Detection of Mycoflora Associated with Different Seeds of Barley (*Hordeum vulgare* L.)

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Abstract

In dry seed inspection, only eight genotypes exhibited light to dark discolouration of seed in the range of 3.0 to 7.0 per cent. The seeds of other two genotypes (NDB-2 and NDB-1173) did not show any discolouration. In standard blotter method, twelve fungal species belonging to nine genera, namely, *Alternaria alternata, A. triticina, Aspergillus flavus, A. niger, Bipolaris sorokiniana, Curvularia lunata, Drechslera graminea, D. spicifera, Fusarium moniliforme, Mucor sp., Penicillium notatum and Rhizopus nigricans* were detected. Genotype RD-2503 was found to harbour all the twelve fungal species. Alternaria alternata was associated with the seeds of all the genotypes tested while A. triticina observed on 7 genotypes. The fungal species B. sorokiniana, C. lunata and D. graminea were associated with 8 genotypes and A. flavus was associated with 7 genotypes. D. spicifera and F. moniliforme were associated with 6 genotypes while Aspergillus niger and Mucor sp. showed association with 5 genotypes. P. notatum and R. nigricans showed less presence. The agar plate method, yielded the same twelve fungal species belonging to nine genera. Genotype RD-2503 harboured maximum number of fungi followed by NDB-1597, RD-2508, RD 2552, NDB-1592, NDB-4, NDB-1, NDB-2, NDB-3 and NDB-1173. Alternaria alternata was observed on seeds of all the genotypes except NDB 1173. *B. sorokiniana* and *C. lunata* were associated with 7 genotypes while *Aspergillus flavus, D. graminea* and *Mucor sp*. were associated with 6 genotypes. *A. niger, F. moniliforme* and D. spicifera were associated with 5 genotypes where as *P. notatum and R. nigricans* showed association with the seeds of 4 genotypes only. Due to treatment of seeds prior to isolations by standard blotter and agar plate methods, not only the colonies of different fungi were reduced. During present investigation, it was observed that more fungal species were associated with shrivelled seeds followed by discoloured ones. Apparently healthy looking seeds showed least fungal association.

Keywords:*Hardeum,vulgare*,mycoflora,,PDA,

Introduction

Barley [*Hordeum vulgare* L.] belongs to family Poaceae. It is popularly known as "Jau" in hindi. It ranks 4th among the major food grain crops after wheat, rice and maize in the world with regard to acreage and production (FAO, 2011). The barley crop in eastern India, suffers from number of fungal foliar diseases, namely Alternaria leaf blotch (*Alternaria alternata*) Kumar and Singh, (1997a), Curvularia leaf spot (*Curvularia lunata*) Kumar and Singh (2002), Net blotch (*Drechslera teres*) Singh and Singh (2006), leaf spot (*Drechslera victoriae*) Kumar and Singh (1997b), spot blotch (*Bipolaris sorokiniana*) Kumar and Singh (1999) and Stripe disease (*Helminthosporium gramineume*) Atheya (1974). Among leaf stripe is one of a serious problem in barley in which the infected plants produce light weight seed, reducing yield 75-80 per cent. However, during commercial harvest this light weight seed is usually lost, resulting in a 100 per cent yield loss per infected plant. Barley leaf stripe caused by the *Pyrenophora graminea*. It was first identified in Montana in 1977. Hence, the present investigation was made to Detection of Mycoflora Associated with Different Seed's of Barley (*Hordeum vulgare* L.).

Materials and Methods

An experiment was conducted in the laboratory of Department of Plant Pathology, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad, Uttar Pradesh. Kumarganj is located in the Indo-Gangetic plains of eastern Uttar Pradesh at latitude 26.47o N, longitude 82.12oE and at altitude of 113m above the sea level. Collected seed samples of barley genotypes listed in table – 1.ISTA (1985) method was followed for testing the samples for the presence of fungal flora, associated with barley seeds, which included four techniques.

