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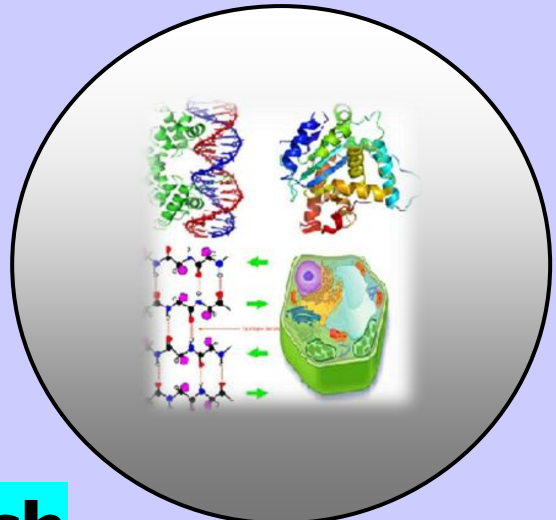

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Occurrence and Diversity of Cultivated Fungi Associated with Soybean Grown in Uzbekistan

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ABSTRACT
Plant-associated fungi are considered an important partner of plants which may affect positively or harm their host. In this study fungal occurrence and diversity of cultivable fungi associated with soybean grown in saline soil, semi-arid climatic region of Uzbekistan was investigated. Soybean (Glycine Max L.) plants were collected from field side of Tashkent province of Uzbekistan that characterized with saline soil and semi-arid climatic region. 47 fungal species belonging to 25 genera were isolated from stem, seeds, pods, leaves and roots of soybean grown in saline soil. The most frequently isolated genera were Fusarium (12%) (F.gibbosum, F. heterosporium, F. oxysporum, F. semitectum, F. solani, F. sporotrichiella), and Aspergillus (10%) (A. fumigatus, A. nidulans, A. niger, A. ochroceus, A. versicolor). The species of Ascohytaso jaecola, Fusarium gibbosum and F. oxysporumwere isolated from all parts of soybean including seed surface, leaves, roots, stem and also seed tissue. About 78% fungal species were found in seed tissue, living as endophytes. This study has provided useful information about the occurrence and diversity of cultivable fungi associated with soybean grown in saline soil of semi-arid condition.
Key words: Soybean, Fungi, Diversity, Salinity and Semi-Arid.

INTRODUCTION
Soybean (*Glycine max*) is an important grain legume crop and an important source of food for man and livestockin many countries including Uzbekistan (Egamberdieva et al. 2015). The

plant-associated microbiome consists of diverse microbial populations living in the roots, and phyllosphere (Berg et al. 2014; Cho et al. 2015; Hashem et al. 2016). Egamberdiyeva et al. (2005a,b) observed a high abundance of bacteria belonging to species *Bacillus*, *Pseudomonas*, *Arthrobacter*, in the rhizosphere of wheat, cotton, and soybean. Although the diversity and function of root-associated bacteria of soybean, including rhizobial diversity are well documented, little is known about the diversity and function of fungi, especially in a hostile environment. Hence, understanding the response of fungal and bacterial communities to alterations in the physiochemical environment of the root system may provide valuable insights into the ecology of plant-associated microbes. Fungi are an important part of the ecosystem, which plays a key role in several ecological processes (Schulz and Boyle 2005). They colonize rhizosphere, phyllosphere as well internal plant tissue, causing damage to the host plant or establish a mutualistic interaction with thehost. Several fungal species can also improve plant growth by protecting plants against soil-borne diseases or various environmental stresses (Khan et al. 2012). Romão-Dumaresq et al. (2016) studied thefungal diversity of sugarcane and observed at least 35 different genera, whereas phylum *Ascomycota* predominated among the fungi identified (96.0%), *Zygomycota / Mucoromycotina* and *Basidiomycota* represented only 2.4% and 1.6% of the community, respectively. It has been also observed that fungal community was influenced by the plant cultivar, and tissue type, e.g. *Cladosporium* (36%), *Alternaria* (13%), *Diaporthe* (9%) and *Epicoccum* (7%) were common genera isolated from soybean (Impullitti and Malvick 2013). Pimentel et al. (2006) isolated 12 genera of fungal endophytes in leaves and stems of soybean grown in Brazil, whereas *Cladosporium* was one of the most frequently identified endophytic fungi. The diversity of fungi associated with soybean grown in Brazil was also studied by de Souza Leite et al. (2013), they isolated and identified the genera *Ampelomyces, Annulohypoxylon, Guignardia, Leptospora, Magnaporthe, Ophiognomonia, Paraconiothyrium, Phaeosphaeriopsis, Rhodotorula, Sporobolomyces*, and *Xylaria* for the first time. However, the composition of fungal community associated with plant depends on thetime of sampling, soil type, plant genotypes and also climatic conditions (Garoe et al. 2012). Therefore, the current exploratory study was designed to evaluate the distribution and diversity of fungi associated with soybean grown in the semi-arid saline soil of Uzbekistan.

