Reprint **ISSN 1923-7766 (Web Version)**

International Journal of Experimental Agriculture

(Int. J. Expt. Agric.)

Volume: 5 **Issue: 2** **March 2015**

Int. J. Expt. Agric. 5(2): 5-14 (March 2015)

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M.A.U. DOULLAH AND K. OKAZAKI

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ISSN-1923-7766 (Online)
Int. J. Expt. Agric. 5(2):5-14(March 2015)

EVALUATION OF RESISTANCE TO DARK LEAF SPOT (*Alternaria brassicicola*) IN *Brassica rapa*

M.A.U. DOULLAH[1]* AND K. OKAZAKI[2]

[1]Associate Professor, Department of Plant Pathology and Seed Science, Sylhet Agricultural University, Sylhet-3100, Bangladesh;
[2]Professor, Department of Agrobiology, Niigata University, Niigata, Japan.

*Corresponding author & address: Dr. Md. Asad-ud-doullah, E-mail: asadmeli@gmail.com
Accepted for publication on 7 March 2015

ABSTRACT

Doullah MAU, Okazaki K (2015) Evaluation of resistance to dark leaf spot (*Alternaria brassicicola*) in *Brassica rapa. Int. J. Expt. Agric.* 5(2), 5-14.

Dark leaf spot (*Alternaria brassicicola*) is an important disease of brassica crops especially in *Brassica rapa*. A total of 281 cultivars of *B. rapa* including *B. napus* and *B. juncea* were screened in two different years by detached leaf and seedling symptom test methods. Two cultivars 'Edononatsu' and 'Saori' exhibited high levels of tolerance in both the years following the two inoculation methods, whereas some cultivars displayed borderline resistance and most of them were susceptible to the disease. A significant correlation (r = 0.52) found in disease severity between two years by detached leaf inoculation test. Cultivars identified as tolerant would be useful in breeding program to develop strains against dark leaf spot disease.

Key words: *screening, disease resistance, dark leaf spot, Alternaria brassicicola, Brassica rapa*

INTRODUCTION

B. rapa (syn. *B. campestris*) L. (AA, n = 10) belongs to family Brassicaceae is widely distributed as green vegetables, root crops and oilseed crops throughout Europe and Asia. Cultivated *B. rapa* appears to have multiple origins i.e., root vegetables derived from biennial ecotypes in western Europe; oil seed crops developed from annual ecotypes in Irano-Turanian region, and foliar vegetables developed in China, from introduced oilseeds (Westman 1998).

The crops suffer from various diseases such as fungal, bacterial, viral and nematode. Alternaria spot is an important and severe seed-borne fungal disease of Brassicas worldwide (King 1994; Rotem 1998; Meah *et al*. 2002). The disease can appear to all cultivated crucifers during any stage of the crop development. Typical symptoms of the disease are dark brown spot on leaf, stem and siliquae, which begin as small brown or black spots that usually enlarge into concentric circles in later stage. Furthermore, they often show chlorotic margins which can reduce the photosynthetic capacity of the plant and lead the premature senescence of the leaves (King 1994; Doullah 2006).

Three different *Alternaria* species *viz. A. brassicicola* (Schw.) Wilt., *A. brassicae* (Beak) Sacc. and *A. raphani* Groves & Skolko are involved in dark spot disease. Above all, *A. brassicicola* is the most virulent and usually predominant among them in commercial seeds (Schimmer 1953; Changris and Weber, 1963; Humpherson-Jons 1985; Richardson 1990; King 1994; Rotem 1998). Neergaard (1945) reported that 90% of various Brassicaceae seeds commercially available were infected by the disease. Eighty eight percent of seed lots produced in the UK (Humpherson-Jones, 1985) and 8% of commercial *B. rapa* seeds in Japan were found to be infected (Tohyama *et al.* 1991). Yield loss up to 30% has been reported in Bangladesh (Howlider *et al.* 1985, Doullah *et al.* 2009) and 35-40% in India (Vishwanath and Kolte, 1999).

The fungus is propagated by asexual spores (conidia) produced in chains (Plate 1). They survive as mycelium or conidia on seeds and in crop debris. Superficial contamination of the seed by the pathogens has been shown to persist for up to two years and internally the fungus can survive for at least 12 years (King 1994). The pathogen can be transmitted to descendants by direct infection to developing seeds in siliquae causing severe damage in seedlings, especially in nursery (Kubota *et al.* 2003).

Dark leaf spot is very difficult to control due to numerous sources of inoculum and wide range of spore dispersal (King 1994). Jasalavich *et al.* (1993) reported that the degree of susceptibility varies among *Brassica* species. Tewari and Mithen (1999) reported that nearly all commercial brassicas are susceptible to *A. brassicicola* and *A. brassicae* and also showed that *Brassica rapa* and *B. juncea* are more susceptible to *A. brassicae* than *B. napus* and *B. carinata*. Resistance to *A. brassicicola* has also been identified by screening genotypes of *B. napus* and *B. oleracea* (King 1994).

