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ORIGINAL PAPER

Screening and genetic studies on resistance to Soil-born Cereal Mosaic Virus (SBWMV) in rye

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Abstract

Due to several reasons soil-borne viruses such as the furoviruses, i. e., cereal mosaic virus (SBCMV) and wheat mosaic virus (SBWMV) as well as the bymovirus wheat spindle streak mosaic virus (WSSMV) gained importance in cereal breeding including rye. High yield losses are recorded, today. Since there is no or little resistance to these viruses in modern rye cultivars, an extended screening for resistance was initiated. In addition to earlier screenings, 37 rye genotypes were tested for resistance. Among them, three genotypes were found with persistent resistance to SBCMV. They belong to *Secale montanum* and *S. vavilovii* species, i. e., wild types of rye. One accession, PC2243 (*S. montanum*), was used as a resistance donor for the present genetic study. In F2 generation, it was observed that resistance to SBCMV is independently inherited from WSSMV. The evaluation of the ELISA values pointed to a 3:1 distribution assuming duplicate dominant epistasis. Molecular marker analysis supports this segregation pattern. By composite interval mapping a QTL on chromosome 2R could be detected. It can be assumed that there is a DNA region of about 13 cM on the long arm of chromosome 2R (2RL) harboring SBCMV resistance with the closest markers *"C9654_1947"* and "*isotig11640"*.Moreover, genotypes with a yellow seed coat showed practically no infection with SBCMV. Thus, the resistance gene could be linked to the allele *an1* determining non expression of anthocyanins. This locus was also mapped earlier on chromosome 2R.

Keywords Rye · *Secale cereale* · *S. montanum* · *S. africanum* · *S. vavilovii* · Furovirus · Soil-borne cereal mosaic virus (SBCMV) · Resistance · Screening · Mapping · Chromosome arm 2RL · Seed color · *An1/an1* locus

Introduction

Since centuries cereals including rye are major crops, particularly in Europe, for feeding, bread making and as renewable resources. Rye cropping on poor, sandy soils and restricted crop rotations promote the growth of soil-borne viruses such as the furovirus cereal mosaic virus (SBCMV) and wheat mosaic virus (SBWMV) as well as the bymovirus wheat spindle streak mosaic virus (WSSMV, see Table 1). Global warming may contribute to the increasing spread of these viruses in the temperate zones of Europe. In the affected areas, yield losses in grain of up to 70% are recorded, at least in wheat (Budge et al. 2008).

Both SBCMV and WSSMV are vectored by the obligate biotrophic root parasite *Polymyxa graminis* (Kanyuka et al. 2003). As spores of *P. graminis* persist in the soil and soil-borne viruses remain viable therein for decades, susceptible cereals cannot be grown on infested fields (Driskel et al. 2004). Because neither chemical control of virus and the respective vector nor crop rotation is effective, breeding of resistant varieties seems to be the only way of plant protection (Ordon et al. 2009).

Since there is no resistance to these viruses present in modern rye varieties, an extended screening for resistant genotypes was initiated years ago (Huth et al. 2007; Kastirr et al. 2006, 2011, 2012; King et al. 2011; Kühne 2009; Ziegler et al. 2015). Only Cadle-Davidson et al. (2006) identified the old U.S. American rye variety "Aroostook" being resistant to WSSMV. However, respective results could not be confirmed under German growing conditions (U. Kastirr,

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personal comm., 2016), maybe due to the genetic heterogeneity of the samples used.

Erath et al. (2016) tested more than 500 accessions of *Secale cereale* and *S. montanum* from 48 different geographical regions in growth chambers with virus-infested soil. Within these genetic resources 35 accessions were detected with a lower infection rate and low virus titre. These genotypes were cultivated on infested fields of five different environments. Two of these regions were mix contaminated by SBCMV and WSSMV and three fields respectively only by SBCMV, SBWMV, and WSSMV. Just one rye population, derived from the combination *S. cereale* var. Lo86 x *S. cereale* var. Moor-Roggen led to a more detailed segregation analysis of F2 to F6 populations showing moderate resistance controlled by quantitative trait loci (QTL) on chromosome 5R (SBCMV) and 7R (WSSMV).

In order to extent search for resistant rye genomes, in 2012 an additional screening was initiated considering genetic tester stocks, wheat-rye amphiploids, and related wild species of rye. Because of the high degree of genetic variability within the populations, supposedly resistant plants were isolated and used for crosses and resistance testing by ELISA. Three genotypes out of 37 turned out to be persistently resistant to SBCMV. The accession PC2243 from *Secale montanum* was used as a resistance donor for the present genetic study.

Materials and methods

Plant material

Out of the cytogenetic tester tsock collection (curator R. Schlegel, 1971–2020), 37 genotypes of a wide range of rye genomes were tested for resistance to soil-borne viruses: diploid *S. cereale* var. Imperial, var. Insave, var. Maton (USA, WSMV res.), var. Petkuser, var. Rosen (Russia > USA), str. Lochow 120-P, str. Lochow KWL1, inbred line; diploid *S. montanum* str. R797, str. R2501 (UK); diploid *S. cereale* var. (unknown origin, Quedlinburg); tetraploid *S. cereale* var. Gorzow, var. Shitomirskaja, var. Sopronyhorpacsi, var. Ukrainskaja, str. 3551/70, str. 3130/69; diploid *S. africanum* str. R1210, diploid; $2R^{afr}$(2D) wheat-rye substitution, *T. aestivum x S. africanum* (kindly provided by Zujun Yang, Chengdu, China); *S. vavilovii*, collected Armenia by the author; *S. cereale*, var. Heines Hellkorn, Trisomic 1R…7R (kindly provided by F. J. Zeller, Weihenstephan, Germany); rye-wheat addition 5D, from *S. cereale* var. Pluto x *T. aestivum* var. Fakon; hexaploid *Triticum turgidocereale* var. Pika, BBAARR; amphi-octoploid *Triticum rimpauii*, *T. aestivum* var. Chinese Spring x *S. montanum*, BBAADDRR, var. *T. aestivum* var. Chinese Spring x *S. cereale* var. Imperial, var. *T. aestivum* var. Chinese Spring x *S. cereale var.* King II, BBAADDRR; var. *T. aestivum* var. Azle x *S. cereale var.* Pearl; Wheat-rye addition $1R^{mo}$, $2R^{mo}$, $4R^{mo}$, $5RS^{mo}$ and $6R^{mo}$ *T. aestivum* var. Chinese Spring–*S. montanum,* 2n = 44.

