Diagnosis of fungi of the genus Fusarium and Alternaria, Bipolaris, causing diseases of sunflower, and immunological methods for the evaluation and selection of genotypes to the pathogens

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Abstract. The distribution of Fusarium, Alternaria and Helminthosporiosis on sunflower crops was studied. The primary mass screening of sunflower cultivars for resistance and endurance to pathogens of rot of sunflower roots and baskets was carried out in laboratory, greenhouse and field conditions. Immunological assessment and selection of varieties resistant to pathogens of sunflower root rot was carried out to a mixture of the most common types of pathogens on an infectious background in laboratory and greenhouse conditions. In an infectious nursery, moderately affected samples are evaluated for the most harmful pathogen species. The dynamics of the development of the disease was studied.

1. Introduction

Sunflower is one of the major oilseed crops cultivated in the World and an important crop for Russia. According to the Ministry of Agriculture, the acreage occupied under sunflower in the Russian Federation since 1990 has increased 2.5 times from 2739 thousand hectares to 7524 thousand hectares in 2016. Among the oilseeds, 70 % of the acreage dedicated to oilseeds accounted for sunflower, 80% of the gross harvest of seeds and 90 % of production of vegetable oils are sunflower [1].

Russia occupies a leading position in sunflower production. According to the Ministry of Agriculture of Russia the share of domestic food products – vegetable oil is 80%. It is connected with the success in seed production. Up to 70 % of crops of sunflower in Russia by elite seeds and F1 hybrids, including, domestic. Production of sunflower oil remained at 3300 tonnes.

However, according to Russian researchers [1-7] over the past ten years the phytosanitary condition of sunflower crops has deteriorated significantly, the cases of violation of crop rotations, tillage systems, application of fertilizers and protection from pests. Crop growth and seed quality significantly reduce diseases of sunflower species composition of pathogens and their degree of harmfulness of the sunflower crops vary not only depending on the environmental conditions, but also under the influence of cultivation technology, the correct choice of varieties. Successful work of breeders significantly reduced...
the severity of Broomrape, downy mildew and rust of sunflower. Effective is breeding to increase resistance of sunflower to the obligate parasites.

At the same time increases the harmfulness of such pathogens as fungi of the genus Fusarium and Alternaria, and Bipolaris. Yield losses from the disease amount to 15-25% [1] and in case of epiphytic disease — up to 50 -70%, lead to the destruction of crops, degrade commercial and sowing qualities of seeds contained in the oil [3].

Fungi of the genus Fusarium and Alternaria, and Bipolaris. striking sunflower for the entire growing season and causing disease in all plants: root rot, baskets, mottling and wilting of plants. These mushrooms have high adaptive capacity have a vast range of host plant are highly pathogens with broad spectrum of activity and have the ability to form resistant forms [3,].

Harmfulness of pathogenic mycoflora is manifested primarily in the reduction of sowing qualities and yielding properties of seeds. Currently among the most widespread and harmful diseases of sunflower-borne seeds are downy mildew (LMR), white, gray, and ashy dry rot, Fusarium, Alternaria blight, Phomopsis blight, verticillus and some others [8]. Research A. D. Cask [7,9] for the determination of pathogenic microflora of sunflower seeds from different zones of hybrid seed production in the Russian Federation it is established that seeds of the first generation of sunflower hybrids grown during three years (2010-2012) in the North Caucasus region (Krasnodar Krai and Rostov oblast), the Central black earth region (Voronezh region) and lower Volga region (Volgograd and Saratov region), pathogenic microorganisms were mainly represented by the pathogens of Alternaria leaf spot and bacterial blight. In a much smaller proportion attended the causative agents of dry rot, scab, white rot and Volgograd. According to the analysis of M. V. Elabora etc., [10] in sunflower seeds that were collected in 2010-2013 in the areas of Krasnodar and Stavropol territories, Voronezh, Volgograd, Penza, Rostov and Saratov regions of the Russian Federation was dominated by small-spore Alternaria species with the dominance of A. tenuissima. These fungi were present in different parts of the seed: the germ, fruit and seed shells. Specialized types of A. helianthi, A. helianthiinficiens and A. protenta are extremely rare. Selected isolates of Stemphylium sp. Ulocladium sp. and B. australiensis also were found. According to Borodin, Kotlyarova (2006) [3,6]. Antonova T. S., et al., (2002) [2], Papricka A. A. and others.(2010-2014 g.) [5,12-14], increasing prevalence and severity of fusariosis in the Krasnodar territory, the Voronezh and Tambov regions.