Naturally evolved resistance systems should provide a promising and readily acceptable means of control. Utilization of host resistance has been recognized as one of the most economic and effective control measures (Su *et al.* 2005). Plant genetic resources are the base for the improvement of crop varieties. Breeding for disease resistance can control crop losses cost-effectively, with minimum effort of growers and in an environmentally benign manner. For these reasons, our present study aimed to screen resistant genotypes of *B. rapa* against *A. brassicicola.*

MATERIALS AND METHODS

Plant material

Genotypes of *Brassica rapa* used in this study were collected from Japanese seed companies, Gene Bank of the

National Institute of Agrobiological Resources, Japan and Bangladesh Agricultural Research Institute, Bangladesh (Table 2). Plants were grown from seeds in soil according to Doullah *et al.* (2006) where 10-cell plastic trays (cell size: 5 cm × 5 cm) were used in a greenhouse (25°C/15°C day/night cycle) and liquid nutrient (N: P_2O_5: K_2O, 0.5:1.0:0.5 g/l; Hyponex, Osaka, Japan) was periodically supplied for plant growth.

Fungal isolates

Alternaria brassicicola isolate Akakura obtained from Doullah *et al.* (2006) was used. Conidia of a 10-day old grown on PDA medium was used for artificial inoculation (Plate 1). The concentration of the conidial suspension at 5×10^4 conidia/ml was adjusted for inoculation.

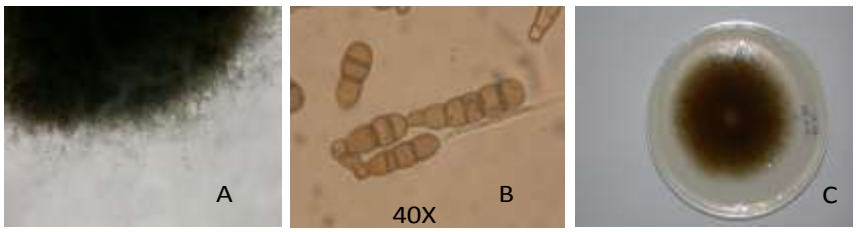

Plate 1. A conidial chains of *Alternaria brassicicola*, B. Conidia of *A. brassicicola*, C. Culture of *A. brassicicola*

Inoculation methods

Detached leaf inoculation test and seedling symptom test method as described by Doullah *et al.* (2006) was used where detached leaves and plants were scored using disease severity index ranged from 1 to10 score (Table 1 and Plate 2).

Table 1. Disease severity rating scale/grade of dark leaf spot disease caused by *Alternaria brassicicola* (Doullah *et al.* 2006)

Disease severity index	Symptoms produced on leaf
1	No spots and no yellow color on the leaf
2	A few pinpoint spots but no yellow
3	Some spots but no large lesions and no yellow
4	Some spots with a few lesions surrounded by light yellow
5-9	Increasing number and size of lesions and yellowing on the leaf
10	Lesions with yellowing on more than 90% of the leaf

Note: Disease severity index: 1–3 Highly resistant, 3.1–4 - Borderline resistant, 4.1–10 Susceptible

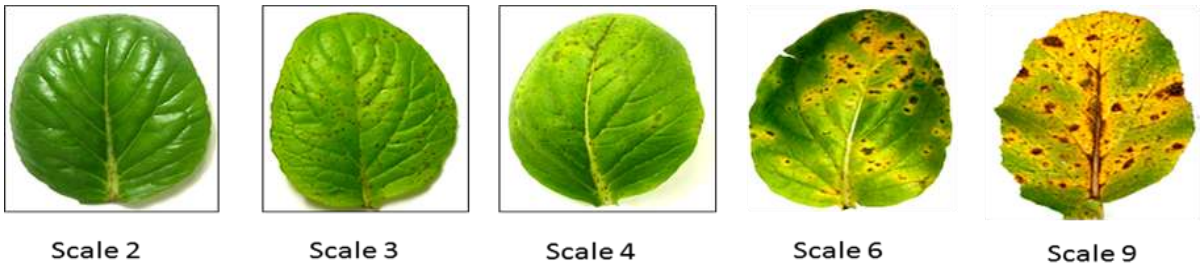

Plate 2. An example of disease severity index of dark leaf spot disease caused by *Alternaria brassicicola* (Doullah 2006)

Evaluation for resistance

A total of 281 cultivars were screened by detached leaf inoculation test in year 2004. In year 2005, 131 cultivars from the total were re-screened by detached leaf and seedling symptom test methods (Table 2 and Table 3). Correlation for disease development between two years was also determined using detached leaf inoculation methods (Fig. 1).

Statistical analysis

The experiment was arranged in a completely randomized design with three replicates (three leaves/plants per replicate) maintaining appropriate controls. Data from the experiments were subjected to analysis of variance (ANOVA) using statistics computer software (ESUMI Co. Ltd., Japan). Coefficient of correlation analysis was used to evaluate relationships using Microsoft Excel.

