SSR MARKERS ASSOCIATED WITH THE RESISTANCE OF RAPESEED TO THE ATTACK OF *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY

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Abstract

Sclerotinia sclerotiorum (Lib.) de Bary is one of the most important pathogens of the rapeseed crop worldwide. Depending of the environmental conditions, the yield losses can get up to 100%. Until now, no oilseed rape cultivars are marked as having resistance to this pathogen. Genetic resistance is considered to be the most efficient way of protecting the plants from this patthogen. The aim of this study was to identify SSR markers for white rot resistance in a collection of 130 rapeseed cultivars, from the Centre for Genetic Resource of Netherlands. There were made correlations between the genotypic and the phenotypic data obtained for the artificial infection with the pathogen and there were revealed 5 SSRs significantly associated with rapeseed resistance to white rot. The artificial infection was made on detached leaves, in controlled environmental conditions. The fungus was grown on solid medium PDA. There were put 2 plug discs of PDA medium with mycelia near the main vein of the leaves. On the control leaf there were put 2 discs of PDA medium, without mycelia. The diameter of the lesions was measured 72 h after inoculation, with a linear ruler. For the genetic analysis, there were used 51 SSR markers, that amplified 139 polymorphic fragments. The fragments ranged between 80 and 340 bp. In order to correlate the data, we used the *ANOVA* method, in the *SPSS* v.13 software. The identification of these SSRs will enhance the breeding for white mold resistance in *Brassica napus* L.

Key words: *rapeseed, SSRs, resistance*

Stem rot of oilseed rape, caused by the fungus Sclerotinia sclerotiorum is one of the most important diseases of the crop and leads to high losses of production worldwide. Depending of the environmental conditions, the yield losses can get up to 100% (Sarahan G.S. et al., 2008).

No oilseed rape cultivars are marked as having resistance to this disease. According to Garg H. et al., (2008), strategies for selecting resistant host are considered the most economic and sustainable control means.

In order to identify the SSRs associated to white rot resistance in rapeseed, we correlated the data obtained for the artificial infection with the pathogen on leaves (Calistru Anca-Elena, 2012), with the genotypic data obtained at the SSR analysis.

MATERIAL AND METHOD

The plant material. The 130 rapeseed genotypes that were tested were provided by the Centre of Genetic Resources of Netherlands.

The artificial infection. Young, fully expanded leaves were detached from plants grown in the controlled environmental room and transferred to the laboratory. There were used 6 leaves for every cultivar, 5 for infection and 1 for control. These leaves were placed in trays with gauze covering the petiole and kept in darkness, at 22 -24° C and humidity of 70 – 80 %. On each leaf, there were put 2 plug discs of PDA medium with mycelia near the main vein. On the control leaf there were put 2 discs of PDA medium, without mycelia. The diameter of the lesions was measured 72 h after inoculation.

DNA extraction. The DNA for each cultivar was isolated using the CTAB protocol, modified by Doyle & Doyle (1987). The quantity and quality of the DNA were determined using agarose gel electrophoresis and spectrophotometer.

DNA amplification. The amplification was made on a LICOR 4200 system.

The SSR analysis. There were used 51 SSR markers.

Data analysis. For the data analysis, it was used the ANOVA method, with the SPSS v.13 software, with a probability P< 0.05%.

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RESULTS AND DISCUSSIONS

The results for the artificial infection are presented in *tab. 1*. The 51 SSRs amplified 139 polymorphic fragments. The fragments ranged between 80 and 340 bp (*tab.2*).

