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Detection of Seedborne Mycoflora in Wheat

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Abstract: Human beings demand for wheat has been significantly increasing over the last decades due to westernization of diet and its consumption. The unique viscoelastic and adhesive properties of gluten proteins facilitate production of wheat and give numerous advantages in commercial industry. Therefore, for desired plant populations and sufficient harvesting the pathogen-free healthy seed is essential. The majority of plant pathogens are seed-borne and fungal pathogens have the most harmful effect on the harvest comparing with bacterial and nematode pathogens. Fungi is the second most widespread wheat pathogen after insects and the amount of seed deterioration rate is too significant for the food industry. For a high harvest rate, the qualified certified seeds are need to be used, therefore stored seeds need to be examined well for pathogens. In our study, we detected the soil wheat diseases and tested various methods for detecting seed fungi present in 8 wheat samples collected from harvested seed loads of irrigated wheat fields from the capital city of Ukraine, Kyiv. The results showed that a total of 7 fungal species including *Fusarium* sp., *A.alternata, A.niger, A.candidus, T.laevis, T.tritici, Penicillium* sp. were isolated and identified from the seeds of two wheat cultivars. The level of infection in tested samples by *T.laevis* were 2.5-5.97%, *T.tritici* were 2.4-6.62%, and *A.niger* in seed samples were 8.43-9.56%. The frequency of *A.alternata* - 7.82%, *A.candidus* - 7.18%, *Fusarium* sp. - 7.52%, and *Penicillium* sp. - 6.46% in the Podolyanka, Natalka seed samples. Totally, fungal generas of *Fusarium* sp., *A.alternata, A.niger, A.candidus, T.laevis, T.tritici, Penicillium* sp were isolated from the wheat seed samples from Kyiv, Ukraine.

Keywords: *Fungi, Seed Borne, Wheat, Pathogen, Diseases.*

I. INTRODUCTION

Historically wheat was one of the major plant species in agriculture field and the archaeological record of wheat cultivation in the regions of the Fertile Crescent are around 9600 BCE proves of its imporrtance. By the data of 2014, the total land area of wheat cultivation is the topmost within the different crops with more than 220 million hectares [11, 12].

By taxonomical classification wheat belongs to genus *Triticum* and the most widely grown and known wheat is *Triticum aestivum*. Wheat is a precious agriculture product widely cultivated mostly for the seeds. Cereal grain is a worldwide popular staple [6, 7] and wheat kernel also a botanical fruit type called a caryopsis [8-10].

For high crop production, the qualified, certified and non-infected seeds are essential. When the infected grains are used as seeds, seed borne diseases are the reason for crop yield decrease and more than that the seeds itself become the cause further disease spreading. Most of the plant pathogens are seed borne and fungal pathogens are the most dangerous, harmful seed borne pathogen to the wheat industry comparing with bacteria and nematode [1, 2].

The fungi are the second most harmful to wheat grains just after insects attack [5].

Various species of fungi such as *Aspergillus, Penicillium, Fusarium* and *Alternaria* are the crucial contamination agents of cereal grains. Seed borne fungi are one of the most crucial biotic constraints in seed production worldwide. They are responsible for both pre and post-emergence death of grains, affect seedling vigor, and thus cause some reduction in germination and also variation in plant morphology [6, 7].

Therefore, before the cultivation, the wheat seeds should be examined for the seed pathogenic agents for preventing further loss of harvest. Since for the long run seeds are the transfer factors of good harvest the seed must be examined precisely [4].

For the detection of theses pathogens, different tests are used and most commonly used traditional methods are visually inspect the seed, wash soak and incubate seeds in suitable conditions. In addition blotter test, embryo count method, filter, and centrifuge extraction techniques are used in seed condition test [3].

Nowadays, advanced molecular techniques are trending for the rapid detection of seed borne pathogens. The advantages of these methods are the results show more specific, fast, reliable and accurate. However, the classic methods are still used because of their low-cost and effective results.

From a dietary point of view, human beings consume cereal plant products as one of main carbohydrate resource

[16], especially whole grain is a salient example which contains both simple and complex carbohydrates [18].

For vegans wheat made products are the best option for protein because about 13% is pure vegetal protein and its number is way high with comparing with other cereals. However content of essential amino acids is not significant high [19, 20].

For the last decades, human beings diet is westernizing and the consumption of processed food is continuously increasing, so the need for wheat cultivation is also steadily rising on world market due to the "glue" proteins which have special effects [17, 18].

However, this "glue" protein is a dangerous agent for people who are sensitive to gluten and most of the diseases caused by different biotic factors [21].

Plant breeding to develop new disease-resistant varieties, along with sound crop management practices, are important for preventing disease [22].

Fungicides, used to prevent significant crop losses from fungal disease, can be a significant variable cost in wheat production. A wide range of organisms infect wheat, of which the important are viruses and fungi [23].

International crop production is constantly increasing for the last 50 years and total number has tripled. Moreover, the data is expected to be rising significantly [15, 16].

