

水稻台中私10號不同生育期對引起淡黃化型白葉枯病之菌株反應¹

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摘 要

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西元 2002 年，在農試所試驗農場進行兩期作水稻臺中私 10 號不同生育期對 2 株分離自葉枯型病株和 3 株分離自淡黃化型病株之白葉枯病菌菌株的反應試驗，分別在插秧時、分蘖期和孕穗期等三個生育期進行接種，插秧時幼苗分別以剪葉及浸根方式接種，分蘖期和孕穗期則以剪葉及噴霧方式接種。結果發現兩期作水稻於插秧時採浸根接種，除第二期作以葉枯型病徵分離之菌株 XM 30 接種後無枯死病株外，其餘處理均可出現 kressek 病徵，剪葉接種則均出現葉枯型病徵，但未見淡黃化型病株。兩期稻作於抽穗時再調查三個生育期接種之發病情形，結果顯示插秧時以浸根與剪葉接種處理均未出現葉枯型病徵，剪葉接種之幼株雖都呈現葉枯病徵，但當罹病葉枯死後，即不再產生葉枯型之新病葉；在分蘖期和孕穗期各以剪葉與噴霧接種處理時，所有菌株於兩期作都出現感病性葉枯型病徵。其中由淡黃化型病株分離之 3 株白葉枯病菌菌株，除第一期作插秧時之剪葉處理未產生黃化株外，其餘在兩期作插秧時和分蘖期之各二種接種處理，至抽穗期調查時都產生淡黃化型病徵；然而，兩期作的孕穗期經噴霧與剪葉處理時，均未出現淡黃化病株。另外，供試之 2 株分離自葉枯型白葉枯病株之菌株，以任何方式接種，均未能在三個生育期造成淡黃化型病徵，顯示菌株間之病原性存在差異性。綜合上述結果顯示，水稻早期於插秧和分蘖期感染才容易產生淡黃化型病徵，至孕穗期感染即不顯現該病徵。kressek 和淡黃化型病徵皆屬於系統性病害，幼苗會產生 kressek，成株則出現淡黃化型。另外，第一期作插秧時低溫接種會引起 kressek 急速萎凋型及淡黃化型病徵，因此推論高溫並非此二型病害發生之必要條件。

關鍵詞：水稻、生育期、白葉枯病、淡黃化型病徵、急速萎凋型病徵、溫度。

前 言

由 *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings *et al.* 所引起的水稻白葉枯病分別有葉片呈典型條狀波浪病斑之葉枯型 (leaf blight)、急速萎凋型 (kressek) 及葉片淡黃化型 (pale yellow leaves) 等三種病徵 (Hsieh 2003; Mew 1987; Ou 1985)。早在 1951 年即由橋岡氏記錄臺灣水稻出現葉枯型白葉枯病 (Hashioka 1951)，而自 1960 年臺中在來 1 號品種推出後，此病害即成爲水稻重

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要病害之一，並以二期作發病較為嚴重。1950 年印尼首先發現“kresek”急性萎凋型病徵 (Reitsma & Schure 1950)，而臺灣則於 1974 年二期作，因推廣嘉農秈 11 號和嘉農秈選 8 號 (Hsieh 2003)，造成中南部地區嚴重發生“kresek”病害。1978 年謝氏報告指出苗期、感病品種、高菌量和高溫等，是發生此病徵型之重要因子 (Hsieh 1978)。直到 1997 年二期作之臺中秈 10 號，首次發現淡黃化型病徵，全葉淡黃化，導致抽穗不良，甚至全株枯死，遂造成彰化縣大村及鄰近鄉鎮地區的重大損失。2000 年在宜蘭地區水稻一期作亦發現該病害，中南部地區第二期作也普遍發生。