RESULTS AND DISCUSSION

A total of 281 *B. rapa* cultivars were screened for resistance to *A. brassicicola* using detached leaf inoculation test in 2004 and randomly selected 131 cultivars (including all types of resistance) in 2005 using detached leaf

inoculation and seedling symptom test according to Doullah *et al*. (2006). Significant differences in resistance were found among these cultivars (Table 2). In year 2004, 21 cultivars showed tolerance and 42 cultivars showed moderate tolerance to the disease. In year 2005, 4 cultivars remained tolerant and 8 displayed moderate tolerance by detached leaf test. Three cultivars were tolerant and 12 cultivars were moderately tolerant to *A. brassicicola* by seedling symptom test (Table 2 & 3). In Brassica species, leaves contain anti-pathogenic substances such as glucosinolates (Meah *et al*. 1988; Milford *et al.* 1989) which make the plant resistant. The total glucosinolate content varies in leaves between cultivars of rape seeds (Ishida *et al*. 2003). This might be the reason for variation of different degree of disease reaction among the genotypes screened. Two cultivars, 'Edononatsu' and 'Saori', were found to be tolerant in both years by the two inoculation methods used (Table 2). Doullah *et al*. (2006) also found Saori and Edononatsu, as partial resistant cultivar to *A. brassicicola* in his preliminary screening test. The detached leaf test has been recommended as the primary screening test to limit the number of *B. rapa* accessions required for the field test to assess dark leaf spot caused by *A. brassicicola* because, the results from the detached leaf test are more consistent under standardized physical conditions (Doullah 2006). For this reason, coefficient of correlation analysis was done using 131 genotypes to evaluate relationships between two years' results using detached leaf test. A significant correlation (r = 0.52) was found between two years (Fig. 1) for disease development by detached leaf inoculation method.

Previously, no *B. rapa* cultivars had been identified with complete resistance to dark leaf spot caused by *A. brassicicola.* In screening for resistant cultivars, 3rd/4th true leaves were collected from 30-day old plants because Doullah *et al*. (2006) found that these leaves showed disease reaction among *B. rapa* genotypes in a reliable manner in their study. Dueck and Degenhardt (1975) suggested that older leaves were more susceptible to Alternaria diseases. Variation in disease severity within all leaf position might be due to the age of leaves. Doullah *et al.* (2006) also clearly demonstrated that older leaves are more susceptible to the disease. Young leaves are less susceptible to the infection compared with aged leaves because *A. brassicicola* has an opportunistic parasitic behavior which requires a weakened plant or plant tissue for infection (Doullah *et al.* 2006). As a result, we identified cultivars 'Edononatsu' and 'Saori' as highly resistant to *A. brassicicola* in both years (as tested by Doullah *et al*. 2006 in his preliminary test) and some other cultivars eg. 'TokinashiTaisai', 'YajimaKabu', 'Purara', 'Norin-F_1-bekana', 'TateiwaKabu' and so on (Table 2) were identified as borderline resistant. Although these cultivars could potentially be used in breeding program to develop strains for dark leaf spot resistance, the highly resistant cultivars became borderline resistant in the inoculation test using relatively older leaves. Thus, it will be necessary to examine the usefulness of these resistance levels under field conditions or in nursery beds where large numbers of seedlings grow under high humidity conducive to dark leaf spot disease.

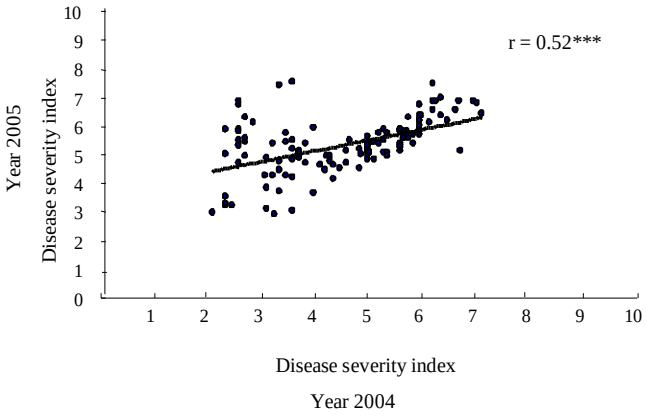

Fig. 1. Correlation between disease severity in two years 2004 and 2005 by detached leaf inoculation test. Asterisks (***) indicate significant correlation at the 0.1% level

Table 2. Mean disease severity index of 281 *Brassica rapa* genotypes using two *Alternaria brassicicola* inoculation methods in two years

