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Evaluation of resistance in rapeseed lines against Sclerotinia rot (*Sclerotinia sclerotiorum*)

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Abstract

Sclerotinia rot (SR) caused by *Sclerotinia sclerotiorum* (Lib) de Bary is a very serious disease of rapeseed in northern Iran. Genotypic diversity of 26 *S. sclerotiorum* isolates collected from rapeseed fields in Mazandaran province was assessed using mycelial compatibility grouping (MCG) using MCG tests, the isolates were grouped into 10 groups that two groups were individual and the remaining eight groups were consisted of two to four isolates. *In vitro*, the reaction of 7 rapeseed [*Brassica napus* (L.)] genotypes was investigated against three MCGs and area under disease progress curve (AUDPC) was calculated. Wersoona and Hayola 401 with 2.90 and 3.05 and Westar with 6.65 exhibited considerably minimum and maximum level of AUDPC respectively. Under field conditions, genotypes Z-15 exhibited high level of tolerance with disease index 54.16, while Sarigol and Z-17 demonstrated to be more susceptible to SR. Reaction of some genotypes such as Z-15 and RGS003 was indicated similar reaction against SR under both *in vitro* and *vivo* conditions.

Key words: *AUDPC, rapeseed, Sclerotinia Sclerotiorum, tolerance*

Introduction

Sclerotinia rot (SR) caused by *Sclerotinia sclerotiorum* (Lib) de Bary is one of the most important fungal diseases in rapeseed and other crops in the world (Zhao *et al*., 2004). The pathogen has wide host range and infects over 400 host plants in 287 genera of 75 families (Boland and Hall, 1994). There are several ways of controlling this disease, but use of resistance cultivars in combination with other cultural and chemical means are prepared (Garg *et al*., 2008). Results of several researchers show considerable differences in reaction of rapeseed cultivars and hybrids. Bardly *et al.* (2006) reported partial resistance in different rapeseed genotypes. Newman and Bailey (1987) also reported significant differences among several rapeseed genotypes.

Dalili *et al.* (2004) evaluated 25 rapeseed genotypes against SR for two years and reported significant difference between genotypes. The MCG technique could be useful in evaluating cultivars for resistance due to its ability to identify variability among pathogen isolates, and the potential for interactions (Agrios, 1999; Shurtleff and Averre, 1997). Greenhouse inoculation studies on canola suggested that some clones of *S. sclerotiorum* are more aggressive in forming lesions (Errampalli and Kohn, 1995; Kimmer, 2003).

Several studies on evaluating soybean resistance to *S. sclerotiorum* demonstrated that there is poor correlation between greenhouse, and field experiments, and also, across field locations (Kim *et al*., 1999; Nelson *et al*. 1991; Wegulo *et al*. 1998). This may also be true, in part for MCG frequencies separating *S. sclerotiorum* isolates from fields (Kull *et al*., 2004). Sclerotinia rot is prevalent on rapeseed in Mazandaran and Golestan provinces of Iran have been reported. Disease incidences of upto 54.4 and 66.8% in Mazandaran and Golestan provinces (Barari *et al*., 2000; Aghajani *et al*., 2009). The present study was under to access taken seven *Brassica napus* genotypes for their

resistance against *S. sclerotiorum in vitro* and *vivo* conditions.

Material and Methods

Fungal isolation

Disease plants were collected from Mazandaran Province in northern Iran, during 2011-2012, infected tissues were surface sterilized in 0.8% sodium hypochlorite for 1 minute, placed on PDA plates, incubated at 22±1 °C in darkness for four days, purified using hyphal tip culture technique and maintained on PDA at 4 °C.

Mycelial Compatibility Grouping

Isolates were manipulated in all possible combinations on PDA-amended with 75 ìl of Wilton's red food coloring (Wilton Crop., USA) (Schafer and Kohn, 2006). All pairings were scored after incubation in the dark at 22±1°C for 7 and 14 days. Pairings were considered and fused and no reaction line was observed within the interaction zone. Incompatible pairings were scored with the presence as an obvious red line on the bottom of Petri dishes, or when a thin to wide band of uniform, aerial mycelium or both was observed between adjacent paired isolates (Kohn *et al*., 1990). To facilitate MCG determination of all isolates, at first a subset of isolates from each field was selected and paired in all possible combinations and representative from each MCG were paired with representatives of each of the other MCGs (Table 1). In the meantime, self-self pairings were performed as well.

