

Screening of Resistance Cultivar to Clubroot Caused by *Plasmodiophora brassicae* for Organic Cultivation of Chinese Cabbage

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We investigated the resistance of 50 commercial Chinese cabbage cultivars against clubroot disease caused by *Plasmodiophora brassicae* in the three difference fields, Suwon, Hwacheon, and Pyeongchang. Wilting symptom on Chinese cabbage was first observed at 15 days after transplanting in Pyeongchang and Hwacheon, while disease symptoms appeared later in Suwon after the rainy season. Among 50 cultivars, eight cultivars, SC26, SC29, SC30, SC31, SC34, SC46, SC47 and SC50 showed highly susceptible symptoms like wilting and heavy root galls in all three fields. Meanwhile, seven cultivars such as SC05, SC06, SC07, SC09, SC11, SC17, and SC36 showed moderate resistance with delayed wilting and few root galls. Only two cultivars, Chuwol (CB22) and Gohyangssam (CB23) were highly resistant to clubroot disease until the harvest season in all of the three fields. These two commercial cultivars may be considered as candidate cultivars for cultivation of organic Chinese cabbage in Suwon, Hwacheon, and Pyeongchang.

Keywords : Bioassay, *Brassica campestris* ssp. *pekinensis*, *Plasmodiophora brassicae*

The clubroot disease, caused by *Plasmodiophora brassicae*, brings serious yield loss, accounting for a 10–15% reduction in yield on a global scale in cruciferous crops including Chinese cabbage, radish, turnip, and cabbage. This is attributed to the increase in population density of the pathogen with their continuous monoculture in intensive production areas (Dixon, 2009; Kim et al., 2003; Yoshikawa, 1981).

In Korea, clubroot disease was first reported in the 1920s, and caused severe economic losses in many Brassica growing areas since the early 1990s. Recently, the disease become a more serious problem in monocropping and extensively cultivated areas including highland areas (Kim and Oh, 1997; Kim et al., 1999).

The obligate biotrophic clubroot pathogen, *Plasmodiophora brassicae*, shows a wide variability in pathogenicity and has an intricate interaction with its host in some parts of the infection process. The disease is easy to inadvertently spread to previously non-infected fields via infected transplanting materials. Also, the disease can be moved long distances, and foreign pathotypes can be introduced (Dixon, 2009; Kim et al., 2003).

To control the clubroot disease, various practical techniques have been tried such as resistant varieties, biological control agents, and crop rotation systems (Donald et al., 2006; Tremblay et al., 1999; Yeoung et al., 2003; Yoon et al., 2010). Chemical control is limited, since the few effective treatments are either banned or too expensive. Because the pathogen survives as resting spores for long time periods in the soil, it is hard to control the disease by cultural practice or agrochemicals (Donald et al., 2006).

Many applications of soil amendments like lime, egg shell, calcium and boron to decrease the pH of the soil were investigated to reduce disease pressure, but these treatments are often not sufficient to keep the crop healthy (Davis and Jones, 2002; Hamilton and Crête, 1978).

Since high-resistance resources have not been found among varieties of Chinese cabbage, several breeding programs have been successfully carried out to develop clubroot resistant (CR) cultivars of Chinese cabbage using CR European turnips (*Brassica rapa* ssp. *rapifera*) as sources of resistance (Hirai, 2006; Lee et al., 2001; Yoshikawa, 1981).

Hereditary clubroot resistance to cabbage crops has been reported but growing resistant *Brassica* cultivars is another widely used alternative (Hirai, 2006). The

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use of disease resistant varieties is one of the most convenient, inexpensive, and environment friendly ways to control plant disease, and plant breeders make extensive use of classically defined *R* genes (Pia et al., 2004; Yoshigawa, 1981).

However, these clubroot resistant (CR) hybrid cultivars have become susceptible to clubroot in some field trials (Pia et al., 2004). Moreover, only a small number of resistant cultivars have been released. Breeding programs aimed at the introduction of resistance to clubroot have been few and the results were often disappointing (Cho et al., 2002; Voorrips, 1995). Yoon et al. (2010) reported that among 34 cultivars and 19 lines of Chinese cabbage, three cultivars, namely, Daetong, Super CR Kimi 85 and Super CR Hiroki, were resistant to clubroot at 60 days after transplanting in the highland field contaminated with *P. brassicae* in Korea.

