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Seedborne Fungi in Hungarian Vetch and Their Transmission to the Crop

Arzu Coşkuntuna and Nuray Özer
Department of Plant Protection, Faculty of Agriculture, University of Trakya, 59030 Tekirdağ/Turkey

Abstract: Isolation were made from original seed stocks of different lines of Hungarian vetch (*Vicia pannonica* Crantz) to determine seedborne fungi. The seed coat, cotyledon and embryo were cultured and the fungi were determined. Newly harvested seeds obtained from the plants grown in the field, after sowing of the original seed stocks, were examined in the similar manner and the variations of incidence of fungi on different seed parts were evaluated. The transmission of the determined fungi on original seed stocks to the plant roots was also investigated in both sterile and field soil. *Aspergillus niger* v. Tieghem was found at the high rates in all parts of original seed samples and in roots of the plants that developed from these seeds in sterile and field soil, differing to the lines. This fungus was re-established to the newly harvested seeds after vegetation period, however its incidence in the tissues decreased at high rates in the most of lines. *Aspergillus alutaceaus* Berk. and Curt was determined in the original seed stocks of some lines, but was not transmitted from seeds to the roots or newly harvested seeds. *Alternaria alternata* (Fr.) Keissler appeared in only newly harvested seeds from the some lines was probably airborne. *F. culmorus* (W.G.Sm) Sacc., *F. equiseti* (Corda) Sacc. and Fusarium oxysporum Schlecht appeared in the roots of the plants grown in sterile or field soil could not develop on original seeds. All data in this study were recorded for the first time in Hungarian vetch.

Key words: Humgarian Vetch, fungi, transmission

INTRODUCTION

Hungarian vetch (*Vicia pannonica* Crantz) is an annual forage legume cultivated for hay, green manure, pasture and seed[1-4]. It is most important alternative forage legumes for drought and cold climatic regions because of its high tolerance to these conditions. Hungarian vetch-cereal rotation has been suggested for high cereal yield in these regions[5-7]. This plant is also capable of improving soil nitrogen by fixing atmospheric nitrogen[8].

Since no information is available for any fungal disease on Hungarian vetch this study was designed 1) to screen the original seed stocks from ten lines for the presence of fungi in the embryo, cotyledon and seed coat, 2) to examine the possible transmission of isolated fungi from original seed stocks to plants in sterilized and in field soil and 3) to evaluate newly harvested seeds after vegetation period for the variations of incidence of fungi in the seed tissues.

MATERIALS AND METHODS

Occurrence of the fungi on Hungarian vetch seeds: Seed stocks from ten Hungarian vetch lines were separately obtained from the Field Crops Department of Tekirdag Faculty of Agriculture and tested for the presence of fungi. First, the seeds were sterilized by dipping them in 1% sodium hypochlorite solution for 3 min, followed by several rinses in sterile distilled water.

They were then soaked in sterile distilled water for 12h, filtered and dried on sterile filter paper. The embryos and endosperms were separated with the sterile scalpel under a stereomicroscope. The dissected seed embryos were placed Individually on potato dextrose agar (PDA) in a glass Petri dish of 10 cm diameter, and the cotyledons in another petri dish. To screen the seed coats, intact seeds, not sterilized, were placed in Petri dishes containing moist filter paper. The experimental unit was a Petri dish containing ten kernel parts and there were ten replicates Petri dishes of each part. All Petri dishes were incubated at 25°C for 10 days in dark, the colonies of fungi which developed around the samples on agar and filter papers were examined and identified under a stereomicroscope.

Seed Transmission of fungi in sterile and field soil: Seed Transmission of fungi was investigated in naturally contaminated seeds (original seed stock) from each line. Ten seeds were sown in 20x24 cm plastic pots containing a sterilized mixture of field soil:manure:sand, 1:1:1 and five such pots were allocated for each line.

Corresponding Author: Mr. Nuray Özer, Department of Plant Protection, Faculty of Agriculture, University of Trakya, 59030 Tekidağ/Turkey Fax: 00 90 282 2931454 E-mail: nurayozer@tu.tzf.edu.tr

Three replicates of the samples of the same original seed stock were sown in an experimental field of the Tekirdağ Faculty of Agriculture. A 3m long bed, 30 cm row distance and 150 seeds m^{-2} sowing rate were used. There were five rows in each bed and seeds were sown manually.

