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New genetic diversity of winter two-row barley lines resistant to Loose smut *Ustilago nuda* (Jensen) Rostrup

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Abstract

The creation of resistant barley cultivars leads to reduction in direct yield losses and limits the accumulation of infection in crops. Studies of new genetic plasma make it possible to find forms that can serve as starting material for the creation of a future disease-resistant cultivar. In the period 2012/2014, in competitive variety experiments, perspective lines of winter two-row barley resistant to loose smut *(Ustilago nuda* (Jensen) Rostrup) were studied. The experiment was based on the method of the Latin rectangle, with a plot of 10 sq m, in four repetitions, with standards Obzor, Emon and Kaskadyor 3. The yield was reported and the results were statistically processed. Grain quality was determined on the basis of the following indicators: 1000-grain weight (g) by weight method, uniformity 1st class (%), expressed as the ratio of the weight of grains remaining on sorting sieves 2.8 mm wide and 2.5 mm to the total weight in a sample of 100 g, protein content (%) (Kjeldahl method), extract content (%) according to EBC (1998). The relationship between grain yield and quality in perspective lines resistant to loose smut *(Ustilago nuda* (Jensen) Rostrup) was studied by correlation and principle component analysis using software SPSS 19.0 and Statgraf 2.1. As a result, winter barley lines resistant to loose smut *(Ustilago nuda* (Jensen) Rostrup) were created. Perspective lines PG 4480, PG 4447 and PG 4628 combine resistance to loose smut *(Ustilago nuda* (Jensen) Rostrup), high productivity and grain quality. They can be successfully used in the barley breeding program as parent components for creating new cultivars.

Key words: two-row barley; genetic diversity; loose smut

INTRODUCTION

The main task of modern barley breeding is to create high-yielding cultivars with very good grain quality characteristics, which are resistant to biotic and abiotic stress factors. In production, cultivar belongs to the important factors with impact on the phytosanitary situation which results in increased significance of the resistant cultivar. Creating resistant barley cultivars leads to reduction in direct yield loss and limits infection accumulation in crops.

The studies of new genetic plasma make it possible to find forms that will serve as starting material for creating a future disease-resistant cultivar (Dimova & Dyulgerova, 2017; Dyulgerova et al., 2016; Al Lawati et al., 2021).

Numerous pathogens attack barley but only a few cause serious damage. Particularly harmful to barley is loose smut caused by *Ustilago nuda* (Jensen) Rostrup. Barley plants are infected during flowering, and the disease appears the following year. Externally, the grain shows no signs of disease, although it carries inside the loose smut mycelium. The life cycle of the pathogen, the spread and harmfulness of loose smut, the methods of artificial infection and their application for the evaluation of cultivars and breeding material, as well as the effect of some factors on the environment and the agro-

technical complex were the subject of studies by a number of researchers from different countries.

(Woldemichael, 2019) It was established that the combination of good productivity and disease resistance in one genotype is more difficult to implement due to complex genetic and correlational dependencies between these traits (Zapryanov, 1990; Navushtanov, 1991; Valcheva, 2000; Mersinkov, 2000).

The value of resistant cultivars is that without the use of additional means of protection, the cultivars reach their biological potential.

The aim of this study was to characterize new perspective lines of two-row barley resistant to loose smut *Ustilago nuda* (Jensen) Rostrup.

MATERIAL AND METHODS

The study was conducted in the experimental field of the Institute of Agriculture in Karnobat, which is located in Southeastern Bulgaria. In the period 2012–2014, perspective barley lines resistant to loose smut were studied in a competitive varietal trial.

The experiment was set by the Latin rectangle method, with a harvest plot of 10 sq. m., in four repetitions, with standards Obzor, Emon and Kaskadyor. The yield was reported and the results were statistically processed. The grain quality was determined on the basis of the following indicators: 1000-grain weight (g) by a weight method, uniformity 1st class (%), expressed by the ratio of the weight of grain remaining on sorting sieves with a width of 2.8 mm and 2.5 mm to the total weight of a 100 g sample, protein content (%) (Kjeldahl method), extract content EBC (%) (1998). The relationship between yield and grain quality in perspective lines resistant to loose smut (*Ustilago nuda* (Jensen) Rostrup) was studied by means of correlation and principal component analysis using SPSS 19.0 and Statgraf 2.1 software products.