This study was carried out to screen resistant cultivars of Chinese cabbage against clubroot disease for safe production of organic Chinese cabbage.

We investigated the resistance of 50 commercial Chinese cabbage cultivars against clubroot disease caused by *P. brassicae* in the three difference fields, Suwon, Hwacheon, and Pyeongchang. The three open fields of Suwon, Hwacheon, and Pyeongchang are usually infected with natural inocula of *P. brassicae*. Legume hairy vetch (*Vicia villosa* Roth) was cultivated in Suwon, while rye grass (*Secale cereale*) was grown in Pyeongchang as green manure crops over the winter season from 2009 to 2011. The whole amount of green manure was thoroughly incorporated into the field soil before the addition of organic fertilizer nutrients. Organic fertilizer (2,500 kg/1000 m²) and mixed expeller cake (1,000 kg/1000 m²) were added in each field.

The physico-chemical characteristics of the three fields were analyzed using the standard soil analysis method. The level of P₂O₄ in the soil was determined by colorimeter. The contents of exchangeable cations K⁺, Na⁺, Ca²⁺ and Mg²⁺ were quantified by atomic absorption spectroscopy (Perkin-Elmer Atomic Absorption Spectrometer 3300, Ueberlingen, Germany). The electrical conductivity (EC) and pH were also determined in a soil to distilled water ratio of 1:5.

A total of 50 commercialized Chinese cabbage cultivars were purchased from ten seed companies such as Nongwoo (17), Danong (10), Hungnong (9), Sakada (4), Syngenta (2), Hannong (2), Jungang (2), Daenong (2), Nonghyup (1), and Asia (1). Those of Chinese cabbage cultivars were used to determine clubroot disease resistance in 2011. After disinfecting with 2% sodium hypochlorite (NaOCl), the seeds were germinated at 28°C in a saturated humidity chamber. The pre-germinated seeds were sown into 72 cells plastic seedling trays and grown for about 35 days in the greenhouse.

Fifteen replicates of 35-day-old Chinese cabbage seedlings per each cultivar were transplanted into the three fields naturally infested with clubroot pathogens. Disease severity was determined for 49 days with 7 days interval after transplanting. The disease severity of clubroot symptoms was assessed by assigning symptom grades based on visual inspection and measurement of healthy and diseased root tissues. Disease severity was classified into four categories according to the following criteria: resistant, no club symptoms; moderately resistant, less than 20.0% disease severity; susceptible, 20.1–66.7% disease severity; highly susceptible, more than 66.8% disease severity.

After treatment of green manure and soil amendments, the physico-chemical properties of the three field soils were determined in Table 1. The soil pH of the three fields was slightly increased for acidic soil after applying of Dolomite (20 kg/1000 m²) and egg shells (25 kg/1000 m²) (Table 1). The pH of the three fields was 5.1 (Pyeongchang), 6.2 (Suwon), and 6.5 (Hwacheon), respectively. The highest EC was found in Hwacheon (151.8 ds/m) followed by Pyeongchang (143.9 ds/m), and Suwon (99.29 ds/m). Concentration of organic material (OM) in the three fields ranged from 1.83% to 2.27%, with Suwon having the highest OM concentration among the three fields. All three open fields had comparably exchangeable cation concentration which was equivalent to what was found in the agriculturally controlled soil for Chinese cabbage. Davis and Jones (2002) reported that the organic amendments, straw and chitin significantly reduced clubroot symptoms compared to other amendments including unamended treatment.

Table 1. Soil characteristics of Chinese cabbage cultivation fields tested for screening of clubroot resistance under the presence of green manure

Location	Green manure		pH (1:5)	E.C (ds/m)	OM (%)	Av. P ₂ O ₅ (mg/kg)	Ex. Cation (cmol/kg)			
	2009	2010					K	Mg	Na	Ca
Suwon	Hairy Vetch	Hairy Vetch	6.2(±0.02)	99.29(±2.4)	2.27(±0.7)	686.5(±0.4)	1.0(±0.6)	2.6(±0.6)	0.1(±0.07)	8.2(±0.08)
Hwacheon	Rye grass	Rye grass	6.5(±0.04)	151.80(±9.1)	1.88(±0.2)	658.9(±0.8)	0.7(±0.1)	1.7(±0.1)	0.1(±0.08)	6.5(±0.05)
Pyeongchang	Rye grass	Rye grass	5.1(±0.02)	143.94(±4.1)	1.83(±0.6)	649.8(±0.4)	0.8(±0.3)	2.8(±0.7)	0.2(±0.08)	9.5(±0.03)



