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Resistance to Brown and Stem Rust in Spring Soft Wheat Varieties in the Arid Climate of Northern Kazakhstan

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Abstract: To create competitive wheat varieties, it is essential to make active use of the world's diversity of genetic resources. The study aims to test the genetic resources of spring soft wheat for resistance to rust diseases. A spring soft wheat collection consisting of 206 samples, was studied in 2020-2022 for resistance to leaf and stem rust, as well as for a complex of valuable agronomic traits. The results of the study identify 13 samples resistant to brown rust and stem rust. The variety Weebill 1 (Mexico) had a high resistance to two diseases and the variety Jacui (Brazil) was also characterized by resistance to these diseases. Three varieties are characterized by high yield: CATBIRD (Mexico), Lutescence 14/10-14 (Russia), and Ana *2/Oc 14//2*Br 35/3/Oc 9015#87 (CIMMYT). Three varieties exceeded the standard for protein content: Ilimenskaya 2 (Russia), Lutescens 128-15 (Russia), and Lutescens 234/11 (Kazakhstan). Based on the level of sedimentation sediment, two samples were above the standard: Ilimenskaya 2 (Russia) and Lutescens 128-15 (Russia). By protein content and sedimentation level, two samples are distinguished: Akmola 2 (Kazakhstan) and Lutescence 234/11 (Kazakhstan). According to the complex of economically valuable characters, three varieties are highlighted: Weebill 1 (Mexico), CATBIRD (Mexico), and Scarlet (USA). The distinguished samples of spring soft wheat are recommended to breeders as sources of resistance to rust diseases in the creation of new competitive varieties adapted to northern Kazakhstan. At present, varieties Weebill 1 (Mexico), CATBIRD (Mexico), and Scarlet (USA) are used as parental forms in the creation of initial material with high yield, grain quality, and resistance to biotic environmental factors.

Keywords: Spring Soft Wheat, Collection, Variety, Brown Rust, Grain Quality

Introduction

Spring soft wheat is the main export crop in Kazakhstan. Kazakhstan is one of the top ten largest exporters of grain globally (Babkenov *et al*., 2020). Every year, Kazakhstan's grain exports to the world's markets are diminishing. The World Grain Council forecasts that in 2021/22, the country will export about 7.1 mln. t of grain compared to 8.1 mln. t in 2020/21 (IGC, 2022). Over the past 5 years, there has been a drop in gross wheat grain harvest from 14.8 mln. t in 2017 to 11.8 mln. t in 2021, with the yield of this crop decreasing from 12.4-9.3 cwt/ha. According to the FAOUN (2022), the average yield of wheat in Kazakhstan between 2011 and 2020 is at a low level, amounting to 11.7 cwt/ha, which is half as much as in Russia-25.3 cwt/ha.

This fact is explained by the arid climate of Kazakhstan, as well as the fact that the cultivated wheat varieties are often unstable in yield formation, grain quality, and susceptibility to rust diseases and septoriosis (Utebayev *et al*., 2016; Babkenova, 2017; Babkenov *et al*., 2020).

In northern Kazakhstan, brown and stem rusts are widespread. Brown rust is caused by *Puccinia triticina* Eriks. The disease mainly affects the leaves of wheat plants. The causative agent of stem rust is *Puccinia graminis f. sp. tritici* (Pgt). The disease affects the leaves, stems, and ears of plants. In this region, epiphyte types of brown rust are observed on wheat crops once every 3-4 years, while stem rust is 2-3 times less frequent (Koishybaev, 2018). When the green parts of plants are affected the assimilating surface decreases, which results in smaller grains and a decrease in the weight of 1,000 grains. Among the varieties of spring wheat grown in northern Kazakhstan, there are almost no varieties resistant to rust diseases (Babkenova *et al*., 2020; Dubekova *et al*., 2021).

Currently, farmers use fungicides to protect wheat crops from rust diseases. However, chemicals are a threat to the environment. In this regard, the selection of wheat varieties resistant to rust is becoming increasingly important (Genievskaya *et al*., 2022).