MATERIAL AND METHODS

Soybean (*Glycine Max* L.) plants were collected from field side of Tashkent province of Uzbekistan during the summer (July 2013 and June 2014). The soil type is calcareous Calcisol having a calcic horizon within 80 cm of the surface. The surface and sub-surface soils are low in organic matter. The climate is semi-arid with mean annual air temperatures of 18^{o}C and 20^{o}C (summer temperature is +35^{o}C), and mean annual rainfalls of 200 mm. Three plants were randomly chosen from field side, and whole plants, including the root systems, were wrapped in plastic bags, brought to the laboratory and stored at 4°C until further processing. For the isolation of fungal strains, plan materials such as stem, leaves, root, seed, and pods were separated, and then the samples were cut into 10 mm pieces and aseptically transferred to Potato dextrose agar (PDA) plates supplemented with streptomycin (100 mg/mL)in three replicates.

The plates were incubated at 30°C for 7 days and monitored daily for fungal growth. As mycelia emerged from the plant tissue, small pieces were transferred to agar plated and purified. The fungal isolates were identified on the basis of morphological characteristics such as colony growth, aerial mycelium, spore morphology, pigment production etc. (Barnett and Hunter1998).

RESULTS AND DISCUSSION

A total forty-seven fungal strains were isolated from various parts of soybean plants. The fungal colonization in plants was influenced by the section of soybean plant. We have focused on seeds, steam, roots, leaves, and pods of soybean. It should be mentioned also that our soybean plants did not show any symptoms of the disease. The leaf was colonized by diverse fungal genera than the root, pod, and seeds of soybean. The most frequently isolated generawere *Fusarium* (12%) (*F. gibbosum*, *F. heterosporium*, *F. oxysporum,F. semitectum*, *F. solani, F. sporotrichiella*), and *Aspergillus* (10%) (*A. fumigatus, A. nidulans, A. niger, A. ochroceus,A. versicolor*) (Table 1).

The high frequency of isolation of *Cladosporium* from soybean has been reported by Impullitti and Malvick (2013). The fungi considered as non-pathogenic, they may produce phytohormone like substance and improve soybean growth and development (Hamayun et al. 2009). In our study, we have isolated *Cladosporiumepi phyllum* and *C. herbarum* from the leaves of soybean.

Rarely occurred species include *Aureobasidium pullulans, Botrytis cinerae, Corynespora cassiicola, Epicoccum neglektum, Erysiphaecommunis f. glycine, Gliocladium roseum, Peronospora manshurica, Phomopsis sojae, Phyllostictasojaecola, Pleosporaherbarum, Pythium debaryanum, Septoriaglycines,* and *Trichotheciumroseum.* The presence of *Epicoccum*, and *Phomopsis* was reported for soybean (Impullitti and Malvick 2013), they could cause stem blight disease (Harrington et al. 2000).

Table 1. Occurrence of fungal genera on different parts of soybean plants.