The method of combination breeding was used to create new lines of barley resistant to loose smut (*Ustilago nuda* (Jensen) Rostrup). Common A x B crosses and a limited number of (A x B) x A backcrosses were performed, with subsequent artificial infections in F3-F4 generations and breeding resistant progeny. In the hybridization were used as mother forms cultivars and breeding lines from Bulgarian breeding and introduction, well adapted to the environmental conditions. Donors of resistance were cultivars Perun, K-8728, K-6823, as well as lines resistant to loose smut, created from sources of resistance and selected from the studies on responses of Bulgarian and introduced barley accessions to loose smut (*Ustilago nuda* (Jensen) Rostrup).

Evaluation and breeding for resistance/susceptibility to pathogens were conducted from the F3-F4 generations obtained through artificial inoculation. The progeny that showed resistance was also evaluated by phenotype. This is followed by reinfection, evaluation and selection of resistant progeny. After a double check for resistance/susceptibility, the selected progeny continued in studies by the general selection scheme. The F1 hybrids were sown by the mother - hybrid - father scheme. The sowing of breeding materials in F1, F2, F3, F4 was done manually, in beds with a length of 1.10 cm, inter-row spacing of 25 cm, in-row spacing of 5 cm and bed spacing of 60 cm. Approved progeny from F3-F4 generations continued testing in the higher breeding steps.

RESULTS AND DISCUSSION

In terms of climate, the region belongs to the transitional continental climate region, with a long and cool spring, dry and hot summer, prolonged and comparatively humid autumn, a snowless winter with great temperature fluctuations and winter erosion. As a whole, the testing period was very different from the monthly temperatures and rainfall typical for the region, which ensured that the perspective lines were studied under conditions of abiotic stress. The weather conditions in 2011/2012 were cold autumn and snowy winter with low negative mean monthly temperatures in January and February, drought at the beginning of the spring vegetation period and abundant rainfall during the period of grain filling and ripening. The economic year of 2012/2013 had a mild and snowy winter, with dry spring with insufficient rainfall and high maximum temperatures. The vegetation period of barley in year 2013/2014 passed through humid and warm autumn, mild winter and abundant rainfall in spring and summer.

In the period 2012-2014, the best lines resistant to loose smut were studied in competition variety

trials. Table 3 presents the data from the phenological observations and evaluations carried out as mean for the study period for the two-row materials. The lines have a high stem ranging from 98.0 cm to 107.0 cm, but in addition, they have very good lodging resistance (rating score 7-9). The heading date of the lines was in the period from 29.04 to 12.05, which is 2 to 3 days earlier or equal to the mean standard. The data on morphological uniformity show that the perspective lines are at the level of the mean standard (rating score 7-9). Disease attack evaluations in field conditions show that the perspective lines manifest good field resistance to loose smut, leaf blotch, leaf rust, net blotch. PG 4461 and PG 4343, which are highly resistant to the four observed diseases, deserve attention.

Data on the productivity of the perspective two-row lines are shown in Table 4. The yield obtained from standards during the study period ranged from 545 kg/da to 563 kg/da, with an average of 554 kg/da for the three standards. The yield of the lines was lowest in 2012, with an average for the group of 557 kg/da, and it was highest in 2013 - 600 kg/da. The total line yield for the period was 583 kg/da, which was higher than the mean standard. The analysis of the results showed that the perspective lines have very good and relatively constant productivity over the years. On average for the three years of the study,

Table 1. Average monthly air temperature (t^0) during barley vegetation in the period 2012-2014

Months	2011/2012	2012/2013	2013/2014	Mean values for the period	Multiannual values 1931-2010	Deviation
X	11.0	16.0	11.9	13.0	12.5	+0.5
XI	4.6	9.2	9.8	7.9	7.1	+0.8
XII	3.9	1.7	1.5	2.4	2.6	-0.2
I	-0.5	2.0	3.4	1.6	0.6	+1.0
II	-1.0	4.4	5.3	2.9	2.2	+0.7
III	6.4	7.0	8.7	7.4	5.3	+2.1
IV	14.0	13.0	11.7	12.9	10.5	+2.4
V	17.0	18.8	15.8	17.2	15.6	+1.6
VI	22.4	21.0	19.9	21.1	19.6	+1.5
VII	25.8	23.1	22.7	23.9	22.0	+1.9

Table 2. Average monthly precipitation amount (mm/m^2) during barley vegetation in the period 2012-2014