有關 *X. oryzae* pv. *oryzae* 引起淡黃化型白葉枯病之致病機制不明，目前尚未有定論。Ou 氏認為係白葉枯病菌在水稻莖基部聚集繁殖至一定濃度，使新葉因養份缺乏而呈現黃化 (Ou 1985)。Mew 氏則認為病原細菌若在木質細胞增殖，將因水份輸導作用受阻而呈現葉片急速萎凋的“kresek”病徵，但淡黃化型病葉並無萎凋，推測葉片黃化缺葉綠素的現象，可能係因細菌產生毒質而造成反光合作用的結果 (Mew 1987)。

淡黃化型病害主要發生於熱帶水稻栽培區，目前世界上有關此病徵型病害的研究甚少，本研究旨在探討水稻在何種生育期被病原侵染後較易出現淡黃化型病徵，並瞭解白葉枯病菌菌株間是否有差異性存在。

材料與方法

供試菌株與水稻種植

自宜蘭採集淡黃化型病株，截取病葉葉鞘近基部之莖節，先 1% 次氯酸鈉表面消毒 3min 一次和無菌水漂洗三次後，以滅過菌之研鉢加無菌水磨細，再以移植環 (transferring loop) 沾其汁液在 Wakimoto's medium (WM) (Ou 1985) 平板劃線，純化分離後依柯霍氏法則 (Koch's postulates) 測定其病原性，再從中選取 3 株菌株 XB 26、XB 28 和 XB 30 供試。另取稻作病害研究室原保存，且自臺中和屏東採集之 2 株葉枯型病株所分離之白葉枯病菌菌株 XF 89-b 和 XM 30，共計 5 菌株進行測試。

田間試驗於 2002 年第一期與第二期作在農試所試驗農場 37 號田區，分別於插秧時、分蘖期和孕穗期等三個不同生長期，各以二種接種方式進行接種。供試稻品種為臺中秈 10 號，採完全區集設計 (Complete Block Design)，每一生長期每一接種處理各三重複，每重複栽植 64 叢 (8 行 × 8 行)，株距 22.5 × 22.5 cm。施肥量均以每十公畝基肥為氮肥 30 kg，磷肥 30 kg，鉀肥 10 kg 的量計算，共分二次施用，栽植期間並依一般稻作栽培方式管理。

不同時期病原菌之接種

取上述 5 株供試菌株，於接種前以 WM 固體培養基更新移植，活化後移入 WM 液體培養基振盪培養 72 hrs，並以無菌水調整其濃度約在 10^{7-8} cfu/mL 作為供試接種源 (Liao & Chien 1982)。

插秧時之秧苗，第一期作為播種後三週，第二期作為播種後二週，分剪葉和浸根二種方式接種，即插秧前秧苗分別先依剪葉法做剪葉接種 (Kauffman *et al.* 1973)，或秧苗先以細菌菌液浸根接種後隨即進行插秧。分蘖期及孕穗期則分剪葉和噴霧二種方式接種，即稻株各依剪葉法做剪葉接種處理 (Hsieh *et al.* 2005)，或以細菌菌液噴佈心葉接種，直至菌液完全佈滿葉片。並以 WM 液體培養基當對照接種處理。每一生長期每一接種處理 64 稻叢，三重複共 192 叢稻株。再各於接種後 30 天依下列方法調查罹病情形。

病害調查方法

針對急性萎凋型病害係調查病株枯死率 (%)；淡黃化型病害為記錄淡黃化莖數；葉枯型病害則係依菲律賓國際稻米研究所 (IRRI) 所訂葉發病面積佔全葉面積之百分率為其罹病指數之判定依據，無病斑為 0 級，發病面積率 1% 以下訂為 1 級，1-5% 為 3 級，6-25% 為 5 級，26-50% 為 7 級，51-100% 為 9 級 (International Rice Testing Program 1980)。

