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Identification of seed-borne pathogens in rice and evaluation of the natural attack

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Abstract: Rice is a main food crop being a host to various pests and diseases that pose a threat to production. Its seeds represent a rich substrate for the development of a number of fungal pathogens. During the period 2023 – 2024, research was conducted on the territory of *Maritsa* Vegetable Crops Research Institute – Plovdiv, in order to isolate and identify seed-borne phytopathogens causing rice diseases, as well as to establish the degree of natural attack on Bulgarian and introduced varieties and lines. As a result, it was found that rice seeds collected from different samples in the Plovdiv region were infected with fungal pathogens of 5 genera: *Alternaria* spp., *Cladosporium* spp., *Fusarium* spp., *Drechslera* spp. and *Sclerophthora* spp. Six types of fungi were identified, among which *Fusarium culmorum* and *Alternaria alternata* were predominant. The species were identified in both years of the study, which was a confirmation for the economic importance and the widespread distribution of the phytopathogens. The current state of resistance to seed-borne pathogens under the conditions of a natural infectious background was determined. Differences in variety susceptibility were established. 23.3% of the studied samples had high and stable field resistance. The lowest attack index is reported for Bogdan and Avalya varieties and line 131 originating in Bulgaria. Pamety Tychina and Fujisaka varieties stand out as the most sensitive when grown in the Plovdiv region. The most variable reaction and unstable resistance were established in Deneb and TR 1759 Turkish varieties.

Keywords: rice; seed-borne fungi; resistance; variety

INTRODUCTION

Rice is known with its productive, nutritional, dietary and economic advantages. It has a wide distribution area and is grown in 114 countries. In Europe the size of the sown and harvested areas is 400-450,000 ha, and production is concentrated in 8 countries (Italy, Spain, Greece, Portugal, France, Bulgaria, Romania and Hungary). On an average, annually about 3 million tons of rice are produced, which provides approximately 70% of the necessary rice for the population of the continent (www.mordorintelligence.com/industry-reports/europe-rice-market). Over the past

ten years, the average size of the sown areas in Bulgaria has been 11539 ha, with an average production of 64222 t (Agrostatistics, 2014 - 2023).

Rice cultivation is often attacked by biotic agents including viruses, bacteria, fungal pathogens, parasitic plants, insects with huge economic and ecological impact (Kumar et. al, 2009; Singh et al., 2020; Senapati et al., 2022; Palei et al., 2024). Taking into account the disease-causing organisms, fungal pathogens pose a greater challenge to the crop production. They attack all plant parts starting from the coleoptile and the emerging root, leaves, nodes, panicle, flower stalks and grain. Globally, about 100 fungal pathogens are

reported to infect rice, over 40 of which are seed-borne (Matsuo et al., 1995; Karov, 2001; Iwuagwu et al., 2018; George et al., 2019; Mahadevakumar and Sridhar, 2021; Ackaah et al., 2023). Fungal pathogens cause seed discoloration and decay, reduce seed germination, viability and storage life, cause changes in the chemical composition of rice and reduce nutritional value and quality (Yonchev, 2010; Uma and Wesely, 2013; Zafar et al., 2014; Tosheva et al., 2019; Ackaah et al., 2023; Dorley et al., 2023). Seed-borne infections cause 50-80% yield loss in rice depending on the applied agronomic techniques, the genus and type of pathogens, and the susceptibility of the variety (Rauf et al., 2020; Wang Bing-Xin et al., 2022; Ackaah et al., 2023). Various studies have shown that the number of seed-borne fungal pathogens and the extent of attack are influenced by location, environmental conditions, time of sampling (during vegetation or during grain storage), varietal susceptibility and the interaction of these factors (Uma and Wesely, 2013; Monajjem et al., 2014; Signaboubo et al., 2016; George et al., 2019; Edun et al., 2019; Korkmaz et al., 2022).

The aim of the present study is to isolate and identify seed-borne pathogens causing rice diseases and to establish the degree of natural attack in Bulgarian and introduced varieties and lines grown in the Plovdiv region.

