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A Baiting Technique for Assay of *Rhizoctonia solani* in Kale Nurseries

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ABSTRACT

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A baiting technique using the cabbage-seed colonization method provided a rapid, sensitive and accurate means of assessing inoculum potential and population of *Rhizoctonia solani* in the soil of kale nursery. The most of *R. solani* from soils was recovered when forty cabbage seeds were evenly distributed in 100-g soil with 12–15% (w/w) water content and incubated for 48 hr at 24 C. The cabbage-seed colonization method was more effective than the multiple-pellet soil sampler method to detect small population of *R. solani* in soils of kale nursery and gave a significant correlation (r = 0.83–0.96, $P < 0.001$) between the amount of inoculum and disease severity of kale seedlings.

Key words: baiting technique, the cabbage-seed colonization method, *Rhizoctonia solani.*

INTRODUCTION

In Taiwan, seedling damping-off caused by *Rhizoctonia solani* Kühn is a serious problem on kale (*Brassica alboglabra* Bailey) in nursery beds. This disease has been found in all major growing areas during summer season since 1990 but is more prevalent in nurseries with a history of continuous kale cropping.

The fungus is difficult to be isolated by means of the usual dilution plate methods (1,9) because it existed in soil as mycelial and sclerotial forms mainly. It is therefore necessary to use some other selective or trapping methods for assaying *R. solani* in soil. Many methods, including plant stem segment colonization (2,6,10), immersion tube colonization (2), debris particle isolation (2), disk-plate method (5) and seed colonization (8), have been used to assay the inoculum potential of the fungus in soils.

In preliminary trials, it was found that the cabbage seeds (*B. oleracea* L. var. *capitata* L.) were colonized by *R. solani* soon after sowing into soil. The objective of the present research is therefore to develop a standardized method for bioassay of *R. solani* in kale nursery soils.

MATERIALS AND METHODS

Soils

The soil was collected from a vegetable growing area at Tali, Taichung. It had a pH of 6.4 and a composition of 84.5% sand, 10.4% clay, 5.1% silt and 1.2% organic matter. It was sieved through a 10-mesh (2-mm) screen and stored at 6% (w/w) water content in polyethylene bags.

Inoculum

R. solani AG-4 isolate RSS-02 was recovered from damping-off seedling of cauliflower (*Brassica oleracea* L. var. *botrytis* L.) and isolates RST-01 and RST-04 were isolated from Chinese kale (*B. alboglabra* Bailey). Their anastomosis groups were determined by pairing with a tester isolate F-8 (supplied by Dr. C. C. Tu & Mr. Y. C. Chang). All isolates of *R. solani* were maintained on potato-dextrose agar (PDA). For soil inoculation, chopped potato-soil inoculum was prepared (7). Infested soils were prepared by mixing 1:100 ratio of inoculum and soil disinfested with hot air (60 C, 30 min).

Assay of inoculum level

The amount of inoculum present in soil was estimated by a cabbage-seed colonization method. Forty cabbage seeds were evenly distributed in 100-g soil with 12–15% (w/w) water content in a 200-ml beaker and covered with a parafilm. After 48-hr incubation at 24 C, seeds were recovered and washed for 3 min with running tap water in a colander. Excess water on seeds was blotted with paper towels. Seeds were then plated on 2% water agar containing 300 μg/ml streptomycin sulfate using 20 seeds/plate. The number of seeds with emerging *R. solani* was determined microscopically after 20–24 hr at 24 C. Cruciferous variety, soil temperature, soil moisture and the length of the incubation period affecting the recovery of *R. solani* (RSS-02, RST-01, and RST-04) in seed baits

were evaluated. Six varieties of crucifers, *i.e.*, cauliflower, cabbage, kale, Pe-tasi [*B. pekinensis* (Lour.) Rupr.], Pak-choi (*B. chinensis* L.), and radish (*Raphanus sativus* L.), incubation temperatures of 12, 16, 20, 24, 28, 32 and 36 C, soil moistures of 2, 6, 9, 12, 15, 18, and 21% (w/w) water content, and incubation periods of 0, 12, 24, 36, 48 and 60 hr were compared to establish a standard procedure for seed colonization by *R. solani*.

