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Micromycetes of Some Legume Crops' Rhizosphere.

Yulia N. Kurkina*, Nguyen T.-L. Huong, Irina V. Batlutskaya, and Aleksandr V. Lazarev.

Belgorod State University, Russia, 308015, Belgorod, street Pobedy, 85.

ABSTRACT

Investigation of complex structure of micromycetes of legume crops' rhizosphere at natural infection background in edaphic-climatic conditions of Belgorod oblast revealed 36 species of micromycetes, with predominant number of *Penicillium* and *Aspergillus* genera. There were 78% of cellulose-digesting micromycetes in soil samples; peptonolytic activity was demonstrated by 42% of them, saccharolytic activity was demonstrated by 14% of them and amylolytic activity – by 8% of them. We have noted negative correlation of *Cunninghamella echinulate* species with the number of *Fusarium*genus (r= -0.647). A number of fungi as a part of typical soil complexes are allergenic for human: deep-colored melaniferous representatives of *Cladosporium* genus, as well as the following species: *Aspergillus fumigatus, A. ochraceus,A. ustus, Bipolarisaustraliensis, Phomamelaena.* The most evident similarity was demonstrated by micromycetes' complexes of peanut, Vigna, yellow clover and crown vetch.

Keywords: complex of micromycetes, legumes, rhizosphere of legumes, allergenic micromycetes.

INTRODUCTION

Fungi are leading component of heterotrophic block of terrestrial eco systems [1]. Composition of micromycetes in soil depends not only on type of soil, but also on the composition of higher plants [2-4]. Phytopathogenic micromycetes lead to crop loss and its pollution by mycotoxins, which are able to preserve at processing of contaminated fruits and be present even in ready products [5-7]. Contemporary models of biological farming implies wide usage of legumes in crop rotation systems, which are not only rich in vegetable protein in food and feed, but is also one of the best preceding crops in conditions of saving fertilizers [8].

Since understanding of complex structure of agrocoenosis' micromycetes is an important components in management of phytosanitary situation, and since there is not enough data about species composition and micromycetes' structure of legumes [9], this defined the aim of this study: to investigate the structure of mycocomplexes of some legume crops' rhizosphere in edaphic-climatic conditions of Belgorod oblast.

THE METHOD

In micro-plot experiments at natural infection background in botanic garden of Belgorod State University (city of Belgorod) the following cultures were seeded: Arachishypogaea, Vigna, common vetch, coronilla, Trifoliumstrepens, Trifoliumrepens, Medicago sativa and Lotus corniculatus, Soil of trial area was ordinary chernozem, medium-textured loam, fine blocky in structural composition, pH of water extract (active acidity) equaled 7,6; pH of salt extract (exchangeable acidity) equaled 6,9.Collection of soil samples from rhizosphere zone was performed with consideration of common requirements in bud formation period and beginning of plants' blossoming of the 2nd year of vegetation (except from annual ones), when root exudation of plants is low [4]. Recovery of fungi from rhizosphere was performed with the help of water outwash method [10].Presence of peptonolytic and saccharolytic activity of fungi was defined in the laboratory, using breeding grounds that contained readily available sugars and peptone (Czapek's medium, sabouraud, meat-and-peptone agar). Taxonomic belonging was defined according to aggregate of cultural and morphological features with the usage of special literature [11-15]. Characteristics of soil complexes' structure of micromycetes were performed on the base of indices of species' abundance and frequency of occurrence. Assessment of similarities, biodiversity and uniformity of micromycetes' complexes were performed on the base of Jaccard's coefficient of community (K_j), Shannon's (H') and Pielou indices (E). With consideration of values of special and temporal occurrence of species in soil complexes, typical species have been distinguished [10, 16].

THE MAIN PART

It has been discovered that species composition of micromycetes' complexes differ between samples of some legume crops' rhizosphere. During blossoming phase, extracted microbiota of learnt legume crops' rhizosphere was presented by 14 genera, 36 species, with prevalence according to species abundance of *Penicillium* Link genus (11 species), as well as *Aspergillus*P. Micheli ex Hallerand *Fusarium* Link: Fr.genera (6 species for each). *Alternaria*Neesand *Trichoderma* Pers. genera included 2 species each, other 9 genera were presented by sole species.