Scientists in Canada, the USA, and Mexico are doing a lot of work to identify resistance genes to various races of phytopathogenic fungi. To date, more than 60 Sr genes have been identified, which determine the resistance of wheat to stem rust, and almost 80 Lr genes-to leaf rust (Ellis *et al*., 2014; McIntosh *et al*., 2017). In addition to the known Lr and Sr genes, the study has hundreds of Quantitative Trait Loci (QTL) for rust resistance identified using clutch mapping (Gebrewahid *et al*., 2020; Kosgey *et al*., 2021; Zhang *et al*., 2019; Rollar *et al*., 2021).

To create varieties of spring soft wheat that stably produce high yields and are adapted to stressful environmental factors, it is essential to actively utilize genetic resources with resistance to rust diseases and a set of economically valuable characteristics in breeding programs.

In this study, sources of resistance to leaf and stem rust with a complex of economically valuable traits adapted to the conditions of Northern Kazakhstan are identified, which is the novelty of this study. The selected sources of resistance to rust diseases are included in the breeding program to create new varieties of wheat as parental forms.

The present study aims to examine the resistance of spring wheat varieties to rust diseases.

Materials and Methods

Location and Period of the Study

A collection of spring soft wheat was studied in 2020-2022 at the plant immunity laboratory of the A.I. Barayev Research and Production Center for Grain Farming. Weather conditions during the growing season of spring wheat in 2020 were described as moderately dry (Hydrothermal Coefficient (HTC) = 0.7), in 2021 as dry (HTC = 0.5), and in 2022 as dry (HTC = 0.6).

Spring Wheat Samples

The study analyzed 206 spring wheat samples of different ecological and geographical origins (Russia-71 samples, Kazakhstan-74 samples, USA-30 samples, Mexico-8 samples, Brazil-2 samples), as well as from the international maize and wheat improvement center (CIMMYT-21 samples). Wheat variety Akmola 2, which is known to be susceptible to brown rust and stem rust, was used as a standard.

The study focused on economically valuable characteristics, including grain yield and the main elements of productivity, the resistance of samples to brown and stem rust, protein content, and the level of sedimentation.

Study Design

Sowing of the collection seedlings was carried out in two replications using an SSFK-7 seeder (Omsk experimental plant, Russia); the area of the plot was 2 m^2 and the sowing date was May 20 in the years 2020-2022. Harvesting was performed with a Wintersteiger Classic combine (Wintersteiger, Austria). The testing of various samples for resistance to brown and stem rust was carried out with infectious backgrounds. In the first stage, the spores of *Puccinia triticina* and *Puccinia graminis* were heated to 45°C for 30 min To determine the viability of the spores, they were kept in a box with high humidity for 360 min contamination of plants normally took place in the evening during the period of booting-ear emergence, with the suspension spraying rate of 8-12 mg of viable spore material per 1 m^2. The studied varieties should be intensively watered to increase air humidity. A mixture of spore material and flour powder (1:100) was prepared and then the plants were sprayed. To create a humid chamber, the experimental plots were covered with a polyethylene film for 12-18 h. During the vegetation period, double records of plant infestation by rust diseases were made. The first control was carried out 8-10 days after inoculation and the next 8-10 days later. The methodology of testing wheat plants for stem rust damage was identical to the methods of research on leaf rust. Artificial infection nurseries for rust diseases were created in accordance with the methods of the State variety testing of agricultural crops (MARK, 2011).

The intensity of stem rust lesions was assessed using a scale developed by Peterson *et al*. (1948). Scales by Stakman and Harrar (1975) were used for the stem rust and brown rust types, respectively. The reaction of the samples to rust infection was tested according to the scale: 0-immune, R-resistant (no signs of disease), MR-Moderately Resistant (singular very small urediniopustules in well-defined chlorotic and necrotic spots), MS-Moderately Susceptible (medium and singular indelible large urediniopustules) and S-susceptible (large pustules, sometimes merging without necrosis but chlorosis is possible). The main parameter was the type of lesion, i.e., qualitative response to pathogen introduction. The biomaterial was a synthetic population of leaf and stem rust developed at the research institute of biological safety problems, Kazakhstan. Protein content was measured using an Infra-Lum FT-10 IR-analyzer (Lumex, Russia). The sedimentation level was determined according to the dodecyl sulfate sedimentation technique. The samples under study were placed in a calibrated and graduated cylinder with a capacity of 10 mL as 0.5 g of whole-milled grains (meal). With added 4 mL of distilled water, the container was sealed with a stopper and shaken 5 times. The resulting mixture was shaken for 120 sec. Then the working solution was prepared: 17 grams of NaC12H25SO4 were added to distilled water, followed by 3 mL of $C_2H_4O_2$. The resulting solution was then added to the test tube and the mixture was stirred for 300 sec. Afterward, the mixture was left to stand for 15 min. Finally, the amount of sedimentation is measured (sediment volume in mL ×10).

