# Advance in research and control of diseases in oilseed rape in China

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**ABSTRACT**

Occurrence of all diseases in oilseed rape in China was to be introduced. Then advance in research and control of main disease, stem rot, caused by *Sclerotinia sclerotiorum* was reported. It includes development of identification methods, conventional breeding and biotechnological breeding for resistance to *S.sclerotiorum;* bacterial and fungal screening, genetic improvement and application for biocontrol of stem rot*;* epidemics and forecasting, and integrated control of stem rot.

**Key words**: Oilseed rape - Main diseases - *Sclerotinia sclerotiorum* - Research advance in China

## DISEASE OCCURRENCE AND ITS DISTRIBUTION

Oilseed rape grown in China is mainly *Brassica napus*, *B.rapa* and *B.juncea*. *B.napus* distributes in all oilseed rape growing regions and takes account of about 80% of total >8M ha planting areas, whereas *B.rapa* and *B.juncea* mainly distributes in Southwest and Northwest regions.

There are more than nineteen diseases appearing on oilseed rape: stem rot (*S.sclerotiorum* ), mosaics (TuMV, TMV, CMV), downy mildew (*Peronospora parasitica*), flowering but no pod setting (Boron deficiency), white blister (*Albugo candida*), leaf spot (*Alternaria brassicae, A. alternate, A. brassicicola and A. raphani*), black rot (*Xanthomonas campestris pv campestris*), soft rot (*Erwinia aroidea*), blackleg (*Phoma lingam)*, damping-off (*Pythium aphanidermatum*), wilt (*Rhizoctonia solani*), bacterial blackspot (*Pseudomonas maculicola*), club root (*Plasmodiophora brassicae*), white spot (*Cercosporella albomaculan*), powdery mildew (*Erisphe polygoni*), anthracnose (*Colletotrichum higginsianum* ), root gall smut (*Urocystis brassicae*), yellow wilt (*Fusarium oxysporum f.sp. conglutinans*), blast (*Thanatephorus cucumerts*). However, disease severity for each disease varied from one region to another. Of these diseases, stem rot, virus diseases and downy mildew are main diseases with more than ten percent of diseased plants, respectively, and more than ten percent of yield loss throughout the country. Stem rot alone caused more than ten percent of yield loss in Yangtze River valley, but yield loss may be up to 80 percent in some areas of the valley in the case of no fungicide application. Leaf spot is one of main diseases in the Northwest regions with more than eight percent of yield loss. Other diseases were severe epidemics only in the areas where weather was favorable to development of a specific disease. The symptom that appears as stunted growth at seedling stage and/or as flowering but no pod setting at flowering stage was caused by Boron deficiency, and the disease was rather severe in the past and now occurs only on Boron-sensitive cultivars or under the condition of long-term drought stress.

## ADVANCES IN SCLEROTINIA DISEASE STUDIES

### Studies on disease resistance to *Sclerotinia*

**Methods of screening for resistance:** Methods studied and used include those of inoculation with mycelia/ascospores or toxin, oxalic acid (OA) (Liu 1999). The former includes natural infection in fields, leaf inoculations with mycelia at the seeding stage, stem inoculations with mycelia at the flowering stage, detached leaf inoculations with mycelia, spray inoculation with ascospores at the flowering stage in greenhouse. The latter includes seedlings fed oxalate through the roots, detached leaves fed oxalate through leaf petioles and cell or callus screening by adding oxalate into media. These methods have been proved of being useful for different kinds of experiments. In our routine resistance identification, each material or variety or population was tested twice or three times using different methods, e.g. tests with OA at the seedling stage and then mycelium inoculation at later stages. However, none of all methods is enough satisfied in keeping the least variations or the homogeneity among plants of a homogenous population. Thus a population identified should have enough number of plants to be inoculated and generally experiments need to be designed in a randomized block form with several replicates.

**Resistance mechanisms and oxalate pathogenesis:** Information on resistance mechanisms is limited. Experiments with rape detached leaves and seedlings fed $^{14}C$-oxalate through petioles and roots, respectively, showed that resistant varieties limited the transportation of OA into intervein cells and the amount of OA absorbed by leaves was less in resistant varieties than in susceptible ones. More than 57% of OA absorbed was metabolized into other organic substances in a form of the higher rate in resistant varieties than in susceptible ones, revealing a mechanism of detoxification in seedlings (Liu *et al* 1998). Pathogen and OA inoculations induced increase of peroxidase and superoxide dismutase, and, in some case, glucosinolates (Li *et al* 1999), but relationship with polyphenol oxidase and phenalalanine ammonia-lysase might be complex and remains to be defined. By molecular marker analysis, some loci, responsible for certain components of glucosinolates, was probably linked with resistance (Zhao *et al*, 2003).

