

Leaf stripe of barley caused by *pyrenophora graminea*, occurrence in barley seeds in Iran

Leila Zare* and Shahla Hashemi-fesharaki

Seed and Plant Certification and Registration Institute (SPCRI), Karaj, Iran

Corresponding author Email: zare_l@yahoo.com

ABSTRACT: In order to evaluate the certified and foundation barley seed contamination with leaf stripe disease, 180 samples from different most important provinces were tested in 2011-2012 in Iran. For isolation of *pyrenophora graminea*, osmotic method was used. The brick-red pigment which changed colour to violet was counted as infected (ISTA, 2012). Results of variance analysis of tests showed significant differences ($P=0.01$) among the provinces. The results showed that the highest infection percent of barley fields to leaf stripe in certified categories were related to Golestan, Qom and Hamedan by 13, 11.17 and 10.67 percent respectively. The lowest infection was belonged to Semnan, Yazd, Zanjan, Ardabil, Mazandaran and Isfahan (less than 1 percent). In foundation (mother) barley seeds Golestan and Qom were placed in one group with the highest infection rate (> 10%) to barley leaf stripe disease. West Azarbayejan, Ardabil, Mazandaran, Isfahan, Hamedan and Semnan were free of disease or less than 1% infection. More research could be done in order to determine economical importance of seed-borne diseases of barley in infested regions since yield losses caused by this fungal pathogens can cause significant yield losses in barley.

Keywords: Barley seed, foundation, certified, *pyrenophora graminea*

INTRODUCTION

Pyrenophora graminea Ito & Kuribayashi [anamorph *Drechslera graminea* (Rabenh. ex. Schlech. Shoem.)] the causal agent of leaf stripe, is exclusively seed borne and grows systemically within the developing barley plant (Mathre, 1997). The fungus survives in kernels as mycelium on the pericarp, and when a barley seed germinates, enters the plantlets through the coleorhizae (Platenkamp, 1976). Fungal hyphae grow intercellularly from the coleorhiza up all sides to the roots and scutellar node, where they initiate shoot infection (Haegi et al., 1998). In many barley growing areas of the world leaf stripe often leads to reduction in yield (Arabi et al., 2004; Porta-Puglia et al., 1986). The disease is particularly acute in Mediterranean's below 12 °C during seed germination promote the infection of rootlet (Arru et al., 2003).

The fungus is seed-borne and overwinters on or in the seed. It grows internally within the infected plant. Spores are produced on the infected leaves and are spread by wind

to nearby healthy heads. The seeds become infected only in the field and are most susceptible during early development of the seed head. Sporulation on the foliage of infected plants is encouraged by high humidity. Infection of seedlings is higher when soil temperatures remain below 15 °C. The teleomorph stage is not important for the epidemics.

This is potentially the most serious seed-borne disease of barley. If seed from affected crops is re-sown without an effective fungicidal seed treatment being applied, the disease can multiply very significantly and produce large yield losses. By re-sown repeatedly, complete crop loss is possible within a few generations of seed multiplication.

Barley stripe can cause significant yield losses in barley, other cereals are not affected. The disease is dangerous for spring barley especially. Yield loss is directly proportional to percentage of infected plants. A 1 % infection causes a 0.7 % direct yield loss. Some crop compensation occurs from neighbouring healthy plants. Decreasing of yield is 5-1 % in general, 70% yield losses is

achieved only sporadically, by high primary seed infestation and good weather condition. Efforts to minimize the impact of leaf stripe have been centered on the use of management strategies such as host resistance, crop rotation, tillage, and fungicide application (Pecchioni et al., 1999; Skou et al., 1994). From a management perspective, the comparison of leaf stripe epidemics across years and locations is necessary to determine the effects of the environment on the efficacy of a given management approach, under similar environmental conditions, and to develop or recommend management strategies or decision thresholds (Boulif and Wilcoxson, 1988; Pecchioni et al., 1996). Zad et.al reported that leaf stripe is one of the most important diseases of barley in Iran especially in Gorgan, Mazandran and near Tehran (Varamin)(Zad, et.al,2002).

