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Greenhouse and field evaluation of rapeseed cultivars and lines for resistance against *Sclerotinia sclerotiorum* (Lib.) de Bary

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Abstract

Sclerotinia stem rot (SSR) of rapeseed caused by *Sclerotinia sclerotiorum* (Lib) de Bary is one of the most important disease of rapeseed- mustard causing significant losses in both quality and quantity of seed. In recent years, the disease has become very serious in northern Iran. In present investigation, 11 rapeseed (*Brassica napus* L.) genotypes were evaluated for their reaction against SSR under greenhouse and field conditions. The results of green house study recorded disease index (DI) of 52.8, 54.5, 55.6, 56.1 and 56.1 respectively, in genotypes RGS003, HAYOLA 401¡SARIGOL¡ RASOPT and ZAR401. Genotypes19SAR-3, RG3OPT, RG3401 and RW19 recorded considerably higher DI 69.4, 66.7, 66.0 and 63.9 respectively. Under field conditions genotypes RASOPT, HAYOLA 401, RGS003, SARIGOL and ZAR401also showed lowest infection percent of 5, 8, 11, 11.5 and 11.5 respectively, while RG3SAR, RW19, RG3401 and 19SAR-3 genotypes proved to be more susceptible to SSR. Reaction of our study showed similar reaction of all genotypes under both greenhouse and field conditions.

Key words: *Sclerotinia sclerotiorum, rapeseed, disease incidence, tolerant*

Introduction

Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum* (Lib) de Bary, is one of the most important disease of rapeseed (*Brassica napus* L.) responsible for causing significant losses of seed yield in the world (Zhao *et al.,* 2004). *Sclerotinia sclerotiorum* is one of the most non- host-specific plant pathogen (Purdy, 1979) infecting over 400 species of plants in 75 different families (Boland and Hall, 1994).

Several important control measures including cultural, biological and chemical have been developed, but use of resistance to SSR will be most useful when applied in combination with other cultural means (Garg *et al.,* 2008). To obtain SSR resistant or tolerant genotypes, to SSR in oilseed rape, oilseed Brassica breeders have been using both morphological (Li *et al.,* 2006) and physiological (Dong *et al.,* 2008) characters of the host genotypes.

Results of several researchers reported considerable differences in reaction between rape-seed cultivars and hybrids (Bailey, 1987; Bardley *et al*., 2006; Grag *et al*., 2010; Lie *et al*., 2006; Lie *et al*., 2009; Sedun *et al*., 1989; Zhao *et al*., 2004). The sclerotinia stem rot of rapeseed is a very serious disease in northern Iran. The present investigation was under taken 11*B. napus* genotypes for their reaction against SSR under greenhouse and field conditions.

Material and Methods

Fungal isolation

Sclerotinia stem rot- infected plant samples were collected from Mazandaran Province in northern Iran, during 2010 - 2011. Pieces of infected plant after surface sterilization in 0.8% sodium hypochlorite for 1 min., were placed on PDA plates and incubated at 22±1 ˚Ñ in darkness for four days. The cultures were purified using hyphal tip culture technique, maintained on PDA at 24±1 ºÑ, and used for inoculating genotypes in the greenhouse.

Evaluation of rapeseed genotypes resistance to *S. sclerotiorum* in greenhouse

Eleven rapeseed lines and cultivar were obtained from the seed and plant improvement Institute of Iran. For planting, plastic pots were filled with autoclaved sandy loam soil. The experiment was performed in a randomized complete design with three replications. The inoculation was carried out using technique of Lewartowska *et al.* (1994) where stems at 20 cm above the soil level, were inoculated at flowering stage with wheat grains overgrown with mycelium of *S. sclerotiorum*; infected grains were covered with moist cotton and attached to stem with parafilm. For each line or cultivar 9 inoculated and 9 non inoculated control plants from 3 replications were used. Disease incidence and intensity were recorded 1-2 weeks after artificial inoculation, using rating from 0 (no stem discoloration) to 5 (discoloration, sclerotia, premature ripening of the whole plant and low seed production). Numerical values of 0, 1.25, 2.5, 3.75 and 5, respectively, were assigned to 1- 5 rating and the disease index (DI) was calculated following the formula of (Dueck *et al*., 1983). Data were analyzed were performed using MS-Excel and MSTAT-C program.

$$\mathrm{DI} = \frac{(1.25*Y2)+(2.5*Y3)+(3.75*Y4)+(5*Y5)}{Total\ of\ \ plants} * \frac{1}{0.05}$$

DI: disease index: Y2, Y3, Y4 and Y5: number of plants with score 2 to 5 respectively

Field evaluation of rapeseed genotypes resistance to *S. sclerotiorum*

The 11 *B. napus* genotypes were evaluated in randomized complete block design with three replications in sclerotia infested soil at Biekol Agriculture Research Station, Neka, Iran, during 2011-12 in plot consisting of four rows of 5 m length. Crop management practices including land preparation, crop rotation, fertilizer, and weed control were followed as recommended the for local area.

Based on the percent disease incidence and intensity the mean degree of infection was calculated following the formula of Krüger(1983), and analyses were performed using MS-Excel and MSTAT-C.

$$\%Q = \frac{(n*0)+(n*10)+(n*20)+...(n*100)}{n}$$

Q= degree of infection: n=number of assessed plants: 0 to 100=% 0f plant affected

Results and Discussion

Evaluation of rapeseed genotypes resistance to *S. sclerotiorum* in greenhouse

Comparison of disease index demonstrated existence of significant differences (P<0.01) between 11 *B. napus* genotypes (Table 1).