Months	2011/ 2012	2012/ 2013	2013/ 2014	Mean values for the period	Multiannual values 1901-2010	Deviation from the mean values
X	149.0	92.1	42.7	94.6	44.3	+ 50.3
XI	0.6	12.9	32.0	15.2	53.7	- 38.5
XII	64.1	100.6	4.1	56.3	51.2	+5.1
I	71.5	46.8	67.6	62.0	36.5	+25.5
II	46.3	49.8	4.8	33.6	35.8	-2.2
III	7.6	46.5	78.7	44.3	34.1	+10.2
IV	47.4	26.8	41.8	38.7	45.3	-6.6
V	117.8	19.5	101.4	79.6	58.5	+21.1
VI	20.6	74.9	89.4	61.6	65.2	-3.6
VII	14.2	16.9	75.5	35.5	49.9	-14.4
Rainfall sum	539.1	486.8	538.0	521.3	474.5	+46.8

Table 3. Agrobiological characterization of perspective lines of two-row barley, resistant to loose smut *(Ustilago nuda),* in the period 2012-2014

Breeding lines	Plant height (cm)	Ear emergence date	Morphological uniformity, rating score (9-1)	Resistance to lodging, rating score (9-1)	Resistance to diseases			
					Loose smut, rating score (1-9)	Leaf blotch, rating score (1-9)	Leaf rust, rating score (1-9)	Net blotch, rating score (1-9)
Obzor	97.6	7-12.05	9	8-9	1	1	3	1
Emon	97.8	3-6.05	9	9	3	3	3	1
Kaskadyor	100.3	1-5.05	8	8	3	5	3	5
Mean standard	**98.6**	**1-12.05**	**8-9**	**8-9**	**1-3**	**1-5**	**3**	**1-5**
Perun	100.7	5-6.05	8-9	8-9	1	3	3	1
PG 4480	102.5	5-8.06	8	8	1	3	1	3
PG 4481	100.0	3-10.05	7-8	8	1	3	1	5
PG 4447	100.0	1-7.05	7-8	8	1	1	1	1
PG 4463	100.5	3-6.05	8	8	1	3	5	5
PG 4461	101.5	1-3.05	7-8	8	**1**	**1**	**1**	**1**
PG 4491	98.0	1-8.05	8	9	1	3	1	3
PG 4622	102.5	1-5.05	8	8	3	1	1	1
PG 4625	112.5	1-3.05	8	8	1	3	3	3
PG 4626	102.5	5.05	7-8	8	1	3	5	5
PG 4648	96.0	10.05	7-8	8	3	1	5	3
PG 4643	107.5	29.04	7-8	8	**1**	**1**	**1**	**1**
PG 4628	103.5	1-5.05	8	8	1	3	5	3
Mean	**101.3**	**29.04-12.05**	**7-9**	**8-9**	**1-3**	**1-3**	**1-5**	**1-5**

Table 4. Yield from perspective lines of two-row barley, resistant to loose smut *(Ustilago nuda),* in the period 2012/2014

Cultivars and lines	2012		2013		2014		Mean for period 2012-2014, kg /da	%
	kg/da	%	kg/da	%	kg/da	%		
Obzor	529	97.06	581	103.20	596	107.78	568	102.53
Emon	571	104.77	512	90.94	506	91.50	529	95.49
Kaskadyor	535	98.17	598	106.33	558	100.90	564	101.81
Mean standard	**545**	**100.00**	**563**	**100.00**	**553**	**100.00**	**554**	**100.00**
Perun	572 cde	104.90	564 fgh	100.10	584 cd	105.50	573	103.40
PG 4480	601 b	110.30	614 cde	109.10	580 cd	104.70	598	108.00
PG 4481	567 bcd	104.02	530 h	94.10	564 d	101.90	553	99.90
PG 4447	530 de	97.30	617 cd	109.60	616 bc	111.40	588	106.10
PG 4463	560 bcd	102.80	605 c-f	107.50	594 cd	107.40	586	105.80
PG 4461	548 cde	100.70	570 e-h	101.20	553 d	99.90	557	100.50
PG 4491	528 de	97.00	555 gh	98.60	536 d	96.80	540	97.40
PG 4622	**647 a**	**118.70**	**691 a**	**122.70**	**665 ab**	**120.30**	**668**	**120.50**
PG 4625	556 cd	102.02	643 bc	114.20	666 **a**	120.30	622	112.20
PG 4626	542 cde	99.40	674 ab	119.80	591 cd	106.80	602	108.70
PG 4648	586 bc	107.60	629 bcd	111.80	614 c	111.00	610	110.10
PG 4643	505 e	92.70	588 d-g	104.40	618 abc	111.70	570	102.90
PG 4628	507 e	93.03	550 gh	97.70	574 cd	103.80	544	98.10
Mean	**557**		**600**		**595**		**583**	
LSD	44.69		46.07		49.17			
VC %	5.62		5.38		5.79			