MATERIALS AND METHODS

The present research was carried out during the period 2023 - 2024. Route surveys of permanent rice cells were conducted on the territory of the Institute of Vegetable Crops „Maritsa” - Plovdiv, where stationary field trials with introduced, and Bulgarian varieties and rice breeding lines are established annually. Infected seeds („arpa” – unpooled rice) were used to establish the presence of surface or systemic pathogens. The isolation and identification of the pathogens was carried out in the Phytopathology laboratory of the Institute of Plant Genetic Resources - Sadovo. Seeds were placed on a nutrient medium - potato-dextrose agar (PDA) and were incubated in a thermostat

at a temperature of 25°C for 14 days. Through digital microscopic observation and morphological assessment of the colonies, seed-borne fungal pathogens were identified, and their determination was based on classifiers (Matsuo et al., 1995; Karov, 2001; Stancheva, 2002; Kuyumdzhieva et al., 2020).

The assessment of resistance was made under field conditions and a natural infectious background. The study included some rice samples from the collection kept in the Institute for Plant Genetic Resources - Sadovo. 28 varieties (6 Bulgarian, 22 foreign) and 2 breeding lines were studied. The degree of attack was established in the vegetation phase of full maturity. The assessment was carried out on the basis of external signs on the rice husks, expressed in different sizes and intensity of spotting. Seeds were divided into four fractions: 0 – visibly healthy; 1 – slightly attacked, 2 – moderately attacked and 3 – heavily attacked and unpollinated. They were determined by weight and the attack index was calculated according to the Mc Kinney formula (1923).

The experimental data processing was carried out using the Microsoft Excel software packages.

RESULTS AND DISCUSSION

The meteorological characteristics are shown in Figures 1-2 and Table 1. The experimental years

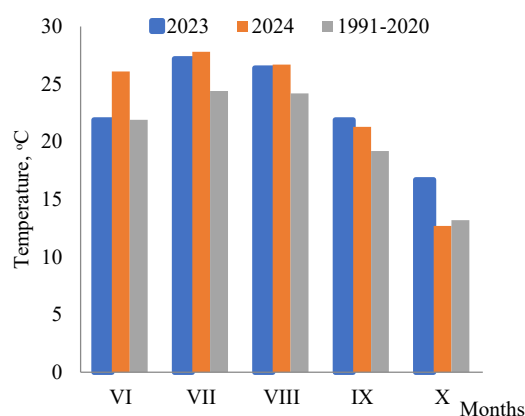


Figure 1. Average monthly air temperature for the Plovdiv region in the period 2023 - 2024, °C

do not differ significantly in terms of temperatures and are distinguished as very warm. The average monthly temperatures in all months except for October 2024 were higher than the average multi-year values by 2.1°C to 4.2°C (Figure 1).

Precipitation during the entire vegetation period except for July 2023 was lower compared to the multi-year period, but it was favourably distributed (Table 1). The experimental year 2024 is characterized as drier than 2023. The vegetation precipitation amount was 54.8 mm. In August, when flowering and pollination occur and in October, when rice ripening and harvesting are observed, the amount of precipitation was below the multi-year values.

The monthly values of relative air humidity during the experimental period changed depending on the amount of precipitation and air temperatures (Figure 2). With the exception of September, the vegetation in 2024 passed under predominant atmospheric drought.

Although the experimental years were characterized as warm and dry, no critical deviations from the main climatic factors were noted during

the individual pheno-phases of crop growth and development.

The isolations carried out under the experimental conditions revealed the presence of fungi from 5 genera: *Alternaria spp.*, *Cladosporium spp.*, *Fusarium spp.*, *Drechslera spp.* and *Sclerophthora spp.* *Alternaria* genus represented 44.4% of all pathogens and was reported with *Alternaria alternata* species. Representatives of *Fusarium* genus made up 25% of all isolates. *Fusarium culmorum* species were observed with a significantly higher percentage (77.7%), which in general causes fusariosis in cereal ears. Next in number was *Fusarium moniliforme* species. The rest of identified seed-borne pathogens were *Drechslera oryzae* (*Helminthosporium oryzae*) (in 16.7% of isolates), *Sclerospora macrospora* (in 8.3% of isolates) and *Cladosporium spp.*, found in 5.6% of isolates. The symptoms caused by these genera are non-specific and are expressed in brown spotting with different intensity and size, accompanied by seed scorching, as well as induction of sterility.