Crt. No.	Cultivar	Lession (mm)	Crt. No.	Cultivar	Lession (mm)
1	CGN17350: (Libritta)	30.83	66	CGN17302: (B. napus group 4)	24.93
2	CGN17310: (Skriverskii)	25.63	67	CGN17303: (B. napus group 5)	26.5
3	CGN17311: (B. napus group 1)	25.7	68	CGN17304: (B. napus group 6)	41.86
4	CGN17312: (Kievskii 216)	35.36	69	CGN17305: (Shen-Li Jutsaj)	21.9
5	CGN17313: (Kievskii 18)	27.83	70	CGN17306: (B. napus group 7)	26.7
6	CGN17314: (Kombi)	33.76	71	CGN17307: (B. napus group 8)	27.06
7	CGN17315: (SKR. II Kormovoi)	24.8	72	CGN18965: (Diadem)	39.76
8	CGN17316: (Uspekh)	32.03	73	CGN18966: (Diamant)	37.06
9	CGN17317: (Blagodatnyi)	29.83	74	CGN18967: (Doral)	30.93
10	CGN17318: (Fedorovskii)	37.16	75	CGN18968: (Doublol)	34.13
11	CGN17319: (Snityskii)	33.13	76	CGN18969: (Eka)	42.76
12	CGN17320: (Diana)	35.5	77	CGN18970: (Elena)	22.66
13	CGN17321: (Ksaverovskii)	34.23	78	CGN18971: (Elvira)	36.93
14	CGN17322: (Kodakskii)	38.7	79	CGN18972: (Erra)	42.33
15	CGN17351: (Lictor)	36.06	80	CGN18973: (Enrol)	42.13
16	CGN17352: (Liglandor)	38.46	81	CGN17323: (Falcon)	31.16
17	CGN17353: (Ligora)	32.36	82	CGN17324: (Fertodi)	26
18	CGN17354: (Lindora)	28.66	83	CGN17325: (Fiona)	26.73
19	CGN17355: (Lingot)	22.66	84	CGN17326: (Gesunder)	35.7
20	CGN17356: (Link)	20.13	85	CGN17327: (Girita)	28.16
21	CGN17357: (Liquanta)	17.2	86	CGN17328: (Glacier)	34.23
22	CGN17358: (Lirabon)	19.6	87	CGN17329: (Gundula)	24.5
23	CGN17359: (Lirajet)	26.83	88	CGN17330: (Hambourg)	34.53
24	CGN17360: (Lirakotta)	32.16	89	CGN17331: (Hambourger)	28.6
25	CGN17361: (Lirama)	38.6	90	CGN17332: (Heimer)	38.16
26	CGN17362: (Lirastern)	24.93	91	CGN17333: (Herkules)	33
27	CGN17363: (Lirektor)	26.5	92	CGN17334: (Hunnia)	34.86
28	CGN17364: (Liropa)	41.16	93	CGN17335: (Jade)	34.83
29	CGN17365: (Madora)	21.23	94	CGN17336: (Janetzkis)	42.16
30	CGN17367: (Maras)	26.36	95	CGN17337: (Jupiter)	36.93
31	CGN17368: (Marens)	25.16	96	CGN17338: (Kurander)	40.83
32	CGN17369: (Marex)	39.8	97	CGN17339: (Lecor)	33.2
33	CGN17370: (Matador)	37.06	98	CGN17340: (Ledos)	30
34	CGN17371: (Mirander)	30.56	99	CGN17342: (Lesira)	22.93
35	CGN17372: (Niederarnbacher)	33.73	100	CGN17343: (Lester)	22.36
36	CGN17373: (Norli)	42.1	101	CGN17344: (Libelle)	18.2
37	CGN17383: (Octavia)	21.73	102	CGN17345: (Liberator)	21.43
38	CGN17374: (Olimpiade)	37.63	103	CGN17308: (Kombainer)	26.8

