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Fungi Associated with Seeds of Barley (*Hordeum vulgare* L.) in Rajasthan and their Phytopathological Effects

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ABSTRACT

Sixty four seed samples of Barley (*Hordeum vulgare* L.) collected from different districts of Rajasthan revealed 35 fungal species of 18 genera in addition to *Alternaria alternata, A. longissima, A. tenussima, Aspergillus candidus, A. flavus, A. fumigatus, A. niger, Bipolaris spicifera, Curvularia lunata, Drechslera graminea, D. tetramera, D. rostrata, Fusarium oxysporum, Fusarium graminearum, Rhizopus nigricans, Trichothecium roseum* which were dominant and affected seed germination, seedling vigour and seedling diseases.

Introduction

Barley (*Hordeum vulgare* L.), a member of the grass family, is a major cereal grain. *H. vulgare*, the fourth important world crop, used for animal feed, beer, and human food was domesticated polyphyletically by humans 10,000 years ago in the Neolithic revolution in at least three centers[1]. Important uses includes use as animal fodder, as a source of fermentable material for beer and certain distilled beverages, and as a component of various health foods. It is used in soups and strews and in barley bread of various cultures[2]. Top 5 barley producers are Russia, Ukraine, France, Germany and Australia. World total production of barley in year 2011-12 is 134 (million metric ton) and in year 2012-13 is 130 (million metric ton) (forecast) source: International grain council[3]. India's total barley production in year 2011-12 is 1620 (1000MT) source: United State department of Agriculture[4]. Barley has high agronomical significance and is sensitive to many diseases (40 pathogens inducing an average loss of $ 252 million *per* year, *i.e.* 19.6% of the average annual value of barley crop)[5]. The seeds of Barley infected and contaminated by various seed-borne fungi while in field or during seed processing or during storage. Seed borne fungi of barley were reported by many researchers[6]. Since no study gives systematic and comprehensive data on fungi associated with barley seeds. An attempt was made to identify different seed mycoflora associated with Barley.

Material and method:

Sixty four seed samples of Barley from seven districts Rajasthan were collected during the crop season 2010-11 were subjected to dry seed examination and incubation tests by standard blotter test and PDA method. For dry seed examination three replicates of 100 seeds per sample taken at random were studied[7]. Percent incidence of different deformities and insect damaged seeds were estimated. For incubation both untreated and 2% chlorine pretreated (300 seeds/ sample) for 5 minutes were sown on moistened blotters and incubated for 8 Days. Twenty six seed samples were studied using dextrose agar (PDA) plates[8]. The discoloured or diseased seeds were also plated separately to identify the potato specific fungi associated with them. Seed germination seedling symptoms and incidence of fungi were recorded (Table1).

Result and Discussion:

Dry seed examination:

Seed samples of Barley collected from 7 districts of Rajasthan revealed both asymptomatic and symptomatic seeds when observed under stereobinocular microscope (Olympus, Germany). Symptomatic seeds showed various kinds of disorders like cracked seeds with black mycelium (0.28-55%), seeds with black and brown discolouration (0.25-45%), seeds with white mycelial growth (0.25-34.75%), shriveled seeds (0.25-3.75%) and broken and insect damaged seeds (2.5-28%).

Cracked seeds with black mycelium on incubation yielded *Alternaria alternata, Bipolaris spicifera, Curvularia lunata, C. pallescens, Cladosporium herbarum, Drechslera graminea, D. rostrata, D. tetramera, Mammaria spp, Nigrospora oryzae, Penicillium spp, Rhizospora oryzae, Epicoccum nigrum.* Seeds with brown and black discoloration were associated with *Alternaria alternata, A. tenussima, A. longissima, Aspergillus flavus, A. niger, A. fumigatus, Bispora catenula, Bipolaris spicifera, Botrytis cineria, Cladosporium cladosporiodes, Cladosporium herbarum, Curvularia lunata, C. pallescens, Drechslera graminea, D. rostrata* and *D. tetramera.* Seeds with white mycelial growth were associated with A*spergillus niger, A. candidus, A. flavus, A. fumigatus, A. sulphureus. Fusarium chlamydosporium, F. oxysporum, F. moniliformae, Rhizopus nigricans, Trichothecium roseum.* Shriveled seeds were associated with *Aspergillus flavus, A. candidus, A. fumigatus, Curvularia lunata, C. pallescens, Penicillium spp., Rhizopus nigricans. Aspergillus niger, A. candidus, A. flavus, A. fumigatus, A. sulphureus, Actinomycetes, Penicillum spp., Rhizopus spp.* were the main causal agent of broken and insect damaged seeds. These seeds were common in samples from all districts.