1. Inspection of dry seed.
2. Standard blotter method.
3. Agar plate method.

1. Inspection of dry seed

Dry seeds were examined for the presence of fungal symptoms like discolouration, deformation and spots on its surface by naked eyes and with the help of magnifying hand lens. Further, these seeds were graded into three categories:

(i) Apparently healthy looking
(ii) Discoloured
(iii) Shrivelled seeds

On the basis of their size, shape and appearance, seeds of each category were further divided into two groups:

(a) Normal seed
(b) Under sized seed

Two hundred seeds from each category were tested by standard blotter method to assess the extent of fungi associated with them.

2. Standard Blotter Method

Three hundred seeds per samples in three replications, each of one hundred seeds were tested by this method. Ten randomly selected seeds were plated on three layered moist blotters at equal distance with the help of sterilized forceps in each Petri dish (9.0 cm diameter). These seeds were then incubated at a temperature of 28 ± 10 C for twelve hours in alternating cycles of light and darkness for 7 days. These plated seeds were examined for the presence of seed- borne mycoflora under stereoscopic binocular and with the help of compound microscope.

3. Agar Plate Method

Three hundred seeds per samples in three replications, each of one hundred seeds were treated with 0.1 per cent Chlorine as described in standard blotter method and plated on PDA at the rate of 5 seeds per Petri dish. The seeds were incubated at 28 ± 10 C for 7 days under twelve hours alternating cycles of light and darkness and examined microscopically for the presence of fungal spores on seeds. Their specific identification was made with the help of standard identification manuals under stereoscopic binocular and compound microscope.

Observation

Mycoflora associated with different categories of seeds:

The seeds of barley genotype RD 2503 showing maximum number of fungal species on blotters, were separated into three categories Viz., apparently healthy looking, discoloured and shrivelled seeds. Further, seeds of all the categories were put under two sets i.e. undersized and normal sized as described earlier. Two hundred seeds of each category were tested in three replications, each treatment having 30 seeds. The observations of the fungi present on the seeds were recorded after seven days of incubation.

Growth habitat and morphological characters of isolated fungi:

The growth characters of each fungal species as it appeared on the seeds were recorded under stereoscopic binocular microscope (6.4 to 40 x magnifications). The cultural and morphological characters of each fungus were studied individually on Potato Dextrose Agar after 7 to 10 days of incubation. The pure culture of each fungus were prepared by single spore and hyphal tip isolations and the morphological characters i.e. shape, size and colour of the structures of fungi were recorded and measured under compound microscope. The size of fungal spores or fructification or mycelia was based on measurements of fifty counts and standard nomenclature for colour according to (Ridgway, 1912). The species of fungus were identified with the help of standard identification manuals or monographs

Results and Discussion

Detection of mycoflora associated with barley seeds was carried out with the help of procedures as described by ISTA (1985) and the findings are being presented:

1. Inspection of dry seeds:

Dry seeds of different genotypes (Table-1) were examined for discolouration of seeds, deformities and presence of fungal fructifications on the seed surface by naked eyes and with the help of magnifying hand lens.

It is evident from Table-1 that the range of light to dark discolouration of seeds was 3.0 to 7.0 per cent. The maximum discolouration was observed on the surface of seeds of RD 2503 (7.0 per cent) followed by NDB-1597 (6.5 per cent). Some other genotypes, namely, NDB-1592 (6.0 per cent), RD 2508 (5.5 per cent), RD 2552 (5.0 per cent), NDB-4 (4.5 per cent), NDB-1 (3.5 per cent) and NDB-3 (3.0 per cent)

exhibited variable discolouration. However, the seed of other genotypes, like NDB-2 and NDB-1173 did not show any discolouration on the seed surface. No fungal fructifications were observed on the seed of any genotype under study. The inert matter in the form of pieces of seeds, husks and leaves were found mixed with seeds

2. Standard Blotter Method:

A perusal of the Table-2 indicates that 12 fungal species belonging to 9 genera were detected on the seeds of different barley genotypes. The detected fungi are *Alternaria alternata, A. triticina, Aspergillus flavus, A. niger, Bipolaris sorokiniana, Curvularia lunata, Drechslera graminea, D. spicifera, Fusarium moniliforme, Mucor sp., Penicillium notatum and Rhizopus nigricans*. RD 2503 was found to harbour maximum number of fungal species (all the 10 fungal species) followed by NDB-1592, NDB-1597, RD 2508, RD 2552, NDB-4, NDB-1, NDB-3, NDB-2 and NDB-1173..

