Field Crops Research 117 (2010) 51–58

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

High level of resistance to *Sclerotinia sclerotiorum* in introgression lines derived from hybridization between wild crucifers and the crop *Brassica* species *B. napus* and *B. juncea*

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ARTICLE INFO

Article history:
Received 29 September 2009
Received in revised form 11 December 2009
Accepted 29 January 2010

Keywords:
Erucastrum cardaminoides
Diplotaxis tenuisiliqua
E. abyssinicum
Brassicas
Introgression lines
Resistance
White mold

ABSTRACT

Sclerotinia rot caused by the fungus *Sclerotinia sclerotiorum* is one of the most serious and damaging diseases of oilseed rape and there is keen worldwide interest to identify *Brassica* genotypes with resistance to this pathogen. Complete resistance against this pathogen has not been reported in the field, with only partial resistance being observed in some *Brassica* genotypes. Introgression lines were developed following hybridization of three wild crucifers (*viz. Erucastrum cardaminoides*, *Diplotaxis tenuisiliqua* and *E. abyssinicum*) with *B. napus* or *B. juncea*. Their resistance responses were characterized by using a stem inoculation test. Seed of 54 lines of *B. napus* and *B. juncea* obtained from Australia, India and China through an Australian Centre for International Agricultural Research (ACIAR) collaboration programme were used as susceptible check comparisons. Introgression lines derived from *E. cardaminoides*, *D. tenuisiliqua* and *E. abyssinicum* had much higher levels ($P < 0.001$) of resistance compared with the ACIAR germplasm. Median values of stem lesion length of introgression lines derived from the wild species were 1.2, 1.7 and 2.0 cm, respectively, as compared with the ACIAR germplasm where the median value for stem lesion length was 8.7 cm. This is the first report of high levels of resistance against *S. sclerotiorum* in introgression lines derived from *E. cardaminoides*, *D. tenuisiliqua* and *E. abyssinicum*. The novel sources of resistance identified in this study are a highly valuable resource that can be used in oilseed *Brassica* breeding programmes to enhance resistance in future *B. napus* and *B. juncea* cultivars against Sclerotinia stem rot.

1. Introduction

Sclerotinia disease, caused by the fungal pathogen *Sclerotinia sclerotiorum*, is a serious threat to oilseed rape production with substantial yield losses recorded worldwide including India, Europe, China, North America and Australia (Li et al., 1999; McCartney et al., 1999; Sprague and Stewart-Wade, 2002; Hind et al., 2003; Koch et al., 2007; Malvarez et al., 2007; Singh et al., 2008). Various methods used for managing Sclerotinia disease include cultural control, chemical control and varietal resistance (Bardin and Huang, 2001). The persistent nature of sclerotia and the wide host range of this pathogen from taxonomically diverse hosts (over 408 plant species) generally render cultural practices such as crop rotation to be ineffective (Williams and Stelfox, 1980; Boland and Hall, 1994). Further, disease management through chemical control is also largely ineffective due to difficulty in timing the fungicide application with the release of ascospores (Bolton et al., 2006). Host resistance offers the only economic and sustainable method for effectively managing this disease (Zhao et al., 2004; Li et al., 2006).

While partial resistance against this pathogen has been observed in certain genotypes of sunflower (*Helianthus annuus*) (Godoy et al., 2005), beans (*Phaseolus coccineus*) (Gilmore et al., 2002), peas (*Pisum sativum*) (Porter et al., 2009), peanut (*Arachis hypogea*) (Cruickshank et al., 2002), or soybean (*Glycine max*) (Hartman et al., 2000), complete resistance has not been reported in the field. Partial resistance was also identified in some of the *Brassica napus* and, to a lesser extent *B. juncea*, genotypes from China (Li et al., 1999, 2006, 2008; Zhao et al., 2004), Australia (Li et al., 2006, 2008) and India (Singh et al., 2008). Although, a

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doi:10.1016/j.fcr.2010.01.013

significant number of at least partially resistant genotypes have been identified, breeding to increase the levels of resistance against Sclerotinia disease, in *B. napus* and/or *B. juncea* has been ineffective. This is mainly because resistance to *S. sclerotiorum* in existing cultivars of *Brassica* and in other cultivated germplasm appears to be of a complex nature, *i.e.*, it can either be monogenic and/or polygenic depending on the different plant species and materials under investigation (Abawi et al., 1978; Baswana et al., 1991; Zhao and Meng, 2003; Zhao et al., 2006). Genotypes with higher levels of resistance are urgently required for inclusion in oilseed *Brassica* breeding programmes to enhance the level of field resistance in cultivated species such as *B. napus* and *B. juncea*.