Incubation test: A total of 35 fungal species belonging to 18 genera, saprophytic as well as pathogenic were observed on Barley seeds in blotter (SBM) and PDA tests (Table 2). The

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fungi encountered in PDA test were mostly common to those observed in SBM. Fungal spp. recorded were Actinomycetes (1-10%) in SBM and in PDA (1-2%), *Alternaria alternata* recorded in untreated (7-68%) and pretreated seeds (3-34%) in SBM samples and in PDA (1-52%), A. longissima recorded (1-20%) in untreated seeds and (1-13%) in pretreated seeds in SBM and (1-2%) in PDA test. *A.tennussima* (1-48%) in untreated, (1-28%) in pretreated seeds in SBM and (1-16%) in PDA. *A. flavus* recorded 5-49%, 4-15% and 1-22% respectively in untreated, pretreated in SBM and PDA test. *A. fumigates* recorded 2-57% in untreated, 1-20% in pretreated in SBM and 1-25 % in PDA. *A. candidus* was found 1-22% in untreated, 1-6% in pretreated in SBM and 1-17% in PDA. *A. niger* recorded in untreated (5-59%) and in pretreated (1-20%) in SBM and in PDA (1-25%). *A. sulphureus* recorded 1-9%, 1-3% and 1-2% respectively in untreated, pretreated in SBM and in PDA. *Bipolaris spicifera* also recorded 1-3% in untreated, 1-3 % in pretreated in SBM and 1-5% in PDA. *Botrytis cineria* recorded 1-15% in untreated, 1-6% in pretreated in SBM and 1-2% in PDA. *Cladosporium cladosporiodes* encountered 1-37%, 1-36% and 1-28% respectively in untreated, pretreated in SBM and in PDA. *C. herbarum w*as isolated 1-26% in untreated, 1-21% in pretreated in SBM and 1-20% in PDA. *Curvularia lunata* encountered 1-57% in untreated, 1-42% in pretreated in SBM and 1-38 % in PDA .*C. pallescens* also recorded 1-18% in untreated, 1-17% in pretreated in SBM and 1-6% in PDA. Fungi such as *Drechslera graminea, D. tetramera, D. rostrata, D. sativum, D. halodes, Fusarium chlamydosporium, F. oxysporum, F. moniliformae, Penicillium spp. R. nigricans, R. oryzae* and *Trichothecium roseum* also recorded in high incidence.

Table 1: Incidence of various seed disorders in Dry seed Examination, Microorganism associated and Seedling diseases caused by them in Standard Blotter Method

Types of seeds deformities	Percent range	Important Microorganism associated with them	Seedling symptoms
Cracked seeds with black mycelium	0.28-55%	*Alternaria alternata*	Browning of transition zone of radical and shoot and brown necrotic spots on the cotyledonary leaves.
		Bipolaris spicifera	Brown flecks, linear streaks and small oval spots
		Curvularia lunata, C. pallescens	Light brown spots on coleoptiles Seedling rot
		Cladosporium herbarum	
		Drechslera graminea *D. rostrata* *D. tetramera*	Brown streaks or spots on the tips or on the margins of the leaves which gradually extended towards the petiole.
		Penicillium spp.	Yellowing of leaves and radical rot
	0.25-45%	*Alternaria alternata* *A. tenussima* *A. longissima*	Browning of radical and base of the shoot and brown necrotic spots on leaves.
		Aspergillus flavus *A. niger* *A. fumigates*	Browning of leaves and basal parts of the shoot
		Bipolaris spicifera	Yellowing of seedling and root rot
		Cladosporium cladosporiodes	Seedling rotting
		C. herbarum	
		Curvularia lunata *C. pallescens*	Light brown spots and streaks on the coleoptiles.
		Drechslera graminea *D. rostrata* *D. tetramera*	Brown streaks or spots on the tips or on the margins of the leaves which gradually extended towards the petiole
Seeds with white mycelial growth	0.25-34.75%	*Aspergillus niger* *A. candidus* *A. flavus* *A. fumigatus* *A. sulphureus*	Browning of leaves and basal parts of the shoot
		Fusarium chlamydosporium *F. oxysporum* *F. moniliformae*	Yellowing of leaves and wilting of seedlings.
		Trichothecium roseum	Seed and seedling rot
Shriveled seeds	0.25-3.75%	*Aspergillus flavus* *A. candidus* *A. fumigatus*	Brown and black lesion on seedlings and browning of basal parts of shoot
		Curvularia lunata *C. pallescens*	Light brown spots streaks on the coleoptiles
		Penicillium spp.	Yellowing of leaves and radical rot
Broken and insect damaged seeds	2.5-28%	*Aspergillus niger* *A. candidus* *A. flavus* *A. fumigatus* *A. sulphureus*	Browning of leaves and basal parts of the shoot
		Penicillum spp.	Yellowing of leaves and radical rot

Table 2: Number of seed samples of barley infected with fungi and percent range of incidence in incubation tests. (64 seed samples studied)