Therefore, Seed health testing to detect seed-borne pathogens is an important step in the management of crop diseases. Little is known about the status of this group of pathogens in barley seed categories such as foundation (mother) and certified in different growing region of Iran.

MATERIALS AND METHODS

A total of 180 seed samples were collected from different locations in certified and foundation barley loads, including 10 provinces (Semnan, Yazd, Zanjan, Qom, Ardabil, Khuzestan, Isfahan, Hamedan, Golestan and Mazandaran) for certified and 8 province (Semnan, Ardabil , Isfahan, Hamedan, Qom , West Azarbayejan and Mazandaran) for foundation after harvest in 2010-2011. At least 5 different locations that is most cultivated areas of barley were considered randomly in each province. A seed sample (1 kg) was taken from each farmer from a seed lot intended for planting based on ISTA rules (ISTA, 2004) kept in a paper envelope and brought to the laboratory for isolation.

For isolation of *Drechslera graminea*, Osmotic method was used. 400 seeds of each sample placed on filter paper's well which is dipped in the sugar solution. Samples incubated for 7 days with alternating bright light (at least 4000 lux) for 16 hours at 26 ± 2 °C and darkness for 8 hours at 22 ± 2 °C. A control sample of seed with known infection incubated under the same conditions as the test samples or other suitable control. For examination the seeds removed and 1% NaOH solution poured onto the filter paper. The brick-red pigment which changed colour to violet was counted (ISTA ,2012). For more information the infected seeds were cultured on PDA.

RESULTS AND DISCUSSION

Certificated and healthy seed is an important input for crop production and hence reduction of yield loss caused by fungi is one way to contribute to the food security in the world. *Pyrenophora teres* and *P. graminea* are seed-transmitted fungi in barley. Plants infected with *P.*

graminea will not give any yield. Infection with seed-borne *P. teres* contributes to yield reduction, especially if plants are infected early. The osmotic method was invented by Joelson in the 1980s (Joelson, 1983). He found that by using a method that is not based on morphological characteristics, costs were lowered as staff input was reduced and throughput increased. The method is based on the ability of *Pyrenophora* spp. to produce brick-red pigments (anthraquinones) on the filter paper by incubation of seeds under certain conditions (correct temperature, bright light and adequate moisture). Our results indicate the incidence and geographical distribution of *pyrenophora graminea* in the certified and foundation barley seed loads in Iran. Results of variance analysis of tests showed significant differences ($P=0.01$) among the provinces in barley leaf stripe disease (Table 1).

The results showed that the highest infection percent of barley fields in certified categories were related to Golestan, Qom and Hamedan by 13, 11.17 and 10.67 percent respectively (Fig.1). Khuzestan by 1.33 percent was between two statistical groups. The lowest infection was belonged to Semnan, Yazd, Zanjan, Ardabil, Mazandaran and Isfahan (less than 1 percent).

In foundation (mother) barley seeds Golestan and Qom were placed in one group with the highest infection rate ($> 10\%$) to barley leaf stripe disease. West Azarbayejan, Ardabil, Isfahan , Mazandaran , Hamedan and Semnan were free of disease or less than 1% infection (Fig 2).

High infection level of disease in Golestan and Qom is related to infected seeds and susceptible cultivars (in Qom) and most important suitable conditions for disease specially in Golestan province.As primary infections can occur if the temperature is over 8 °C at germination. The optimum range for the development of the disease is 12 - 16 °C. Cold humid soils at emergence and in the first growing stages favour the disease which all of these environment were in these areas. The disease is particularly acute in Mediterranean's below 12 °C during seed germination promote the infection of rootlet (Arru et al., 2003). So if seed is saved and re-sown repeatedly, complete crop loss is possible within a few generations of seed multiplication.