Reactions of rapeseed cultivars and lines to *S. sclerotiorum in vitro*

In this study, three isolates from three different mycelial compatibility groups, MCG2 (isolate No. 21 from Sari), MCG5 (isolate No.16 from Neka) and MCG10 (isolate No.26 from Qaemshahr) were used. In order to assess cultivars, rapeseeds leaves at 6 to 8-leaf stages were placed onto sterilized moistened filter paper in a Petri dish (15 cm diam.). A mycelial agar plug (3 mm diam.) from the edge of a 3-day-old colony on PDA was placed face-down onto the leaf surface, avoiding major veins (Godoy *et al*., 1990). The inoculated leaves were incubated at room temperature and lesion diameters measured after 24, 48 and 72 h. The area under disease progress curve (AUDPC) was calculated as follows:

$$\text{AUDPC} = \sum_{i=1}^{n_{i-1}} \frac{y_i + y_{i+1}}{2} (t_{i+1} - t_i)$$

AUDPC=Area Under Disease Progress Curve

$(t_{i+1} - t_i)$= The distance between the two sampling

$(y_i + y_{i+1})$ = Sum of lesion diameter of the two sampling

Area under disease progress curve was analyzed statistically using MSTAT-C program with completely randomized design (CRD), and AUDPC was examined using analysis of variance (ANOVA), and the Duncan's Multiple Range Test (DMRT) was employed to determine significant ($P< 0.01$) differences among mean values.

Reactions of rapeseed cultivars and lines to *S. sclerotiorum in vivo*

The reaction of seven *B. napus* genotypes was evaluated in randomized complete block design with three replications at Biekol Agriculture Research Station, Neka, Iran, during 2012-13 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 for the local area.

The inoculation was carried out using technique of Lewartowska *et al*. (1994) where, stem of rapeseed was inoculated at flowering stage with wheat grains overgrown with *S. sclerotiorum* mycelium of isolate No. 21. Infected grains were covered with moist cotton and attached to stem with parafilm. For each genotype or cultivar, 9 inoculated and 9 non-inoculated control plants from 3 replications were used. Disease severity were recorded before physiological ripening, 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 using MSTAT-C program.

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

Results and Discussion

Assessment and Comparison of MCG Diversity

Compatibility was based on mycelial fusion, whereas continuity and incompatibility was based on the failure of the two colonies to fuse reflected by the formation of a strip of mycelium or aerial mycelia at the interaction zone (Kohli *et al*., 1992). Three levels of incompatibility were distinguished. Level 1 incompatibility was designated with the presence of a sharp distinct thin band of mycelia in the interaction zone; Level 2 incompatibility meant presence of clarify reaction line visible abundant tufts and white patches of aerial mycelia at the interaction reaction zone at the colony surface; and Level 3 incompatibility (100% incompatible) occurred when a red reaction line was observed between the interfering paired isolates.

Ten mycelial compatibility groups were identified among 26 isolates collected from three cities Neka, Sari and Qaemshahr cities in northern of Iran. MCG 2, 8 and MCG 1, 3, 6, 9 consisted of two and three isolates, respectively; MCG 4 and 7 each included four isolates. The remaining 2 isolates (3 and 26) were compatible only with themselves (Fig. 1). The MCG analysis of *S. sclerotiorum* populations in this study showed that in a given location there was a heterogeneous mix of MCGs, (Table 1).Investigated *S. sclerotiorum* isolates grouped through MCG, isolate code, and location

This study demonstrated mycelial compatibility groups commonly shared between different fields suggestion movement isolate due to propagules variable environmental conditions (Kull *et al*., 2004). The results also indicated a high level of MCG diversity. Genetic exchange, meiotic recombination (Carbone *et al*., 1999), mitotic recombination, transitory selection, selective neutrality, diversifying