Origin[1]	Cultivar	Average diseaseseverity index[2]		
		1st Year	2nd Year	
		Detached[3]	Detached	Seedling[3]
Nih	Tuyazakikomatsuna	2.38	4.95	6.22
Nih	**Edononatsu**	**2.13**	**2.89**	**2.22**
Nih	CR Kanki No. 100	2.75	5.39	6.11
Nih	KokeshiKabu	3.75	-	-
Nih	Norin F_1nekana	2.38	3.17	3.44
Mu	Honnkonnsaishin	2.63	6.67	5.33
Mu	Aishinbeni 1 Gobenikabu	6.00	5.61	4.89
Mu	Harunoutahakusai	2.63	5.22	5.33
Mu	Natsumaki 13 Goukokabu	5.63	5.72	4.89
Mu	Hakutakakokabu	4.38	4.56	6.00
Mu	NatsushomiAokou	4.38	-	-
Ish	Cr Seiga 75	4.38	-	-
Ish	Gakuyou	8.25	-	-
Ish	Reihou	7.13	-	-
Ish	Matsunami	8.38	-	-
Ish	Kinami 90	2.63	4.65	5.34
Ish	Touga	3.38	4.39	5.33
Ish	Cr Seiga 95	2.75	5.50	6.00
Ya	Kiyorokobi	2.63	5.44	4.78
Ya	Eigyoku 65	2.63	5.27	5.45
Ya	Eikei	2.75	4.89	5.33
Ya	Tyoraku	4.00	-	-
Ya	Makomana	3.63	4.11	4.56
To	Ayumi	3.50	5.39	5.56
To	**Saori**	**2.50**	**2.84**	**3.00**
To	Bisai	3.38	4.67	3.44
To	Shironoyuki	5.13	-	-
To	Fukutoku	3.88	5.28	4.22
Sa	Yukikaze	3.63	7.45	4.33
Sa	Kuromizuki	5.00	5.17	4.89
Sa	Hanakazaki	5.75	5.72	5.33
Sa	CR Kyo Fu	4.50	-	-
No	Utage	2.88	6.06	4.22
No	Harumaki 1 go	4.63	-	-
No	Nozaki 123	2.75	6.22	4.78
No	Mochina	5.11	5.39	4.89
No	Ootakana	4.88	5.11	5.78
Wa	Wakazishi	4.63	-	-
Wa	CR Kyouyou	3.38	7.33	4.78
Wa	crTamachan	4.38	-	-
Wa	Maruchan	4.67	5.45	5.67
Wa	Niihao 4 go	5.13	5.39	4.55
Wa	Seiichiro	3.63	5.11	3.56
Ma	CR Kisaku 80	3.13	-	-
Ma	Rakusai	3.38	3.61	4.11
Ma	CR Kyounoharu	2.63	5.78	5.11
Ma	Tsugaru beni	5.88	5.61	3.78
Ma	CR Azikurabe	5.25	-	-

Cont. Table 2

Origin[1]	Cultivar	Average diseaseseverity index[2]		
		Year 2004	Year 2005	
		Detached[3]	Detached	Seedling[3]
Tok	Hanae	5.63	5.06	5.44
Tok	Tamae	6.38	6.28	5.78
Tok	CR Kaiou	4.63	-	-
Tok	CR Saitaikai	7.00	6.78	6.11
Tok	Yuziro	3.63	5.44	5.11
Tok	Tokiziro	4.38	-	-
Tok	Sharaku	3.50	4.22	3.89
Ta	Osome	5.63	5.17	5.78
Ta	Shoten	3.25	4.22	3.00
Ta	Gokurakuten	3.50	5.67	5.11
Ta	Taibyohikari	5.67	5.72	5.33
Ta	Suwan	5.75	5.61	5.90
Ta	Harue	6.75	5.06	4.22
Fu	Koaki	2.38	3.22	3.33
Fu	Kimuchiasu	6.22	7.39	6.78
Nan	Harupinnatsu	2.63	6.78	6.00
Nan	Mana	4.25	-	-
Nan	Medaka	2.63	5.72	5.33
Nan	Fantomu 85	7.05	6.72	6.44
Nan	Aporo 60	6.72	6.78	6.45
Nan	Shirafuzi	5.38	5.00	4.89
Ni	Purara	3.11	3.17	3.00
Ni	Yoko	4.63	-	-
Ni	Kounan	5.00	5.06	4.56
Tak	Harumaki you to	5.78	6.22	5.89
Ka	Kuronami	2.38	3.44	3.89
Ka	Fuzishiro	3.88	4.61	5.22
Ka	Kiryoko 70	6.28	6.78	5.67
Ka	Ougetsu 77	6.38	6.94	6.22
Ka	Irodori	5.50	-	-
Asa	Marubasantousai	3.63	-	-
Asa	Yukishirotaisai	4.13	-	-
Asa	Nikanmokutaisai	5.00	5.33	4.45
Asa	Osakashirona	5.63	5.78	5.56
Asa	Banseikomatsuna	3.75	5.06	4.89
Asa	Banseishirokukisenkin	3.38	-	-
Asa	Marubamibuna	3.13	3.78	4.45
Asa	ShigatsuShirona	5.00	5.56	5.33
Asa	Hiroshimana	3.38	-	-
Asa	Nozawana (S-2)	6.00	5.83	5.33
Asa	ShinseimiikeTakana	4.00	-	-
Asa	Hakarashina	4.38	-	-
Asa	Kikarashina	6.18	6.06	4.67
Asa	ShokuyoNanohana	3.63	-	-
Asa	Bitaminna	4.75	-	-
Asa	Hakuchoi	4.00	-	-
Asa	Saishin	5.61	5.78	5.56
Asa	Kousaitai	6.00	6.11	5.44
Asa	Taasai	4.38	-	-
Asa	Katsuona	5.25	-	-
Asa	Odakana	4.75	-	-