不同生長期對不同菌株反應之分析

比較在不同生長期接種不同病徵型所分離之菌株時，水稻呈現白葉枯病病徵反應之異同，並分析最適產生淡黃化型病徵之生長期及菌株。

結 果

插秧時分剪葉和浸根接種處理。其中剪葉接種處理，經 30 天後，發現 5 株白葉枯病菌株 XB 26、XB 28、XB 30、XF 89-b 和 XM 30 均可使水稻出現感病性葉枯型病徵，其罹病度在第一期作分別為 9、7、7、7 和 3 級，第二期作 5 菌株都達 9 級；浸根接種處理，於接種 30 天後，均未發生葉枯型病徵，但有造成急性萎凋，5 菌株引起病株之枯死率，第一期作分別為 55.4、41.5、45.7、35.5 和 0.3%，第二期作各為 41.9、26.1、19.1、30.7 和 0%。兩期作之二種接種處理，經 30 天後均未出現淡黃化型病徵 (表 1)。於抽穗期再調查時，苗期之剪葉和浸根處理，兩期作中 5 菌株接種區水稻都未發現葉枯型病徵 (表 2)，其中 3 株自淡黃化型病株分離之菌株 XB 26、XB 28、XB 30 接種，除第一期作之剪葉處理不產生淡黃化型病徵外，其餘各處理區均會出現，即第一期作浸根處理區，3 株分離自淡黃化型病株之菌株分別使水稻產生 48、30、70 枝淡黃化莖數，第二期作之剪葉和浸根處理區分別得 2、3、7 和 15、36、30 莖淡黃化株 (表 3)。

分蘖期行剪葉和噴霧接種處理。經 30 天後調查發現，5 菌株均引起水稻出現感病性葉枯型病徵，第一期作罹病度分別為 9、6、7、7、5 級和 7、5、7、5、3 級，第二期作各為 9、9、9、9、5 級和 6、5、7、7、3 級 (表 2)。其中 3 株自淡黃化型病株分離之菌株都可引起水稻產生淡黃化型病徵，剪葉和噴霧處理於第一期作各得淡黃化莖數 120、24、61 和 73、2、59 枝，第二期作各得 51、36、47 和 7、11、25 枝；另 2 株葉枯型菌株於兩期作三個生長期之各二種處理接種區，則均未出現淡黃化株 (表 3)。

孕穗期亦分剪葉和噴霧接種處理。經 30 天後調查，發現 5 菌株均可造成感病性葉枯型病徵，其罹病度於第一期作分別為 7、5、5、6、3 級和 6、6、6、5、0 級，第二期作分別為 7、9、9、9、5 級和 6、5、5、5、3 級 (表 2)。但此二種接種處理，5 菌株都未能使水稻產生淡黃化病徵 (表 3)。對照組以 WM 營養液處理，三個時期剪葉結果都未出現任何白葉枯病病徵。

討 論

本研究於田間以 3 株分離自淡黃化型病徵及 2 株原保存分離自葉枯型病徵之白葉枯病菌株，分別於插秧時、分蘖期和孕穗期等 3 個生育期接種，測試對兩期作水稻臺中秈 10 號之致病反應，以瞭解水稻何時感染最易發生淡黃化型病徵。結果顯示急性萎凋型 "kresek" 和淡黃化型病徵都屬系統性病徵，幼苗感染會產生 kresek 病徵，成株則出現淡黃化型。且此淡黃化型病徵都出現在新葉，孕穗期接種就不產生，亦即早期感染容易發生此病徵型病害。又由第一期作插秧時浸根接種會引起

稻株發生 kresek 病徵；成株出現淡黃化型病株，顯示高溫並非 kresek 及淡黃化型病徵發生之必要因子。

兩期作插秧時之剪葉處理均可引起感病性之葉枯型病徵；浸根處理，除第二期作 1 株原保存菌株接種後未發現枯死病株外，其餘各菌株均使稻株黃化後隨即萎凋呈現 kresek 病徵。水稻抽穗時調查插秧時、分蘖期及孕穗期等三個生育期接種之發病情形，顯示插秧時以剪葉與浸根接種處理均未出現葉枯型病徵，剪葉處理幼株雖有呈現葉枯病徵，但當罹病葉枯死後，稻株即未再出現葉枯型的新病葉；分蘖期和孕穗期之剪葉及噴霧接種處理，發現所有菌株均使兩期作水稻呈現感病性葉枯型病徵。又其中 3 株分離自淡黃化型病株之菌株，除第一期作插秧時之剪葉處理不產生淡黃化株