Comparison with multiple-pellet soil sampler method

Inoculum density of *R. solani* in infested soil was diluted with soil disinfested with hot air to achieve 7 ratios (1:0, 1:2, 1:10, 1:20, 1:100, 1:1000, and 0:1) of infested to total soil, then the soil was moistened to about 15% (w/w) and placed on *Rhizoctonia* selective medium (7) with a multiple-pellet soil sampler (4). For each inoculum density, fifteen 120- to 130-mg of soil pellets were deposited on the selective medium in each of six 9-cm-diameter petri dishes. Petri dishes containing soil were incubated in the dark at 24 C for 24–48 hr and colonies of the fungi were counted. Four replicate experiments were conducted to determine recovery percentage of *R. solani* (RST-01, RST-04 & RSS-02) in the seven inoculum levels. Each inoculum concentration was also sampled with the cabbage-seed colonization method. Then, a recovery efficiency was compared between the two methods.

Seedling disease assay and field soil assay

Seedling infection was determined by planting surface-sterilized seeds of kale in the infested soils in pots (10-cm diameter). Ten seeds were planted in each of four replicates and the plants were maintained for 25 days in the greenhouse at 28–32 C. Disease severity was expressed as percentages of seedlings with pre-emergence and post-emergence damping-off. The effectiveness of the cabbage-seed colonization for monitoring naturally infested soil was evaluated in 12 soil samples from six nursery beds in Tali and Taichung with kale seedlings with and without damping-off. One hundred and twenty cabbage seeds were used for each soil sample. Statistical analyses were run on the SAS/STAT system for personal computers (SAS Institute Inc., Cary, NC27511, USA). The identity of the pathogen was verified by reisolating the fungus from diseased plants on water agar and confirming its features microscopically.

RESULTS

Factors affecting seed colonization

Cruciferous varieties: At the end of 2 days incubation, the seeds were removed, washed, plated out and examined as previously described. Cabbage and pe-tasi had the highest percentage of colonization by *R. solani*. Cauliflower and kale were intermediate and radish was comparatively poorly colonized by RST-01, RST-04, RSS-02 (Table 1). Radish was commonly colonized by *Mucor* and *Fusarium* contaminants. Therefore, cabbage seed was chosen as the most suitable bait for *Rhizoctonia* assay from soils in this study.

Soil temperatures: The best recovery of *R. solani* (AG-4) occurred at 24 C (Fig. 1). At 12–16 C and 32–36 C, *R. solani* (AG-4) apparently grew very slowly and less frequently colonized cabbage seeds during the 2-day incubation period. The relationship of soil temperature (X) with cabbage seeds colonized by *R. solani* (Y) was quadratic in infested soil. The equations were $\hat{Y} = -180.1 + 21.1\ X - 0.43\ X^2$ ($r = 0.98$, $P < 0.001$) for RST-01, $\hat{Y} = -230.0 + 23.4\ X - 0.47\ X^2$ ($r = 0.99$, $P < 0.001$) for RST-04, and $\hat{Y} = -263.7 + 28.9\ X - 0.60\ X^2$ ($r = 0.97$, $P < 0.001$) for RSS-02, respectively.

Soil moistures: Best colonization of the cabbage seeds by *R. solani* (AG-4) (RST-01, RST-04, and RSS-02) was obtained in the soil water content (WC) between 12 and

TABLE 1. Comparative efficiency of six cruciferous seeds for baiting *Rhizoctonia solani* (AG-4) (isolates RST-01, RST-04, and RSS-02)

Seeds	Colonization (%)		
	RST-01	RST-04	RSS-02
Cauliflower	66 a[1]	60 b	80 b
Cabbage	66 a	77 a	96 a
Kale	57 ab	59 b	88 b
Pe-tasi	61 a	74 a	92 a
Pak-choi	46 c	60 b	67 c
Radish	50 bc	45 c	62 c

