Identification of resistance sources within lentil accessions collected from the Australian Gene Bank, was performed through conventional screening techniques. A total of sixty five lentil genotypes were evaluated for the resistance of *ascochyta* blight at two locations of naturally hot spot area. These all materials were introduced from Australia and all the tested genotypes were well germinated. Augmented design with two replications was used. The spacing was 20 cm between rows with 4m row length. After germination, observation was recorded regularly for the appearance of *ascochyta* blight and severity. The disease severity was recorded three times at different growth stage every seven to ten days intervals using (1-9) point disease ratings scale. According to Hussain disease data were recorded following 1-9 scoring scale at 15 days interval. For *Ascochyta* blight: 1 = No lesions visible (highly resistant), 3 = Few scattered lesions seen after careful searching (resistant), 5 = Lesions, common and easily observed, but little defoliation. Only in one or two patches in plot (moderately resistant), 7 = Lesions very common and damaging (Susceptible), 9 = Lesions extensive many plants killed (highly susceptible) (Hussain et al., 2008). Test genotypes were further categorized for their reaction to AB infection on the basis of Gowen et al. (1989) scale, according to this scale; 1-2 = Highly resistant (HR); 2-4 = resistant (R); 4-6=moderately resistant (MR); 6-7= moderately susceptible (MS); 7-9= susceptible (S); and 9-10=highly susceptible (HS).

RESULT AND DISCUSSION

This study was conducted under natural infection conditions in the field. The climatic conditions during the experiment were favorable for the development of fungal (*Ascochyta* blight). Results of disease reaction of germplasm accessions have been summarized in Table 1 and 2. A wide range of variation in disease reaction was observed among lentil genotypes. Maximum number of the genotypes were susceptible to

Ascochyta blight (*Ascochyta lentis*), whereas minority of genotypes showed resistant or moderately resistant reaction against *Ascochyta* blight (*Ascochyta lentis*). In the present study, a significant variation among the genotypes of lentil against to *Ascochyta lentis* was observed. Phenotypically, variation in resistance level among the tested genotypes was grouped according to their reaction to *Ascochyta lentis*. Disease symptoms on different accessions ranged from small flecks (resistant) to extensive lesions on both leaves and stems with death of some plants (highly susceptible). Among 65 lentil genotypes tested for resistance to *A. lentis*, none was immune or highly resistant; 7 (507, 611, 859, 1720, 109058, 123452, and 123514) were resistant, sixteen were moderately resistant and one, 10 and 30 lines were moderately susceptible, susceptible and highly susceptible, respectively at Alem tena. Among 65 tested genotypes, 1 was resistant, and 34 showed an average reaction while the rest ranged from susceptible to highly susceptible. Some lines were resistant at one location, but not at others. This suggests the presence of pathogen variability or different pathogen inoculum [24].

Table1: Reaction of *Ascochyta* blight on lentil genotypes in 2019/20 cropping season at Alem tena

Disease reactions	Genotypes	Final PSI (%)
Resistant (7)	507,611,859,1720, 109058, 123452, 123514	33.3%
Moderately resistant (16)	96, 921, 73968, 109051, 123533, 123798, 600, 615, 712, 857, 911, 5418, 69537, 112082, 123469, 123652, 123801	14.3 - 20.0 %
Moderately susceptible (1)	506	24.3 - 30.0 %
Susceptible (10)	94, 171, 301, 504, 641, 914, 915, 68520, 71438, 123499	33.3- 50.0%
Highly susceptible (30)	ICC 3421, ICC 7184, ICC 11121, ICC 637, ICC 9586, ICC 15606, ICC 7441,, ICC 1098, ICC 12851, ICC 9402, ICC 7867, ICC 8058, ICC 11498, 597, 190, 207, 298, 341, 342, 606, 640, 642, 920, 1735, 2167, 4369, 4849, 4851, 4853, 5233, 70175, 71436, 71437, 71442, 71489, 75947, 75972,76141, 107896, 109050,	88.9-100

115077, 115201,
123483

Table2: Reaction of *Ascochyta* blight on lentil genotypes in 2019/20 cropping season at Minjar

Disease reactions	Genotypes	Final PSI (%)
Resistant (1)	123801	33.3%
Moderately resistant (8)	96, 507, 642, 712, 859, 112082, 123452, 123514	14.3 - 20.0 %
Moderately susceptible (1)	298, 640, 1720	66.7 %
Susceptible (10)	504, 611, 73968, 75947, 109050, 109051, 109058, 115201, 123469, 123483, 123533, 123798	77.8 %
Highly susceptible (41)	301, 341, 94, 171, 190, 207, 342, 506, 597, 600, 606, 615, 641, 857, 911, 914, 915, 920, 921, 1735, 2167, 4369, 4849, 4851, 4853, 5233, 5418, 68520, 69537, 70175, 71436, 71437, 71438, 71442, 71489, 75972, 76141, 107896, 115077, 123499, 123652	88.9-100

Reactions observed on lentil lines starting from 21 days after planting from 1 (no visible lesion) to 9 (plant death). In addition to leaf lesions, extensive defoliation and stem girdling was observed on most of the susceptible lines. Symptoms differed mainly in the extent of lesions. Disease symptoms were observed first on the most vulnerable lines. To compare the resistance of the different lines, the mean disease scores were computed for each line and expressed as a percent of severity index. On the basis of disease indices, the lentil lines were divided in to five groups i.e. resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible (Table 1 and 2).

Lentil breeding for resistance to *ascochyta* blight has been the objective of international and national breeding programs. The lentil genotypes those showed resistant and moderately resistant disease reaction against to *ascochyta* blight would be further tested in wide representative agro ecologies in the preliminary variety trial or in national variety trial [25-30].

CONCLUSION

Forward research would be focused on the study of variability among the pathogen for their pathogenic behavior and other

morphological traits which is vital for the development of breeding strategy to evolve genotypes with durable disease resistance. Success in breeding improved crop cultivars is determined by the amount of increased genetic variability among the elite cultivars achieved through the introduction of germplasm from a wider genetic background into the cultivated background for resistance to disease and increased yield potential. Unless, the amount of genetic variability existing between the Ethiopian lentils parents that are selected for hybridization revealed similar. Identification and characterization of lentil genotypes for the source of multi-disease resistance would be continues work in research program [31-38].

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