



Research Article

Evaluation of Dry Pea Germplasm against *Ascochyta* Blight Disease under Green House Condition

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Abstract

Dry peas (*Pisum sativum* L.) are grown in the world for its utility in variety of delicious food items. Diseases like blight caused by *Ascochyta pisi* causes severe yield losses in its production. The current research was aimed at evaluation of response of one hundred and three dry pea germplasm genotypes against blight disease under the controlled environmental conditions in plastic tunnel. A highly susceptible check variety was planted after every two test germplasm genotypes. Disease was induced by artificial spraying of *Ascochyta pisi* culture. The experiment was conducted in Pulses Research Institute (PRI), Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan from 2018 to 2020. The data was recorded according to the disease rating scale, only one germplasm genotype was found highly resistant while three germplasm genotypes shown resistant response against the disease. Two germplasm genotypes showed moderately resistant response. Thirteen germplasm genotypes exhibited susceptible response. Eighty-four germplasm genotypes showed highly susceptible response against the disease. This study indicates that most of germplasm genotypes found susceptible against *Ascochyta* blight, but the research activity is useful to find out resistance sources of dry pea against blight disease.

Keywords: Pea, *Pisum Sativum* L., *Ascochyta* Blight, Germplasm, Resistant.

Introduction

Dry Pea (*Pisum Sativum* L.) is one of the oldest food crops which belong to the leguminous family. It is a cool season crop and is grown in many countries of tropical and subtropical region including Burma, India, Pakistan, Peru, Ecuador, Columbia, Morocco and Ethiopia. The legume crops have ability to fix atmospheric nitrogen which increases their ecological importance, especially in soil fertility restoration (Pachev *et al.*, 2011, Butnariu, *et al.*, Butu, *et al.*, 2015, Barbat, *et al.*, 2013 and Butnariu, *et al.*, 2015a). The addition of this crop balances the different nutrients level in the soil which became deficient due to cereal based cropping (Christine *et al.*, 2016, Rodino, *et al.*, 2014, Butnariu and Coradini, 2012). Peas are of two types including summer annual and winter annual legume. In Pakistan, dry pea is grown as a winter crop in the plains of all four provinces. It is also grown as a summer crop in the highlands of KPK. It can be grown in both, rain fed and irrigated regions. The area of field pea cultivation in Pakistan is about 45.30 thousand hectares with average production of 658 kg ha⁻¹ (Anonymous, 2015-16).



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Ascochyta blight caused by *Ascochyta pisi* is a major disease of leguminous crops and potentially is an important limiting factor in yield of dry peas. It attacks the plant at vegetative and seedling stage which causes poor plant growth and reduces its yield. The disease appears regularly in alarming epidemic form both in barani and irrigated areas of Pakistan. *Ascochyta* blight occurs in epidemic form during the years receiving more than 13 cm rainfall. The pathogen infects all the above ground parts of the plant and is perpetuated from season to season through the disease debris lying in the field from previous year (Maden,1987). The fungus perpetuates rapidly on dry peas crop in rainy season with 85-98% humidity and temperature of 20°C (Chauhan and Sinha,1973).

Ascochyta blight (*Ascochyta pisi*) is the most serious disease of dry peas, it causes considerable degradation in quality and the yield of the crop stand. It has been reported to cause 50-70% crop losses at favorable environmental condition for the disease development (Malik and Bashir, 1984). *Ascochyta* Blight has also been reported from Latin America (Kaiser, 2000) and North Africa (Akeem, 1999). *Ascochyta* blight is one of the most important yield limiting factors in Australia and Canada potentially affecting 95% of the area sown by dry peas (Gan *et al.*, 2006). Disease epidemics in Pakistan as well as in different parts of the world have also been reported (Kausar, 1965).