The presence of these fungal genera and differences in species composition have also been established in other studies (Gopalakrishnan et al., 2010; Zafar et al., 2014; Naher et al., 2016; Quintana et al., 2017; Mahadevakumar and Sridhar, 2021; Shamsi et al., 2023; Haider et al., 2024). Although similar studies have been conducted in different rice-producing countries, the results obtained are related to the identification of seed-borne fungal pathogens in a specific geographical area (Central Southern Bulgaria, Plovdiv) and the update of the existing information.

The resistance assessment conducted with 30 rice samples under natural infection conditions

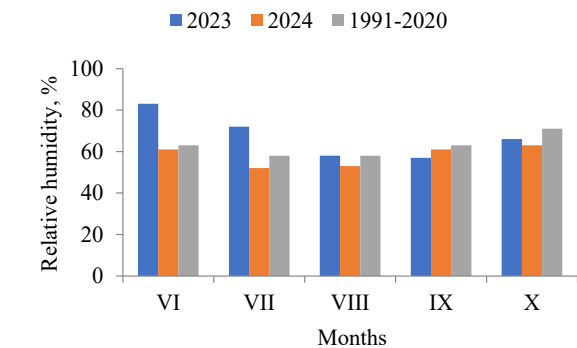


Figure 2. Relative air humidity in %

Table 1. Amount of precipitation and its distribution by month for the region of the city of Plovdiv during the period 2023 - 2024

Month	VI	VII	VIII	IX	X	ΣVI - X
Precipitation amount, mm (2023)	108.3	20.4	25.3	35.8	3.6	193.4
Precipitation amount, mm (2024)	2.1	22.5	9.8	20.4	–	54.8
Multi-year value, mm (1991-2020)	68.2	63.6	48.1	51.0	54.6	285.5

Table 2. Reaction of rice types to seed-borne pathogens under natural infection conditions

Variety/Line	Origin	2023					2024				
		Degree of attack (fraction), %				Attack index	Degree of attack (fraction), %				Attack index
		0	1	2	3		0	1	2	3	
Avalya	Bulgaria	79.2	11.3	3.8	5.7	9.0	87.5	4.9	2.9	4.7	6.2
Iskra	Bulgaria	66.0	18.9	5.7	9.4	14.6	67.3	16.3	4.1	12.3	15.4
Bogdan	Bulgaria	84.5	6.7	4.4	4.4	7.2	83.3	10.4	2.1	4.2	6.8
IPGR Viki	Bulgaria	60.5	11.6	9.3	18.6	21.5	66.5	11.6	9.3	18.6	21.5
Dunav	Bulgaria	67.6	18.9	8.1	5.4	10.3	69.2	21.2	3.8	5.8	11.6
Zhana	Bulgaria	67.9	21.4	7.1	3.6	11.6	55.8	13.2	4.8	26.2	25.3
Line 131	Bulgaria	78.8	12.1	3.0	6.1	9.1	84.5	4.6	1.8	9.1	8.8
Line 168	Bulgaria	75.6	13.3	4.4	6.7	10.6	70.0	16.6	9.4	4.0	11.6
Osmanchik 97	Turkey	49.4	24.5	6.8	19.3	24.0	61.2	22.1	4.5	12.1	16.8
Deneb	Turkey	75.5	16.2	2.7	5.4	9.5	59.8	3.4	1.1	35.6	28.1
Efe	Turkey	48.7	34.6	6.3	10.4	19.6	78.6	8.0	5.2	8.2	10.0
TR 1759	Turkey	33.9	33.1	12.8	20.2	29.8	79.0	5.8	6.1	9.1	11.3
TR 1279	Turkey	67.9	17.1	7.0	8.0	13.8	70.8	7.0	3.6	18.6	17.5
Trakya	Turkey	64.7	17.6	5.9	11.8	16.2	59.7	24.2	3.2	12.9	17.3
Gala	Turkey	64.9	21.1	7.0	7.0	14.0	56.4	30.6	6.5	6.5	15.8
Krasnodarsky 424	Russia	52.2	23.9	15.2	8.7	20.1	52.1	25.0	8.3	14.6	21.4
Belozerniy 3223	Russia	58.1	30.2	6.7	5.0	14.7	66.3	29.3	2.1	2.3	10.1
Pamety Tychina	Russia	41.2	21.4	13.4	24.0	30.1	46.7	7.9	20.4	25.0	30.9
Dvros 2568	Russia	58.6	20.9	9.0	11.5	18.2	54.3	29.9	6.9	8.9	17.6
Volgo-donskiy	Russia	55.3	29.8	7.0	7.9	16.9	58.8	35.3	1.7	4.2	12.8
Gritna	Italy	36.8	37.8	9.6	15.8	26.1	47.5	32.9	6.2	13.4	21.4
Ribello	Italy	51.2	23.4	7.9	17.5	22.9	39.5	33.5	7.9	19.1	26.7
Torio	Italy	54.2	17.7	8.9	19.2	23.3	46.4	27.2	10.8	15.6	23.9
Bonni	Italy	45.5	18.2	12.2	24.1	28.7	63.5	11.4	6.8	18.3	19.9
Rocca	Italy	65.5	18.3	7.3	8.9	14.9	70.8	2.9	2.9	23.4	19.7
Rodio	Italy	45.9	24.4	13.9	15.8	24.9	47.8	25.4	10.1	16.7	23.9
Tramonto	Italy	75.3	7.2	5.7	11.8	13.5	81.8	5.1	5.6	7.5	9.7
Carmen	Italy	77.2	8.6	7.5	6.7	10.9	65.1	7.1	4.5	23.3	21.5
Hokusetsu	Japan	71.1	14.5	9.4	5.0	12.1	66.1	14.9	10.7	8.3	15.3
Fujisaka	Japan	27.7	44.9	13.6	13.6	28.2	46.4	14.3	8.9	30.4	30.8
Min.		27.7	6.7	2.7	3.6	7.2	39.5	2.9	1.1	2.3	6.2
Max.		84.5	44.9	15.2	24.1	30.1	87.5	35.3	20.4	35.6	30.9
Average		60.0	20.7	8.1	11.3	17.5	63.4	16.7	6.1	13.9	17.7
S		14.6	9.1	3.4	6.1	6.9	12.8	10.4	3.9	8.5	6.9
CV, %		24.4	43.9	41.7	54.3	39.6	20.2	62.3	63.9	61.2	38.9