Cont. Table 2

Origin[1]	Cultivar	Average diseaseseverity index[2]		
		Year 2004	Year 2005	
		Detached[3]	Detached	Seedling[3]
Asa	Michiru	3.13	4.83	4.56
Asa	Asahi kounigohakusai	3.38	-	-
Asa	Houfuhaksai	6.22	6.78	4.22
Asa	Gokunaseashihakusai	3.00	-	-
Asa	Sho ho	4.25	-	-
Asa	A-1 Gona	4.00	5.89	5.34
Wa	Kikou 85	5.98	6.28	5.78
Wa	Winta	6.67	6.50	5.33
Wa	Sutato 60	6.22	6.50	6.00
GB 25846	Wasena	2.38	5.83	4.78
GB 25847	Inakashu	4.63	-	-
GB 25848	Shakushina	6.00	6.11	5.11
GB 25849	Mie Zairai	5.63	-	-
GB 25850	HakusuiZairai	5.38	-	-
GB 25851	Oukabura	5.63	-	-
GB 25852	WaseNatane	4.63	-	-
GB 25854	Tottori Zairai	4.88	-	-
GB 25855	Motoippon	5.06	5.00	5.00
GB 25856	Chabo	5.38	5.67	5.67
GB 25857	Kato Zairai	5.33	5.00	5.11
GB 25860	WaseAkana	5.75	-	-
GB 25863	Wasena-1	6.25	-	-
GB 25864	Miyagi Wase	4.63	-	-
GB 25865	WaseZairai	6.00	5.78	6.34
GB 25866	Wasena-2	7.25	-	-
GB 25867	AkatsukaWase	6.25	-	-
GB 25868	Ishikawa Zairai	5.88	5.33	4.89
GB 25869	Yokkaichi Akadane	7.50	-	-
GB 25870	Hatana 4	5.63	-	-
GB 25871	HikiZairai	5.75	-	-
GB 25872	YashuuZairai	5.50	-	-
GB 25873	KumaZairai	3.75	-	-
GB 25874	Yokkaichi Marubashu	6.01	6.28	5.78
GB 25875	NishikinoZairai	3.25	5.33	4.78
GB 25876	Saga Zairai	4.25	4.89	5.56
GB 25877	Nagasaki Zairai	4.13	-	-
GB 25878	Hokkai	7.00	-	-
GB 25881	EnumaZairai	5.63	-	-
GB 25883	IyohiKabu	5.13	-	-
GB 25892	Washu 1	4.38	-	-
GB 26129	Sendai Bashouna	5.38	-	-
GB 26130	Ooba Takana	5.25	-	-
GB 26131	Karashina	5.38	-	-
GB 26132	Ha Karashina	5.33	5.78	5.33
GB 26133	Ki Karashina	6.63	-	-
GB 26134	Yamashiona	5.00	5.28	5.33
GB 26135	Kairyoukatsuona	7.63	-	-
GB 26136	Kumamoto Karashina	5.50	-	-
GB 26137	Okinawa Karashina	5.63	5.33	5.78
GB 90743	Unknown	5.13	-	-
GB 26797	Kanazawa Aomaru	5.38	-	-
GB 26799	Kohime	5.13	-	-
GB 26800	KoiwaiKabu	5.88	-	-
GB 26801	Shimofusakabu	3.63	4.78	5.78
GB 26802	Hinokuchi	3.75	5.00	5.00
GB 26803	SomeyaKanamachi	5.63	-	-