Its characteristic symptoms include slightly sunken, tang coloured lesions with definite dark brown margins on leaves, stems and pods. These lesions are circular on leaves and pods and elongate on stems, small black pycnidia are often present. Sometimes disease also attacks at base of plant (Bretag *et al.*, 2006). Dry peas blight is mainly controlled by fungicides. As the fungicides are not eco-friendly and increase inputs cost when applied on larger area, therefore these are not recommended. The best long-term strategy for against *Ascochyta* blight disease is the development of *Ascochyta* blight resistant pea varieties (Bretag *et al.*, 2006). Hence, resistant or tolerant varieties of dry peas may be the most effective tool to control dry peas blight (Gan *et al.*, 2006, Ilyas *et al.*, 2007). Genetic studies revealed that resistance of dry peas against *Ascochyta* blight disease is due to either a single dominant gene or recessive gene (Reddy & Singh, 1993). Thus, the present study was conducted to identify the new sources of resistance to develop blight resistance in dry pea cultivars.

Methodology

Preparation of Inoculum of Pathogen

Isolation and Purification of Pathogen: Blight affected pods were taken from the disease infected plants grown in the pluses Research Institute Faisalabad seeds were removed aseptically which were further placed in petri plates on dry peas seed meal agar medium having 20g of Agar, 20g glucose, 20g gram seed meal and 940ml of sterilized water (Ilyas and Iqbal, 1986). The colonies of *A. pisi* developing out of blighted seeds were isolated and purified by spore streak method (Phatak,1986). The purified culture was maintained at 5°C until used for further studies.

Mass Culturing and Inoculum Spray: Dry pea seeds were soaked in tap water for 5-7 h which was then boiled for 20-30 minutes. The seeds were then surface dried and placed on paper. 350-400 seeds were put in polypropylene bags (12x15cm). Bags were sealed by placing cotton plugs and tied with rubber bands and then placed them in autoclave @15psi for 25-30mins. Bags were inoculated by 8mm sized mycelial plugs from pure culture. Streptomycin (10mg/bag) was added in bags for reducing bacterial contamination and bags were placed in incubator at 20±5°C for two weeks for complete development of conidia (Ilyas and Khan,1986). Inoculum from bags was stirred in water to prepare spore suspension which was passed through muslin cloth for purity. The filtered solution was used for spraying purpose as an inoculum.

Screening of Dry Peas Genotypes against Blight Disease

The present study was conducted in 2016-2018. One hundred and three (103) genotypes/lines of dry peas were tested against blight disease by artificially inoculating the germplasm genotypes under plastic tunnel where day and night temperature ranged from 8-25 °C and humidity was maintained above 85% by sprinkling fresh water. Each germplasm genotypes was sown in a single row subplot of two meter length with 30cm row to row distance and 15cm plant to plant distance. The experiment was conducted in Pulses Research Institute (PRI), Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan from 2018 to 2020. The germplasm was sown in RCBD design with Each germplasm genotype was sown in a single row subplot of two-meter length with row to row distance of 30 cm and plant to plant distance of 15 cm. A highly susceptible check line peas-2009 was planted after every two test germplasm lines. At adult plant stage i.e.at flowering and early pod formation, the test lines were sprayed every day with the spore suspension of *Ascochyta pisi* (1×10^5 spores/ml) until the appearance of disease on the susceptible check pea-2009 and its death.

Disease Rating

The data regarding disease screening of dry pea lines/varieties against the *Ascochyta* blight were assessed based on 1-9 disease rating scale (Pande *et al.*, 2011). Where 1= no visible symptoms, 2= minute lesions prominent on the apical stem, 3=lesions up to 5mm in size and slight drooping of apical stem, 4= lesions obvious on all plant parts and clear drooping of apical stem, 5=lesions on all plants parts, defoliation initiated, breaking and drying of branches slight to moderate, 6= lesions on all aerial parts, defoliation, broken, dry branches common, some plants killed, 7=lesions as in 5, defoliation, broken, dry branches very common, up to 25% of plants killed, 8= symptoms as in 7 but up to 50% of the plants killed and 9= symptoms as in 7 but up to 100% of the plants killed.

Result and Discussion

The screening of 103 examined genotypes (Table1) revealed that one line exhibited resistant and only three lines exhibited moderately resistant response. While 2 lines were found moderately susceptible, thirteen lines susceptible and eighty-four lines showed highly susceptible type of reaction in the present study.

Table1. Response of genotypes/lines after artificial inoculation with *Ascochyta* blight.