Statistical Analysis

Mathematical and statistical processing of the results was performed via variance and correlation analysis in Excel (Microsoft, USA).

Results

The study of 206 collection samples on infectious grounds showed 13 samples to be resistant to rust diseases. High resistance to brown and stem rust was found in the variety Weebill 1 (Mexico) and resistance was demonstrated by Jacui (Brazil) (Table 1). Moderate resistance to brown and stem rust was detected in 11 samples: Scarlet, ZAK, Erythrospermum 25787, WAKANZ, Ana *2/Oc 14//2*Br 35/3/Oc 9015#87, CATBIRD, Lutescence 128-15, Lutescence 14/10-14, Ilimenskaya 2, Lutescence 316/16 and Lutescence 234/11. The standard cultivar Akmola 2 was characterized by susceptibility to leaf and stem rust (Fig. 1).

Yields of the studied samples ranged from 1.25-2.83 t/ha (Table 2). The average yield was 1.90 t/ha. The standard variety Akmola 2 had an average yield of 2.83 t/ha. None of the studied samples exceeded the standard variety in yield. Three varieties, however, were at the same level as the standard Akmola 2: CATBIRD (Mexico), Lutescence 14/10-14 (Russia), and Ana *2/Oc 14//2*Br 35/3/Oc 9015#87 (CIMMYT).

The structure analysis reveals that three varieties were the most short-stemmed: Weebill 1 (Mexico), WAKANZ (USA), and Jacui (Brazil). The following three varieties were marked by high numbers of grains and grain weight per ear: CATBIRD (Mexico), Lutescence 14/10-14 (Russia), and Ana *2/Oc 14//2*Br 35/3/Oc 9015#87 (CIMMYT). Four varieties were distinguished as those with a high mass of 1,000 grains: Scarlet (USA), CATBIRD (Mexico), Lutescence 14/10-14 (Russia), and Erythrospermum 25787 (Russia).

Fig. 1: Leaf of the wheat variety Akmola 2, affected by stem rust on an artificial infectious background

Table 1: Response of the studied samples to brown and stem rust (data of 2020-2022)

		Brown rust		Stem rust	
Sample title	Origin	Reaction type	Intensity, %	Reaction type	Intensity, %
Akmola 2 (st)	Kazakhstan	S	70	4	70
Weebill 1	Mexico	0	0	0	0
Jacui	Brazil	R	10	R	5
Scarlet	USA	0	0	MR	5
ZAK	USA	R	10	MR	10
Erythrospermum 25787	Russia	R	5	MR	20
WAKANZ	USA	MR	5	MR	10
Ana *2/Oc 14//2*Br 35/3/Oc 9015#87	CIMMYT	MR	5	MR	5
CATBIRD	Mexico	MR	10	MR	5
Lutescence 128-15	Russia	MR	10	MR	5
Lutescence 14/10-14	Russia	MR	10	MR	5
Ilimenskaya 2	Russia	MR	5	MR	20
Lutescence 316/16	Kazakhstan	MR	5	MR	20
Lutescence 234/11	Kazakhstana	MR	10	MR	20

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Table 2: The yield of the studied samples and their constituent structural elements (data for 2020-2022)