Cont. Table 2

Origin[1]	Cultivar	Average disease severity index[2]		
		Year 2004	Year 2005	
		Detached[3]	Detached	Seedling[3]
GB 26804	Kamo	6.25	-	-
GB 26805	Hakabu	5.88	-	-
GB 26808	Natsu Yutaka	5.63	-	-
GB 26809	Yutaka 2	5.25	-	-
GB 26810	WaseKanamachi	4.89	4.95	5.78
GB 26812	HonbeniOomaru	3.63		-
GB 26813	Oonaga	6.67	6.50	5.45
GB 26814	Azuma Chuukabu	5.63	-	-
GB 26815	Shiromine	5.88	-	-
GB 26816	Hakata Koroge	4.63	-	-
GB 26817	Yonago Aka Kabu	5.50	-	-
GB 26818	KisobeniKabu	6.00	6.67	6.00
GB 26819	Sapporo murasaki	4.33	4.89	5.33
GB 26820	OonoBeni	5.50	-	-
GB 26821	Yukijirushi kairyou	5.00	4.95	5.00
GB 26822	Shin OonoBeni	4.38	-	-
GB 26823	ToyosatoKabu	6.50	-	-
GB 26824	TateiwaKabu	4.00	3.61	4.56
GB 26825	OchiboKabu	4.38	-	-
GB 26826	Zaruishi	3.13	3.06	3.44
GB 26827	Atsumi	4.22	4.39	4.45
GB 26828	Yorii	5.75	-	-
GB 26829	Koukei 2	4.25	-	-
GB 26843	Kanazawa AoKabu	5.63	-	-
GB 26844	Yamauchi	5.11	5.39	4.45
GB 26845	OonoBeni	5.00	-	-
GB 26846	KodakariKabu	5.63	-	-
GB 26847	Kidaao	4.63	-	-
GB 26849	Tokyo Naga	6.75	-	-
GB 26850	Kisobeni	6.25	-	-
GB 26851	Habirona	6.00	-	-
GB 26852	KanamachiKokabu	4.88	-	-
GB 26853	Tokyo Naga Kabu	5.38	-	-
GB 26854	GensukeKabu	5.00	-	-
GB 26855	Yoshino Kabu	6.38	-	-
GB 26856	KisobeniKabu	6.50	6.11	6.44
GB 26857	Inekokina	6.13	-	-
GB 26859	Fukushima Na	5.63	-	-
GB 26861	Narusawa Na	3.75	4.83	5.56
GB 26862	HidaBeni	5.38	-	-
GB 26863	Owari	5.63	5.22	6.33
GB 26864	OwariKabu	5.38	-	-
GB 26866	Hinona	6.38	-	-
GB 26867	Tennouji	5.00	5.11	4.56
GB 26868	ShougoinOomaru	5.38	-	-
GB 26869	Sugukina	5.23	4.83	4.44
GB 26870	TennoujiKabu	4.25	5.28	4.67
GB 26871	TogariKabu	3.75	-	-
GB 26872	HikoneKabu	5.63	-	-
GB 26873	Honbeni Aka Daikon	5.88	-	-
GB 26874	Yajimakabu	3.28	2.83	3.22
GB 26876	MarubaTennouji	4.00	-	-
GB 26878	ImaichiKabu	5.00	-	-
GB 26879	Oouchi	5.00	-	-
GB 25968	Oukana	3.75	-	-
GB 25969	Wakana	4.33	4.67	4.22

Cont. Table 2

Origin[1]	Cultivar	Average disease severity index[2]		
		Year 2004	Year 2005	
		Detached[3]	Detached	Seedling[3]
GB 25970	GokuwaseNatanena	4.63	5.06	5.78
GB 25972	Sangatsuna	6.75	-	-
GB 25973	Wakana	5.33	5.44	4.44
GB 26061	TokinashiTaisai	3.63	2.95	3.67
GB 26062	Sendai Yukina	5.67	5.50	5.00
GB 26063	Nagaokana	5.63	-	-
GB 26064	Yayoi Komatsuna	5.78	5.50	4.67
GB 26065	UzukiKomatsuna	6.63	-	-
GB 26066	NikanmeYukijiroTaisai	6.00	-	-
GB 26067	YukijiroTaisai	-	4.56	4.22
GB 26068	ShinbanseiAburana	4.88	4.44	3.67
GB 26069	Bitamin Na	5.88	-	-
GB 26070	Irakabu	5.00	4.78	4.89
GB 26086	KuroMizuna	6.00	-	-
GB 26087	SensujiKyouna	6.63	-	-
GB 26089	OkuteSensujiMizuna	4.38	4.06	4.89
GB 26090	WaseMibuna	4.63	4.61	4.11
GB 26091	OkuteMibuna	5.00	5.22	4.55
GB 26092	WaseSensujiKyoumizuna	5.38	4.89	3.67
GB 26687	ShimoyamaChitose	7.00	-	-
GB 26688	Matsushima Shin 2	6.00	-	-
GB 26689	BanseiTougou	4.50	4.44	4.67
GB 26690	4 N Nozaki	6.13	-	-
GB 26691	Nozaki 2	5.00	5.22	5.33
GB 26692	Nozaki Harumaki 1	6.00	5.95	5.67
GB 26693	Marubasantousai	6.50	-	-
GB 26694	NishiaraiSantousai	6.88	-	-
GB 26695	Gokuwase Asahi	5.75	-	-
GB 26696	Aichi	6.25	-	-
GB 26697	Kintai	6.13	-	-
GB 26698	Senryou	5.67	5.50	4.67
GB 26699	Kairyou Kyoto	7.75	-	-
GB 26700	KyouChitose	6.50	-	-
GB 26701	Chitose	6.00	-	-
GB 26702	GokuwaseChiifu	5.63	-	-
GB 26703	Matsushima Oogata 2	6.63	-	-
GB 26705	Wase Kanazawa	6.00	-	-
GB 26706	Kyoto 3	6.75	-	-
GB 26707	KairyouShinsei	7.88	-	-
GB 26708	Kaga	5.50	-	-
GB 26709	Kogane	5.22	5.67	5.22
GB 26710	HankekkyuuSantou	6.25	-	-
GB 26711	KuriharaSantou	6.13	-	-
GB 26712	OogataShiodome	5.13	4.78	4.55
GB 26713	Kyoto 2	6.38	-	-
GB 26714	HagoromoKashinSantou	7.25	-	-
GB 26715	Kashin	7.25	-	-
GB 26716	Mikawa	7.00	-	-
GB 26717	HankekkyuuSantousai	6.88	-	-
GB 26718	ChirimenHakusai	5.88	-	-
GB 26719	Hiratsuka	5.00	5.06	4.55
GB 26720	KuriharaKashin	5.06	5.00	5.11
GB 26721	KairyouChitoseHakusai	6.13	-	-
GB 26722	Minato Bekana	6.13	-	-
GB 26723	Kashinsantou	5.50	-	-
Hok	Nozawana (S-2)	7.13	6.34	6.11