Protective measures to reduce the harmfulness of alternariosis, fusariosis and helminthosporiosis on sunflower almost never performed, and pre-sowing seed disinfection is ineffective against all types of manifestations of the disease. To hold selections need to hard infectious background under favorable for reproduction and development of the pathogen conditions. This allows you to select a selection form field resistance to obtain stable and high yields seeds of good quality. For the selection and development of methods of artificial inoculation that best reflects the natural infection of sunflower plants by pathogens fungi of the genus Fusarium, Alternaria, Bipolaris we have studied the biological characteristics of fungi, their prevalence, pathogenic and phytotoxic properties, interactions of species with each other, as well as the sources of infection and types of disease caused by these pathogens [15-17].

When creating maps of distribution of Fusarium wilt pathogens on the territory of the Russian Federation [18] studied the species composition, intraspecific variability of fungi-morphological cultural and pathogenic properties and toxine producing ability, the adaptability of the species to climatic and edaphic factors [19-24]. The assessment methodology and selection of genotypes, a system for creating infectious backgrounds on the basis of a stable collection strains with known characteristics [25].

The aim of our study was to determine an effective method of artificial infection of plants, the evaluation and selection of breeding material sunflower with resistance to fungi of the genus Fusarium and Alternaria, Bipolaris.

2. Methods and materials
The research was conducted in laboratory and field conditions on crops of sunflower (Central field RAMS, VNIIF, growing season 2015-2019).
The starting material was seeds and diseased sunflower plants, fungi of the genus Fusarium (Fusarium oxysporum (Schlecht.) Snyd.et Hans., F. sporotrichiella nom.nov.Bilai., F. moniliforme Sheld., F. culmorum (W. G. Sm.) Sacc., F. sambucinum Fuck., F. semitectum Berk.et Rav., Alternaria sp and Bipolaris sorokiniana (Sacc) from the national collection of fungi RAMS, VNIIF.

Immunological evaluation of sunflower for resistance to alternariosis, Fusarium and Helminthosporium fungi were carried out in laboratory and field conditions against the background of artificial infection. In laboratory conditions evaluated the infection of plants by pathogens on seedlings of sunflower with the same vigour. For this, the sunflower seeds were put on filter paper to 25 pieces (roll method) in triplicate, and each option has inoculable 5 ml aqueous suspension of conidia of the same species under study mushroom. The concentration of conidia was 15 - .20 dispute in the field of vision of microscope at the magnification of 100 - 120 x 10^-6. Rolled in rolls and were grown for 5 days at a temperature of 25C. Served as control seedlings grown in distilled water. Take into consideration the number of rotten and sprouted seeds was measured by the length of the germ and the Central root. The data were processed by variance analysis.

In field conditions, development of Fusarium, and Helminthosporium fungi alternariosis studied in all phases of the ontogenesis of plants. The defeat of the root system, the leaves, stem and baskets were studied during the growing season on a natural background, as well as the defeat of the leaves, and stalk of sunflower was evaluated on infectious background. The inoculation of sunflower was carried out in the evening. Optimal infection load was worked out previously at the grain. Leaves of sunflower were inoculable by applying a micellar masses of the fungus on the surface of the leaf blade (10-15 grams) in different phases (before budding) and hermetically closing the sheet package. Baskets of sunflower was inoculable in the phase of Bud formation in two ways: application of micellar weight of mushroom (10-15 grams) in the center of the emerging baskets and inoculation (spraying) 5- 7 ml aqueous suspension of conidia of the fungus (concentration 3500-4000 spores/ml). Infection of the stem was performed by introducing a spore suspension with a syringe in the stem or surface coating of fungus on suspension caused by scalpel scraping. In all embodiments, the control used distilled water.

Visual assessment of immunological reaction of plant and the differentiation stage of resistance on leaf surface and stems was carried out after 10-15 days and on the basket of sunflower in the phase of maturation for the dissemination of lesions in points: 0 – the symptoms of the disease symptoms are absent; 1 – mild lesion - lesion area is 10% of the surface of the baskets; 2 – secondary lesion – area lesion occupies up to 40% of the surface of the baskets; 3 – severe lesion – affected a large part of the basket, the pathogen penetrates the seeds and parenchyma baskets.