1. Means ($n = 6$) in the same column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

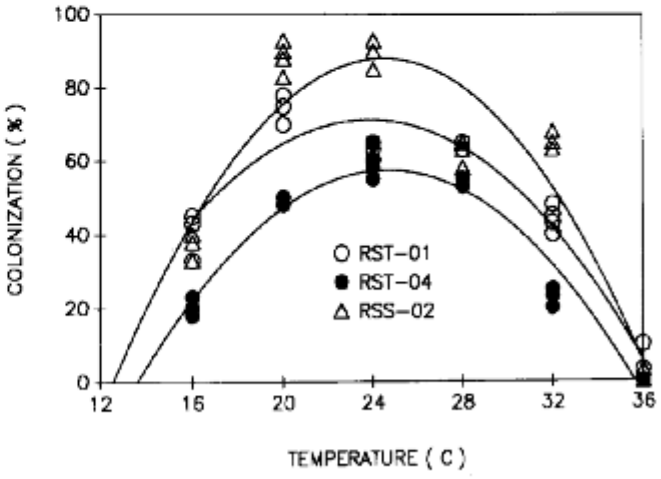

Fig. 1. Effect of temperatures on colonization of cabbage seeds by isolates RST-01, RST-04, and RSS-02 of *Rhizoctonia solani* (AG-4).

15%. Less frequency of colonization by *R. solani* occurred when soil moisture was adjusted to below 9% or above 18% (Fig. 2). The relationship of soil moisture (X) with cabbage seeds colonized by *R. solani* (Y) was quadratic in infested soil. The equations were $\hat{Y} = -13.2 + 11.6\ X - 0.45\ X^2$ ($r = 0.95$, $P < 0.001$) for RST-01, $\hat{Y} = -24.1 + 13.8\ X - 0.56\ X^2$ ($r = 0.97$, $P < 0.001$) for RST-04, and $\hat{Y} = -32.4 + 19.6\ X - 0.8\ X^2$ ($r = 0.97$, $P < 0.001$) for RSS-02, respectively.

Incubation period: A high frequency of cabbage seed colonization by *R. solani* (AG-4) with relatively little competition from other organisms occurred at 36–60 hrs (Fig. 3). A stable and maximum colonization was reached after 48-hr incubation. The relationship of incubation period (X) with cabbage seeds colonized by *R. solani* (Y) was quadratic in infested soil. The equations were $\hat{Y} = -1.68 + 1.65\ X - 0.02\ X^2$ ($r = 0.98$, $P < 0.001$) for RST-01, $\hat{Y} = 2.42 + 2.61\ X - 0.03\ X^2$ ($r = 0.99$, $P < 0.001$) for RST-04, $\hat{Y} = 3.11 + 3.15\ X - 0.03\ X^2$ ($r = 0.99$, $P < 0.001$) for RSS-02, respectively.

Comparison between the cabbage-seed colonization and multiple-pellet soil sampler methods

The cabbage-seed colonization method was more effective than the multiple-pellet soil sampler method to detect small population of *R. solani* (AG-4) in the infested soils (Table 2). When inoculum level of RST-04 and RSS-02 was at 1:100 (potato-soil inoculum: hot air-disinfested soil), *R. solani* was not recovered by the multiple-pellet soil sampler method. However, by the cabbage-seed colonization method 8–15% of the pathogen were recovered.

Relationship between the amount of cabbage-seed colonization and the percent damping-off of kale seedlings

An increase in *R. solani* (AG-4) infestation in soil enhanced both the colonization activity of this fungus and their pathogenic effect on kale seedlings (Table 3). There was significant correlation ($r = 0.83$–0.96, $P < 0.001$) between the percent damping-off and the percentage of cabbage seeds colonized.

Application of cabbage-seed colonization technique to naturally infested soil

The cabbage-seed colonization technique was used to monitor the survival of *R. solani* in six nursery beds in Tali and Taichung. About 15–25% of cabbage seeds were colonized by *R. solani* (AG-4) in soil where damping-off of kale seedlings occurred. However, *R. solani* was not recovered in soil from beds where damping-off symptoms were lacking.

DISCUSSION

A particular crop seed susceptible to certain *Rhizoctonia* strains may be used to measure them in the soil (2,8). Pan (8) reported that beet seed was a very useful bait for trapping *R. solani* but beet seed is not a common crop in Taiwan. In our study, we found cabbage seed could be used in place of beet seed and was found to be the best bait among six cruciferous seeds (Table 1) for detecting *R. solani* in soils.