Cont. Table 2

Origin[1]	Cultivar	Average disease severity index[2]		
		Year 2004	Year 2005	
		Detached[3]	Detached	Seedling[3]
Unknown	ShikizakiHanana	5.75	-	-
Kob	AkakuraKabu	-	7.22	6.78
Kyo	Satomaru	-	7.97	7.33
Ta	ShirokukiSeusuzi	-	4.90	5.22
Hok	NikanmeTaisai	-	6.22	7.99
BARI	BARI 12	6.13	-	-
BARI	BARI 9	6.13	-	-
BARI	BARI 8	6.63	-	-
BARI	BARI 6	7.00	-	-
BARI	SS-75	4.38	-	-
BARI	TS-72	5.88	-	-
BARI	Tori-7	5.13	-	-
BARI	BARI 11	4.88	-	-
BARI	Doulat	5.13	-	-
	LSD[4] (p = 0.05)	0.16	0.16	0.16

[1] To = Tohuku Seed Co., Japan; Nih = Nihonnorin Seed Co., Japan; Ni = Nitto Seed Co., Japan; Ta = Takii Seed Co., Japan; Ish = Ishii Seed Co., Japan; Ka = Kaneko Seed Co., Japan;Hok = Hokuetsu Seed Co., Japan; Kob = Kobayashi Seed Co., Japan; Kyo = Kyowa Seed Co., Japan; Sa = Sakata Seed Co., Japan;Tak = Takayamu Seed Co., Japan; Tok = Tokita Seed Co., Japan; Ya = Yamata Seed Co., Japan; Asa = Asahinouen Seed Co., Japan; Ma = Marutane Seed Co., Japan; Mu = Musashi Seed Co., Japan; Nan = Nanto Seed Co., Japan; No = Nojaki Seed Co., Japan; Fu = Fukudane Seed Co., Japan; Wa = Watanabe Seed Co., Japan; GB = Gene Bank of National Institute of Agrobiological Resources, Japan and BARI = Bangladesh Agricultural Research Institute, Bangladesh.

[2] Cultivars with a disease severity index of 1–3 are classified as resistant, those having an index of 3.1–4 are borderline resistant, and those having an index of 4.1–10 are classified as susceptible to the disease.

[3] Detached: Detached leaf inoculation test; Seedling: Seedling inoculation test.

[4] LSD (p = 0.05): Least Significant Difference.

Table 3. Screening result of 281 vegetable and oil seed *Brassica rapa* cultivars including *B. napus and B. juncea*

Year	Inoculation methods	
	Detached leaf inoculation test	Seedling inoculation test
Year 2004		
Resistant (1.0-2.0 grade)	21	-
Borderline resistant (3.1-4.0 grade)	42	-
Susceptible (4.1-7.0 grade)	195	-
Highly susceptible (7.1-10.0 grade)	23	-
Total	281	-
Year 2005		
Resistant (1.0-2.0 grade)	5	3
Borderline resistant (3.1-4.0 grade)	7	13
Susceptible (4.1-7.0 grade)	110	111
Highly susceptible (7.1-10.0 grade)	5	-
Total	127	127

CONCLUSION

A detail screening test was done against dark leaf spot disease caused by *Alternaria brassicicola* in *Brassica rapa* crops. Two cultivars “Edononatsu” and “Saori” showed high level of tolerance in both the years following two different inoculation methods while some other cultivars displayed different levels of disease severity against the disease.

ACKNOWLEDGEMENT

This work was supported by Ministry of Education, Culture, Sports, Science and Technology, Government of Japan (Monbukagakusho-MEXT).