Plants from each seed sample, grown in the pot, were harvested during eighth month of growth. In the field, 50 plants from each bed were harvested during the same period by taking 10 plants from a randomly selected length of each row. They were removed from the soil and 1-2 mm pieces of roots were placed on the agar plate and identification of the colonies was done after 10 days of incubation at 25°C.

The other plants remained in the field until the pods on the lower parts of the plant become yellow in color. After this period, the seeds were removed from the Pods and were examined again using the method above mentioned for the isolation from original seed stocks.

The variations of the incidence of fungi in different parts of newly harvested seeds were determined by calculating the percentage of increase or decrease in incidence, comparing with the original seed stock for each line.

The optimum temperature and relative humidity during the sterile soil experiment was 20°C and 55%, respectively; that during the field experiment was 11.5°C and 84.4%.

RESULTS AND DISCUSSION

Occurrence of the fungi on original and newly harvested seeds of Hungarian vetch lines: Two species of fungi were identified in original seed of Hungarian vetch and their tissues (Table 1). Among them, *A. niger* was isolated from all samples and seed parts especially in the lines 31, 36, 62, 69, 72 and 82. However in the line 28, the highest rate of contamination of the seed coat was by *A. niger*. The rates of the embryo and cotyledon contaminated with this pathogen were higher in the lines 36 and 72 than in those of other lines. *A. alutaceaus* was isolated at low rates from samples from some of lines. Both fungi were identified for the first time on Hungarian vetch seeds and their parts.

A. alternate and *A. niger* were identified when the newly harvested seed samples from the same lines were examined after vegetation period (Table 1). *A. alternate* was assumed to be an airborne contaminant since it was not detected in the samples from original seed stocks. Among the fungi determined in the seed parts, only *A. niger* had the ability for re-establishment to the newly harvested seeds from most of the lines. However this pathogen could not re-established to the newly harvested seeds when the seed samples from the lines 28 and 39 with its seed coat and cotyledon infection, respectively, were used in the field experiments. New infection of embryo and cotyledon by this fungus was observed in the seed samples from lines 23 and 30. In the other lines, *A. niger* was present in the all parts of newly harvested seeds such as in original seed stocks.

Variations in the incidence of fungi on newly harvested seeds: Since only *A. niger* re-established to the newly harvested seeds of the lines, the variations in the incidence of fungi detected on the parts of the seeds were evaluated for this fungus. The incidence of *A. niger* on the parts of newly harvested seeds from the lines generally decreased at high rates. The highest percentage of decrease (100%) in the incidence of the fungus on newly harvested seeds was detected in the seed coat and cotyledon for the seed samples from the lines 28 and 39 respectively. However, there were high percentages of decrease in its incidence of all parts of newly harvested seeds from the line 36. In contrary, the incidence of *A. niger* on all the parts of newly harvested seeds from the line 72 increased although the increases were at low rates. But the highest rate of increase (75%) in its incidence on newly harvested seeds was determined on seed coat of the seeds from line 82. Hungarian vetch lines behaved quite differently with regard to seed transmission, due to probably differences in the host-pathogen interaction as informed by Neergaard[9].

Seed transmission of fungi to the plant roots: In the all samples of lines, except for line 28, *A. niger* was isolated from the roots of the plants grown in field soil (Table 2). *Fusarium spp.* Which were not determined in the parts of original seed stocks *F. culmorum*, *F. equiseti* and *F oxysporum* were isolated from the roots of plants from the lines grown in both soil. The highest rate of infection of root was by *F. equiseti* in most of lines. *F. oxysporum* anked second in incidence on Hungarian vetch roots in sterile or field soil. *F. culmorum* was isolated from the sample of lines 31, 36, 39 and 62 in both soil. None of *Fusarium spp.* was isolated from newly harvested seed after vegetation period (Table 1), although they could be transmitted from original seed stock to the root of the plant in sterile and field soil. The inhibitation effect of *A. niger* on *Fusarium spp.* on agar plate was showed in the previous experiment for onion seedborne fungi[10]. In the present study, we also thought that *Fusarium spp.* .might be seedborne but inhibited by *A niger* in artificial media.

If seed, contaminated by pathogen that is also soil borne, is sown in non infested soil, the pathogen then can be transferred to the field and invade to the host as exemplified by the soil invaders during plant growth[9].