Table-1: Dry seed examination for discolouration:

S. No.	Genotypes	Per cent discoloured seeds
1.	RD-2503	7.0
2.	RD-2508	5.5
3.	RD-2552	5.0
4.	NDB-1	3.5
5.	NDB-2	0.0
6.	NDB-3	3.0
7.	NDB-4	4.5
8.	NDB-1173	0.0
9.	**NDB-1592**	6.0
10.	**NDB-1597**	6.5

Alternaria alternata was observed on the seeds of all the genotypes in the range of 3 to 22 per cent. Its incidence was maximum on RD 2503 followed by NDB-1597 and NDB-1592 and minimum on NDB-1173. *A. triticina* was observed on 7 genotypes, its maximum presence was recorded on RD 2503 and minimum on RD 2552 and the range of incidence was 3 to 10 per cent. *Aspergillus flavus* was observed on 7 genotypes being, maximum on RD 2503 and minimum on NDB-1 in the range of 2 to 14 per cent. Colonies of *Fusarium moniliforme* were observed on RD 2503, RD 2508, RD 2552, NDB-4, NDB-1592 and NDB-1597 in the range of 2 to 13 per cent with maximum incidence on RD 2503 and minimum on NDB-4. The *fungal species Bipolaris sorokiniana*, *Curvularia lunata* and *Drechslera graminea* were observed on 8 genotypes with range of 2 to 9, 1 to 5 and 1 to 8 per cent, respectively. *Drechslera spicifera* was observed on 6 genotypes within range of 1 to 7 per cent.

Aspergillus niger and Mucor sp. were associated with 5 genotypes within range of 2 to 11 and 2 to 5 per cent, respectively. *Penicillium notatum* and *Rhizopus nigricans* showed less presence on seeds of different genotypes within range of 1 to 2 and 1 to 6 per cent, respectively.

Table-2: Percent seeds* of different barley genotypes associated with individual fungal species as detected by standard blotter method.

S. No.	Fungal species	Genotypes										Number of genotypes associated with fungus	Range
		RD-2503	RD-2508	RD-2552	NDB-1	NDB-2	NDB-3	NDB-4	NDB-1173	NDB-1592	NDB-1597		
1.	Alternaria alternata	22	16	16	11	13	8	18	3	18	20	10	3-22
2.	A. triticina	10	7	3	6	7	6	6	0	0	0	7	3-10
3.	Aspergillus flavus	14	6	8	2	0	0	5	0	12	10	7	2-14
4.	A. niger	8	2	0	6	0	0	0	0	6	11	5	2-11
5.	Bipolaris sorokiniana	9	4	3	5	2	0	5	0	6	7	8	2-9
6.	Curvularia lunata	3	1	3	0	0	3	5	1	2	4	8	1-5
7.	Drechslera graminea	8	6	5	4	0	1	3	0	5	7	8	1-8
8.	D. spicifera	7	5	4	1	0	0	0	0	6	5	6	1-7
9.	Fusarium moniliforme	13	7	5	0	0	0	2	0	9	12	6	2-13
10.	Mucor sp.	5	3	2	0	0	0	0	0	2	5	5	2-5
11.	Penicillium notatum	2	0	0	0	0	0	1	0	1	2	4	1-2
12.	Rhizopus nigricans	6	0	0	0	0	0	0	0	1	4	3	1-6
Total number		12	10	9	7	3	4	8	2	11	11	-	-