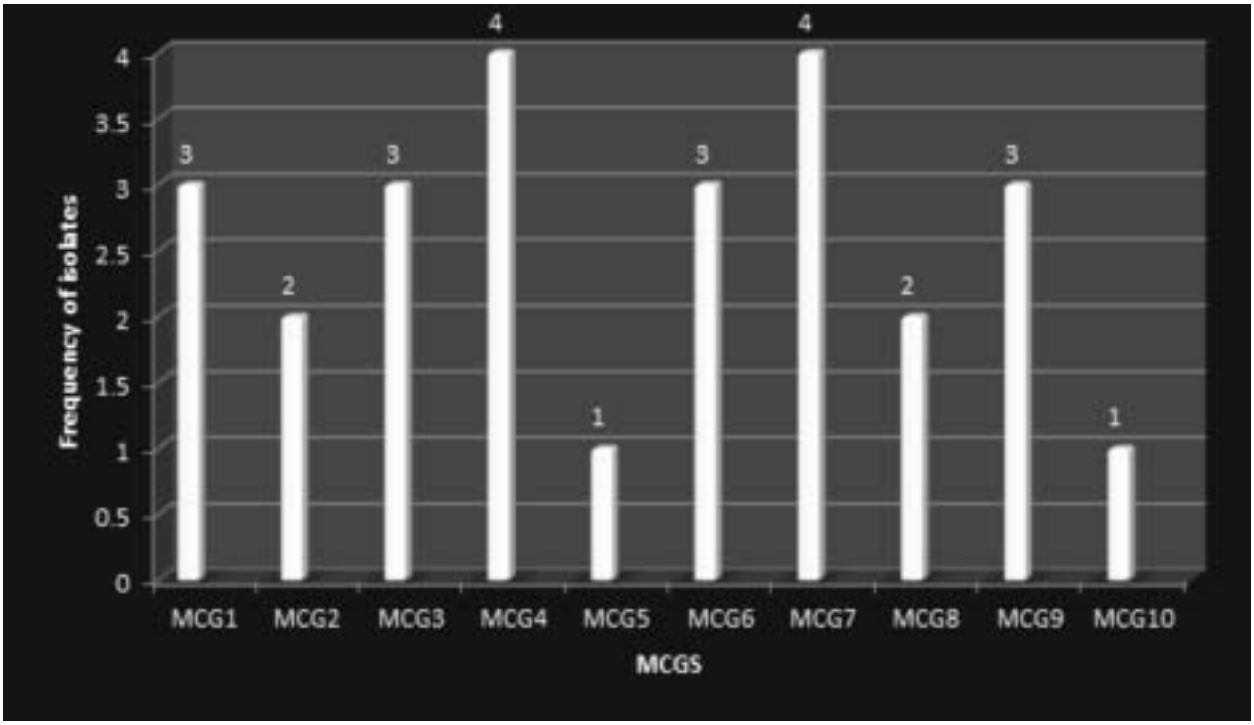

Figure 1. Histogram of the 10 mycelial compatibility groups (MCGs) showing frequency of isolates.

Isolates	Location	MCGs	Isolates	Location	MCGs
1	Sari	1	17	Qaemshahr	6
2	Sari	1	18	Qaemshahr	6
13	Qaemshahr	1	9	Sari	6
3	Sari	2	19	Qaemshahr	7
21	Sari	2	20	Qaemshahr	7
4	Sari	3	10	Sari	7
5	Sari	3	22	Neka	7
14	Qaemshahr	3	23	Neka	8
6	Sari	4	11	Sari	8
7	Sari	4	24	Neka	9
8	Sari	4	25	Neka	9
15	Qaemshahr	4	12	Sari	9
16	Qaemshahr	5	26	Neka	10

selection (Kohli *et al.*, 1992), infrequent outbreeding in *S. sclerotiorum* isolates, immigration of strains from other sites (Glass and Kuldau, 1992), could account for the existence of MCG diversity.

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

In this study, AUDPC for three *S. sclerotiorum* isolates on seven rapeseed genotypes were calculated, the data were statistically analyzed using MSTAT-C program (Table 2). Results showed significant differences among the isolates placed in different MCG groups. Isolates 21 and 16, with 2.22 and 2.28 AUDPC, and isolate 26 with 1.71 AUDPC were placed in Group A and B, respectively (Table 3).

Table 2: Variance Analysis of AUDPC caused by different MCGs of *S. sclerotiorum* on different genotypes of rapeseed *in vitro*

Source of variances	Degree of freedom	Sum of square	Means of square	F -Value
Isolates	2	5.507	2.754	15.821**
Genotypes	6	7.939	1.323	7.6051**
Isolates × Genotypes	2	2.340	0.195	1.1209 ns
Error	63	10.691	0.174	-------

ns: No significant; **: Significant at 1% probability level; Coefficient of Variation: 1.45%

Evaluation of rapeseed genotypes resistance against different MCGs of *S. sclerotiorum* showed a significant difference among the genotypes. Duncan grouping test results showed that genotype R-24 with a value of 2.61 indicated the highest AUDPC and was placed in group A, and followed by P-26, Z-17, HAYOLA 401, Sarigol and RGS003. Genotype Z-15 with an area of 1.53 showed the lowest AUDPC and was placed in group C (Fig. 2).