The mycelium survives in the seed and causes the primary infection in the seedling when the seed germinates in the soil. Conidia may occur on the lesions and contaminate the neighboring seedlings. Persistence of the mycelium in the stubbles can cause secondary contaminations of the leaves but these lesions usually reach only 2 - 3 cm.Because of the strictly seed-borne nature of this disease, a healthy seed is extremely important. It is achieved either by seed production in semiarid areas without sprinkler irrigation, or by seed treatment. Efforts to minimize the impact of leaf stripe have been centered on the use of management strategies

such as host resistance, crop rotation, tillage, and fungicide application (Pecchioni et al., 1999; Skou et al., 1994).

Our results indicate the incidence and geographical distribution of the barley leaf stripe in the irrigated barley certified and foundation seeds sites of Iran. More research could be done in order to determine economical importance of this disease of barley in infested regions since yield losses caused by the fungal pathogen is not yet

known in Iran. Hence, there is need for reducing the pathogenic fungi in produced seeds by applying different control options. Seed-borne fungi are the easiest pathogens to be controlled through treatment of seed using suitable chemicals and biological compounds. In addition, using standard stores for preserving seed and planting resistant cultivars for protecting the infection level below damage threshold have been recommended.

Table1. Variance analysis of barley infection to leaf stripe between provinces.

Source of variables	Seed categories	DF	Mean Square
Province	certified	9	309.98**
	Foundation	6	2631.62**
Error	certified	53	
	Foundation	62	
total	certified	71	
	Foundation	59	

** Significant at 0.01 confidence level

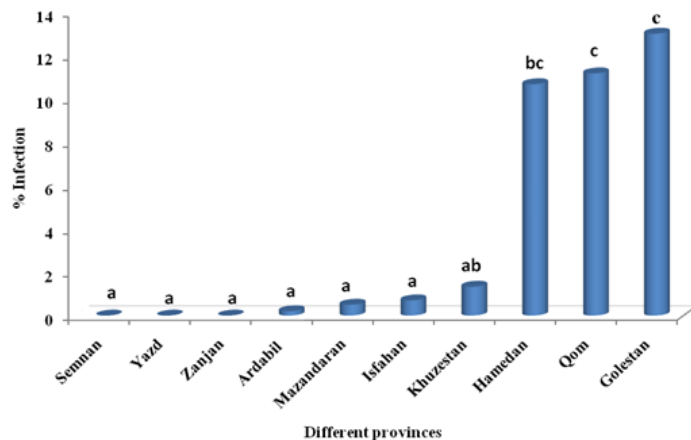


Figure 1. Means Comparison of *pyrenophora graminea* Infection in certified barley seeds Means in the same columns are not significantly different according to DMRT (P=0.01)

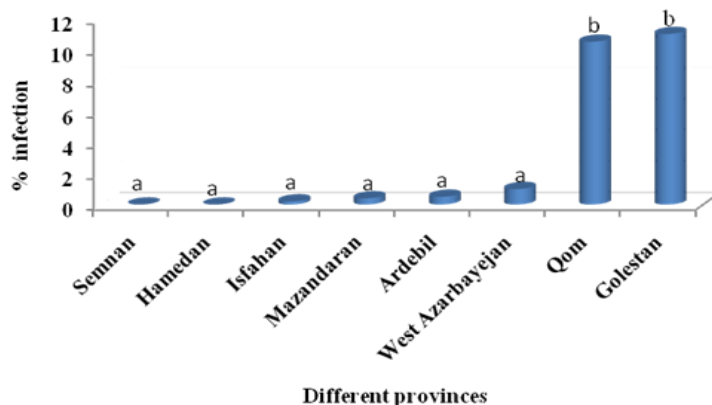


Figure 2. Means Comparison of *pyrenophora graminea* Infection in foundation barley seeds Means in the same columns are not significantly different according to DMRT (P=0.01)

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