Screening for resistance to stem canker (*Leptosphaeria maculans*) in *Brassica napus* genetic resources.

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ABSTRACT

Leptosphaeria maculans causes stem canker of oilseed rape (*Brassica napus*), a disease of major importance worldwide. Due to inefficient control with fungicides, and insufficient cultural management of the disease, genetic resistance is paramount to control *L. maculans*. Here, 453 accessions of *B. napus*, mainly from the IPK GeneBank, were investigated for the occurrence of major resistance genes *Rlm1*, *Rlm2*, and *Rlm4*, as well as novel resistance sources, using genetically improved strains of the fungus harbouring as few corresponding avirulence genes as possible, and fully virulent isolates.

Major resistance genes (*R*) were rarer in frequency and diversity in spring-type cultivars as compared to winter types. In the former, 65.7% of the accessions were fully susceptible to all isolates, whereas only 12.2% of the winter types were devoid of at least one *R* gene. In spring cvs, the most common *R* gene, *Rlm4* was found in 26.6% of the accessions, whereas the other R genes were rare. In winter types, the most common R genes were *Rlm2* (more than 49.2-54.0% of the accessions) and *Rlm4* (26.4-27.3% of the genotypes). In winter types however, the improvement of the quality of oils, through the generation of single- and double-low genotypes improved the homogeneity of the cvs, whereas it impoverished *R* gene diversity, including the loss of complete resistance that was harboured by 16.3% of the less advanced accessions, and a reduction in the number of accessions harbouring *Rlm1*. Field resistance could be related to the presence of *Rlm1* in France, where the corresponding avirulent race is prevalent, and to the occurrence of resistance to virulent isolates in Germany. In contrast, a few genotypes that harboured no identified, or few, *R* genes were also shown to display a high level of resistance in the field.

Key words: oilseed rape, stem canker, gene-for-gene resistance, genetic resource

INTRODUCTION

Stem canker of crucifers, also termed blackleg or Phoma of crucifers (*Leptosphaeria maculans)*, is the most ubiquitous disease of oilseed rape worldwide and its incidence increased dramatically with the development of oilseed rape culture in all parts of the world (West et al., 2001). Dissecting the genetic control of specific interactions on the pathogen side is a prerequisite to generate strains of the pathogen harbouring as few avirulence (Avr) genes as possible in order to accurately identify specific resistance genes that may be present in Brassica genotypes, and identify non-redundant novel resistance sources (Balesdent et al., 2002). It is the objective of the present study to use these genetically characterized isolates to draw a picture of the genetic diversity, expressed in terms of R genes, that may be present in large genotype collections of Brassicas such as the Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) collection. In addition, such a screening also aimed at uncovering novel R sources, not currently used in Europe, that may be a novel resource for breeders.

MATERIALS AND METHODS

Plant Material. 453 *Brassica napus* accessions were analysed in the current study. Of these, 402 originated from the Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) Genebank. The accessions are separated in three types of *B. napus* genotypes : (i) spring genotypes (BNS), which are widely grown in Canada and Australia of which the most recent (double-low type) are termed “canola”, (ii) “old” winter types (BNN), i. e. displaying a high level of both erucic acid and

glucosinolates in the oils, and (iii) improved winter type cvs or lines (BNQ) corresponding to recently or currently used cvs displaying either a high level of glucosinolates only (single low genotypes) or low level of both erucic acid and glucosinolates (double-low genotypes). Most of the accessions (78.1%) originated from Europe with a large representation of genotypes from Eastern Europe countries (32.4%).

Fungal isolates. For R gene identification, isolates for screening were chosen so that they contain only one Avr gene, whenever possible. As a consequence, all differential isolates were single ascospore isolates originating from *in vitro* crosses. Reference differentials used here were v11.1.1 as a virulent control representative of the main race of the fungus at the E.U. scale (Volke, 1999); v11.1.2; v23.2.1 (Balesdent et al., 2001) and 7.6.27 (Ansan-Melayah et al., 1998-Table 1). Finally, isolate BBA62908 was used as an avirulent control (Table 1).

Isolates were inoculated on cotyledons of *B. napus* accessions as previously described (Ansan-Melayah et al., 1995). Plants were incubated in a growth chamber at 16 °C (night)-24 °C (day) with a 12 h photoperiod. Symptoms were scored 14 to 21 days after inoculation using the IMASCORE rating scale comprising six infection classes (IC), where IC1-3 are resistance responses and IC4-6 are susceptibility symptoms (Balesdent et al., 2001). Resistance screening data of IPK accessions will be available on the IPK web site (http://skyrix.ipk-gatersleben.de).

RESULTS

Most of the 134 BNS-type genotypes (65.7%) were fully susceptible to all four isolates (Table 1). No consistent resistance to v11.1.1 was observed but four accessions nevertheless showed heterogeneous resistance to v11.1.1. In two cases, this was due to very heterogeneous behaviour from one plant within the accession to the other, with both heterogeneous resistance to the BBA62908 isolates and delayed susceptibility to other isolates. In two cases however, a little number of plants within the accession were fully resistant (HR-type) to all differential isolates.