Scale	Disease Reaction	Pulses Research Institute, Faisalabad	
		Year (2016-2018)	
1	Highly Resistant (HR)	DP-2	
3	Resistant (R)	DP-11, DP-12, DP-55	
5	Moderately resistant (MR)	DP-30, DP-35	
7	Susceptible (S)	DP-1, DP-3, DP-4, DP-5, DP-13, DP-14, DP-15, DP-31, DP-32, DP-33, DP-34, DP-46, DP-50	
9	Highly Susceptible (HS)	DP-5, DP-6, DP-7, DP-8, DP-9, DP-10, DP-16, DP-17, DP-18, DP-19, DP-20, DP-21, DP-22, DP-23, DP-24, DP-25, DP-26, DP-27, DP-28, DP-29, DP-30, DP-35, DP-36, DP-37, DP-38, DP-39, DP-40, DP-41, DP-42, DP-43, DP-44, DP-45, DP-47, DP-48, DP-49, DP-51, DP-52, DP-53, DP-54, DP-56, DP-57, DP-58, DP-59, DP-60, DP-61, DP-62, DP-63, DP-64, DP-65, DP-66, DP-67, DP-68, DP-69, DP-70, DP-71, DP-72, DP-73, DP-74, DP-75, DP-76, DP-77, DP-78, DP-79, DP-80, DP-81, DP-82, DP-83, DP-84, DP-85, DP-86, DP-87, DP-88, DP-89, DP-90, DP-91, DP-92, DP-93, DP-94, DP-95, DP-96, DP-97, DP-98, DP-99, DP-100, DP-101, DP-102, DP-103	

DP = Dry peas

During study, it was found that most of the genotypes were highly susceptible to susceptible. This shows that one line of dry peas germplasm is highly resistant to blight disease. In Pakistan, present available germplasm is mostly susceptible against dry peas blight. Iqbal *et al.*, (2010) screened out one hundred and forty-five genotypes against *Ascochyta* blight and *Fusarium* wilt disease. Most of the genotypes showed susceptible to highly susceptible reaction. Similarly, Bokhari *et al.*, (2011) evaluated the resistance level of ten cultivars of chickpeas and observed that maximum number of varieties were susceptible under field conditions. The resistant varieties found in this study can further be exploited in breeding programmes for the development of disease resistant commercial cultivars after determining their genetics. A thoroughly study on the number of genes conferring resistance against *Ascochyta* blight, their nature and diversity is essential for exploiting a particular resistance source in resistance breeding programme. *Ascochyta* blight resistance is a complex endeavor suggesting that there is a range of different resistant sources with different genes of resistance (Collard *et al.*, 2003). Genetic studies revealed that resistance of chickpea against *Ascochyta* blight disease is due to either a single dominant gene or recessive gene (Reddy and Singh, 1993)

To date the different varieties of different research institutes are mostly susceptible to present races of *Ascochyta* disease (Ghanzanfar *et al.*, 2010). Previously a number of chickpea resistant lines/ cultivars have been identified against *Ascochyta* blight at national and international levels. Pyramiding of different resistance genes into commercial cultivars may facilitate building up the level of resistance and increasing the durability of resistance in the commercial cultivars. (Tekeoglu *et al.*, 2000)

Like this, the moderately resistant varieties found in this study can further be exploited in breeding programs for the development of disease resistant commercial cultivars after determining their genetics. A thoroughly study on the number of genes conferring resistant against *Ascochyta* Blight, their nature and diversity is essential for exploiting a particular resistance source in resistance breeding program. *Ascochyta* blight resistance is a complex endeavor suggesting that there is a range of different resistant source of different resistant genes (Collard *et al.*, 2003). Since the host plant resistance is not stable due to emergence of new pathotypes of *A. rabiei*, therefore, identification of resistant sources against the prevalent pathotypes/isolates should be considered. The present study was conducted to identify the new sources of resistance to develop blight resistant chickpea cultivars (Collard *et al.*, 2003). The frequency of highly resistant lines is generally very low. This indicates the high aggressiveness or relatively narrow diversification of genetic materials studied.

Conclusion

It can be concluded from this study that frequency of resistance is very low; this is due to high variability and aggressiveness of the pathogen. The moderately resistant source found in this study can be utilized in breeding program for the development of new commercial varieties.

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