Table 3 – Mean comparison of the AUDPC caused by different MCGs on different genotypes of rapeseed *in vitro*

No. of isolate	AUDPC	α =0.01
21	2.226	A
16	2.287	A
26	1.716	B

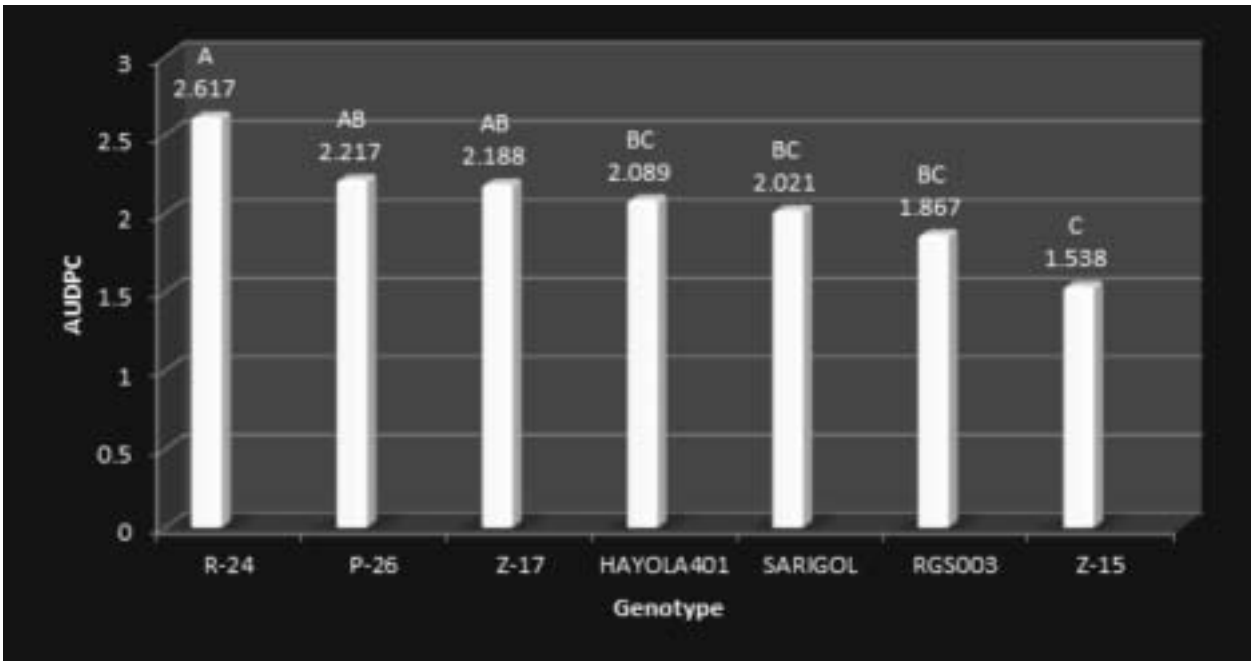

Figure 2 - The mean area under the disease progress curve rapeseed genotypes

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

Field evaluation demonstrated significant (P<0.01) differences in mean degree of infection between seven rapeseed genotypes (Table 4). Results showed that genotypes Z-15, P-24 and P-26 with 54.16, 58.33 and 60.46 values, respectively had significantly lower mean disease indices (Fig 3). Statistically, Z-15 was significantly more resistant to *S. sclerotiorum* than all other genotypes. The genotypes Sarigol and Z-17 with mean disease index of 75 were found to be significantly more susceptible than most other genotypes.

Table 4. Variance Analysis of Disease index on different genotypes of rapeseed caused by *S. sclerotiorum in vivo*

Source of variances	Degree of freedom	Sum of square	Means of square	F Value
Replication	3	106.57	35.52	0.68^{ns}
Genotype	6	1591.68	256.28	5.06^{**}
Error	18	15.72	0.87	_

^{ns}: No significant; **: Significant at 1% probability level; Coefficient of Variation: 11.25%

In vitro and *vivo* evaluation results showed that, none of the rapeseed genotypes tested showed complete resistance to *S. sclerotiorum*. The genotypes, however, differed significantly in their reaction to SR. Researchers from Australia, china and France have also reported partially resistant genotypes in *B. napus* lines (Lie *et al*., 2006, Lie *et al*., 2009, Zhao *et al*., 2004). Zhao and Meng (2003) reported to have identified the loci associated with partial resistance to *S. sclerotiorum* in rapeseed. Newman and Bailey (1987) also reported absence of complete resistance to SSR in rapeseed cultivars. Bradley et al. (2006) observed absence of complete resistance against *S.sclerotiorum* in canola genotypes, although significant differences between genotypes were observed.