Table 1. A synthetic view of the occurrence of resistance genes in the accessions analysed here.

				Interaction phenotype when inoculated with : [a]				
			Suceptibility to all isolates	v11.1.1 [b] *(avrLm1 avrLm2 avrLm4)*	BBA62908 *(AvrLm1 AvrLm2 AvrLm4)*	v11.1.2 *(AvrLm1 avrLm2 avrLm4)*	v23.2.1 *(avrLm1 avrLm2 AvrLm4)*	7.6.27 *(AvrLm1 AvrLm2 avrLm4)*
	Nb			**134**	**123**	**124**	**124**	**26**
	C[c]		88	124	101	113	101	24
BNS	I	Hom		0	18	1	11	2
	I	Het.		10	11	10	22	0
	Nb			**153**	**125**	**125**	**126**	**75**
	C		17	128	17	87	91	29
BNN	I	Hom		6	66	3	12	13
	I	Het.		19	42	35	23	33
	Nb			**166**	**164**	**163**	**163**	**146**
	C		22	164	22	137	120	58
BNQ	I	Hom		0	88	11	11	43
	I	Het.		2	54	15	32	45

[a] accessions showing complete resistance to all isolates (i.e. 10 BNS accessions, 25 BNN accessions and 2 BNQ accessions), are not included in these columns.

[b] Isolate names and, within brackets, genotype at the AvrLm1, AvrLm2 and AvrLm4 loci. *AvrLmi*, presence of the avirulent allele at the AvrLm*i* locus, *avrLmi*, presence of the virulent allele at the AvrLm*i* locus.

[c] C, compatible interaction : number of accessions displaying susceptibility when inoculated with the corresponding isolate; I, incompatible interaction : number of accessions displaying either an homogeneous resistance response (Hom.) when inoculated with the corresponding isolate or an heterogeneous resistance response (Het.) when inoculated with the corresponding isolate. Accessions resistant to v11.1.1 are also resistant to all 4 differential isolates, and are excluded from the number of accessions reacting to BBA62908, v11.1.2, v23.2.1 and 7.6.27.

Only 17 of 153 BNN-type genotypes (11.1%) were fully susceptible (Table 1). Complete resistance to all isolates including v11.1.1 was not uncommon, being present as a homogeneous trait in six accessions from New Zealand, Germany and the UK (Table 1). It was also observed as a heterogeneous trait in 19 accessions. In this latter case, resistance was expressed either as an HR

to all isolates (6 accessions), and often in very few plants of the accessions, e. g. 3 plants out of 23 in accession CR310, or as an heterogeneous resistance expressed as a delayed expression of susceptibility in 13 accessions. Twenty-two of the 166 BNQ-type genotypes (13.2%) were fully susceptible (Table 1). Resistance to all isolates including v11.1.1 was only observed in two East German and one Canadian accession where it was expressed as a heterogeneous delayed susceptibility, i. e., general resistance (Table 1).

DISCUSSION

The present study is the first one to investigate genetic diversity of R genes in a large collection of *B. napus* genotypes, using genetically characterized *L. maculans* strains. This allowed us to postulate the presence of resistance genes *Rlm1*, *Rlm4*, *Rlm2*, as well as presence of novel resistance corresponding to none of these R genes.

This analysis pointed out that many *B. napus* cvs are heterogeneous for the presence of R genes. Heterogeneity for the presence of R genes was mainly observed in BNN genotypes, and was strongly reduced in BNQ genotypes. Correlatively, diversity of R genes was also reduced in BNQ genotypes as compared with BNN genotypes. One of the main observations was the low R gene diversity and amount within genotypes of spring cvs as compared to winter genotypes. The main R gene, *Rlm4*, occurs quite commonly in Australian genotypes. In contrast to spring genotypes, we observed a great diversity in the R genes that may be harboured by winter genotypes, including *Rlm1*, *Rlm2*, *Rlm4*, and novel resistance. In addition, very few of the winter-type *B. napus* were completely devoid of R genes, mainly in the case of BNN genotypes. Association of more that one gene in a single genotype (or in different plants within a single accession) was not uncommon and was observed in ca. 13.3 and 13.6% of the BNN and BNQ accessions, respectively.

The gain of homogeneity in terms of R genes when BNN genotypes were improved to generate BNQ cvs, seems to have a counterpart in terms of diversity of available sources of resistance. Resistance to v11.1.1, that was present in a non-negligible number of BNN accessions, either as general resistance or as a major gene resistance, was absent as a major gene in BNQ. In all cases, this finding has one practical important consequence as it suggests that resistance sources for improved cvs may be easily identified (and transferred) from BNN resources to currently used cvs.

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