In legume crops' rhizosphere 3 species appeared to be dominant ones:(*Aspergillus terreus*Thom*, Fusariummerismoides*Corda,*F.solani*(Mart.) Sacc.). The following species were defined as frequently occurred ones: *Alternariatenuissima* (Kunze) Wiltshire, *Aspergillus terreus* Thom*, Fusarium chlamydosporium* Wollenweber&Reinking, *F.merismoides, F. semitectum*Berk. &Ravenel, *Penicillium cyclopium* Westling,*Trichodermakoningii* Oudem.*, T. lignorum*(Tode) Harz. Rare ones were represented by 17 species:*Alternariaalternata*(Fr.) Keissl., *Aspergillus niger*Tieghem, *A. ochraceus*Wilh.*, A. terreus*Thom*, A. ustus*(Bainier) Thom & Church*, Candida albicans*(C.P. Robin) Berkhout*, Cunninghamellaechinulata*(Thaxter) Thaxter*, Fusariumgraminearum*Schwabe,*F.merismoides*Corda,*Penicilliumdecumbens*Thom, *P. digitatum*(Pers.) Sacc.,*P. funiculosum*Thom, *P. lanosum* Westling, *P. martensii*Biourge, *P. viridicatum*Westling*, Phomamelaena*(Fr.) Mont. &Durieu, *Rhizopusmicrosporus*Tiegh.Sporadic ones were represented by 23 species of solid micromycetes: *Actinomucorelegans*(Eidam) C.R. Benj. &Hesselt.,*Alternariaalternata*(Fr.) Keissl.,*Aspergillus flavipes*(Bainier&Sartory) Thom & Church*, A. fumigatus* Fresen.*, A.niger*Tieghem*, A. ochraceus*Wilh.,*Bipolarisaustraliensis*(M.B. Ellis) Tsuda&Ueyama,*Cladosporiumepiphyllum*(Pers.) Nees*, Cunninghamellaechinulata*(Thaxter) Thaxter,*Fusariumchlamydosporium* Wollenweber& Reinking, *F.merismoides* Corda, *F. semitectum*Berk. &Ravenel, *F. subglutinans*(Wollenw. &Reinking) P.E. Nelson,*Mucorstrictus*Hagem,*Penicilliumcyaneofulvum*Biourge,*P.cyclopium*Westling, *P. decumbens* Thom, *P. expansum*Link, *P. frequentans*Westling, *P. funiculosum*Thom, *P. purpurogenum*Stoll, *Rhizopus microspores* Tiegh., *Verticillium album* (Preuss) Pidopl.

In general, for the complex of soil micromycetes of learnt legume crops 2 to 7 typical species have been defined, which corresponds to 71% of microbiota's species diversity for Vigna, vetch, medic, 60% for peanut, 47-43% for coronilla, Trifoliumstrepens, bird's foot and 29% for clover.

It should be noted that phytopathogenic species of *Fusarium merismoides*was found in dominating species range (Vigna) and frequent species (peanut, vetch, coronilla, medic), and rare one (Trifoliumstrepens) and sporadic species (clover). In addition, other species of this genus are widespread, like *F. chlamydosporium* (frequently seen under bird's foot and clover), *F. semitectum* (frequently seen species in medic complex), *F. graminearum* (rare at Vigna) and *F. solani* (dominant under vetch); some of them are dangerous sincethey cause almost symptom-free fusarium diseases and produce hazardous toxins.

Among typical species, generally in rare range, species of *Aspergillus* and *Penicillium*genera are detached.

In the rhizosphere of all studied perennial grasses *Rhizopus microspores* species is presented. In the rhizosphere of annual plants, we have not detected the majority of species from *Aspergillus*, *Penicillium*genera, as well as species of *Actinomucorelegans*, *Bipolarisaustraliensis*, *Candida albicans*, *Cunninghamellaechinulata*, *Fusarium chlamydosporium*, *F. semitectum*, *F. subglutinans*, *Phomamelaena*, *Trichoderma lignorum*, which were detected in the rhizosphere of perennial legumes. While *Cladosporiumepiphyllum*species was detected only under annual species of legumes (peanut and vetch) in sporadic range, *Verticillium album* species was detected to be sporadic only in soil samples under bird's foot.
Analysis of Jaccard's coefficient of community showed that the most vivid similarity was in mycocomplexes of legume grasses, like: clover with Trifoliumstrepens (K_j = 29%); vetch with Vigna, Trifoliumstrepens and coronilla (21% for each). There is a similarity of complexes of soil micromycetes of peanut with mycocomplexes of other plants, with annual Vigna and vetch (20% each), and also with Trifoliumstrepens (17%) and medic (15%). Far less similarity was between soil mycocomplexes of annual vetch with perenn8ial plants; clover (7%), medic(6%) and Trifoliumstrepens (4%). There was no similarity detected between bird's foot with annual legumes. For the rest of the pairs of compared samples, values of Jaccard's coefficient of community were in the range of 8-15%.