Table 1. Analysis of variance for disease index for 11 rapeseed genotypes in greenhouse

KValue	Source	Degrees of Freedom	Sum of Squares	Mean Squares	FValue
1	Replication	2	50.146	25.073	0.9248^{ns}
2	Genotypes	10	1085.874	108.587	4.0050^{**}
3	Error	20	542.261	27.113	
	Total	32	1678.281		

^{ns}: No significant

**: Significant at 1% probability level

Coefficient of Variation: 8.56%

The results showed that genotype RGS003 had the lowest (52.8) and genotype 19SAR-3 the highest (69.4) disease index; RG3OPT and RASRG3 although had disease index of 66.7 and 67.2 respectively, the differences were statistically not significant from 19SAR-3 (Fig 1).

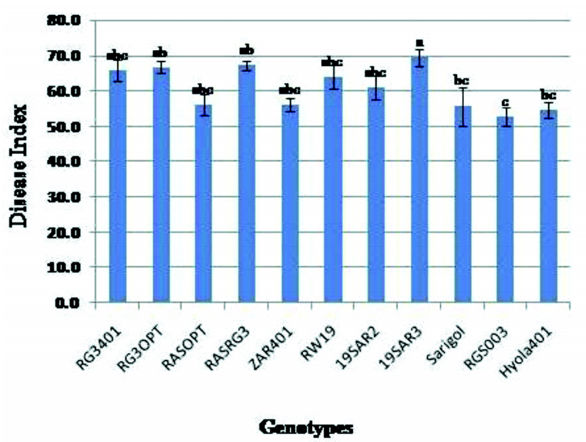

Fig 1. Mean comparison of disease index in different genotypes of rapeseed to SSR in the greenhouse

Field evaluation of rapeseed genotypes resistance to *S. sclerotiorum*

Field evaluation demonstrated significant (p<0.01) differences in mean degree of infection between 11 rapeseed genotypes (Table 2).

Results showed that RASPOT, HAYOLA 401 and RGS003 genotypes had significantly lower mean degree of infection 5.8 and 11 respectively (Fig 2). Statistically, RASPOT was significantly more resistant to *S. sclerotiorum* than all other genotypes. The genotypes RW19 and RG3401 with mean degree of infection 20 and 19 respectively, were found to be significantly more susceptible than most other genotypes.

In our greenhouse evaluation studies, none of line rapeseed genotypes showed complete resistance to *S.sclerotiorum*. The genotypes, however, differed significantly in their reaction to SSR. Researcher

Table 2. Analysis of variance for infection degree in 11 rapeseed genotypes under field condition

KValue	Source	Degrees of Freedom	Sum of Squares	Mean Squares	FValue
1	Replication	3	4.424	1.475	0.4414^{ns}
2	Genotype	10	836.811	83.681	25.0434 **
3	Error	30	100.243	3.341	_

^{ns}: No significant; **: Significant at 1% probability level ; Coefficient of Variation: 13.88%

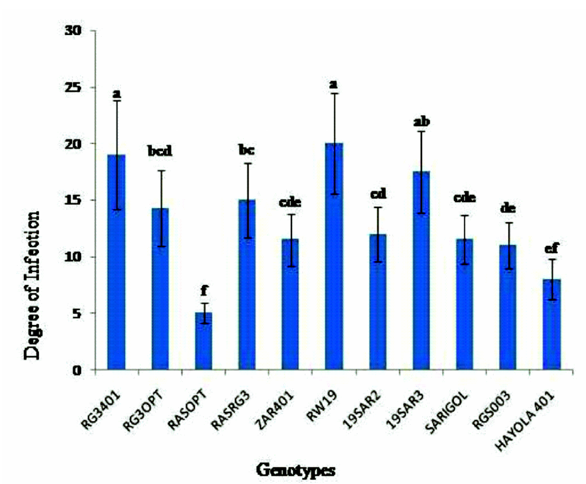

Fig 2. Mean comparison of degree of infection in different genotypes of rapeseed under field condition

from Australia, china and France reported partially resistant to SSR in *B. napus* lines (Li *et al*., 2006, Li *et al*., 2009, Zhao *et al*., 2004). Zhao and Meng (2003) have reported to have identified the loci was associated with partial resistance to *S. sclerotiorum* in rapeseed. Newman and Bailey (1987) also reported absence of complete resistance to SSR in rapeseed cultivars. Bardley *et al.* (2006) observed absence of complete resistance against *S.sclerotiorum* in canola genotypes, although significances differences between genotypes were observed.

Khot *et al*.(2011) reported reaction of *B. napus* 436554, 458940, 169080, 286418 and 633198 similar to that of the partially resistance check cultivar Hayola 357. Li et al. (2009) in their field evaluation studies in Western Australia reported significant differences among 95 *B. napus* and *B. juncea* genotypes for their reaction against *S. sclerotiorum*.

Garg *et al.* (2010) evaluated several Brassica genotypes for their reaction against *S. sclerotiorum* and also reported complete, although partial resistance in some was observed. Dalili *et al*. (2004) in their two years' filed evaluation studies of 25 rapeseed genotypes, reported cultivars Foseto and Eboni with the lowest and highest susceptibility DI of 48.86 and 67.94, respectively. Fernando and Wu (2001) found rapeseed cultivar Zhongyou 821 to be highly tolerant compared cultivar Wester for their reaction against SSR.

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