By the data of 2018, the world total wheat production was around 0.74 billion tons and it was the second most popular plant cultivation number, but by the trade mean wheat is the highest in the world [13, 14].

80% of the total wheat cultivation is produced in just 10 countries (Ukraine, et.al) from all around the world (FAO, 2003).

II. MATERIALS AND METHODS

The climate of agriculture area of Kyiv is freezing during winter season and warm during summer. The climate becomes warmer to the southern part of the capital. The amount of precipitation is moderately high with 610 mm and air humidity is usually high with an average of 4 years is about 75% in the summer and 80-90% during the winter. Although, sometimes the air may become dry with humidity level drops to 12%. In our experiment, a total of 8 seed samples (Smuglyanka, Podolyanka, Sotnitsya, Natalka, Podarock Podillya, Favoritka, Lymerivna, Novokievskaya) were tested from different regions of Ukraine. Samples of two wheat cultivars including Podolyanka, Natalka tested by the international seed testing association (ISTA). The seeds isolated and washed, frozen and then the pathogenic fungi were identified by embryo count assay.

III. RESULTS AND DISCUSSION

Wheat fungi in normal conditions do not have pathogenic activity, but when conditions and time is right, the fungi growth increases. The metabolites secreted by fungi have negative effects on seed quality in every stage of development. Some fungi species synthesize mycotoxins and in high concentrations does have toxic effects on mammals [24].

Commonly five bunt and smut diseases are often associated with wheat (*Triticum* sp.). These are common bunt (*Tilletia sp*), dwarf bunt (*T.controversa*), Kamal bunt (*T.indica*), loose smut (*Ustilago tritici*), and flag smut (*Urocystic agropyri*) [25].

A study in Canada on seed microflora presented that 59 species and research in Pakistan revealed 17 genera and 45 species of fungi. In Kerman province, Iran the investigation of mycoflora of stored grain and the fungi showed 9 species and 15 fungal species were determined in objects [1].

Within smut fungus the *T.indica* has is well-known quarantine pest. The pathogen is regulated by the European Union (EU, 2000) and by other European and Mediterranean Plant Protection Organization (EPPO) member countries [26].

Different bunts have spores similar morphology, so it is troublesome to identify pathogen species, especially when fungi found away from host origin. During the harvest, wheat and weeds attach with the harvester and spores on these weeds can be spread onto the wheat. Thus a washing test applied to wheat grain to collect all spores on wheat plants [27].

A study by a Ukrainian team lead by Novokhatka in 1990 revealed that base of hybrids between susceptible varieties and new sources of resistance. 4 different new genes were found and identified as resistance genes against *T. caries* [*T. tritici*], *T.laevis* and *T. contraversy*. The experiments were under artificial infection with a local bung sample from forest steppe region of Ukraine [28].

In another study by Netherland researchers, the fungi were used as inoculum *Penicillium* sp., *Alternaria* sp., yeasts, and bacteria were isolated from deteriorated seeds and embryos samples. According to results, the above mentioned fungi have been the reason for seed contamination [29].

Researchers from Algeria led by Nouari Sadrati (2013) made a screening test agent with antimicrobial effects on different fungi and isolated totally more than 20 endophytic fungi and 23 endophytic Actinomycetes species, respectively [30].

Another research completed in Argentina by researchers of the National University of La Plata (2002) indicates that from 450 incubated wheat leaf parts totally

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three bacterial isolates and 130 fungal isolated were obtained. From those isolates, 19 fungal species were identified [31].

For isolation of fungi, different techniques are used and researchers from Pakistan approached with ISTA technique to test 12 wheat (*Triticum aestivum* L.) seed samples and different seed borne fungi species [32].

In our study, the taxonomy classification starts with the fungi kingdom and follow with dothideomycetes class for *A.alternata*; eurotiomycetes class for *A.candidus, A.niger, Penicillium*; exobasidiomycetes class for *Tilletia*; sordariomycetes class for *Fusarium.*

Total of 7 fungal species including *Fusarium sp*., *A.alternata, A.niger, A.candidus, T.laevis, T.tritici, Penicillium sp*. were isolated Ukrainian samples and tested for fungi identification (Table 1, 2).

The final results show that the species of *Tilletia* (*T.laevis*, *T.tritici*) teliospores were from 1 to 37 spores in each wheat seed.

Results illustrated a moderate range of infection level of *T. laevis* was as following, in the Smuglyanka seeds 3.32–4.25%; Podolyanka 3.89-5.97%; Sotnitsya 3.13%; Natalka 5.38%; Podarock Podillya 2.72–2.94%; Favoritka 2.67– 3.39%; Lymerivna 2.6%; Novokievskaya 2.5–3.01%.

The *T. tritici* fungi infection rates are at the Podolyanka seeds 6.62%; Sotnitsya 3.34 to 4.54%; Natalka 4.26 to 4.93%; Podarock Podillya 2.4%; Favoritka 2.67%; Lymerivna 2.42 to 2.56%; Novokievskaya 3.84%.