表 1、水稻台中秈 10 號插秧時接種白葉枯病菌，一個月後各型病害之發病情形

Table 1. Effect of inoculation with *Xanthomonas oryzae* pv. *oryzae* at different growing stages on occurrence of three types of bacterial wilt diseases on rice Taichung Sen No. 10 one month after transplanting

Isolates	Kresek (% of died seedlings)		Leaf blight (disease severity index)		Pale yellow leaves (no. of affected stems) affected	
	So ^z	Cu	So	Cu	So	Cu
First crop season in 2002						
Pale-yellow-leaf isolates						
XB 26	55.4	0	0	9	0	0
XB 28	41.5	0	0	7	0	0
XB 30	45.7	0	0	7	0	0
Leaf blight isolates						
XF 89-b	35.5	0	0	7	0	0
XM 30	0.3	0	0	3	0	0
CK (WM) ^y	0	0	0	0	0	0
Second crop season in 2002						
Pale-yellow-leaf isolates						
XB 26	41.9	0	0	9	0	0
XB 28	26.1	0	0	9	0	0
XB 30	19.1	0	0	9	0	0
Leaf blight isolates						
XF 89-b	30.7	0	0	9	0	0
XM 30	0	0	0	9	0	0
CK (WM)	0	0	0	0	0	0

^z Totally 192 plants of Taichung Sen No. 10 rice were inoculated. Inoculation methods: So, the rice roots were soaked in bacterial suspension; Cu, a half of leaves was cut by scissor infested with bacterial suspension.

^y CK, the controls were inoculated by root dipping and leaf-clipping method without pathogen.

外，其餘插秧時和分蘖期之各二種處理水稻，於兩期作中都產生淡黃化型病徵；孕穗期二種處理於兩期作接種後均未見淡黃化病株。原保存之 2 株白葉枯病菌株，在三個生育期之各二種接種處理，均未使水稻出現淡黃化病徵，據此得知菌株間之病原性應存有差異性。

由試驗得知，水稻淡黃化型病徵均出現於新葉上，孕穗期進行接種時，即不再顯現此病徵型，顯示早期感染才容易發生淡黃化型病徵。又苗期之剪葉和浸根接種處理及對照區，到成株均未產生葉枯型白葉枯病（表 2），顯示試驗期間之氣候環境不適合葉枯型白葉枯病自然發生；但有出現淡黃化病株，由此推論插秧時浸根處理之未枯死稻株，其中部分秧苗根被感染，雖未產生急性萎凋型

表 2、不同生育期接種白葉枯病菌對水稻台中秈 10 號抽穗期發生葉枯型病徵之影響

Table 2. Effect of inoculation stages and inoculation methods on appearance of leaf blight symptoms caused by *Xanthomonas oryzae* pv. *oryzae* on rice Taichung Sen No. 10 at rice booting stage

Isolates	Disease severity index at					
	Seedling stage		Active tillering stage		Booting stage	
	So ^z	Cu	Sp	Cu	Sp	Cu
First crop season in 2002						
Pale-yellow-leaf isolates						
XB 26	0	0	7	9	6	7
XB 28	0	0	5	6	6	5
XB 30	0	0	7	7	6	5
Leaf blight isolates						
XF 89-b	0	0	5	7	5	6
XM 30	0	0	3	5	0	3
CK (WM) ^y	0	0	0	0	0	0
Second crop season in 2002						
Pale-yellow-leaf isolates						
XB 26	0	0	6	9	6	7
XB 28	0	0	5	9	5	9
XB 30	0	0	7	9	5	9
Leaf blight isolates						
XF 89-b	0	0	7	9	5	9
XM 30	0	0	3	5	3	5
CK (WM)	0	0	0	0	0	0

^z Totally 192 plants of Taichung Sen no 10 rice were inoculated. Inoculation methods: So, the rice roots were soaked in bacterial suspension; Sp, the leaves of rice were sprayed with bacterial suspension; Cu, a half leaves of rice were cut by scissor infested with bacterial suspension.