Four mapping populations segregating for SBCMV and WSSMV were established from combinations (a) *Secale cereale* var. Imperial x *S. montanum*, (b) *S. cereale* var. Imperial x *S. africanum*, (c) *S. cereale* var. Inbred line (KWL1) x *S. montanum*, and (d) *S. cereale* var. Imperial x *S. vavilovii.* However, in this paper the first of the four combinations (a) is considered only, and exclusively for SBCMV.

After crossing and production of F1 plan ts two mapping populations of 202 and 133 F2 plants were established, resp. The individual seedlings, separated by four classes of different seed color (cf. Table 4), were planted in September 2018 on a SBCMV- and WSSMV-infested field plot at Thören (Lower Saxony). In addition, a replicated population with 133 plants was used for planting in September 2019 on a SBCMV- and WSSMV-infested field at Walternienburg (Saxony-Anhalt). Because of the high degree of phenotypic variation and mild symptom development, no phenotypic trait assessment was performed.

ELISA

The virus concentration in parents and progeny was determined by semi-quantitative DAS-ELISA (double antibody sandwich enzyme-linked immunosorbent assay) according to Clark and Adams (1977). In March 2019 and March 2020, the youngest leaves of plants were collected. The extracted leaf sap from 0.1 g leaf tissue was diluted 1:20 with extraction buffer and added to microtitre plate wells (Nunc Maxisorp, San Diego, CA, USA), which were coated with specific antibodies against SBCMV or WSSMV, respectively. The extinction (optical density, OD) was measured at 405 nm.

Table 1 Classification of important soil-borne viruses in rye transmitted by fungal vector *Polymyxa graminis* Ledingham (Kühne 2009)

Polymyxa viruses	Code	Virus group	Cereal hosts
Wheat spindle streak mosaic virus	WSSMV	Bymovirus	wheat, triticale, rye
Wheat yellow mosaic virus	WYMV		
Soil-borne wheat mosaic virus	SBWMV	Furovirus	wheat, triticale, rye
Soil-borne cereal mosaic virus	**SBCMV**		
Chinese wheat mosaic virus	CWMV		

Table 2 Results of pre-screening of rye genotypes for resistance against soil-borne viruses (+ = susceptible, 0 = resistant). Greenhouse and field testing are included for SBCMV, SBWMV WSSMV between 2012 and 2018. Selected entries. Complete Table 2a can be obtained from the authors

No	Material	Phenotype	
		Resistant	Susceptible
PC0272	*S. cereale* var. Imperial, diploid		+ (SBCMV, 2013) [1]
PC1947	*S. cereale* var. Insave, diploid		+ (SBWMV, 2013) [3]
PC2245	*S. cereale* var. Maton (USA), diploid (WSMV res.)		+ (SBCMV, 2012) [1]
PC0373	*S. cereale* var. Petkuser, diploid	0 (SBCMV, 2013) [1]	+ (SBWMV, 2015) [2] + (WSSMV, 2015) [2]
PC2107	*S. cereale* var. Rosen (Russia > USA), diploid		+ (SBWMV, 2013) [3]
PC2244	*S. cereale* str. Lochow 120-P, diploid		+ (SBCMV, 2012) [1]
PC2238	*S. cereale* str. Lochow KWL1, inbred line, diploid		+ (SBCMV, 2015) [5]
PC2243	*S. montanum* str. R797, diploid	0 (SBCMV, 2012)[1]	
PC2247	*S. montanum* str. R2501 (UK), diploid		+ (SBCMV, 2012) [1]
PC2329	*S. cereale* var. (unknown origin, Quedlinburg), diploid		+ (SBCMV, 2013) [1]
PC2323	*S. cereale* var. Gorzow, tetraploid		+ (SBWMV, 2013) [3]
PC2321	*S. cereale* var. Shitomirskaja, tetraploid		+ (SBWMV, 2013) [3]
…			
PC2209	*S. cereale* str. 3130/69, tetraploid		+ (SBWMV, 2013) [3] + (SBCMV, WSSMV 2013) [4]
PC2328	*S. africanum* str. R1210, diploid	0 (SBCMV, 2013, 2014, 2015) [1]	
PC2327	$2R^{afr}$(2D) substitution; wheat-rye substitution, *T. aestivum x S. africanum*	0 (SBCMV, 2012) [1]	+ (SBCMV, 2015) [2] + (SBWMV, 2015) [2] + (WSSMV, 2015) [2]
PC2364	*S. vavilovii*, collected Armenia by the author	0 (SBCMV, 2015) [4]	
PC0196	*S. cereale*, Trisomic 1R		+ (SBCMV, 2013) [1]
PC0197	*S. cereale*, Trisomic 2R		+ (SBCMV, 2013) [1]
PC0198	*S. cereale*, Trisomic 3R		+ (SBCMV, 2013) [1]
…	…	…	…
PC2230	Wheat-rye addition $1R^{mo}$ *T. aestivum* var. Chinese Spring – *S. montanum,* 2n = 44		+ (SBCMV, 2012) [1]
PC2231	Wheat-rye addition $2R^{mo}$ *T. aestivum* var. Chinese Spring – *S. montanum,* 2n = 44		+ (SBCMV, 2012) [1]
PC2233	Wheat-rye addition $5RS^{mo}$ *T. aestivum* var. Chinese Spring – *S. montanum,* 2n = 44		+ (SBCMV, 2012) [1]
PC2234	Wheat-rye addition $6R^{mo}$ *T. aestivum* var. Chinese Spring – *S. montanum*, 2n = 44		+ (SBCMV, 2012) [1]
Σ 37		3	34

