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

CERCETĂRI PRIVIND IDENTIFICAREA DE MARKERI PENTRU SSR ASOCIATI CU REZISTENȚA RAPIȚEI LA ATACUL DE *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY

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Abstract. *White rot, caused by Sclerotinia sclerotiorum (Lib.) de Bary is an important pathogen of the Brassica napus crop. The most efficient way of protecting the rapeseed plants from this patthogen is through genetic resistance. The aim of this study was to identify SSR markers for white rot resistance in a collection of 130 rapeseed cultivars from the Centre of Genetic Resources of Nerherlands. The correlations made between the genotypic and the phenotypic data previously obtained for the artificial infection with the pathogen, revealed 10 SSRs significantly associated with rapeseed resistance to Sclerotinia sclerotiorum (Lib.) de Bary. The identification of these SSRs will enhance the breeding for white mold resistance in Brassica napus L.*
Key words: *rapeseed, SSRs, resistance*

Rezumat. *Putregaiul alb, provocat de Sclerotinia sclerotiorum (Lib.) de Bary este un pathogen important pentru specia Brassica napus. Cea mai oficientă metodă de protecție a plantelor de rapiță împotriva acestui patogen este prin intermediul rezistenței genetice. Scopul acestui studiu a fost identificarea de markeri pentru SSR asociați cu rezistența la putregai alb, în cadrul unei colecții de 130 de cultivare de rapiță provenită de la Centrul pentru Resurse Genetice al olandei. Corelațiile realizate între datele genotipice și cele fenotipice obținute anterior în urma realizării infecției artificiale au evidențiat 10 markeri pentru SSR semnificativ asociați cu rezistența rapiței la Sclerotinia sclertiorum (Lib.) de Bary. Identificarea acestor markeri va sprijini ameliorarea speciei Brassica napus L., în ceea ce privește rezistența la putregai alb*
Cuvinte cheie: *rapiță,markeri pentru SSR, rezistență*

INTRODUCTION

There is a concerted effort among rapeseed breeders to reduce the losses caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary.

Depending of the environmental conditions, the yield losses can get up to 100% (Sarahan et al., 2008). Until now, no oilseed rape cultivars are marked as

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having resistance to this pathogen. Strategies for selecting resistant host are considered the most economic and sustainable control means (Garg et al., 2008).

In order to identify the SSRs associated to white rot resistance in rapeseed, we correlated the data previously obtained for the artificial infection with the pathogen (Calistru, 2012), with the genotypic data obtained at the SSR analysis. There were found 10 SSRs significantly associated with the resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary.

MATERIAL AND METHOD

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

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.

There were used 51 SSR markers, that amplified 139 polymorphic fragments. The fragments ranged between 80 and 340 bp (Table 1).

Table 1

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				

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

RESULTS AND DISCUSSIONS

From the data analysis, there were identified 10 SSRs significantly associated with rapeseed resistance to the pathogen (Table 2).

Table 2

The SSRs significantly associated with the resistance to white rot

Crt. No.	Primer	R	R^2	Adjusted R^2	p	Significance
1.	Na10B11_204	0.209	0.044	0.036	0.017	***
2.	Ol10C10_200	0.217	0.047	0.04	0.013	***
3.	Ol10C10_204	0.422	0.178	0.172	0	***
4.	Ol10D01_270	0.183	0.034	0.026	0.037	***
5.	Ol10E12_280	0.238	0.057	0.049	0.006	***
6.	Ol11B05_140	0.21	0.044	0.037	0.016	***
7.	Ol10G06_165	0.177	0.032	0.024	0.043	***
8.	Na12B07_137	0.237	0.056	0.049	0.007	***
9.	Na12A02_150	0.186	0.035	0.027	0.034	***
10.	Ol11H02_210	0.243	0.059	0.052	0.005	***

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. The *Ol11H02* marker has been perviously used by Hasan et al. (2006), in researches regarding the genetic diversity in rapeseed. Also, Tommasini et al., (2003) used the *Na12A02* marker in experiments in order to evaluate some qualitative traits in the *Brassica napus* species.

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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