Sample title	Yield, t/ha	Plant height, cm	Number of grains, pcs.	Grain weight per ear, g	Weight of 1,000 grains, g
Akmola 2 (st)	2.83	61	35.3	1.31	38.3
Weebill 1	2.14[a]	46	29.2	1.07	36.6
Jacui	1.25[a]	50	21.5	0.62	27.2
Scarlet	2.22[a]	56	26.1	1.11	42.2
ZAK	1.31[a]	53	18.1	0.65	36.7
Erythrospermum 25787	2.17[a]	66	27.4	1.07	38.9
WAKANZ	1.47[a]	49	22.5	0.73	32.9
Ana *2/Oc 14//2*Br 35/3/Oc 9015#87	2.44	55	39.0	1.22	31.0
CATBIRD	2.79	57	35.8	1.39	38.9
Lutescence 128-15	2.23[a]	60	31.8	1.10	34.7
Lutescence 14/10-14	2.71	81	35.1	1.35	38.5
Ilimenskaya 2	1.57[a]	63	24.4	0.79	32.2
Lutescence 316/16	2.14[a]	79	31.8	1.06	33.1
Lutescence 234/11	2.28[a]	74	32.7	1.14	34.9
Mean	1.90[a]	59.9	27.1	0.95	34.4
LSD_{05}	0.422	-			

Note: [a] – $p<0.05$

Table 3: Protein content and SDS-sedimentation level of flour in the studied samples (data of 2020-2022)

Sample title	Protein content, %	Volume of sedimentation, mL
Akmola 2 (st)	14.2	75.0
WEEBILL 1	14.0	66.0
Jacui	13.1	46.0
Scarlet	14.4	72.0
ZAK	12.8[a]	58.0
Erythrospermum 25787	12.5[a]	62.0
WAKANZ	12.9[a]	53[a]
Ana *2/Oc 14//2*Br 35/3/Oc 9015#87	15.3	48.0
CATBIRD	14.4	64.0
Lutescence 128-15	13.4	78.0
L.14/10-14	12.2[a]	56.0
Ilimenskaya 2	13.3	88.0
Lutescence 316/16	14.4	68.0
Lutescence 234/11	14.7	75.0
Mean	13.7	62.6
LSD_{05}	1.2	21.5

Note: [a] – $p<0.05$

The protein content is one of the main indicators defining the quality of wheat grain. This parameter varied from 12.2-15.3% in the studied samples, with an average of 13.7% (Table 3). The standard variety Akmola 2 had a protein content of 14.2%. Five samples exceeded the standard in this parameter: Ana *2/Oc 14//2*Br 35/3/Oc 9015#87 (CIMMYT), Lutescence 234/11 (Kazakhstan), CATBIRD (Mexico), Lutescence 316/16 (Kazakhstan) and Scarlet (USA). Another important parameter is the level of sedimentation, which characterizes the quality of gluten. This parameter varied from 46-88 mL in the examined samples. The standard variety Akmola 2 had a value of 75 mL. Three varieties surpassed the standard by this indicator: Ilimenskaya 2 (Russia), Lutescence 128-15 (Russia), and Lutescence 234/11 (Kazakhstan). Based on the protein content and sedimentation level, two samples stood out: Akmola 2 (Kazakhstan) and Lutescence 234/11 (Kazakhstan).

Discussion

The results of the study indicate 13 samples that demonstrate resistance to brown and stem rust in field conditions in northern Kazakhstan. These 13 samples have not been distributed in spring wheat crops in the north of Kazakhstan, as they are collecting samples and are studied by scientific organizations. In this region, collection samples of wheat were studied for productivity and resistance to rust. In 2018-2019, 184 spring wheat accessions were studied in the field, as well as using Genome-Wide Association Analysis (GWAS). Testing identified 80 promising lines of durum wheat against a local reference variety (Anuarbek *et al*., 2020). In the study by Gultyaeva *et al.* (2020), a collection of 21 spring durum wheat samples was studied; three lines characterized by resistance to three types of rust were identified: Hordeiforme 178-05-02, Hordeiforme 05-42-12 and Hordeiforme 1591-21. In 2016-2019, 120 collection samples of wheat were studied. The study identified two effective genes providing resistance to leaf rust (Lr19 and Lr26), as well as a stem rust resistance gene (Sr31) (Morgounov *et al*., 2020). In the study by Kokhmetova *et al*. (2016), 30 varieties of winter wheat were studied and it was found that the Lr26 and Lr1 genes did not provide resistance to leaf rust; the effective genes included Lr34, Lr37, Lr19, and Lr68. Thus, in the studies conducted, there are conflicting data on effective leaf rust resistance genes. Also in these works, the emphasis is either on productivity or rust resistance. For the effective creation of new varieties of wheat in breeding programs, it is necessary to have germplasm not only resistant to diseases but also a complex of economically valuable traits adapted to the conditions of the region. In our studies, this is the main priority.