Fungalgenera	Seedsurface	Stem	Leaf	Root	Pods	Seedtissue
Alternaria alternate (Fr) Keiss.	+	+	+	-	+	+
A. humicola Oud.	-	-	+	-	+	+
*A. tenuis*Nees.	-	-	+	-	+	+
Ascohytasojaecola Abr.	+	+	+	+	+	+
A. phaseoforum Sacc.	-	-	+	-	-	+
Aspergillus fumigatus	-	-	+	+	+	+
A. nidulans (Eid) Wint.	-	-	+	+	-	+
*A. niger*v. Tiegh	+	-	+	+	+	+
A. ochroceus Wilhelm	-	-	-	-	+	+
A.versicolor (Vuillemin) Tirab.	-	-	+	-	+	+
Aureobasidium pullulans (De Bary)	-	-	-	-	-	+
Botrytis cinerae (Pers.)	+	+	+	-	+	+
Cercosporasojina Hara	+	+	+	-	+	+
C. kikuchii Mats et Tom Gard.	-	-	+	-	-	+
Chaetomiumglobosum Kunze.	-	-	-	+	-	+
C. spirale Zopf	-	-	-	+	-	+
Cladosporiumepiphyllum (Pers.)	-	-	+	-	-	+

C. herbarum (Pers.) Link	-	+	+	-	+	-
ColletotrichumglycinesHori	+	+	+	-	+	+
Colletotrichumtruncatum (Schwein.) Andrus & W.D. Moore	-	+	-	-	-	+
Corynesporacassiicola (Berk. & M.A. Curtis) C.T. Wei.	+	-	-	-	-	-
Epicoccumneglektum Desm.	-	-	-	+	-	+
Erysiphecommunis f. glycine Jacz.	-	+	+	-	+	-
Fusariumgibbosum App. et Wollenw. Emend Bilai	+	+	+	+	+	+
F. heterosporium Nees.	+	-	-	+	-	+
F. oxysporum Schlecht.	+	+	+	+	+	+
F. semitectum Berk. & Ravenel	-	-	-	+	-	-
F. solani (Mart.) Sacc	+	+	-	+	-	+
F. sporotrichiella Bilai.	+	-	-	-	-	+
Gliocladiumroseum (Lk) Thom	+	-	+	+	-	+
Mucor adventitious	-	-	-	+	-	+
M. circenelloides Tiegh.	-	-	-	+	-	+
M. hiemalis Wehmer	-	+	+	+	+	-
M.plumbeus Bonord.	-	-	-	-	+	-
Penicilliumgranulatum Bainier.	+	-	-	-	-	+
P. chrysogenum Thom	+	-	-	-	+	+
P. stipitatus Thom	-	-	-	-	-	+
Peronosporamanshurica Naum.	+	-	+	-	+	+
Phomopsissojae Lehman	-	+	+	-	-	-
Phyllostictasojaecola Mass.	-	+	+	-	-	-
Pleosporaherbarum (Pers.) Rabenh.	+	-	+	-	-	+
Pythiumdebaryanum Hesse	+	+	+	-	-	-
Septoriaglycines Hemmi.	+	+	-	-	-	-
Trichotheciumroseum (Pers.) Link	-	-	-	+	-	+
*Verticilliumlateritium*Berk.	-	-	-	+	-	+
V. dahliae Kleb	-	-	-	+	-	+
Whetzeliniasclerotiorum (Lib.) Korf & Dumont	-	+	-	-	+	+
Total fungal species: 47	**20**	**18**	**26**	**19**	**21**	**38**

Pimentel et al. (2006) also observed *Colleto trichum* in leaves of soybean grown in Brazil. In this study soybean was grown in thesalinesoil of the semi-arid climatic region, thus the fungal diversity may differ from other regions. The species of *Ascohytasojaecola*, *Fusarium gibbosum,* and *F. oxysporum*were isolated from all parts of soybean including seed surface, leaves, roots, stem and also seed tissue. About 78% fungal species were found in seed tissue, living as endophytes, whereas less fungal species found in root and steam. Russo et al. (2015) studied a fungal diversity of soybean grown in Argentina, and found a greater number of fungal endophytes from stem tissues than from leaves and root tissues. The most frequently isolated species was *Fusarium graminearum* and the least isolated one was *Scopulariopsis brevicaulis*. It is known that endophytic fungi may enhance plant growth through modulation of plant physiology and induced stress tolerance of plants (Cho et al. 2005).Thus, identified species could be important for developing biotechnological approaches in the management of soybean disease. This study has provided useful information about the occurrence and diversity of cultivable fungi associated with soybean grown in the saline soil of semi-arid condition. They may have beneficial interactions with soybean or cause various disease.

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