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Dry Rot Causing Species of Fusarium Prevalent in Republic of Tatarstan.

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ABSTRACT

Fusarium species are the most frequently fungal pathogens of potato worldwide. In this study we isolated *Fusarium* species causing dry rot in potatoes that were grown in Republic of Tatarstan in 2014. Isolated species were identified as *Fusarium oxysporum* (4 strains), *Fusarium solani* (1), *Fusarium avenaceum* (1)*, Fusarium tricinctum* (1)*, Fusarium sambucinum* (1) and *Fusarium redolens*(1) by sequencing the ITS regions of rRNA. Variable degrees of pathogenicity were observed with the collected *Fusarium* isolates after artificial inoculation of healthy potato tubers. *Fusarium oxysporum* strains (MG2, NK3, MG1) were the most aggressive and virulent followed by *Fusariumsolani* NZ1*, Fusarium tricinctum* SA1and *Fusarium sambucinum* NK2*, Fusarium avenaceum* NK1 were the least pathogenic. The results of the identification of prevalent pathogenic *Fusarium* isolates from potato tubers can contribute to development of regional strategies for controlling the disease development in this area.

Keywords*:* potato, *Fusarium,* potato fungal diseases, dry rot, pathogenicity

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INTRODUCTION

Phytopathogenic fungi are the most distributed and aggressive plant pathogens causing significant crop losses of economically important crops. Yield losses of the most important crops such as rice, wheat, corn, soybeans and potatoes caused by the phytopathogenic fungi can reach 125 million tons per year [1].

Fusarium is a genus of fungi that contains many agronomically important and dangerous plant pathogens responsible for severe vascular wilts, rots of roots, tubers, bulbs and corms.

Fusarium species are mainly distributed in the soil and able to survive in the form of spores up to 30 years, even under the most difficult climatic conditions [2, 3]. Pathogens can penetrate into the plant through roots and the lower part of steam. Tubers can be also infected during storage. Potato tubers losses during storage attributed to the dry rot have been estimated to average 7-11%, and under high temperature and humidity can reach 30-50% [4, 5]. Some species of the genus *Fusarium* may also produce different mycotoxins, which can cause serious human diseases [6, 7].

Several *Fusarium* pathogens are widely distributed in the natural environment but different species are occurred with maximal frequency in various geographic areas. [8] Therefore, the identification and characterization of *Fusarium* species predominating in each region are required to develop the effective plant protection approaches against dry rot. The application of morphometric and molecular methods aloow the more accurate identification of species. This in turn enables us to investigate a diversity, as well as ecological and biochemical aspects of the adaptation and dissemination of pathogenic fungi [9].
This study aimed to isolate and identify fungi associated with potato dry rot in Republic of Tatarstan and characterize the pathogenicity of the isolated pathogens.

MATERIALS AND METHODS

Isolation of fungi. The rot disease-affected tubers of potato cultivars Udacha and Arosa were collected from the fields of Tatar Agriculture Research Institute, Kazan, Russia (latitude: 55°24’16” N, longitude: 49°33’01” E) and used for isolation of fungi. The potato tubers with dry rot symptoms were washed under running tap water and air dried. Then the tubers were sterilized with 1.5% sodium hypochlorite solution 3 min and rinsed three times in sterile water. The 2x2 cm pieces were excised with a sterile scalpel from the affected area of each tuber and plated on Czapek agar and potato glucose agar (PGA) in Petri plates. Plates were incubated at 28°C, 50-60% relative humidity for 7 days. Fungal colonies were purified several times by serial transfers on Czapek agar. All isolates were identified using morphological characteristics of colony and conidia including pigment of colony, size and shape of conidia, and other morphological structures, according to published descriptions [10].

Molecular identification of fungal isolates. Identification of fungi was performed by the method of ITS (internally transcriber spacers) region sequencing of the fungal 5.8S rRNA genes [11]. The fungal DNA was extracted from 500 µg of mycelia according to extraction method described by Liu et al [12]. PCR amplification and electrophoretic analysis PCR products were performed according to the method described previously [13]. The PCR products sequenced using primers ITS1F and ITS4 (Joint Stock Company Syntol, Moscow, Russia) in Interdisciplinary Center of Shared Facilities, Kazan Federal University (KFU ICSF). The sequenced products were analyzed by NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) against the GenBank database.

Pathogenicity test. The disease-free potato tubers (*Solanumtuberosum* L., Udacha) were used for this experiment. The healthy tubers and uniform in size (80–100 g) were washed, surface sterilized in 1.5% sodium hypochlorite solution for 3 min, rinsed with sterile distilled water three times and dried. Then the tubers were inoculated artificially by 7-day-old pure culture of fungi containing active mycelium [7]. Tree tubers used for each fungal strain. All the infected potato tubers were incubated in black polyethylene bags in the dark at 20-22°C for 2 weeks. As a control, tubers were wounded with a sterile cork borer only. Following incubation, the width of the rot area was measured. At least three independent experiments were conducted to evaluate the phytopathogenic poitential of fungi tested.