Crt. No.	Cultivar	Lession (mm)	Crt. No.	Cultivar	Lession (mm)
39	CGN17375: (Olymp)	41.6	104	CGN17346: (Liborius)	32.83
40	CGN17377: (Panter)	42.03	105	CGN17347: (Librador)	38.6
41	CGN17379: (Perle)	30.5	106	CGN17348: (Libraska)	24.96
42	CGN18948: (Andol)	25	107	CGN17349: (Libravo)	26.56
43	CGN18950: (Arabella)	26.1	108	CGN13914: (Cascade)	41.46
44	CGN18955: (Bienvenu)	35.3	109	CGN13915: (Bridger)	21.6
45	CGN18956: (Brilland)	28.3	110	CGN06869: (Kromerska)	26.43
46	CGN18957: (Bristol)	33.83	111	CGN06870: (Slapska)	28.23
47	CGN18958: (Buko)	25.23	112	CGN06871: (Mestnij)	40.23
48	CGN18959: (Capricorn)	31.1	113	CGN06872: (Trebieckska Krajova)	37.23
49	CGN18960: (Cobra)	32.53	114	CGN06874: (Niemierczanski)	30.83
50	CGN18961: (Collo)	38.83	115	CGN07227: (Jet Neuf)	33.8
51	CGN17380: (Planet)	33.6	116	CGN07228: (Rafal)	42.9
52	CGN17381: (Prominj)	35.5	117	CGN13912: (Expander)	22.36
53	CGN18974: (Ridana)	34.23	118	CGN11012: (Mansholts Hamburger)	40.96
54	CGN18975: (Samourai)	40.16	119	CGN11013: (Primor)	42
55	CGN18976: (Score)	37.83	120	CGN11014: (R-33)	42.03
56	CGN18977: (Silesia)	38.76	121	CGN13913: (Rapol)	41.16
57	CGN19951: (Silvia)	34.96	122	CGN06877: (Dublianskij)	32.9
58	CGN19952: (Sollux)	28.66	123	CGN06879: (Vinnickij 21)	30.76
59	CGN19953: (Susana)	22.66	124	CGN06880: (Mytnickij)	22.86
60	CGN19955: (Tamara)	21.63	125	CGN06881: (Vinnickij 15/59)	21.93
61	CGN19956: (Tapidor)	18.6	126	CGN06882: (Nemercanski 1)	16.86
62	CGN19957: (Tor)	19.3	127	CGN06883: (Nemercanskij 2268)	19.5
63	CGN19959: (Veronika)	26.56	128	CGN06884: (Podol'skij Mestnij)	27.16
64	CGN17300: (B. napus group 2)	33.53	129	CGN06885: (Skziverskij)	32.16
65	CGN17301: (B. napus group 3)	38.6	130	CGN06886: (B. napus group 9)	38.6

Table 2

The results of the SSR analysis

Crt. No.	Primer	No of bands	Allelle size	Crt. No.	Primer	No of bands	Allelle size
1	CB-10065	2	210-230	27	Na12-A01	3	155-165
2	Na10-G08	3	310-340	28	Ol10-D03	3	155-235
3	Ol10-B02	2	80-170	29	Ol10-F02	1	155
4	Na12-C01	3	40-110	30	Na14-G06	2	240-245
5	BRMS-30	2	210-220	31	Ol11-B05	3	140-160
6	Na10-D11	2	218-220	32	Ni2-C12	1	80
7	CB 10536	2	145-150	33	Na12-B11	1	130
8	Ol10-E12	1	280	34	Ol13-E08	2	170-190
9	MD 60	2	180-190	35	Ol10-G06	3	130-165
10	CB 10028	5	170-255	36	OL10-E05	6	130-170

11	CB10206	2	240-245	37	OL13-F08	2	140-145
12	CB 10437	1	190	38	Na12-B07	7	130-147
13	Cb 10097	2	210-220	39	Ra12-E12	5	150-240
14	CB 104347	2	220-230	40	Na12-A02	5	150-226
15	Na12-H06	3	210-265	41	Na12-B05	2	220-230
16	CB 10611	2	170-190	42	HMR416	4	240-265
17	BRMS 20	1	200	43	Ra2-F11	5	210-245
18	Ol10-D08	2	180-185	44	Ol11-H02	2	200-210
19	BRMS 309	4	200-230	45	Na10-C01	1	100
20	Na10-B11	4	200-240	46	HMR354	6	260-315
21	Na12-D08	4	90-145	47	Na14-G10	2	170-180
22	OL10-C10	6	190-280	48	HMR562	2	210-215
23	Ra2-F04	2	110-150	49	HMR585	5	170-195
24	Na14-H12	1	257	50	Na12-G05	3	120-230
25	Ol10-D01	2	270-275	51	CB10536	2	145-150
26	CB10600	1	310				

From the data analysis, there were identified 10 SSRs significantly associated with rapeseed resistance to the pathogen (tab. 3).

The R^2 indicates the rate of the phenotypic variation given by the considered marker. The value of $p < 0,05$ indicates that the marker is significant for the resistance to the pathogen.

Table 3

The SSRs significantly associated with the resistance to white rot

Crt. No.	Primer	R	R2	Adjusted R2	p	Semnif.
1.	Na12H06_210	0.234	0.025	0.047	0.008	***
2.	BRMS20_200	0.189	0.036	0.028	0.032	***
3.	Ol10E05_135	0.184	0.034	0.026	0.036	***
4.	Na12B07_145	0.221	0.049	0.041	0.011	***
5.	Na12A02_226	0.260	0.068	0.060	0.003	***

CONCLUSIONS

The results obtained indicate that these markers can be successfully used in researches, in order to identify QTLs for the rapeseed resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary.

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