**Resistance characteristics and inheritance:** Investigations were done to characterize relation of resistance with other characters. The results showed that correlations were significant between resistance and each of cold tolerance, virus disease resistance, lodging resistance, stem hardness, branching type, flower size, flowering date, date of flower disappearance and maturation date. The evaluations of germplasm collections indicated that there is wide variation in these materials. The resistance is heritable. The experiments of genetics in different group showed that resistance to both the pathogen and OA was mainly controlled by additive and dominant genes. The broad and narrow heritabilities of resistance to the pathogen, in term of disease severity or lesion size, were up to 0.40 and 0.75 in diallel mating design experiments whereas those of resistance to OA were up to 0.42 and 0.78 (Liu 1999). Furthermore resistant parents generally had positive additive effect on resistance whereas dominant effects of resistant varieties varied in different crosses. Analysis of conditional variance suggested that inheritance of resistance to lesion spread was similar. In recently years, research on resistance molecular marker showed four to 16 loci associated with resistance. Zhao and Meng used 128-$F_{2:3}$ family analysis, and showed a total of 13 loci identified by one-way ANOVA and six QTLs detected with MapMaker-QTL (Zhao *et al*, 2003). We screened more than 700 random primers to develop SCAR and found a stable and distinct marker (619bp) of at lease four markers (Liu *et al*, unpublished data).

**Resistance improvement:** There were about 6000 rapeseed collections primarily identified for resistance. Resistance levels were defined as relative based on a control (different controls for *B.napus,B.rapa and B.juncea*) known as mid-resistance. The results showed that 60% of the collections was at low resistance/susceptibility levels, and about 10% was high resistance (Zhou *et al* 1994). These relatively high resistance collections were further tested and population-selected. By such selection, lines which resistance was much improved were ZhongRVS-1, Zhong R-888 and ZhongR-783. Resistance is one of main breeding objectives. Breeding methods include hybridization and single or population selection, recurrent selection, somatic mutation and gene transformation. There are a number of resistant varieties released such as Zhongyou821, Hua shuang No3 and ZhongshuangNo9. One fourth of planting area in China was Zhongyou821 during ten years of from 1988 to1998 mainly due to its resistance. Cell and gene engineering has become important approaches for resistance improvement. A successful example is *in vitro* mutation-selection at haploidy level via microspore culture (Liu 1999). Two lines which resistance was great improved were M083 and M004. Transgenes such as β-1,3-glucanase, chitinase and maize transposon Ac could increase resistance (Guo *et al*. 2001, Z.H.Chen, personal communication).

**Biological control of *Sclerotinia* disease**

**Mycoparasites against *Sclerotinia*:** Investigation of mycoparasites on sclerotia showed that a large number of fungi can parasitize sclerotia. However, most effective parasites are *Trichoderma viride, T. hazianum, T.hamatum, Gliocladium catenulatum and Coniothyrium minitans*. One strain of *T viride* was mutated of resistance to a fungicide, carbendazim. It had a stable performance in both lab and fields. During years of from 1989 to 1994, control effects in the 40 field trials were from 40% to 84% when the fungal formulations were applied into soil or sprayed (Zhou *et al*, 1994)

**Bacteria for biocontrol:** Bacteria with activity of degrading oxalic acid and PGPR function have been isolated from soil or stem rot lesions using selective media. These bacteria are mainly *Pseudomonas and Bacillus. P.alcaligenes* inhibited significantly mycelial growth and reduced disease severity by 40%-70% in past three year field trials in three locations. Treatment of rape seeds with *P.alcaligenes* increased seedling biomass by 36% 90 days after sowing. The bacterial strain could colonize in rape roots. A DNA fragment has been cloned and transformed into E.coli. The transformants had significant inhibition activity against *Sclerotinia* mycelium (Hu *et al* 2001).

**Epidemiology, forecast and Integrated control of Sclerotinia disease**

Epidemiological factors include effective rainfall (irrigation), variety resistance, plant development stage, amount of field inoculum, plant vigor, micro-environment, and temperature etc. These factors interacted each other. Of them, effective rainfall and variety resistance would be main factors in China. Mathematic models were established based on 30 year data for long-term disease forecast. Based on the long-term, mid-term and short-term forecast, disease decision-support systems were constructed to deploy resource and manage the disease (Zhou *et al* 1994). The application of the systems could control the disease up to 85% reduction.

## FUTURE PERSPECTIVES

In China, future research may focus on resistant improvement, biocontrol and effective disease forecast. Traditional breeding methods are less effective and modern technology will be more applied. Resistance evaluation methods still need improvement to meet requirement of precious evaluation. Gene improvement of biocontrol microbes would have its bright future. Molecular diagnostic techniques of disease will allow to more precious forecast of disease.

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