SBCMV = Soil-borne cereal mosaic virus (Furovirus)

SBWMV = Soil-borne wheat mosaic virus (Furovirus)

WSSMV = wheat spindle streak mosaic *virus* (Bymovirus)

1 = Greenhouse testing with contaminated soil from Bormkoppel (Cashagen)

2 = Field testing at locations with contaminated soil (Gödnitz, Thören, Eickleoh, Heddesheim, Westerrade, Schleesen; two replications on each site; each replication included 10 single plants)

3 = Field testing with infested soil at Heddesheim (two replications)

4 = Field testing with infested soil at Gödnitz (two replications)

5 = Field testing with infested soil by KWS Lochow GmbH, Dr. Schmiedchen

DNA extraction and molecular analysis

Genomic DNA was extracted from F2 plants including parental individuals with a silica-membrane technology according to a Macherey & Nagel NucleoSpin 96 Plant II DNA extraction kit (Saghai-Maroof et. al. 1984, Anonymous 2019a).

Marker data were obtained by using a KWS custom 10 k Infinium iSelect single nucleotide polymorphism (SNP) array according to the supplier guidelines. From the array,

Table 3 WSSMV and SBCMV infestation (ELISA titre) in parental plants *S. cereale* var. Imperial (PC272) and *S. montanum* (PC2243) at the test site Thören in 2019 and Walternienburg in 2020 revealed by ELISA

Parents	WSSMV	SBCMV	
	2019	2019	2020
S. cereale var. Imperial (PC272)	0.02	**4.89**	**0.21**
	0.03	**3.78**	**4.88**
	0.01	**3.14**	**0.19**
	0.01	**4.89**	**0.11**
			4.88
			4.88
			0.09
			2.59
			4.88
S. montanum (PC2243)	0.01	0.04	0.07
	0.01	0.03	0.04
	0.01	0.03	0.03
			0.04

*exceeded threshold of infestation 0.10

public available markers (Martis et al. 2013; Bauer et al. 2017) have been used in combination with a KWS internal combined genetic map.

QTL analysis was conducted with R (R Core Team, Anonymous 2019b) using the library R-QTL (Broman et al. 2003). Markers were removed by using a minor allele frequency of 1% and if they had more than 50% missing data. Only one marker was kept in case of co-segregation. Individuals with more than 50% missing data after marker QC were removed. Phenotypic values of parental components were not considered.

Reported QTL detecting methods are SIM and CIM. Both approaches use a genetic map, in contrast to single marker regression (results not shown). SIM (simple interval mapping) scans for QTL between adjacent markers with the drawback that markers outside the interval can influence the result, e.g., caused by genetic background. CIM (composite interval mapping) includes the genetic background by applying intervals with cofactors used as regressors.

Results

Screening for new sources of resistance

Over eight years, 37 different rye genomes from distant gene pools were examined. It was carried out both under controlled conditions in the greenhouse and mostly in fields

Table 4 SBCMV infestation (ELISA titre) in F2 plants of combination *Secale cereale* var. Imperial (PC272) x *S. montanum* (PC2243) at the test site Thören 2019 revealed by ELISA (missing numbers are missing data evaluations). Selected entries. Complete table 4 can be obtained from the authors

F2 Plant	ELISA titre
001 g	0.02
002 g	0.03
003 g	0.02
004 g	0.02
005 g	0.02
006 g	0.05
007 g	0.04
009 g	0.02
010 g	0.02
011 g	0.03
012 g	0.02
017 g	0.02
018 g	0.02
019 g	0.02
020 g	**4.88**
021 g	0.02
022 g	0.04
023 g	0.03
024 g	**0.16**
025 g	0.02
026 g	0.02
027 g	**2.02**
028 g	0.03
030 g	0.07
031 g	0.04
032 g	0.03
033 g	0.02
035 g	**0.13**
036 g	0.02
037 g	0.03
…	
038 g	0.02
046 g	0.02
…	
047 g	0.03
079 y	0.03
080 y	0.03
081 y	0.02
082 y	0.02
083 y	0.02
084 y	0.03
085 y	0.03
086 y	0.03
087 y	0.03
088 y	0.03
090 y	0.03

Table 4 (continued)

F2 Plant	ELISA titre
091 y	0.03
092 y	0.03
093 y	0.02
094 y	0.03
095 y	0.02
096 y	0.06
097 y	0.02
098 y	0.02
099 y	0.02
100 y	0.06
101 y	0.03
102 y	0.04
103 y	0.03
104 y	0.02
105 y	0.02
106 y	0.03
107 y	0.03
108 gy	**0.48**
109 gy	0.06
110 gy	0.07
111 gy	**4.89**
112 gy	**0.38**
113 gy	**0.21**
116 gy	0.10
…	
117 gy	0.10
118 gy	0.07
119 gy	0.08
146 gy	0.03
147 gy	0.03
148 gy	**4.89**
149 gy	**0.10**
150 gy	0.03
151 gy	0.02
152 gy	0.03
153 gy	0.02
154 b	0.02
155 b	0.03
156 b	0.03
157 b	**3.06**
158 b	0.02
159 b	0.02
162 b	0.02
163 b	0.02
165 b	0.02
166 b	**0.63**
167 b	0.08
168 b	0.02
…	
169 b	0.04