The lowest infection rate of seeds by fungi species were *T.laevis* with 2.5–5.97%, *T.tritici* with 2.4–6.62% in Novokievskaya, Podarock Podillya, and Podolyanka seed samples (Table 1).

№	Variety	Identified species	Mean rate of seed infection in each sample (%)
1	Smuglyanka	*T. laevis*	4.25
		T. laevis	3.78
		T. laevis	3.32
2	Podolyanka	*T. laevis*	3.89
		T. laevis	5.97
		T. tritici	6,62
3	Sotnitsya	*T. laevis*	3.13
		T. tritici	3.34
		T. tritici	4.54
4	Natalka	*T. laevis*	5.38
		T. tritici	4.26
		T. tritici	4.93
5	Podarock Podolya	*T. laevis*	2.72
		T. tritici	2.4
		T. laevis	2.94
6	Favoritka	*T. tritici*	2.67
		T. laevis	2.67
		T. laevis	3.39
7	Lymerivna	*T. tritici*	2.42
		T. tritici	2.56
		T. laevis	2,6
8	Novokievskaya	*T. laevis*	3.01
		T. laevis	2.5
		T. tritici	3.84

Table 1: Incidence and Frequency of Bunt and Loose Smut Fungi Associated With Wheat Seed

The seed infection rate was highest between 5-14 days after inoculation. However, infection started at 3rd day and ended on 21st day after inoculation. Result examination valued by the microscopic analysis by Schilder and Bergstrom in 1933. By other researchers data the seed infection time changes by environment conditions of humidity, temperature and inoculum density, and seed development stage. Especially, the wheat seed is highly vulnerable to *P.tritici* repent infections at every seed developing every stage [33].

Bishaw (2004) reported that Syrian wheat sample infection with a common bunt and loose smut were 68% and 14%, respectively. *T. laevis* and *T. tritici* were not dominant in Syrian region. Only 3% of samples showed contamination of both pathogens. The highest severity detected with 60% incidence in the bread wheat cultivar Mexipak and a 33% incidence showed in the durum wheat landrace cultivar Shyhani (Mamluke et al., 1990). *Fusarium graminearum* found as main disease in East Azarbaijan, Khorasan Razavi regions of Iran and the *T.caries*, *T.leavis* were the main infection agents examined by different methods. In Ethiopia, various seed borne fungus genera like *Cochliobolus sativum, Fusarium sp.* and

Septoria sp. were detected with different rates with 84%, 31%, 74%, 13%, 52% and 31% [34].

Saprophytic or weakly pathogenic fungi including *Alternaria, Helminthosporium, Curvularia, Stemphylium, Rhizopus, Cladosporium, Aspergillus, Penicillium, Gonatobotrys and Nigrospora* is found in wheat seeds. (Bhutta et al.1999). Hajihasani et al. reported an experiment explains that the average contamination level of the seeds was determined for *A.niger* 10.6%, *A.candidus* 4.7%, *A.flavus* 3.1%, *Penicillium sp*. 11.1%, *Mucor sp*. 3.2% and *Rhizopus sp*. 3.3% [1].

Culture of freshly harvested wheat using agar plate method, eight genera and 13 species were isolated (Abdul Rehman et al, 2011).

Another way of identifying the fungus species is agar method, in 2011 Abdul Rehman and others have identified more than 13 species of *Aspergillus sp.*, *Fusarium sp.*, *Mucor* sp., *Rhizopus* sp., and *Curvularia sp.* These species were isolated from four different districts of Pakistan. One of the main points of this research was the *Absidia* sp. found in seeds stored just for 3-6 months. Besides *Absidia* the genuses like *Rhizopus* sp., *Aspergillus* sp. were dominantly spread throughout the storage seeds with 20-24%. Among of *A.alternata* in Chakwal region was low due to the antagonist species like *Penicillium* sp. [5].

In our study, the average contamination rate of the Podolyanka was determined for *A.alternara* 7.82%; *Fusarium sp*. 7.52%; *A.niger* 9.56% and in the Natalka seed samples *A. candidus* 7.18%; *Penicillium sp.* 6.46%; *A.niger* 8.43%.

The species of *A.niger* was the highest identified fungi in all seed test samples (Table 2).

№	Variety	Identified species	Mean rate of seed infection (%)	Mean of regional infection (%)
1	Podolyanka	*A. alternata*	7.82	68.6
		Fusarium sp.	7.52	69.8
		A. niger	9.56	62.7
2	Natalka	*A. candidus*	7.18	68.2
		Penicillium sp.	6.46	70.6
		A. niger	8.43	61.7

Table 2: Seedborne Fungi Isolated from Wheat Seeds and their Infection Rate

IV. CONCLUSIONS

This study reports on the results of studies to identify pathogens of different varieties of wheat and evaluate various methods for the detection of pathogenic fungi present in wheat samples.

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