A method of field disease nursery equipped with a water spray system for identification of resistance to *Sclerotinia sclerotiorum* in oilseed rape

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Abstract

The disease caused by *Sclerotinia sclerotiorum* is a major disease in oilseed rape worldwide. Use of resistance to the disease is a main measure for control of the disease. To effectively evaluate and select resistance, a new, sensitive and reliable method for resistance identification in fields was developed. Significantly correlated, reproducible data across different experiments and across replicates (blocks) of each experiment were obtained by using the method.

Key words: Oilseed rape, *Sclerotinia sclerotiorum*, resistance, identification, new method

Introduction

The ascomycete fungus *Sclerotinia sclerotiorum* has been reported to infect more than 400 plant species, including agronomically important crops, such as soybean, sunflower and oilseed rape (*Brassica napus*) (Boland & Hall, 1994). On oilseed rape, it causes rot of leaves, stems and pods, resulting in a tremendous seed yield loss in China. Because application of fungicides to the crop for control of the disease is expensive, can be ineffective due to difficulty in applying sprays to thick canopies and a lack of suitable forecasting methods to enable timely application, use of resistant varieties become a major measure of the disease control (Liu et al. 2005). Furthermore, due to the economic and environmental concerns associated with fungicides, selection of resistant cultivars is a major priority for canola breeding.

Precondition for resistance breeding is that there is a efficient method by which consistent, precise and reliable results can be obtained in differentiation of differences in resistance existing in different accessions or breeding lines in which such differences are usually small. Several methods have been used to identify resistance to *S. sclerotiorum* in soybean, sunflower, and common bean (Hartman et al., 2000; Kim & Diers, 2000; Nelson et al., 1991; Vuong et al., 2004; Wegulo et al., 1998). In oilseed rape, the methods were developed for the assay under controlled conditions and field tests, respectively (Liu et al, 2005, 1998, Zhao et al. 2004). The former includes the detached leaf inoculation, oxalate resistance test using detached leaves, seedlings, isolated cell, calli or germinated seeds, and methods in glasshouse using stem inoculation, leaf inoculation or ascospore/mycelial spray at flowering time. The latter includes natural infection, sclerotia-seeded semi-natural field evaluations, stem inoculation with match stuck with mycelia or infected wheat seeds or infected petals.

Unfortunately, all methods developed in different host species are not satisfied in term of their reproducibility, preciseness, simplicity, and inexpensive nature, especially under field conditions, in oilseed rape. It is common for responses of cultivars to vary among methods and experiments (Wegulo et al., 1998). For resistance identification in fields, the major problems encountered are: 1) How to maintain the uniform environment around plants and across plots/replicates and uniform inoculum in a field are a challenge because infection and development of the disease are highly dependent upon the environment in which they interact. 2) Differences in resistance between accessions or varieties are small and thus an "appropriately" sensitive method is needed to differentiate the differences. Here we introduce the term "disease pressure" as a criterion for the sensitivity. It is a potential for disease occurrence determined by amount of inoculum and conditions required for disease infection and development. An appropriate disease pressure (not too high and not too low) is necessary to differentiate differences in resistance between accessions or lines, especially when the differences are not big. Too high pressure will result in all accessions or lines having the same severity and too serious disease, even all die. Too low pressure will also result in all accessions or lines having the same severity, but too little disease. Therefore, critical measures we need take are to ensure an appropriate disease pressure. Failure to maintain the uniform environment, the uniform inoculum and the appropriate disease pressure will result in an inconsistent data which can not be used for QTL mapping work and selection for inheritable resistance.

Objectives of this study were to develop and test a disease nursery method to characterize resistance to *S. sclerotiorum* in *B. napus* in fields.

Materials and Methods

Construction of artificial disease nursery: The disease nursery is equipped with a spraying system in a 1440M^2 field and in a top waterproofed house (Fig.1). The sprayers were hanged at 2.2 M away from the ground and each spraying range is 4 M in diameter. Water is sprayed in very fine droplets like smoke. *S. sclerotiorum* inoculum had been maintained in the fields by growing oilseed rape consecutively (left sclerotia in the field) and placing two sclerotia in each row before sowing in each of the previous five seasons. To remove emergence of voluntary seedlings after sowing, the field is plugged and irrigated three times during May to September after previous harvest. Throughout flowering time (from the beginning to the end of flowering), the penstocks were lifted for spraying. Each spray lasted 40 seconds and six times a day if no rain.

Fig. 1 Disease nursery equipped with spraying system in an open field (left) and a top water-proofed house

Experiment materials: In the seasons of 2004-2005 and 2005-2006, in total 275 *Brassica* lines plus 2 resistant controls were tested in three experiments. These lines came from the China National Rapeseed Variety Regional Trials and the ACIAR project. All materials were sown on 30 September and the field management was followed as normal farm practice except for no fungicide application.