Table 4 (continued)

F2 Plant	ELISA titre
182 b	0.02
183 b	**0.91**
184 b	0.02
185 b	0.02
186 b	**4.89**
187 b	0.05
188b	0.03
189 b	**0.17**
190 b	0.04
192 b	0.03
194 b	0.02
195 b	**0.48**
196 b	0.02
198 b	0.03
199 b	0.02
200 b	0.04
202 b	0.04
Observed#Σ 170	151:**19**
Expected	
1:2:1 (3:1)	127.5:42.5
9:3:3:1 (15:1)	159.4:10.6

*Description of seed color (g-green, y-yellow, gy-green/yellow, b-brown)

that are contaminated with the various viruses, which were characterized in several reports (Kastirr 2004; Kastirr und Ziegler 2018). However, those test sites showed great variability in the infestation of cereal plants. Of course, this made genetic analysis more difficult (see Table 2).

The results obtained over the years are summarized in Table 2. Since sometimes rye populations classified to be resistant had to be declared as susceptible at other testing locations, it was extremely difficult to select suitable populations with virus resistance for a subsequent genetic analysis.

In the case of rye, three accessions turned out to be resistant after repeated studies, i.e., the wild rye *Secale montanum* (PC2243), *S. africanum* (PC2328), and *S. vavilovii* (PC2364). Since the allogamous rye generally has a high degree of genetic variability, the three isolated populations were further separated and increased by self-pollination. Among the cultivated rye, *S. cereale*, no resistant source was identified during these studies (cf. Table 2).

Virus infestation

Leaf symptoms (cf. Figure 1) of SBCMV could not be estimated in all plants, however, sporadically. Therefore, ELISA was used as main criterion for virus infestation of tillering plants. During ELISA testing the titre threshold was set to

Table 5 Chi^2 testing of distribution between infested (C1) and resistant (C2) plants expecting 3:1 segregation

	Observed		Expected		Chi^2	
Year	2019	2020	2019	2020	2019	2020
C1	151	104	127.50	99.75		
C2	19	29	42.50	33.25		
	170	133	170.00	133.0	17.32	0.72
Null hypothesis is rejected in 2019, i. e. no relationship to 3:1 segregation						
Null hypothesis is accepted in 2020, i. e. relationship to 3:1 segregation is likely						
				P=0.1, df=1	2.30	
Considering 10% false-negative plants in 2019 then null hypothesis is accepted, i.e. 3:1 distribution is likely						

0.1 to characterize infected plants. On the test site Thören, in 2019 there was little infestation for WSSMV that even the parental lines of *S. cereale* var. Imperial and *S. montanum* did not show any reaction (Table 3). It was decided to only consider the infestation with SBCMV.

The first comparison of the viral infestation of young plants showed that the WSSMV did not cause any significant symptoms at the chosen location of Thören. Even the parental genotypes PC272 and PC2243 did not differ (Table 3). The F2 analysis at the Thören location was therefore not suitable for the WSSMV study and was thus no longer considered. However, some plants were found to be susceptible to WSSMV, but not to SBCMV. This means that the susceptibility or resistance is independently determined for WSSMV and SBCMV.

SBCMV

The evaluation of the ELISA values shows that in 2019 19 of the 170 genotyped plants exhibit a clear infection to SBCMV and 151 none. (Table 4). When this distribution of 151r: 19s is compared to a 3r:1s expectation (= 127.5r: 42.5s), then just a slight association can be considered. However, the observed distribution matches better with a 9:3:3:1 or 15r:1s distribution (= 159.4r: 10.6s) assuming duplicate dominant epistasis, suggesting *A* epistatic to *B* and *b*, *B* epistatic to *A* and *a*.

The statistical analysis applying Chi^2 test did not clearly confirm neither a 3r:1s nor a 15r:1s distribution (cf. Table 5 and 6) in 2019. By the Chi^2 values of 17.32 (3r:1s) and 7.03 (15r:1s) the null hypothesis can be rejected within the confidence level of $P = 0$, df = 1 and $P = 0.0081$, df = 1, respectively.

However, when false-negative plants taken into account that occur with about 10% and more in 2019, then the Chi^2 values change to $Chi^2 = 2.3$, $P = 0.1294$, for a 3r:1s segregation and $Chi^2 = 51.84$, $P = 0$, for 15r:1s segregation. Because of various environmental conditions and inhomogeneous *Polymyxa* spatial distributions false-negative plants are always found in these field trials. Thus, a Chi^2 value of 2.3 ($P = 0.1294$, df = 1) would indicate a 3r:1s segregation rather than a 15r:1s, respectively. This suggestion fits to the replication of the study in 2020 with a more homogenous virus infestation as well as the molecular study.

Mapping analysis

Based on the ELISA scores given in Table 4 the molecular data were associated with the phenotypic result. The prerequisite for this study was a clear differentiation of the ELISA scores for SBCMV between the parents PC272 and PC2243 (cf. Figure 2).