Statistical analysis was performed using the software package SPSS 12.0. Standard deviation (σ) was calculated and the results were considered significant when $\sigma \leq 10\%$.

RESULTS

In this work nine fungi isolates from the rot disease affected tubers of potato cultivars Udacha and Arosa were isolated (Figure 1a). According to the results of morphometric analysis the isolates have been classified as members of the genus *Fusarium* (Figure 1b, 1c). Molecular genetic identification method based on the sequencing of genes 5.8S rRNA and the BLAST analysis using NCBI database (http://www.ncbi.nlm.nih.gov/) allowed us to determine fungal isolates to the species level. Four isolates were identified as different strains of *Fusarium oxysporum* (99-100% sequence similarity with the reference strains). Other isolates were identified as single strains of *Fusarium solani, Fusarium avenaceum, Fusarium tricinctum, Fusarium sambucinum* and *Fusarium redolens* with 99-100% homology.

Isolates from affected potato tubers were assessed for the ability to cause dry rot in disease-free potato tubers after artificial inoculation of the pathogen. Figure 2 shows typical symptoms of dry rot disease on potato tubers infected with the mycelium of strain *F. oxysporum* MG2. As can be seen from Table, all of the selected *Fusarium* strains cause the different affect upon the potato tubers depending upon strains after infecting and subsequent incubation of healthy tubers. After 14 days incubation, the diameter of affected area for infected tubers ranged from 2.2 to 20.2 mm, indicating different levels of virulence of isolated strains. Strains *F. oxysporum* NK3, MG1 and MG2 most actively caused the dry rot (diameter of the affected area was 18.2, 17.5 and 20.2 mm, respectively). Strains *F. solani* NZ1 (15.5 mm) and *F. tricinctum* SA1 (14.7 mm) also demonstrated high pathogenicity. Strains *F. oxysporum* ID1, *F. redolens* NZ2, *F. sambucinum* NK2, and *F. avenaceum* NK1 demonstrated low phytopathogenic activity (average lesion sizes of infected potato tubers were 8.3, 6.4, 3.2 and 2.2 mm respectively).

Thus, four from nine *Fusarium* isolates collected from dry rot infected tubers of potato cultivars Udacha and Arosa were identified as *F. oxysporum* species and three of them demonstrated high degree of pathogenicity after artificial inoculation of potato tubers. The results obtained indicate the predominant occurrence of *F. oxysporum* strains in the region studied.

DISCUSSION

In recent decade, different pathogens causing potato tuber infections, particularly fungi of genus *Fusarium*, have become widely spread in the Republic of Tatarstan [14]. Many species of this genus are pathogens affecting different economically important crops [15, 16]. It was shown that potato dry rot can cause more than thirteen different *Fusarium* species [17].

Fusarium species, causing potato plant diseases, can vary significantly according to the geographical distribution and pathogenic properties. It has been reported that in Iran and China dry potato rot is mainly caused by *F. solani* and *F. sambucinum* [5, 6]. In Great Britain, the potato fusarium diseases are primarily caused by four species: *Fusarium avenaceum, Fusarium coeruleum, Fusarium culmorum* and *Fusarium sambucinum* [18]. *Fusarium avenaceum* is the dominant species in other European countries, affecting potato tubers during their storage. *Fusarium* species are hemibiotrophic pathogens, and most species are associated with different plants. The occurrence of certain *Fusarium* species is determined by the climatic conditions in the region, and their prevalence depends on annual changes of metereological indexes. It has been found that just 1-4 *Fusarium* species can be dominant in each particular area [19]. In Russia, the species of the genus *Fusarium* are the most important pathogens of plant diseases in Siberia, in the Non-chernozem zone and the central part of European Russia, the North Caucasus, as well as in Stavropol region [20]. The regions with more humid and warmer conditions are characterized by prevalence of: *F. culmorum, F. sporotrichiella, F. oxysporum*. [21].

The results obtained indicate that *Fusarium oxysporum* is the main pathogen responsible for dry rot disease of the potato in the region studied of the Republic of Tatarstan. The representatives of this species have been described earlier as the most common plant pathogens found in warm conditions. Thus, the increase in the number and variety of fungi of genus *Fusarium* pathogens of potato dry rot - in the Republic of Tatarstan in recent years, and particularly the aggressive strains of *F. oxysporum* may be due to changes in agro-climatic conditions - increased temperature and aridity typical of this region in the last decade [14]. The results obtained suggest the importance of monitoring the pathogenic fungi in order to develop effective plant protection methods and agricultural activities in different areas.

CONCLUSION

Thus, 9 isolates of fungi identified by their morphological and cultural properties as the representatives of the genus *Fusarium* were isolated from potato tubers with dry rot symptoms. Molecular genetic identification based on genes 5.8 S RNA homology allowed to identify the isolates to the species level. 4 of 9 strains were identified as strains of *F. oxysporum*. Analysis of the pathogenic potential of the isolates by infecting the healthy potato tubers showed that three strains of *F. oxysporum* demonstrated the most aggressive properties. This indicates the predominance of these *Fusarium* species among the pathogenic species associated with potato tuber dry rot in the studied region.

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