Experiment design: The experiment was in a randomized block design with three blocks and a plot size of 1.33m×2.0m (ca 33 plants). At maturity, disease severity was assessed on a 0-4 scale and disease index (DI) was calculated as DI = 100$\sum$(i n_i)/(N k), where i is a disease severity score on the 0-4 scale (Liu et al. 2005), n_i is number of plants with each score, N is total number of plants assessed and k is the highest score (here k = 4). Statistical analysis for these experiments was done using the DPS program (China Agricuture Press). In this report, relative resistance index (RRI) was used to categorize resistance catalogs (high, medium and low resistance, and low, medium and high susceptibility). RRI = ln((100 - DI_{ck})/DI_{ck}) - ln((100 - DI_m)/DI_m), where ln is the natural logarithm, DI_{ck} is the disease index of the control (Zhongyou 821), and DI_m is that of the line evaluated (Liu et al. 2005).

2. Results and Discussions

In this study, disease pressure can be represented by percent diseased plants of susceptible to middle resistant varieties and frequency distribution of plants (plots) with various disease severity of varieties with different resistance. In the test of the disease nursery in the season of 2004-2005, percent plants with lesions in the resistant control Zhongyou 821 is about 54.0%, and percent plants with lesions in all 90 lines ranged from 3.3% to 100%. According to previous studies, empirically only experiments with more than 15% of percent plants with lesions are effective for data analysis and inference. Thus, in this experiment, the disease pressure in the nursery is high and appropriate to differentiate differences in resistance of these lines tested.

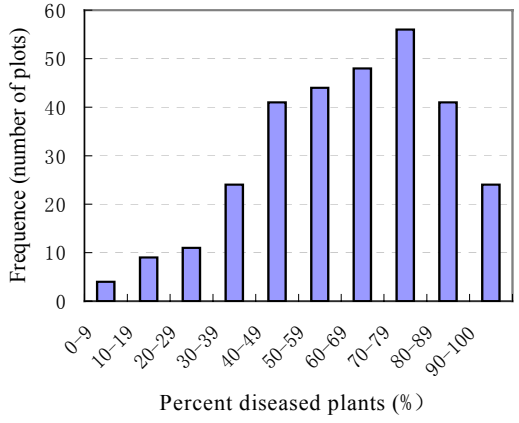

Fig.2 Variation in percent plants with *Sclerotinia sclerotiorum* lesions in a disease nursery experiment in 2004-2005

The above conclusion is supported by variations in resistance detected by the disease nursery method. Variations in both percent plants with lesions and disease indice are great, ranging from 29.3%-100% for percent plants with lesions and 18.3%-85.3% for disease index (Fig.2 and 3; Table 1 was not presented). Differences in the two disease parameters between lines were significant (P<0.05). At the reasonable disease pressure, distribution of number of plots with various disease indice is normalized while the frequency distribution of percent diseased plants was positively skewed. This indicated that most of lines are relatively at the middle level of resistance. If we plotted number of field plots against RRI, results are similar.

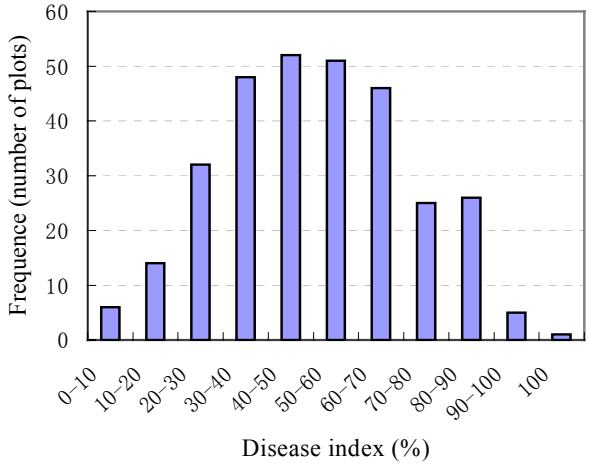

Fig.3 Variation in *Sclerotinia sclerotiorum* disease index in a disease nursery experiment in 2004-2005

Consistency or reproducibility of the experiments can be checked with correlation between replicates of each experiment and between the years in which a set of common lines were tested. Between three replicates, either percent diseased plants or disease index or RRI correlated significantly (P<0.01) in an experiment of the 2004-2005 season. Other two experiments produced similar results and one with greater correlation coefficients in 2005-2006.

Table 2 Correlation between replicates of a randomized block experiment for resistance evaluation against *Sclerotinia sclerotiorum* (Significant level: $P_{0.05}$=0.195,$P_{0.01}$=0.254,df=102)

	Percent diseased plant		Dis ease index		Relative resistance index	
Replicate	R2	R3	R2	R3	R2	R3
R1	0.51	0.46	0.54	0.65	0.44	0.43
R2		0.33		0.46		0.32

In the experiments of the years of 2004-2005 and 2005-2006, there were some common lines to allow analysis of correlation between the three experiments of the two years. Correlation coefficients were 0.685 between the 2004-2005 experiment and experiment 1 of 2005-2006 (df=22, $P_{0.05}$=0.388), 0.781 between the 2004-2005 experiment and experiment 2 of 2005-2006, and 0.828 between the experiment 1 and experiment 2 in 2005-2006 (Table 3 was not presented). All coefficients are significant. A large number of reports as well as results in this study showed that resistance exists in oilseed rape accessions or cultivars. The challenge is to develop a sensitive and reliable method to identify the resistance. Here the method we presented is reliable and sensitive, and useful for resistance and genetic research in a high throughput, inexpensive way. This method provides a technique to maintain an appropriate disease pressure that is lack in other methods of field resistance evaluation.

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