Table 1: The incidence of fungi detected in different parts of original and newly harvested seeds of different hungarian vetch lines and varieties in the incidence of *A. niger*

		Percentage of the parts of original (A) and newly harvested seeds (B) contaminated with				Variations in the incidence of *A. niger*	
		(A)		(B)			
Line	Seed part[a]	*Aspergillus alutaceaus*	*Aspergillus niger*	*Alternaria alternata*	*Aspergillus niger*	Percentage of decrease	Percentage of increase
23	Emb.	0	0.0	0	28	0.0	0.0
	Coty.	0	0.0	10	9	0.0	0.0
	SC	0	26.0	0	12	53.8	0.0
28	Emb.	0	0.0	0	0	0.0	0.0
	Coty.	0	0.0	0	0	0.0	0.0
	SC	2	69.0	0	0	100.0	0.0
30	Emb.	0	0.0	0	11	0.0	0.0
	Coty.	0	0.0	0	9	0.0	0.0
	SC	5	11.0	0	13	0.0	15.4
31	Emb.	0	9.0	0	5	44.4	0.0
	Coty.	0	11.0	0	13	0.0	15.4
	SC	0	15.0	0	15	0.0	0.0
36	Emb.	0	63.0	30	7	88.9	0.0
	Coty.	2	44.0	7	19	56.8	0.0
	SC	3	37.0	46	11	70.3	0.0
39	Emb.	0	0.0	0	0	0.0	0.0
	Coty.	0	2.0	0	0	100.0	0.0
	SC	0	0.0	0	0	0.0	0.0
62	Emb.	0	31.0	0	28	9.7	0.0
	Coty.	0	34.0	0	36	0.0	5.5
	SC	0	30.0	0	32	0.0	6.2
69	Emb.	0	20.0	0	22	0.0	9.1
	Coty.	0	23.0	0	15	34.8	0.0
	SC	0	9.0	0	18	0.0	50.0
72	Emb.	0	55.0	0	60	0.0	8.3
	Coty.	0	46.0	0	53	0.0	13.3
	SC	0	39.0	0	55	0.0	39.1
82	Emb.	0	2.0	0	1	50.0	0.0
	Coty.	0	1.0	0	2	0.0	50.0
	SC	0	1.0	0	4	0.0	75.0

[a]Emb., embroyo; Coty., cotyledon; Sc, seed coat

Table 2: The incidence of fungi on the root of Hungarian vetch developed from original seed stocks in sterile and field soil

		Percentage of roots bearing			
Line	Type of soil	*Aspergillus niger*	*Fusarium culmorum*	*Fusarium equiseti*	*Fusarium oxysporum*
23	Sterile	8.0	0	24.0	2.0
	Field	16.0	0	28.0	12.0
28	Sterile	0.0	0	12.0	4.0
	Field	0.0	0	36.0	6.0
30	Sterile	16.0	0	20.0	8.0
	Field	12.0	0	18.0	2.0
31	Sterile	8.0	12.0	8.0	4.0
	Field	4.0	24.0	8.0	4.0
36	Sterile	8.0	8.0	20.0	16.0
	Field	8.0	4.0	16.0	12.0
39	Sterile	16.0	8.0	1.0	0.0
	Field	4.0	6.0	2.0	0.0
62	Sterile	24.0	16.0	40.0	8.0
	Field	12.0	4.0	24.0	4.0
69	Sterile	32.0	0	28.0	8.0
	Field	12.0	0	12.0	6.0
72	Sterile	8.0	0	4.0	8.0
	Field	2.0	0	6.0	4.0
82	Sterile	16.0	0	4.0	8.0
	Field	4.0	0	4.0	4.0

Original seed stock of Hungarian vetch lines are probably naturally contaminated by *Fusarium spp*. And these species may be transferred from seeds to soil, which are expected as this possibility. However, *A. niger* and *F. oxysporum* are natural components of the soil mycoflora of our experimental field[10]. Thus they were probably transmitted from both seed and soil to the Hungarian vetch roots.

In conclusion, all of the determined fungi occurred commonly a natural contaminant of seeds. Thus *F. culmorum, F. equiseti and F. oxysporum* likely could be transmitted from seeds to the roots of the plants in sterile and field soil. *A. niger* had the ability for the re-establishment to the newly harvested seeds of Hungarian vetch from all lines except for line 28 and 39. Its incidence on newly harvested seeds from the lines generally decreased. A. alternata was isolated only from newly harvested seeds from some lines, which indicates that these seeds might have been contaminated by air.

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