showed that all varieties and lines were infected but at different levels (Table 2). The dynamics of change in the average value of the attack index was expressed by the coefficient of variation (CV) – 39.6% in 2023 and 38.9% in 2024. The magnitude of the coefficient of variation exceeded

20%, which indicated that the group of studied varieties and lines was highly heterogeneous with respect to this indicator.

Differentiation in the reaction of the studied varieties and lines was also observed when assessing the individual fractions with different

levels of attack, with a tendency towards lower variation in fraction 0 (healthy seeds).

During the experimental period under the conditions of a natural infectious background, including a number of pathogens - 16 of the samples exhibited a stable level of resistance with unidirectional results. Seven of the studied genotypes (23.3%) were resistant, with varieties and lines originating from Bulgaria prevailing in this group. Bogdan, Avalya and line 131 were with the lowest attack index. The same percentage of samples exhibited average sensitivity. Pame-ty Tychina and Fujisaka showed sensitivity and highest attack score.

The phytopathological assessment data showed that 14 varieties exhibited variable resistance. In 30% of the samples a variation in their reaction from low to moderate attack values was observed. Greater sensitivity was reported in 10% of the varieties, with the attack index ranging from high to moderate values. The Turkish varieties Deneb and TR 1759 had the most variable reaction and unstable resistance.

CONCLUSIONS

It was established that rice seeds collected from different samples in the Plovdiv region were infected with fungal pathogens of 5 genera: *Alternaria* spp., *Cladosporium* spp., *Fusarium* spp., *Drechslera* spp. and *Sclerophthora* spp.

Six species of fungi parasitizing rice seeds were identified, among which *Fusarium culmorum* and *Alternaria alternata* were predominant. The species were found in both years of study, which confirms the economic importance and widespread distribution of phytopathogens.

The current state of resistance to seed-borne pathogens under the conditions of a natural infectious background was determined. Differences in varietal susceptibility were reported. 23.3% of the studied samples had high and stable field resistance. Bogdan, Avalya and line 131, originating in Bulgaria had the lowest attack index. Pamety Tychina and Fujisaka varieties stood out as the most sensitive when grown in the Plovdiv

region. Immune and highly susceptible varieties and lines were not identified.

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