Fig. 1. Symptom grades of clubroot, resistant (A, SC22), moderately resistant (B, SC50), susceptible (C, SC48) and highly susceptible (D, SC50) of Chinese cabbage cultivars based on disease severity of clubroot caused by *Plasmodiophora brassicae* at 35 days after transplanting. HR: healthy root, CR: clubbed root.

Also, the effects are probably due to a combination of pH effects on the clubroot disease and nutrients effects on the Chinese cabbage.

Clubroot-infected Chinese cabbage plants are usually wilted and dwarfed compared to healthy plants. When infected plants are pulled out of the soil, the root system shows typical gall formation. At maturity, the galls turn brown and a large portion of the infected roots remains under ground when the plants are harvested (Fig. 1).

Among 50 Chinese cabbage cultivars, eight cultivars (SC26, SC29, Sc30, Sc31, SC34, SC36, SC47, and SC50) were first observed with wilting symptoms 15 days after transplanting in the Pyeongchang and Hwacheon areas. The cultivars showed disease severity of more than 66.8% and highly susceptible symptoms such as wilting and heavy root galls. Symptoms of wilting and root galls were observed later in Suwon after the rainy season (Table 2, 3).

The clubroot disease is difficult to control in Chinese cabbage cultivation because the pathogen survives in the soil for a long time as resting spores. For cultural control practices, crop rotation is the most frequently recommended management strategy. Crop rotation is an

effective management when combined with other treatments or in fields where *Brassica* crops have not been grown (Kim et al., 2003; Tremblay et al., 1999). The disease is especially serious in organic *Brassica* crops (Murakami et al., 2002). The resting spores of *P. brassicae* may remain capable of initiating new infections for seven years or longer in the absence of host plants, as well as a motile zoospore that can swim in wet soils. More than 97% of total populations of clubroot pathogens are distributed within the 5 cm depth of top soil (Kim et al., 2000).

Among the 50 cultivars of Chinese cabbage tested in this study, only nine cultivars showed low disease severity (0–20.0%) of clubroot 49 days after transplanting in the three open fields. Seven cultivars, SC05, SC06, SC07, SC09, SC11, SC17, and SC36, showed the moderate resistance scored as disease severity of less than 20.0% and delayed wilting symptoms and rare root galls. Only, two cultivars, Chuwol (CB22) and Gohyangssam (CB23) showed high resistance to clubroot disease until the harvest season in all of the three fields. These results indicate that two commercial cultivars may be useful candidate's cultivars for organic Chinese

Table 2. Clubroot disease severity of 50 commercialized Chinese cabbage cultivars in 2011

Cultivars	Severity of clubroot disease (%) ^a			Cultivars	Severity of clubroot disease (%)		
	Hwacheon	Suwon	Pyeongchang		Hwacheon	Suwon	Pyeongchang
SC01	20	20	40	SC26	100	66.7	100
SC02	13.3	0	26.7	SC27	0	40	20
SC03	13.3	13.3	40	SC28	13.3	0	26.7
SC04	20	0	20	SC29	100	66.7	100
SC05	20	20	13.3	SC30	100	66.7	100
SC06	20	13.3	20	SC31	100	26.7	80
SC07	13.3	13.3	20	SC32	53.3	13.3	66.7
SC08	100	46.7	53.3	SC33	53.3	13.3	66.7
SC09	6.7	13.3	20	SC34	100	26.7	100
SC10	33.3	33.3	66.7	SC35	80	66.7	20
SC11	13.3	6.7	20	SC36	6.7	6.7	20
SC12	53.3	33.3	80	SC37	13.3	0	33.3
SC13	66.7	40	80	SC38	66.7	40	100
SC14	13.3	26.7	40	SC39	6.7	20	33.3
SC15	33.3	6.7	66.7	SC40	26.7	33.3	80
SC16	33.3	6.7	80	SC41	26.7	53.3	93.3
SC17	6.7	13.3	20	SC42	66.7	53.3	100
SC18	33.3	20	100	SC43	66.7	20	100
SC19	46.7	13.3	100	SC44	33.3	20	40
SC20	33.3	33.3	20	SC45	33.3	46.7	53.3
SC21	40	20	26.7	SC46	80	53.3	86.7
SC22	0	0	0	SC47	100	46.7	80
SC23	0	0	0	SC48	66.7	46.7	66.7
SC24	60	13.3	100	SC49	66.7	46.7	66.7
SC25	26.7	0	80	SC50	100	66.7	100