3. Results and discussion
Fusariosis, alternariosis and helminthosporiosis common on sunflower crops everywhere. Diseases caused by these pathogens develop on the plant throughout the growing season and damaging all its organs. Fungi of the genus Fusarium in plants of sunflower, causing different types of disease symptoms: the rot baskets, roots, rotting of seeds, seedlings, wither, lose leaves and stems. The most important rot of head, roots and wilting are manifested in the reduction to 20% of the energy of germination, germination of seeds and their yields. The most common sunflower species are F. oxysporum and F. sporotrichiella, F. moniliforme, F. culmorum, F. sambucinum. The main causative agents of root rot and wilt of sunflower are F. oxysporum. Infection occurs through the soil in the initial period of vegetation. The dominant species causing Fusarium rot baskets are F. sporotrichiella and F. moniliforme. Infection baskets in natural conditions occurs aerogenic way during flowering. Fungi of the genus Alternaria sp and Bipolaris sp are present in the soil, allocated from the affected sunflower plants under favorable climatic conditions may cause substantial harm to the safety and quality of seed and products.

Primary mass screening of sunflower genotypes for resistance, and resistance to pathogens and rot the roots of sunflower should be carried out in laboratory, greenhouse and field conditions. Rapid assessment of the infected 5-day seedlings of sunflower and experiments in the greenhouse will hold the primary screening of plants for resistance to defeat pathogenic fungi. Field trials on infectious
backgrounds will allow to obtain the seeds of the most resistant forms and to assess the economic profit and loss.

The technique has been developed for the artificial infectious backgrounds. To create artificial infectious backgrounds should be small isolated areas small sunflower crops. For the evaluation of lesions of the root system more efficient to make infectious material accumulated by solid state cultivation on a sterile milled wheat grain simultaneously with the seeding rate of 100 g/m2. For production of infectious material it is important to use a stable collection of strains of pathogens with known characteristics for pathogenicity and toxicity.

Immunological evaluation and selection of genotypes resistant to causative agents of root rot of sunflower in infectious nurseries was carried out according to the following scheme:

1. Preliminary evaluation of accessions to a mixture of the most common types of pathogens at the infectious background in laboratory and greenhouse conditions.
2. Moderately affected (lose 50%) pathogens samples to evaluate some of the most harmful species in the infectious nursery in the phase of germination (phase 2) and early Bud formation (phase 6).
3. Exploring the dynamics of diseases on the cultivars of sunflower of the most harmful species in the infectious nursery.

The defeat of plants by inoculation of the leaf surface and the stem is of local character and the absence of favorable conditions for the development of the pathogen has no direct effect on defeat of sunflower. Evaluation of lesions of stem and leaf must be conducted in the phase of beginning of flowering (phase 8).

Inoculation baskets of sunflower plants before flowering aqueous suspension of the pathogen provides a sufficiently intense lesion and a significant difference in the assessment and selection of genotypes to Fusarium, Helminthosporium rot, alternariosis and baskets. Assessment of lesion baskets of sunflower it is necessary to conduct the maturation phase (phase 12).

The emergence and development of the pathological process is the result of the interaction between host plant and pathogen, and the impact of environmental factors. Distribution and harmfulness of a particular type of the causative agent vary not only depending on the climatic condition of the year, vegetation period, but also the location of crops in the field, which cultivated sunflower.

Species composition of pathogens and their degree of harmfulness of the sunflower crops vary not only depending on the condition of the environment, but also under the influence of cultivation technology, the correct choice of varieties.

4. Conclusion

These experiments showed that the dynamics of infection during the growing season and the crop are important criteria in the selection of varieties that preserves the productivity in terms of epiphytotic pathogen.

Resistance of sunflower to study imperfect fungi is nonspecific, and is measured at the penetration of the pathogen, its rate of spread and damage tolerance of plants. In the laboratory the resistance of plants to pathogens is determined by parameters such as percent germination, average length of primary root, average length of sprouts, evaluated the response of plants to the impact of spore suspension of the pathogen of the disease; in the field - for the score of the lesion, the percentage of plant death, the intensity of lesions, as well as the dynamics of disease development and yield reduction of infectious background, calculated as a percentage of control.

On the stability effect of morphological characteristics of crops and varieties: the structure of the root system, plant height, structure and position of the baskets. Evaluation of genotypes on the hard infectious background allows you to select and select the form of plants and varieties with predictable resistance and resistance to pathogens under different environmental conditions.

Selection of the fungi of the genus Fusarium and Alternaria should be done in the direction of obtaining tolerant varieties or varieties with the field, the long-term stability, which is controlled by many genes.
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