Table 6 Chi^2 testing of distribution between infested (C1) and resistant (C2) F2 plants expecting 15:1 segregation

	Observed		Expected		Chi^2	
Year	2019	2020	2019	2020	2019	2020
C1	151	104	159.37	124.7		
C2	19	29	10,63	8.3		
	170	133	170.00	133.0	7.03	55.07
Null hypothesis is rejected, i. e. no relationship to 15:1 segregation						
				P = 0.1, df = 1	54.81	
Considering 10% false-negative plants then null hypothesis is rejected, i.e., 15:1 distribution is not likely						

Table 7 SBCMV investigation for QTL. Detailed marker results for QTL detected by CIM (2R) and SIM (3R). Each column represents one marker. In rows are the parents on top and aggregated statistics of individuals with ELISA scores above (susceptible) or below (resistant) of 0.1. The Markers C9654_1947 and isotig11640 are most likely linked to the resistance locus. A = homozygote, H = heterozygote

		SBCMV	02R									03R					
Secale_Imperial		4,17	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Secale_Montanum		0,03	H	H	H	H	B	B	H	H	H	H	B	B	B	B	-
			Contig939	isotig04562	C9654_1947	C12339_800	Contig1592	isotig05078	isotig05079	isotig11640	C19562_671	isotig18779	C6404_695	isotig10820	C27126_442	C6792_261	C20763_308
LOD	SIM											0,84	1,87	1,37	2,57	2,57	0,94
LOD	CIM		1,80		5,00	0,10	1,15	1,54	1,54	5,10	0,23						
%Missing			0,0	0,0	0,0	0,0	1,2	0,0	0,0	0,6	0,0	0,6	0,0	0,0	0,0	42,9	49,4
%A	larger 0.1 (n = 18)		50,0	83,3	83,3	33,3	50,0	55,6	83,3	55,6	22,2	61,1	38,9	44,4	27,8	27,8	44,4
%B			0,0	0,0	0,0	5,6	16,7	16,7	0,0	0,0	27,8	5,6	22,2	16,7	22,2	22,2	5,6
%H			50,0	16,7	16,7	61,1	33,3	27,8	16,7	44,4	50,0	33,3	38,9	38,9	50,0	11,1	5,6
%A	smaller 0.1 (n = 152)		58,3	72,8	71,5	25,2	34,4	34,4	70,9	52,3	18,5	49,0	19,2	24,5	13,9	13,9	28,5
%B			1,3	0,0	0,0	5,3	15,9	15,2	0,7	0,7	23,2	4,0	29,8	27,8	37,7	37,7	15,9
%H			40,4	27,2	28,5	69,5	48,3	50,3	28,5	46,4	58,3	46,4	51,0	47,7	48,3	4,6	6,6

Table 8 Seed color and mean SBCMV infestation (ELISA titre) in F2 plants of combination *Secale cereale* var. Imperial (PC272) x *S. montanum* (PC2243) at the test site Thören revealed by ELISA analysis

	Seed color			
	Green	Yellow	Green-yellow	Brown
Nr. Plants considered (Total = 170)	48	45	39	38
Mean ELISA titre	0.18	0.03	0.34	0.29

Altogether 8,950 molecular markers were available, from which 6,362 were mapped across the seven rye chromosomes. For the study on SBCMV totally 987 cleaned and translatable markers were included: 1R = 133, 2R = 135, 3R = 133, 4R = 162, 5R = 200, 6R = 152, 7R = 72, i. e., about 141 per chromosome. With the exception of chromosome 7R, there is a balanced distribution across the genome (cf. Fig. 3).

Applying these 987 markers and composite interval mapping (CIM) a QTL on chromosome 2R was detected (cf. Table 7 and Fig. 4). In this comparison, the phenotypic results of the parents were not included because of the allele effects that are highly influenced by the A-parent (*S. cereale* var. Imperial). The differences for the allele frequencies between groups are larger or smaller 0.1, i.e., they are rather small (Table 7).

Taking all restrictions into account, it can be assumed that there is a genetic stretch of about 13 cM (between 114 and 127 cM) on the long arm of chromosome 2R critical for the SBCMV resistance in this particular population (Fig. 4).

Fig. 1 Chlorotic streak symptoms of soil-borne cereal mosaic virus in rye, *Secale cereale*. *Source:* U. Kastirr

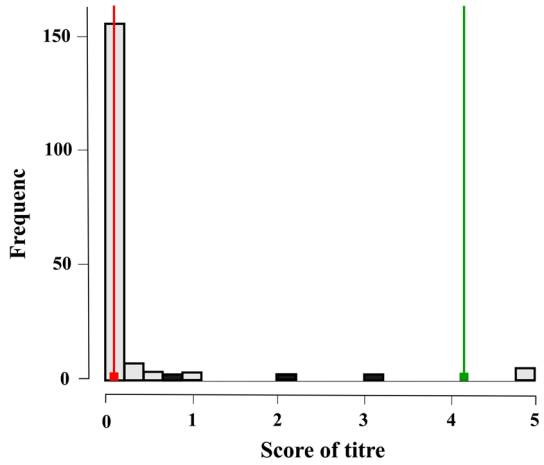

Fig. 2 Phenotypic distribution of ELISA scores of SBCMV in the F2 population from the cross *Secale cereale* var. Imperial and *S. montanum.* The parental means are highlighted (green = Imperial and red = *S. montanum*)

The markers "*C9654_1947"* and "*isotig11640"* are the peak markers within this region (Table 7).

Additional calculations, such as a reduced ABH matrix with expected segregation patterns only or transformed phenotypes, where scores larger 0.1 have been coded as 1 and smaller 0.1 as 0 to simulate the non-linearity and/ or sensitivity of the ELISA test, did not improve the significance of the results. The application of the basic local alignment search tool (BLAST) for known wheat markers to SBCMV did not show a clear overlapping with the detected region on 2R. The molecular investigation also revealed a higher heterozygosity for the donor parent *S. montanum* as compared to the recipient parent "Imperial" (Table 7) that is in general agreement with morphological observations.

This can be explained by the fact that the variety "Imperial" variety has been propagated over several generations through self-pollination, while the *S. montanum* accession resulted from a sample that was once collected with two multiplications under isolated conditions.

Variation of seed color

It is known that the color of the rye caryopses can vary from yellow, green, brown to violet (Schlegel 2013). In the present investigation, the female cross parent "Imperial" had green and the male parent, *S. montanum*, brownish grains. The F1 grains were dark green throughout. But in the F2 generation there was a clear segregation of the seed color.