The obtained results are consistent with the GRIS catalog (Genetic Resources Information System for Wheat and Triticale). Specifically, resistance to brown and stem rust in the variety Jacui is explained by the presence of genes Sr8, Lr13, and Lr34, in WEEBILL 1-gene Lr14b, in Scarlet and ZAK-Lr adult resistance and in wakanz-MR resistance to Lr (GRIS, 2022). For the other varieties, the GRIS catalog contains no information, since these specimens are recent and have not been fully studied.

According to our data, the standard variety Akmola 2 and other samples from Kazakhstan produced yields of 2.14-2.83 t/ha, while varieties of foreign origin have a productivity of 1.25-2.79 t/ha, lagging behind the local breeding varieties by 10-30%. This can be explained by the fact that local varieties are more adapted to the soil and climate of northern Kazakhstan, which agrees with the results obtained by other scholars (Shamanin *et al*., 2021; Morgounov *et al*., 2022).

In our studies, the protein content of Kazakh varieties reaches about 14.2-14.7% and meets the requirements for high-quality wheat. This conclusion is confirmed by other researchers who have studied wheat varieties in northern Kazakhstan (Dashkevich *et al*., 2022; Utebaev *et al*., 2022).

In breeding programs, when creating varieties of spring soft wheat resistant to rust diseases, in addition to disease resistance, much attention should be paid to yield and grain quality (Tembo, 2021). Thus, we recommend using genetic resources with high yield, grain quality, and resistance to diseases as source material for the creation of new competitive spring soft wheat varieties. According to the results of our research, Weebill 1, Scarlet, and CATBIRD can be such variety samples.

The variety Weebill 1 (Mexico) is highly resistant to brown and stem rust. Its yields average 2.14 t/ha in three years, significantly lower than the standard variety Akmola 2, but above average overall. The variety is marked by short stems of 46 cm. Weebill 1 delivers satisfactory results by the number of grains in an ear-29.2 pcs., by the weight of grains per ear-1.07 g, and by the weight of 1,000 grains-36.6 g. The variety shows decent indicators of grain quality: Protein content-14.0% and the volume of sedimentation-66 mL.

The variety of scarlet is highly resistant to brown rust and moderately resistant to stem rust. The variety has an above-average yield of 2.22 t/ha and a high 1,000-grain weight of 42.2 g. Moreover, Scarlet tests well in grain quality with a protein content of 14.4% and 72 mL of sediment.

The variety CATBIRD belongs to the moderately resistant varieties in resistance to brown and stem rust. However, the variety has a high yield-2.79 t/ha, on the same level as the standard variety Akmola 2. This variety shows good grain quality indicators: Protein content-14.4% and the volume of sedimentation 64 mL.

Conclusion

The study conducted in 2020-2022 identifies 13 samples resistant to brown and stem rust. High yield is demonstrated by three varieties: CATBIRD (Mexico), Lutescens 14/10-14 (Russia), and Ana *2/Oc 14//2*Br 35/3/Oc 9015#87 (CIMMYT). In terms of protein content and sedimentation level, two samples stand out: Akmola 2 (Kazakhstan) and Lutescens 234/11 (Kazakhstan). Based on the complex of economically valuable characters, three varieties are distinguished: Weebill 1 (Mexico), CATBIRD (Mexico), and Scarlet (USA).

The highlighted samples of spring soft wheat are to be recommended to breeders as sources of resistance to rust diseases and yield and grain quality when creating new competitive varieties adapted to northern Kazakhstan.

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Author’s Contributions

All authors equally contributed to this study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

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