Khot *et al*. (2011) reported reaction of *B. napus*

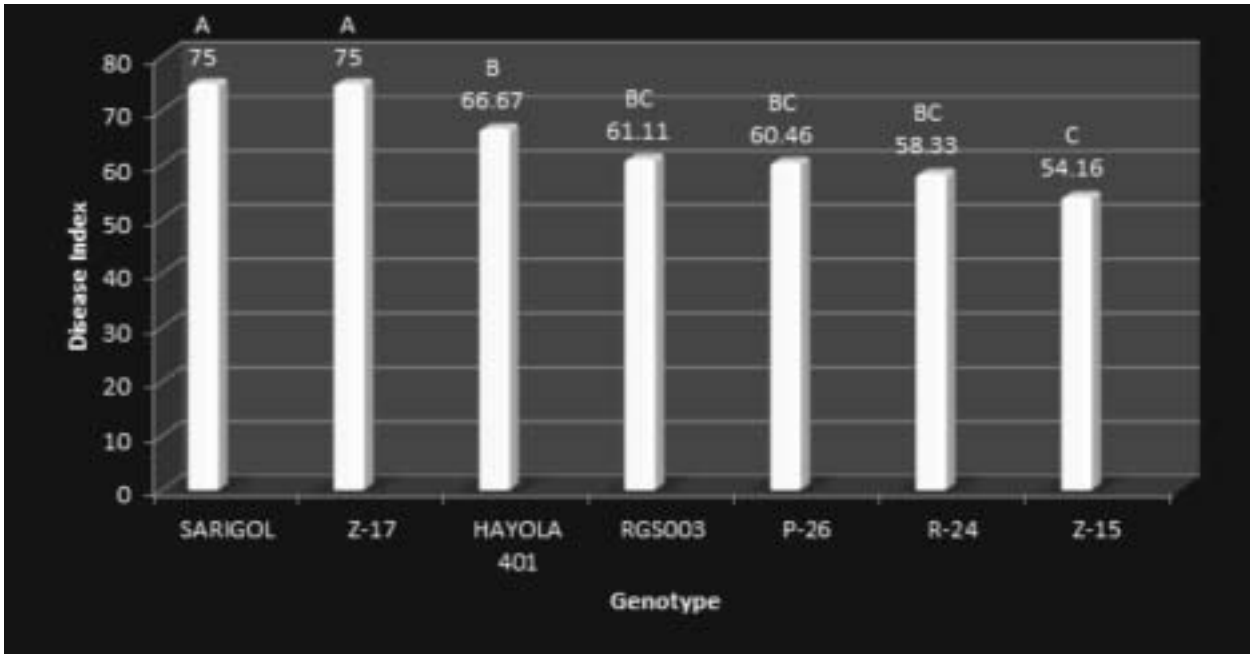

Fig 3. Mean comparison of disease index in different genotypes of rapeseed caused by *S. sclerotiorum in vivo*

genotypes 436554, 458940, 169080, 286418 and 633198 similar to that of the partial resistance check cultivar Hayola 357. Lie *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 partial resistance in some. 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 to cultivar Wester for their reaction against SSR.

Few reports regarding variation in *S. sclerotiorum* aggressiveness on rapeseed are available. A report by Price and Calhoun (1975) compared pathogenicity of 14 *S. sclerotiorum* isolates on 11 different hosts and showed that a variation in the degree of pathogenicity is dependent on host species. On the basis of a detached celery petiole assay, 50 MCGs were identified from 160 Argentinean isolates (Durman et al., 2003) and differences in aggressiveness among MCGs groups were reported. By using a limited-term, plug inoculation technique, Kull *et al.* (2004) reported that aggressiveness varied between isolates and MCGs from different locations, but not in MCGs produced from isolates originating from infections in single fields.

This genetic association of low aggressiveness and high pervasiveness supports the popular idea held by evolutionary biologists that pathogens can evolve to become harmless, more deadly, or anything in between depending on the forces guiding natural selection; such forces can pull the pathogen in opposite directions at the same time, creating an evolutionary tradeoff between fecundity and infectivity factors (Kohn, 1995).

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