Therefore it seemed reasonable to consider this phenotypic variation in relation to the resistance against SBCMV.

Thus, four groups of approximately the same size were created for the colors yellow, green, green-yellow, and brown (cf. Fig. 5). They were sown separately and later compared with the ELISA results. Plants susceptible to SBCMV (ELISA scores > 0.10) occur with different frequencies between the classes of seed color (Table 4) and can be distinguished if the ELISA scores of the four groups are averaged. The highest ELISA scores are found among the green-yellow and brown-colored seeds. These differences from the purely yellow and green seeds are statistically significant (Tables 8, 9 and 10). It shows that the plants with the yellow seed coat show practically no infection with SBCMV.

Discussion

Screening

Both earlier screenings of rye genomes and the current study indicate that little genetic variability can be expected in adapted rye (*Secale cereale*), although rye generally shows a high degree of variability as it is a typical open-pollinator. The first studies by Erath et al. (2016) as well as the present results show that significant resistance can only be found in wild rye, particularly in the perennial *Secale montanum* and *S. africanum* as well as within the annual *S. vavilovii*. While *S. montanum* and *S. africanum* belong to the genome complex of *S. strictum*, *S. vavilovii syn. S. ranicum* are declared to be part of the *S. cereale* genome complex (Hammer et al. 1987). Further screenings should therefore focus on accessions from these genome complexes. However, this does not render breeding of resistant rye varieties easier. The critical genes have first to be transferred to the modern varieties and/ or inbred lines via laborious introgressions and back crosses. However, the results indicate a monogenic, dominant inheritance, which is favorable for hybrid rye breeding.

Molecular study

As stated above, it is likely that the molecular markers "C9654_1947" and "isotig11640" on chromosome 2RL are closely linked to resistance against SBCMV. The region covers about 14 cM of the DNA and segregates according to a monogenic mode of inheritance. In order to support this assumption, the investigation of a corresponding physical stretch of around 30 Mbp is recommended to detect a candidate gene and/or genes. For a repeated study, the population size should be enlarged to increase the chance for susceptible individuals. The precision of the ELISA testing together with an optimized experimental design toward uniform SBCMV infestation may contribute to this approach.

Cytological studies should also be involved because the donor genome of *Secale montanum* differs by two reciprocal translocations involving the chromosomes 2R, 6R, and 7R (cf. Figure 6). In F1 plants, a hexavalent chromosome configuration is usually formed during metaphase I of meiosis.

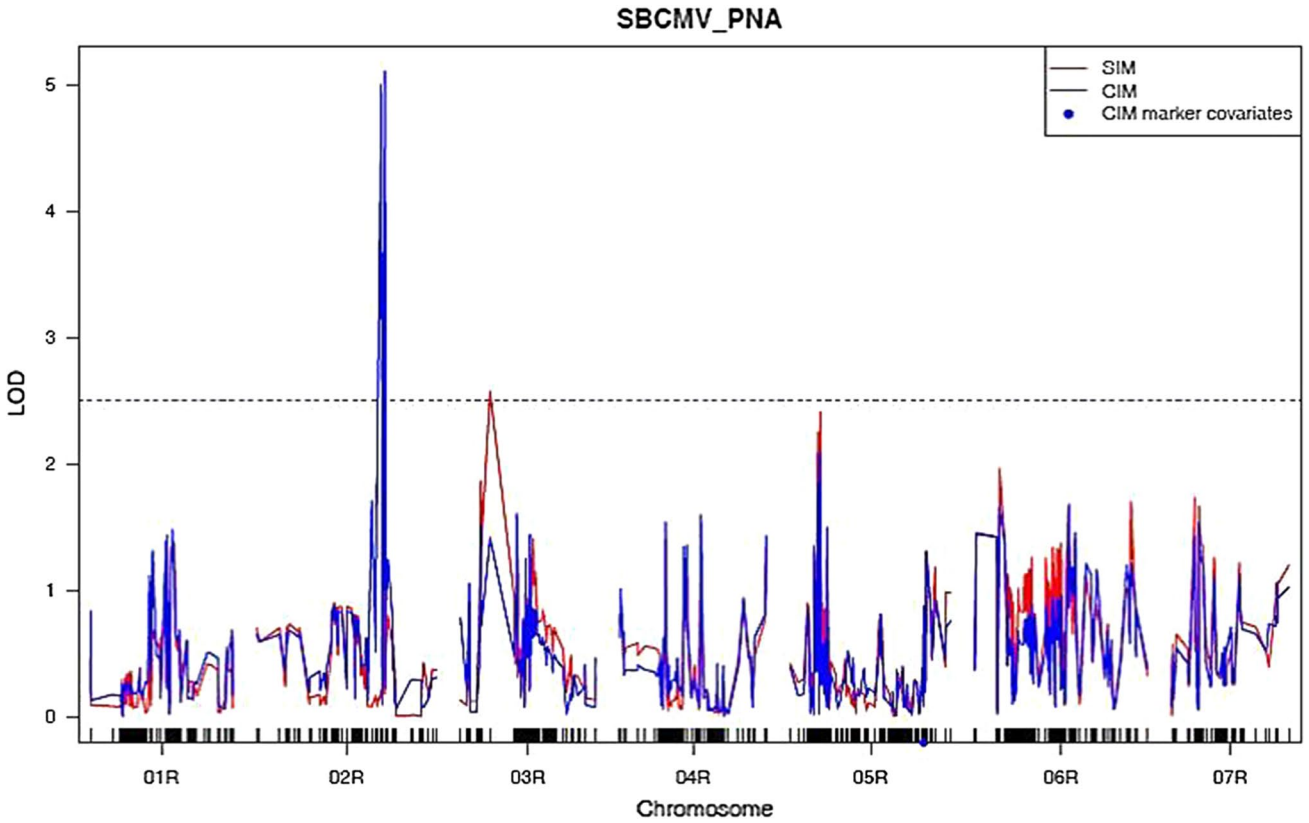

Fig. 3 LOD curves of SBCMV across chromosomes with the highest peak on chromosome 2R. Phenotypic results of parents are not included