^y CK, the controls were inoculated without pathogen.

表 3、不同生育期接種對水稻台中秈 10 號抽穗期發生淡黃化型病徵之影響

Table 3. Effect of inoculation stages and inoculation methods on appearance of pale yellow leaf symptoms caused by *Xanthomonas oryzae* pv. *oryzae* on rice Taichung Sen No. 10 at rice booting stage

Isolates	Percentage of yellowing stems at					
	Seedling stage		Active tillering stage		Booting stage	
	So ^z	Cu	Sp	Cu	Sp	Cu
First crop season in 2002						
Pale-yellow-leaf isolates						
XB 26	25.0	0	38.0	62.5	0	0
XB 28	17.2	0	1.0	12.5	0	0
XB 30	36.5	0	30.7	31.8	0	0
Leaf blight isolates						
XF 89-b	0	0	0	0	0	0
XM 30	0	0	0	0	0	0
CK (WM) ^x	0	0	0	0	0	0
Second crop season in 2002						
Pale-yellow-leaf isolates						
XB 26	7.8	1.0	3.6	26.6	0	0
XB 28	18.8	2.0	5.7	18.8	0	0
XB 30	15.6	3.6	13.0	24.5	0	0
Leaf blight isolates						
XF 89-b	0	0	0	0	0	0
XM 30	0	0	0	0	0	0
CK (WM)	0	0	0	0	0	0

^z Totally 192 plants of Taichung Sen No. 10 rice were inoculated. Inoculation methods: So, the rice roots were soaked in bacterial suspension; Sp, the leaves of rice were sprayed with bacterial suspension; Cu, a half leaves of rice were cut by scissor infested with bacterial suspension.

^y CK, the controls were inoculated with distilled water by leaf-clipping method.

病徵，但病菌於莖基部繼續繁殖聚積相當菌量，到成株雖遇氣候乾燥不適合葉枯型白葉枯病發生，卻可能產生淡黃化型病徵。由此更驗證 kresek 型與淡黃化型病徵均屬系統性病害 (Goto 1964)，唯在幼苗產生 kresek，成株則出現淡黃化型。



圖 1、台中秈 10 號水稻白葉枯病之三種病徵型（由上而下依序為葉枯型、kresek 急性萎凋型與淡黃化型）。

Fig. 1. Three types of symptoms of rice bacterial blight caused by *Xanthomonas oryzae* p. *oryzae* on rice Taichung Sen No. 10. Different disease symptoms showed as leaf blight (upper), kresek, the seedling blight or wilt phase of the syndrome (middle), and pale-yellow leaves (lower).