REFERENCES

Changris W, Weber GF (1963) Three *Alternaria* species pathogenic on certain cultivated crucifers. Phytopath. 53, 643-648.

Doullah MAU (2006) Screening and analysis of resistance to dark spot (*Alternaria brassicicola*) and black rot (*Xanthomonas campestris* pv. *campestris*) diseases in brassica crops. Ph. D. thesis, Niigata University, Niigata, Japan. pp. 127.

Doullah MAU, Meah MB, Mohsin GM, Hassan A, Ikeda T, Hori H, Okazaki K (2009) Evaluation of resistance *in Brassica rapa* to dark pod spot (*Alternaria brassicicola*) using the *in vitro* detached pod assay. SABRAO *J. Breed. Genet*. 41,101-113.

Doullah MAU, Meah MB, Okazaki K (2006) Development of an effective screening method for partial resistance to *Alternaria brassicicola* (dark leaf spot) in *Brassica rapa*. *European J. Plant Path*. 116, 33-43.

Dueck J, Degenhardt K (1975) Effect of leaf age and inoculum concentration on reaction of oilseed *Brassica* spp. to *Alternaria brassicae*. (Abstract) Proceedings of the American Phytopathol.Soc. 2, 59.

Howlider MAR, Meah MB, Jalaluddin M, Rahman MA (1985) Effect of fungicides in reducing intensity of Alternaria blight of mustard. *Bangladesh J. Agric. Sci*. 10, 41-46.

Humpherson-Jones FM (1985) The incidence of *Alternaria* spp. and *Leptosphaeria maculans* in commercial brassica seed in United Kingdom. Plant Path. 34, 385-390.

Ishida M, Takahata Y, Kaizuma N (2003) Simple and rapid method for the selection of individual rapeseed plants low in glucosinolates. Breed. Sci. 53, 291-296.

Jasalavich CA, Seguin-Swartz G, Vogelgsang S, Petrie GA (1993) Host range of *Alternaria* species pathogenic to crucifers. *Canadian J. Plant Path.*15, 314-315.

King SR (1994) Screening, selection, and genetics of resistance to *Alternaria* diseases in *Brassica oleracea*. Ph.D. Dissertation, Cornell University, Ithaca, New York.

Kubota M, Abiko K, Nishi K (2003) Effect of cultivation conditions of cabbage plugs on sooty spot disease. Bulletin National Ins. Vege. Tea Sci., Japan 2, 1-8.

Meah MB, Hau B, Siddique MK (2002) Relationships between disease parameters of *Alternaria* blight (*Alternaria brassicae*) and yield of mustard. *J. Plant Dis. Prot*. 3, 243-251.

Meah MB, Howlider MAR, Alam MK (1988) Effect of fungicide spray at different times and frequencies on *Alternaria* blight of mustard. *Thai J. of Agril. Sci*. 21, 101-107.

Milford GFJ, Fieldsend JK, Porter AJR, Rawlinson CJ (1989) Changes in glucosinolate concentrations during the vegetative growth of single and double low cultivars of winter oilseed rape. Aspects Appl. Bio. 23, 83-90.

Neergaard P (1945) I. Alternaria. Danish species of Alternaria and Stemphylium. Einar Munksgaard, Cophenhagen, Denmark. 87-232.

Richardson MR (1990) An annotated list of seed-borne diseases, 3rd ed. CAB International Mycological Institute, Kew, Survey, England and ISTA, Zurich, Switzerland.

Rotem J (1998) The Genus *Alternaria*: Biology, Epidemiology, and Pathology. APS Press, the American Phytopath. Society, Minnesota, USA, pp. 326.

Schimmer FC (1953) *Alternaria brassicicola* on summer cauliflower seed. Plant Path. 2, 16-17.

Su CC, Wan J, Zhai HQ, Wang CM, Sun LH, Yasui H, Yoshimura A (2005) A new locus for resistance to brown plant hopper identified in the *indica* rice variety DV85. Plant Breed. 124, 93-95.

Tewari JP, Mithen RF (1999) Diseases. *In:* Gomez-Campo C (ed.) Biology of Brassica coenospecies. Elsevier, Amsterdam, The Netherlands.

Tohyama, Akira, Keisuke K, Seung HY, Mitsuya T (1991) 1. *Alternaria* on cruciferous plants. 2. *Alternaria* species on seed of Chinese radish and their pathogenicity. Transaction of the Mycological Society of Japan 32, 247-258.

Vishwanath, Kolte SJ (1999) Methods of inoculation for resistance to *Alternaria* blight of rape seed and mustard. *J. Mycol. Plant Path*. 29, 96-99.

Westman AL (1998) Molecular marker variation and disease reaction to *Alternaria brassicicola* and *Xanthomonas campestris* pv. *Campestris* in *Brassica nigra* and other crucifer species. Ph.D. thesis, Cornell University, Ithaca, New York, 159 pp.