line PG 4622 realized 668 kg/da and exceeded the standard by 20.50%, and based on the LSD values, in all three years it fell into group a and ab.

Data on the grain quality of the perspective two-row barley lines are presented in Table 5. During the study period, the 1000-grain weight of the perspective lines had a mean value of 45.27 g, whereas the lines varied from 41.50 g to 51.50 g. The average of the perspective lines during the period was 46.46 g of 1000-grain weight, which defines them as lines with large grain. Their uniformity 1st class varied in the range from 84.8% to 95.5%. The mean value was lower compared to the value of the standards. The protein content of the two-row barley lines ranged from 10.25% to 13.90%, with mean value of 12.36%. Suitable for brewing grain are the low-protein (up to 12.0%) lines PG 4480 (12.00%), PG 4626 (11.68%) and PG 4628 (10.25%). High protein content is characteristic for lines PG 4481 (13.80%), PG 4491 (13.90%) and PG 4622 (13.25%), whose grain can be used as feed. The extract content mean for the studied lines was 76.6% compared to 78.2% for the mean standard, which shows that the group as a whole falls behind on this indicator. Among, them there were also those that showed a high extract content of over 80%. Line PG 4628 had an extract of 80.1% and was distinguished for brewing purposes by a good combination of the investigated quality indicators.

To create perspective barley lines combining high productivity with disease resistance, it is important to achieve a good balance between yield and grain quality. The established negative correlations between yield - quality - resistance to biotic factors, very often may impede breeding and make it almost impossible to create a genotype of high productivity, good grain quality and disease resistance.

Therefore, the relationship of yield and grain quality in the created perspective barley lines, resistant to loose smut (*Ustilago nuda*), was also studied in this investigation by means of principle component analysis. In the studied lines, two main components were extracted, which determined 72.79% of the total variation (Table 6).

Grain yield and protein content were associated with the first principal component (Table 7). High positive values of both indicators indicated a positive relationship between them, which determined

Table 5. Grain quality of perspective lines of two-row barley, resistant to loose smut *(Ustilago nuda),* in the period 2012-2014

Breeding lines	1000-grain weight (g)	Uniformity 1st class (%)	Protein content (%)	Extract content (%)
Obzor	46.30	89.5	12.57	77.2
Emon	45.50	95.6	11.74	78.0
Kaskadyor	44.00	94.6	11.50	79.5
Mean standard	**45.27**	**93.2**	**11.93**	**78.2**
Perun	44.50	91.9	12.14	76.2
PG 4480	44.80	90.2	12.00	73.6
PG 4481	51.00	92.9	**13.80**	75.4
PG 4447	44.00	84.8	12.32	75.4
PG 4463	49.50	86.0	12.72	76.1
PG 4461	51.50	95.2	12.18	73.0
PG 4491	50.00	93.5	**13.90**	75.6
PG 4622	41.50	88.2	**13.25**	75.8
PG 4625	46.50	89.2	12.36	77.7
PG 4626	**44.50**	**95.9**	**11.68**	**78.7**
PG 4648	46.00	92.8	13.50	71.2
PG 4643	**47.00**	**88.3**	**12.32**	**80.5**
PG 4628	**48.00**	**94.5**	**10.25**	**80.1**
Mean	**46.46**	**91.6**	**12.36**	**76.6**

Table 6. Principal component values for perspective lines of two-row barley, resistant to loose smut *(Ustilago nuda),* in the period 2012-2014

Principal components	Weight	%	Cumulative %
PC 1	2.00	40.06	40.06
PC 2	1.63	32.73	72.79

Table 7. Yield and grain quality values of perspective lines of two-row barley, resistant to loose smut *(Ustilago nuda),* in the period 2012-2014

Indicators	PC 1	PC 2
Yield	**0.59**	0.28
1000-grain weight	-0.28	-0.62
Grain uniformity 1st class	-0.49	-0.14
Extract content	-0.38	**0.50**
Protein content	**0.43**	-0.51

the creation of a genotype with high yield and high protein content. For two-row barley, this relationship is suitable for feed cultivars. When selecting genotypes for brewing grain with low protein content and high yield, the effect will be minimal. The second main component was related to the extract content. On this component, the value of the protein content indicator was high and negative. This suggests that among the studied group of perspective lines there were those with a favorable combination of low protein and high extract content, suitable for brewing purposes.