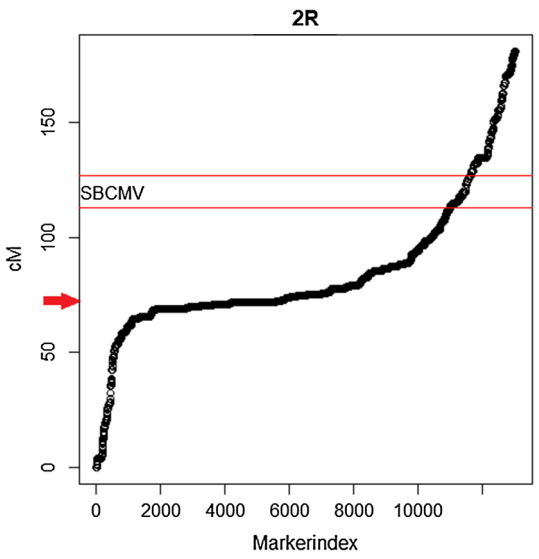

Fig. 4 Distribution of mapped markers by Bauer et al. (2017) across chromosome 2R and the SBCMV resistance candidate markers at about 125 cM and centromere position at about 60 cM

This may lead to a non-regular distribution of chromosomal segments in F2 generation and can make the chromosomal assignment of the markers difficult.

Also in the experiment carried out by Erath et al. (2016), no resistance genes have so far been identified that can be used in rye breeding. Additional characterization of the DNA segment on chromosome 2R therefore offers the chance to describe such a gene and to create suitable markers for it.

Seed color

The color of the rye grains depends on the combination of the color, thickness, and transparency of the seed coat (pericarp), and of the color of the aleuron layer. It may vary between bright yellow, dark yellow, bright red-brown, brown, dark brown, and violet. Rye with brown and black grains or seeds with brown tips are not suitable for practical utilization. Steglich and Pieper (1922) were the only ones who described black xenia in crosses with "Pirnaer Roggen", and even its 3:1 F2 segregation. The various colors are thus determined by different genes.

The relationship between yellow-grained F2 plants and resistance to SBCMV found in this study is at least worth

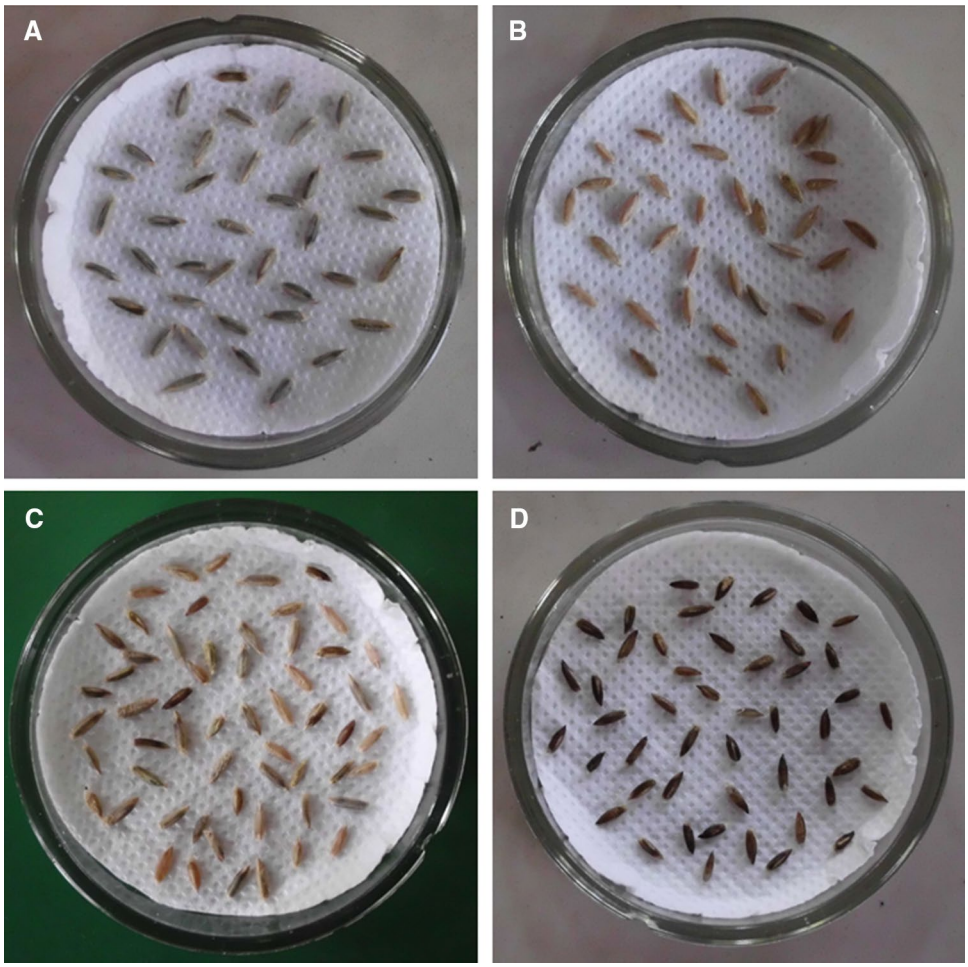

Fig. 5 Separated types of seed color in F2 population from *Secale cereale* var. Imperial x *S. montanum*, 2017; green **A**, yellow **B**, green-yellow **C**, and brown **D**