Lack of effective resistance to Sclerotinia disease in cultivated species has stimulated the interest of researchers towards exploitation of wild crucifer species to diversify the existing gene pool. Higher levels of resistance against Sclerotinia have already been reported in the secondary gene pool of bean (Abawi et al., 1978; Gilmore et al., 2002; Schwartz et al., 2006), wild *Helianthus* species (Seiler, 1992; Gulya et al., 2009) and in a *Pisum* core collection (Porter et al., 2009). Several successful attempts have been reported to introgress the resistance from the secondary gene pool of bean (*Phaseolus vulgaris*) into the cultivated bean species through interspecific hybridization followed by backcrossing (*e.g.*, Schwartz et al., 2006; Singh et al., 2009). Introgression of genomic segments responsible for resistance against Sclerotinia from wild to cultivated species of sunflower has been attempted in the past (*e.g.*, Ronicke et al., 2004). Despite the Brassicaceae family comprising of a wide array of different species, to date, it appears that only two wild crucifers, *Capsella bursa-pastoris* (Chen et al., 2007) and *Erucastrum gallicum* (Lefol et al., 1997a; Seguin-Swartz and Lefol, 1999), have been previously reported to show high levels of resistance against Sclerotinia disease. Although introgressive hybrids were successfully obtained between different *Brassica* (*B. rapa* and *B. napus*) species and *Capsella bursa-pastoris* (Chen et al., 2007), it remains to be confirmed if the introgression of resistance against *S. sclerotiorum* from *E. gallicum* into cultivated species has in fact been accomplished (Lefol et al., 1997a,b; Seguin-Swartz and Lefol, 1999). There remains substantial potential both to identify wild crucifers with high levels of resistance to Sclerotinia disease and for its successful introgression to the cultivated species.

Three wild crucifers, *viz. Erucastrum cardaminoides*, *Diplotaxis tenuisiliqua* and *E. abyssinicum*, have been identified with very high levels of resistance against *S. sclerotiorum* (S.S. Banga, unpublished data). The aim of this study was to introgress the genomic segments responsible for resistance against *S. sclerotiorum* from these three wild species into the cultivated germplasm. This paper reports the results of screening highly fertile (S_4/S_5) introgression lines of *B. juncea* and *B. napus* derived from three different wild *Brassica* species, *viz. E. cardaminoides*, *D. tenuisiliqua* and *E. abyssinicum*, against *S. sclerotiorum*.

2. Materials and methods

2.1. Plant materials

2.1.1. Experiment 1

Introgression lines, carrying alien genomic segments, were developed for *B. juncea* and *B. napus* by introgression from three wild crucifers *viz. E. cardaminoides* ($2n = 18$), *D. tenuisiliqua* ($2n = 18$) and *E. abyssinicum* ($2n = 32$). The general outlines of breeding schemes to introgress genomic segments from wild crucifers into these cultivated species are detailed in Figs. 1–3. These lines were developed by S. S. Banga (surin11@rediffmail.com) and co-workers, Punjab Agricultural University from identified wild crucifers by synthesizing intergeneric hybrids, *E. cardaminoides*/*B. rapa* Chandra et al. (2004), *E. cardaminoides*/*B. nigra* Chandra et al. (2004), *B. napus*/*E. cardaminoides*, *B. juncea*/*D. tenuisiliqua*, and *B. juncea*/*E. abyssinicum*. Chromosome doubling was achieved using colchicine in cross combinations involving monogenomic *Brassica* species to restore seed fertility. In crosses of wild crucifers with digenomics, chromosome doubling was not required as these were partially fertile. The synthetic amphiploids or the trigenomic hybrids were subsequently used as pollen/seed parents to hybridize with cultivated digenomics, *B. juncea* or *B. napus* (Figs. 1–3). This was followed by three to four generations of selfing using the single pod descent method. Special attempts were

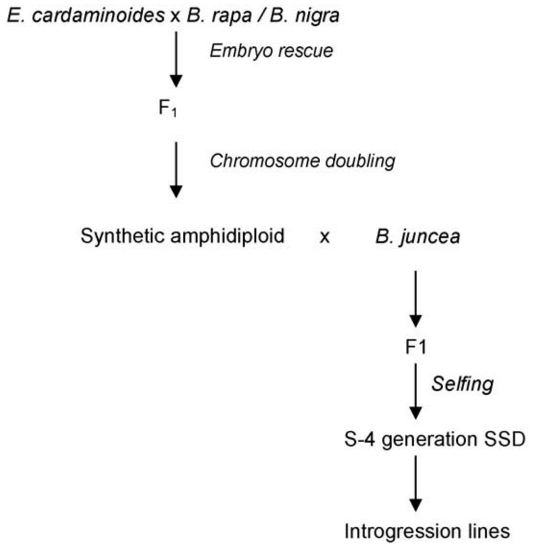

Fig. 1. The general breeding scheme used to introgress segments of the genome from wild species *Erucastrum cardaminoides* into cultivated lines of *Brassica juncea*.

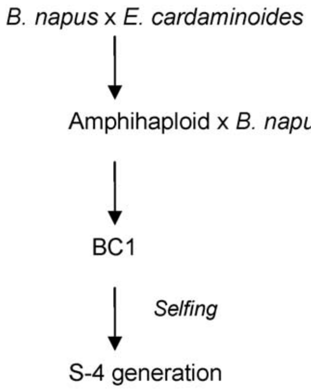

Fig. 2. The schematic representation for developing introgression lines by crossing *Brassica napus* with *Erucastrum cardaminoides*.

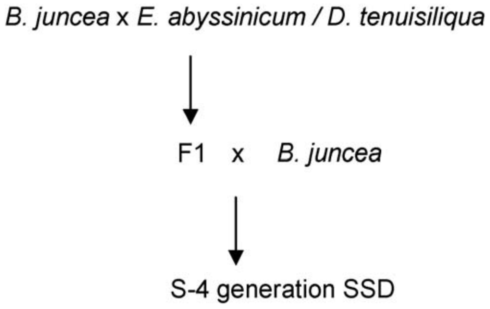

Fig. 3. The schematic representation for the development of introgression lines from wild crucifers *Erucastrum abyssinicum* and *Diplotaxis tenuisiliqua*.