^aThe disease severity of clubroot symptoms was rated by assigning symptoms based on visual inspection, as well as by measurements of healthy and diseased plants. The data was mean of 15 plants per cultivar.

Table 3. Grade of 50 commercialized Chinese cabbage cultivars based on the disease severity of clubroot in 2011

Clubroot resistance (Disease severity)	Chinese cabbage cultivars		
	Hwacheon	Suwon	Pyeongchang
Highly susceptible ($\geq 66.8\%$)	SC08, SC26, SC29, SC30, SC31, SC34, SC35, SC46, SC47, SC50	—	SC12, SC13, SC16, SC18, SC19, SC24, SC25, SC26, SC29, SC30, SC31, SC34, SC38, SC40, SC41, SC42, SC43, SC46, SC47, SC50
Susceptible (20.1–66.7%)	SC10, SC12, SC13, SC15, SC16, SC18, SC19, SC20, SC21, SC24, SC25, SC32, SC33, SC38, SC40, SC41, SC42, SC43, SC44, SC45, SC48, SC49	SC08, SC10, SC12, SC13, SC14, SC20, SC26, SC27, SC29, SC30, SC31, SC34, SC35, SC38, SC40, SC41, SC42, SC45, SC46, SC47, SC48, SC49, SC50	SC01, SC02, SC03, SC08, SC10, SC14, SC15, SC21, SC28, SC32, SC33, SC35, SC37, SC39, SC44, SC45, SC48, SC49
Moderate resistant (0.1–20.0%)	SC01, SC02, SC03, SC04, SC05, SC06, SC07, SC09, SC11, SC14, SC17, SC28, SC36, SC37, SC39	SC01, SC03, SC05, SC06, SC07, SC09, SC11, SC15, SC16, SC17, SC18, SC19, SC21, SC24, SC32, SC33, SC36, SC39, SC43, SC44	SC04, SC05, SC06, SC07, SC09, SC11, SC17, SC20, SC27, SC25, SC36
Resistant (0%)	SC22, SC23, SC27	SC02, SC04, SC22, SC23, SC25, SC28, SC37	SC22, SC23

cabbage cultivation and will be further tested *in vivo* (Table 2, 3).

The pathogenic diversity of clubroot disease is one of the best challenges to breed resistant Chinese cabbage varieties. As an example, if resistant Chinese cabbage varieties are grown continuously in the same field, they are likely to change into susceptible progenies (Cho et al., 2002). Also, the level of disease resistance observed depends not only on the combination of host and pathogen genotype but on environmental factors such as soil pH and moisture content and population density of the *P. brassicae* (Davies and Jones, 2002; Hamilton and Crête, 1978; Kim et al., 1999, 2000). In our results, we were able to select two commercial cultivars, 'Chuwol' and 'Gohyangssam', with a high level of resistance to *P. brassicae*. They scored 0% clubroot disease incidence, and showed no sign or symptoms of the disease in 49 days after transplanting in three open field. Yoon et al. (2010) reported that, among 53 Chinese cabbage cultivars and lines, several Chinese cabbage cultivars, including 'Gohyangssam', were found resistant to clubroot disease 60 days after transplanting. Also, Jo et al. (2010) reported that 25 CR Chinese cabbage cultivars, including 'Chuwol', were highly resistant to clubroot disease in 35 days after inoculation using soil-drenching method. CR resistant varieties of Chinese cabbage differently showed the resistant level, susceptible, moderate resistant, and resistant depending on the cultivated area and season (Kim et al., 2003).

We confirmed Chuwol and Gohyangssam as clubroot resistant cultivars in this study. Both cultivars are medium-size Chinese cabbage and have yellow inner leaves. While more genetic and epidemiological studies will still be conducted, we recommend these two selected resistant cultivars as candidates for organic Chinese cabbage cultivation.

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