Shannon's biodiversity indices in all studied soil samples were not very high and they varied from 0.26 (clover) to 2.44 (coronilla). Soil mycocomplexes under coronilla and medic were characterized by the highest biodiversity (H' equaled 2.44 and 1.74, respectively) and included 15 and 10 species of fungi, respectively. Somehow lower value of Shannon's index (1.61) for soils of vetch (7 species of fungi), probably, was explained by prevalence of *Trichoderma koningii*, fungi in the complex, which was represented by antagonists of phytopathogenic fungi.

Analysis of values of Pielu uniformity index demonstrated yet less scales – from 0.1 (clover) to 0.9 (coronilla). More uniformity characterized mycocomplexes of vetch and medic (E=0.8), peanut (E=0.7), less uniformity characterized vigna and Trifoliumstrepens(E=0.5), bird's foot (E=0.3).

Correlational analysis detected a range of negative connections. Thus, the number of genera in soil sample were negatively connected with the number of *Fusarium* genus (r= -0.318). Presence of *Trichoderma koningii* genus negatively correlate with the number of species from *Aspergillus* (r= -0.571) and *Penicillium* (r= -0.342), genera, and *Cunninghamellaechinulata* species – with the number of *Fusarium* species (r= -0.647) and representatives of *Trichoderma lignorum*(r= -0.333).

It is known that in soil fiber is mainly decomposed by cellulose-digesting micromycetes from *Alternaria,Aspergillus, Cladosporium, Fusarium, Penicillium, Trichoderma* genera[9], which composed the majority in soil samples (78%). Peptonolytic activity was demonstrated by 42%, saccharolytic activity was demonstrated by 14% of them and amylolytic activity – by 8% of fungi.

Analyzing the presence of cellulolytic fungi in soil complexes per each species of plants, we have discovered that their maximal presence (100%) was typical for soil samples from annual peanut and vigna. The number of such species decreases in the following row: vetch (87%),Trifoliumstrepens(86%), medic (80%), clover (71%), coronilla (69%), bird's foot (50%). Thus, decomposition of cellulose inn soil with annual legumes and Trifoliumstrepens is happening more intensively that under other studies species.

A range of fungi in composition of typical types of soil complexes is allergenic for human: deep-colored melaniferous representatives of *Cladosporium* genus, as well as the following species: *Aspergillus fumigatus, A. ochraceus,A. ustus,Bipolarisaustraliensis, Phomamelaena.* [17]. The largest amount of such species was found in soils under vigna and Trifoliumstrepens (29 and 27% respectively), and the least amount was found under coronilla (6%).

CONCLUSION

In general, the most biodiverse mycocomplexes were the ones that were under coronilla and medic, and the ones with the biggest uniformity were the ons under coronilla, vetch and medic. The most significant similarity belonged to mycocomplexes of clover and Trifoliumstrepens, which, according to classification of C. Linnaeus, refer to one genus (*Trifoliumrepens* L. and *T. agrarium* L., respectively). Fungi of *Trichoderma*genus, with anti-fungal activity, are frequently seen in soils under vetch and Trifoliumstrepens. Decomposition of cellulose is happening more intensely in soil under annual legumes, Trifoliumstrepens and medic. Observes bio specificity of soil fungi groups under investigates legumes, may be connected with the type of living form and root system, as well as with composition of intra-vital exudation of plants.

SUMMARY

The most frequently seen species in complexes of micromycetes of studied legumes' rhizosphere were *Penicillium* and *Aspergillus* genera. Mycocomplexes of legumes' rhizosphere were presented with usual for many soil types of fungi, among which destructors of carbohydrate-containing substrates. However, there is a species specificity of groups of soil fungi under studied legume plants. The largest similarity of soil complexes of peanut and vigna, Trifoliumstrepens and coronilla, medic with the majority of studied legumes require consideration of their correct placement in crop rotation. Presence of majority of legume phytopathogenic fungi in soil samples from *Fusarium* genus necessitate conduction of regular phytosanitarianinvestigations of crops.

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