* Number of seed tested: 30

3. Agar plate method:

A perusal of Table-3 indicates that 12 fungal species belonging to 9 genera were detected on the seeds of different barley genotypes. The detected fungi are *Alternaria alternata, A. triticina, Aspergillus flavus, Aspergillus niger, Bipolaris sorokiniana, Curvularia lunata, Drechslera graminea, D. spicifera, Fusarium moniliforme, Mucor sp., Penicillium notatum* and *Rhizopus nigricans*..Genotype RD 2503 was found to harbour maximum number of fungi followed by NDB-1597, RD 2508, RD 2552, NDB-1592, NDB-4, NDB-1, NDB-2, NDB-3 and NDB-1173. *Alternaria alternata* was observed on the seeds of all the genotypes except NDB-1173 in the range of 3 to 20 per cent. Its incidence was maximum with genotype RD 2503 followed by NDB-1597, NDB-1592, RD 2508, RD 2552, NDB-4, NDB-1, NDB-3 and NDB-2. *Bipolaris sorokiniana* and *Curvularia lunata* were next dominating fungal species observed with 7 genotypes in the range of 2 to 8 and 2 to 6 per cent, respectively. Maximum presence was recorded with RD 2503.

Table-3: Per cent seeds* of different barley genotypes associated with individual fungal species as detected by agar plate method.

S. No.	Fungal species	Genotypes										Number of genotypes associated with fungus	Range
		RD-2503	RD-2508	RD-2552	NDB-1	NDB-2	NDB-3	NDB-4	NDB-1173	NDB-1592	NDB-1597		
1.	*Alternaria alternata*	20	15	12	9	3	8	10	0	16	18	9	3-20
2.	*A. triticina*	9	5	4	4	0	0	4	0	0	0	5	4-9
3.	*Aspergillus flavus*	12	6	6	0	0	0	3	0	9	10	6	3-12
4.	*A. niger*	8	3	1	0	0	0	0	0	4	6	5	1-8
5.	*Bipolaris sorokiniana*	8	4	7	3	0	0	6	0	5	2	7	2-8
6.	*Curvularia lunata*	6	3	2	3	0	0	4	0	3	5	7	2-6
7.	*Drechslera graminea*	9	5	2	0	4	0	0	3	0	8	6	2-9
8.	*D. spicifera*	7	5	3	0	0	0	0	1	0	2	5	1-7
9.	*Fusarium moniliforme*	9	7	1	0	3	5	0	0	0	0	5	1-9
10.	*Mucor sp.*	4	1	3	0	3	0	0	0	2	2	6	1-4
11.	*Penicillium notatum*	3	0	0	0	0	0	2	0	3	3	4	2-3
12.	*Rhizopus nigricans*	5	0	0	0	0	2	0	0	3	3	4	2-5
Total number		12	10	10	4	4	3	6	2	8	10	-	-

The other fungal species showed less presence on

* Number of seed tested: 300

Mycoflora associated with different grades of seeds

On the basis of general appearance, the seeds of genotype RD 2503 were divided into 3 grades for the determination of fungi associated with them as under:

1. Apparently healthy looking seeds.
2. Discoloured seeds.
3. Shrivelled seeds

Two different sets i.e. (a) under sized and (b) normal sized seeds were separated from each grade. Thus finally graded seeds were processed for the detection of mycoflora associated with them by standard blotter method.

A perusal of the Table-3 indicates that maximum number of fungi were detected from the shrivelled seeds followed by discoloured and apparently healthy looking seeds. Twelve fungal species were detected from shrivelled seeds while 9 to 10 and 6 to 8 fungi were detected from the seeds of discoloured and healthy looking grades, respectively. The healthy looking undersized seeds showed the presence of 8 fungi while normal seeds showed the presence of 6 fungi.

Shrivelled seeds showed higher association with fungi than normal ones of each grade. *Curvularia lunata, Drechselra graminea, D. spicifera, Mucor sp, Penicillium notatum* and *Rhizopus nigricans* were not detected from normal seeds of healthy looking seeds while in undersized seeds *Drechslera graminea, Mucor sp., Penicillium notatum* and *Rhizopus nigricans* were not detected.

Mucor sp., *Penicillium notatum* and *Rhizopus nigricans* were not detected form normal seeds of discoloured seeds while *Rhizopus nigricans* was detected with rest of 9 fungi except *P. notatum* and *Mucor sp.* in undersized seeds.

Alternaria alternata, A. triticina, Aspergillus flavus, A. niger, Bipolaris sorokiniana and *Drechslera graminea* were detected from the seeds of all grades.

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