Figure 1 shows a projection of the yield and the studied indicators of grain quality in the factor space. The vector of the protein content indicator has the longest length, indicating that it has a decisive role in the total variation. It is also the vector that forms an acute angle with the yield vector, which indicates a direct relationship between the indicators. In the negative quadrant of the vector

Table 8. Correlations between yield and grain quality of perspective lines of two-row barley, resistant to loose smut *(Ustilago nuda),* in the period 2012-2014

Indicators	1000-grain weight	Uniformity 1st class	Extract content	Protein content
Yield	-0.578*	-0.475	-0.249	0.239
1000-grain weight	1	0.217	-0.170	0.231
Grain uniformity 1st class		1	0.084	-0.270
Extract content			1	-0.574*

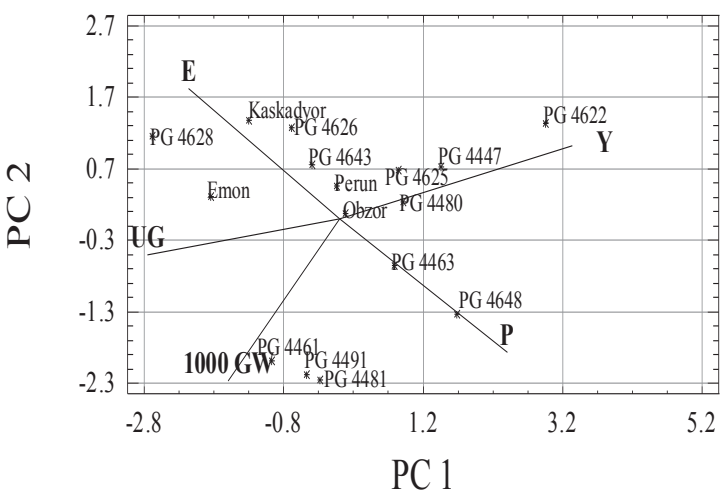

Figure 1. Projection of yield and grain quality of perspective lines of two-row barley, resistant to loose smut *(Ustilago nuda)*

space fall the vectors of uniformity 1st class and 1000-grain weight. Their location proves that they are negatively related to yield in the studied group of lines. The indicators of protein content and extract content depend on the year conditions, which is evidenced by the high negative values of PC 1 for the extract and PC 2 for the protein. In the positive quadrant of the vector space around the yield vector fall the highest yielding lines of two-row barley. These are PG 4480, PG 4625, PG 4447 and PG 4622. PG 4463 and PG 4648 also have a high yield, but their location shows a strong dependence on the year conditions. At the level of the standards were PG 4628, PG 4626 and PG 4643. Low productive were PG 4461, PG 4491 and PG 4481 (Figure 1).

The established correlations also speak for the relationship between the yield and grain quality indicators in the perspective two-row barley lines (Table 8). There is a proven negative high correlation between yield and 1000-grain weight (r= - 0.578*). Analyzing the absolute values of the indicator, it can be said that the created two-row barley lines have a high 1000-grain weight, higher than the mean standard (Table 5). Comparing 1000-grain weight and yield data makes is clear that high-yielding lines are the ones that have a 1000-grain weight lower than the group mean values (Table 4). A high negative correlation (r= - 0.574*) between extract content and protein content was found in the studied group of perspective brewing barley lines. This relationship is very favorable for brewing grain lines in which low protein and high extract content are preferred. The established correlation also confirms the studies by other researchers (Valcheva & Valchev, 2012).

CONCLUSIONS

In conclusion, we can summarize that winter barley lines resistant to loose smut have been created. Perspective lines PG 4480, PG 4447 and PG 4628 combine resistance to loose smut, high productivity and grain quality. They can be successfully used in the breeding process as parental components and to create new barley cultivars.

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