Using infection of kale seedlings to monitor biocontrol or chemical control requires considerable space in greenhouse. Furthermore, seedling infection requires a longer exposure period than cabbage-seed baiting. Therefore, cabbage-seed colonization was exploited to solve these two problems before research was extended to the greenhouse and the field. The bioassay with cabbage seeds is a convenient and efficient method for detecting the inoculum potential of *R. solani* in kale nursery soils.

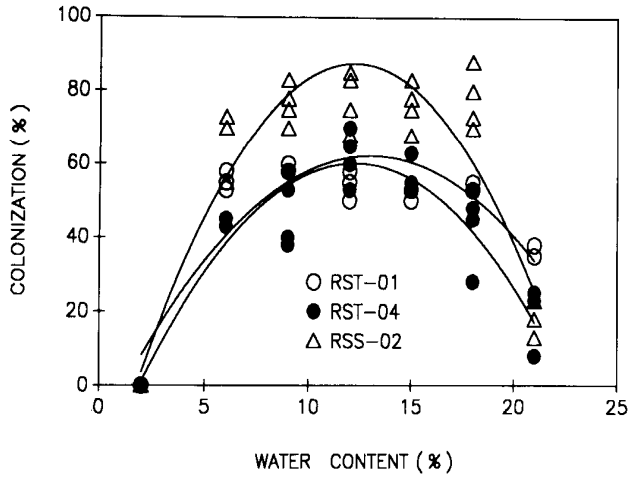

Fig. 2. Effect of soil moistures on colonization of cabbage seeds by isolates RST-01, RST-04, and RSS-02 of *Rhizoctonia solani* (AG-4).

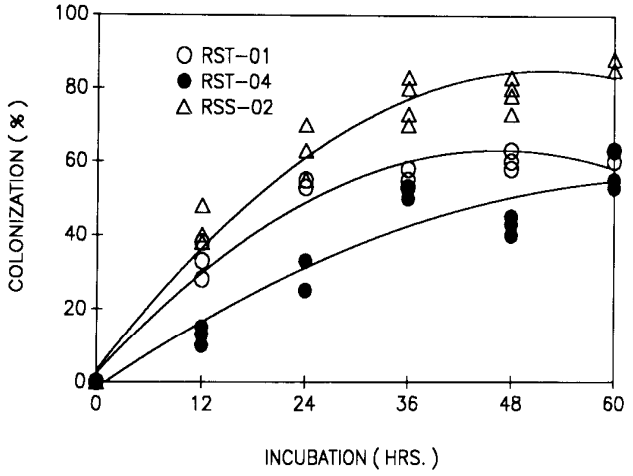

Fig. 3. Colonization of *Rhizoctonia solani* (AG-4) (isolates RST-01, RST-04, and RSS-02) on cabbage seeds after different incubation periods at 24 C.

TABLE 2. Sensitivity of the cabbage-seed colonization and multiple-pellet soil sampler methods for recovery of *Rhizoctonia solani* (AG-4) (isolates RST-01, 04 and RSS-02) from soils infested with different inoculum density of each isolate at 24 C

Inoculum level[1]	*Rhizoctonia* recovered (% of seeds or samples)					
	RST-01		RST-04		RSS-02	
	SCM[2]	MPSM	SCM	MPSM	SCM	MPSM
1:0	100[3]	48	98	48	100	52
1:2	95	48	98	45	100	38
1:10	63	52	60	32	85	8
1:20	40	28	30	27	50	3
1:100	10	13	8	0	15	0
1:1000	5	0	0	0	8	0
0:1	0	0	0	0	0	0

1. Ratio of potato-soil inoculum to soil disinfested with hot air (60 C, 30 min).
2. SCM: the cabbage-seed colonization method; MPSM: the multiple-pellet soil sampler method.
3. Mean values were obtained from four replicates with 40 seeds each.