Name of the fungi	Blotter test				PDA	
	Untreated seeds		Pretreated seeds			
	Samples infected	% range	Samples infected	%range	Samples infected	% range
Actinomycetes	16	1-10	9	1-10	6	1-2
Alternaria alternata	56	7- 68	48	3-34	46	1-52
A. longissima	21	1-20	18	1-13	10	1-2
A. tenussima	37	1-48	30	1-28	19	1-16
Aspergillus candidus	12	1-22	6	1-6	10	1-17
A. niger	56	5-59	43	1-20	18	1-25
A.flavus	64	5-49	56	4-15	26	1-22
A. fumigatus	48	2-57	39	1-20	20	1-25
A. sulphureus	12	1-9	5	1-3	1	1-2
Bipolaris spicifera	8	1-3	4	1-3	3	1-5
Bispora catenula	2	1-2	-	-	-	-
Botrytis cineria	7	1-15	3	1-6	1	1-2
Cladosporium cladosporiodes	19	1-37	15	1-36	16	1-28
C. herbarum	12	1-26	9	1-21	5	1-20
Curvularia lunata	58	1-57	49	1-42	18	1-38
C. pallescens	12	1-18	8	1-17	5	1-6
Chetomium spp.	1	1-2	-	-	1	1-2
Cephalosporium spp.	2	1-3	-	-	1	1-2
Drechslera graminea	17	1-46	14	1-40	14	1-39
D. tetramera	58	1-52	49	1-37	36	1-39
D. rostrata	27	1-64	25	1-59	21	1-43
D. sativum	9	1-38	5	1-36	5	1-28
D. halodes	14	1-15	10	1-26	7	1-26
D.dematioidea	1	1-5	-	-	1	1-2
Epicoccum nigrum	2	1-2	-	-	-	-
Fusarium chlamydosporium	7	1-15	5	1-17	5	1-10
F. oxysporum	29	1-17	25	1-12	22	1-10
F. moniliformae	13	1-26	11	1-22	5	1-11
F. graminarum	1	1-2	1	1-2	1	1
Mammaria spp.	1	1-3	-	-	-	-
Nigrospora oryzae	2	1-2	-	-	-	-
Penicillium spp.	26	1-14	9	1-8	13	1-36
Rhizopus nigricans	31	1-15	5	1-6	9	1-21
R. oryzae	7	1-6	5	1-6	4	1-3
Trichothecium roseum	38	1-27	19	1-21	9	1-12

The other minor fungi A*lternaria dianthicola, Aspergillus nidulans, A. ochraceous, Bispora catenula, Chetomium spp., Cephalosporium spp., Drechslera dematiodea Epicoccum nigrum, Fusarium graminarum, Mammaria spp.*and *Nigrospora oryzae*, which were also listed in Table 2. A general assessment of the total seed borne inoculums revealed that samples from Bharatpur, Alwar, Jaipur, Dholpur and kota mostly showed heavy inoculum and greater incidence of fungi. This may be due to more reverine areas and with more rainfall, hence high humidity is in general, which favours the sporulation of fungus.

Effect of Sodium Hypochlorite pretreatment on seed borne fungi in SBM: In standard blotter method both untreated and seeds pretreated with sodium hypochlorite were used. In general, 1% concentration of available chlorine was found to increase the seed germination without affecting the incidence of the pathogenic seed borne fungi, but the incidence of saprophytic fungi was greatly reduced and their growth and sporulation on seed surface rendered sparse due to seed treatment[9]. The fungi such as *Bispora catenula, Chetomium spp. cephalosporium spp., Epicoccum nigrum, Nigrospora oryzae* and *Mammaria spp.* that occurred in low incidences were completely inhibited after chlorine pretreatment in SBM.

Phytopathological effects: Fungi associated with seeds affected germination as well as vigour and also produced symptomatic seedling. In 64 seed samples studied, germination ranged from 1-100% in untreated and pretreated seeds in SBM. The fungi which commonly affected seed germination were spp. of *Alternaria, Aspergillus, Bipolaris, Curvularia, Drechslera, Fusarium, and Trichothecium.*

Most of the fungi like *Alternaria alternata, Aspergillus flavus, Curvularia lunata, Drechslera tetramera, Drechslera graminea,* caused serious seed diseases and produced infected seedlings. Seed infection by *Alternaria alternata* caused browning of radical and base of the shoot. Seed contamination with *Aspergillus flavus* caused brown to black lesions on seedlings. Seed infected by *Bipolaris spicifera* caused brown flecks, linear streaks and small oval spots. *Fusarium spp. caused* yellowing and drying of seedlings[10] and *Drechslera graminea* caused leaf stripe disease. The disease is widely distributed in most barley growing areas, where it causes serious damage and yield losses[11],symptoms appear as long yellow stripes extending the entire length of the leaf and the stripes later become dark brown with chlorotic margins[12]. .*D. tetramera* and *D. rostrata* results in 1-3 mm long coalescing lesions with extensive necrosis. Variations in symptoms expressed by diseased plants may lead to an improper diagnosis. These variations can result from a couple of factors. It is possible that there is more than one problem present, and in some cases there may be more than one pathogen infecting a plant. Symptoms associated with these infected plants may be significantly different from the symptoms expressed in response to each of the different pathogens acting separately[13].

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