Table 9 F test for mean SBCMV infestation (ELISA titre) of different seed colors in F2 plants of combination *Secale cereale* var. Imperial (PC272) x *S. montanum* (PC2243)

Variability	SQ	FG	F
Total	109.50	169	
Between groups	6.85	3	2.28
Residue	102.65	166	0.61
			3.74***

$F_{theo\ 166}5\% = 1{,}98$; $F_{theo\ 166}1\% = 2{,}61$; $F_{theo\ 166}0.1\% = 3{,}35 < < < F***$ 3.75 significant at 0.01%

Table 10 t-Table for mean SBCMV infestation (ELISA titre) of different seed colors in F2 plants of combination *Secale cereale* var. Imperial (PC272) x *S. montanum* (PC2243)

	Green (0.18)	Yellow (0.03)	Green-yellow (0.34)
Brown (0.29)	0.11	0.26**	0.05
Green (0.18)	–	0.15	0.16
Yellow (0.03)		–	0.31***

mentioning (Fig. 7). While Rümker and Leidner (1914) observed a monogenic dominant inheritance from green (dominant) to yellow (recessive) seed color in rye, later, Sturm et al. (1981) localized the gene for green color seed (*An1*) on chromosome 2R (Schlegel 2020).

Thus, the recessive allele for the seed color on chromosome 2R (*an1*) could be related to the locus for resistance to SBCMV. Further investigations are initiated in order to clarify this linkage.

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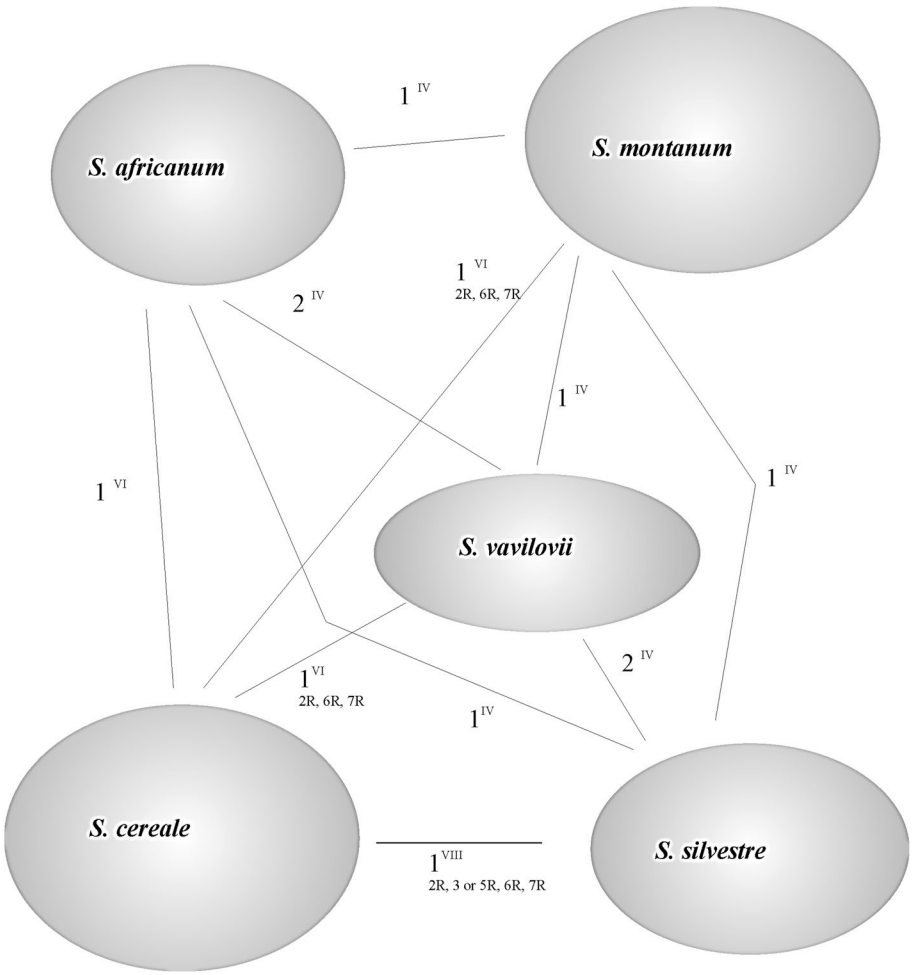

Fig. 6 Schematic drawing of cytological differences between rye species by the presence of interchanges of chromosomes (IV = quadrivalent, VI = hexavalent, VIII = octovalent); between *Secale cereale* and *S. montanum* 1 hexavalent is common involving chromosomes 2R, 6R, and 7R. Source: R. Schlegel (2013)

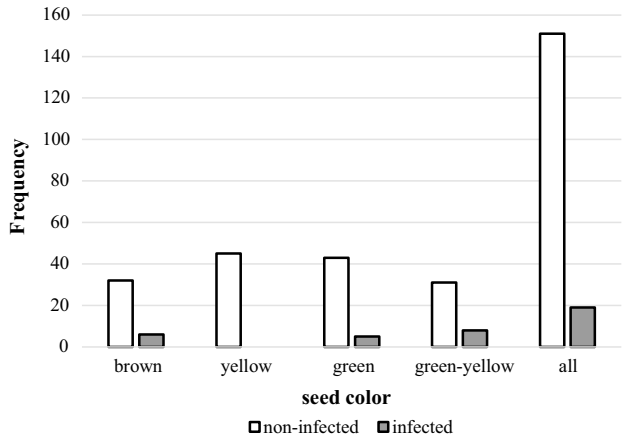

Fig. 7 Observed frequencies of SBCMV infection, estimated by ELISA in F2 plants of combination *Secale cereale* var. Imperial (PC272) x *S. montanum* (PC2243) at the test site Thören, 2019

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