TABLE 3. Colonization of cabbage seeds by *Rhizoctonia solani* compared with damping-off of kale seedlings in soil with various concentrations of inoculum of *Rhizoctonia solani* (AG-4) (RST-01, 04, and RSS-02)

Inoculum level[1]	RST-01		RST-04		RSS-02	
	Seed colonization (%)	Seedling[2] damping-off (%)	Seed colonization (%)	Seedling damping-off (%)	Seed colonization (%)	Seedling damping-off (%)
1:0	100	100	100	96	100	100
1:2	98	100	97	96	100	100
1:10	64	93	63	80	87	85
1:20	41	90	30	59	53	65
1:100	10	64	7	56	18	44
1:1000	6	4	0	2	4	4
0:1	0	0	0	0	0	0
Correlation between seed colonization (X) and seedling damping-Off (Y)	$Y = 24.74 + 0.87\ X$ ($r = 0.83$, $P < 0.001$)		$Y = 25.35 + 0.76\ X$ ($r = 0.85$, $P < 0.001$)		$Y = 9.25 + 0.92\ X$ ($r = 0.96$, $P < 0.001$)	

1. Ratio of potato-soil inoculum to soil disinfested with hot air (60 C, 30 min).
2. Includes preemergence and postemergence damping-off.

The multiple-pellet soil sampler method was reported to be both accurate and convenient for estimating the quantity of *R. solani* in soil (4). The sensitivity of the cabbage-seed colonization method to detect the presence of *R. solani* was compared with that of the multiple-pellet soil sampler method. Results (Table 2) indicate that the cabbage-seed baiting was able to detect lower levels of *R. solani* (AG-4) than the pellet soil sampler. The detectable level of propagules of *R. solani* in the soil by the latter method is limited by the number of replicates, by the size of the pellets (4) and by the selectivity of the growth medium (6). Many soil microorganisms are competitive saprophytes (3) and a prolongation of the incubation period allows them to physically obscure colonies of *R. solani* on the assay plates (8). Growth of microorganisms other than *R. solani* on the medium used by Ko and Hora (7) cannot be eliminated completely, and delaying the recording of *R. solani* growth beyond 24 hr allows some microorganisms such as *Trichoderma* and *Fusarium* to overgrow *R. solani*, making quantitative assessment difficult (2). The cabbage-seed baiting technique, on the other hand, limits competitive saprophytic pressure of other fungi and thus has an advantage over the multiple-pellet soil sampler method.

Parallel determinations of colonization of cabbage seeds and disease severity of *R. solani* (AG-4) on kale seedlings at various inoculum concentrations in soil indicate a high degree of correlation between the amount

of damping-off and the percentage of cabbage seeds colonized. These results suggest that the cabbage-seed colonization method can be widely used in both ecological studies and measurements of fungicide effectivenes on *R. solani* (AG-4) in field soils.

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摘　　要

黃振文、楊尚勳. 1992. 芥藍苗床立枯絲核病菌的偵測技術. 植病會刊 1:26-30. (台中市　國立中興大學植物病理學系)

芥藍幼苗立枯病主要是由立枯絲核病菌 (*Rhizoctonia solani*) (AG-4)所引起。在土壤中，十字花科蔬菜種子被本菌纏據 (colonization)之百分率，以甘藍種子最佳且穩定，其次爲包心白菜、花椰菜與芥藍等種子。由土壤偵測本菌的方法是：1)利用燒杯盛裝100公克之帶菌土壤後，隨即調整土壤水分含量爲12–15% (w/w)，2)把40粒甘藍種子均勻與土壤拌合，並在24 C培育48 hr，3)從土壤中回收種子並以清水漂洗後，放在2% 水瓊脂(含300 ppm鏈黴素)平板上，4)在24 C經20–24 hr，即可檢視 *R. solani* (AG-4)纏據種子的百分率。在七種不同菌量密度的土壤中，*R. solani* (AG-4)纏據甘藍種子的百分率與芥藍立枯病的發生率間呈直線迴歸正相關 (r = 0.83–0.96, $P < 0.001$)。這種甘藍種子的誘釣法，可快速、敏感且準確的預估 *R. solani* (AG-4)在芥藍苗床的菌體密度與爲害芥藍的百分率。

關鍵字：偵測技術、芥藍種子誘釣法、立枯絲核菌 (*Rhizoctonia solani*)。