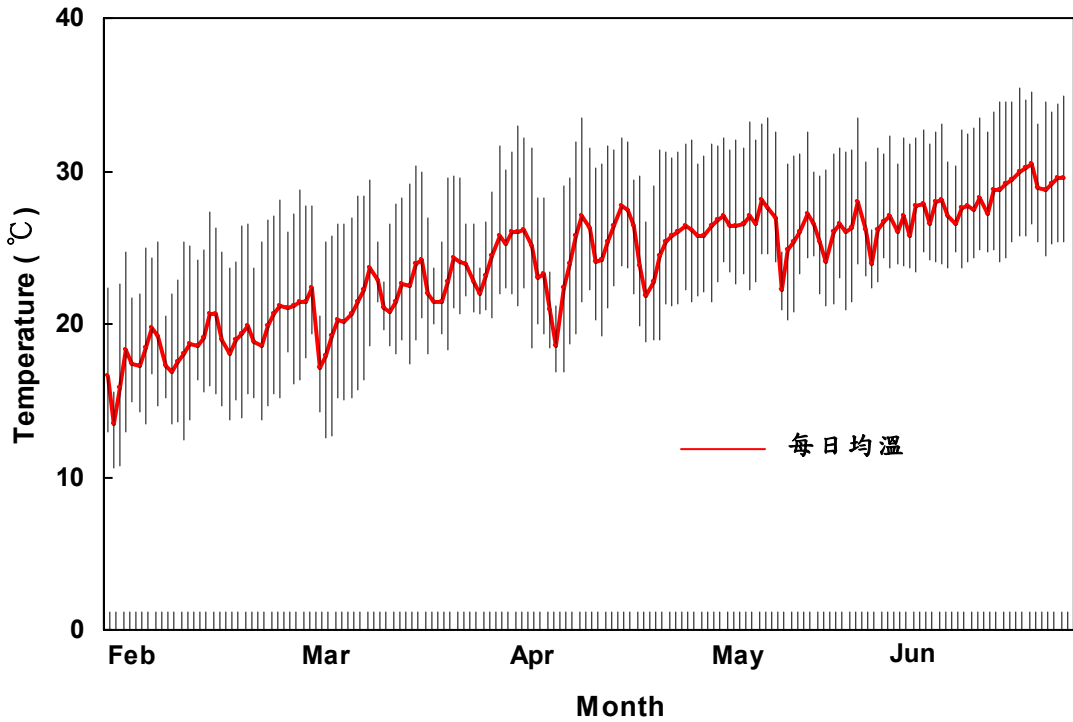


圖 2、台中霧峰地區 2002 年 2 月至 6 月的溫度變化。
Fig. 2. Temperature fluctuation at Wufeng, Taichung during February to June in 2002 .

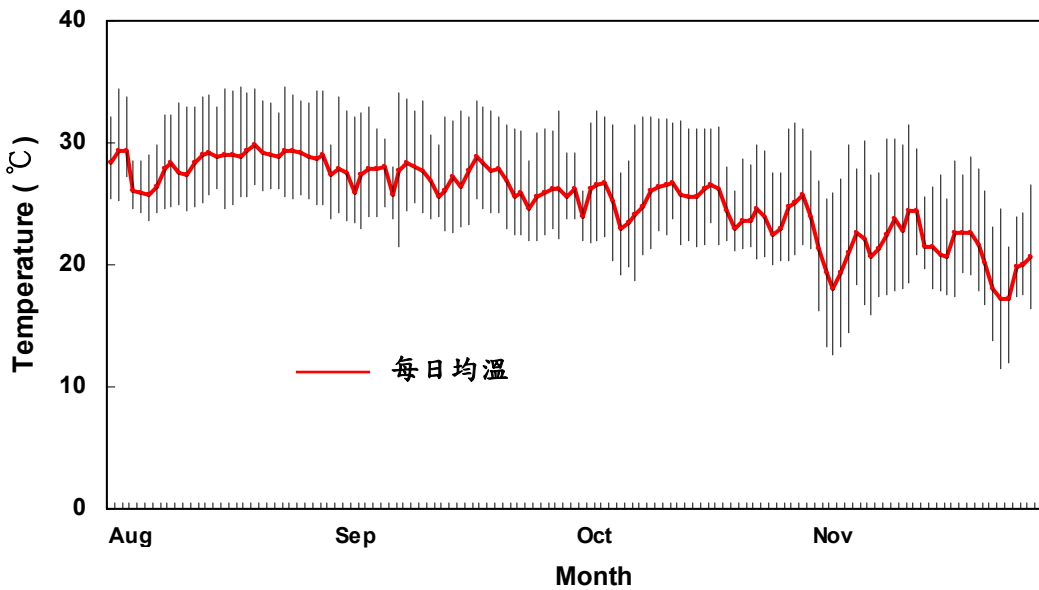


圖 3、台中霧峰地區 2002 年 8 月至 11 月的溫度變化。
Fig. 3. Temperature fluctuation at Wufeng, Taichung during August to November in 2002.

另外，一般都認為 kresek 和淡黃化型病徵皆屬熱帶型病害，即在高溫下才會產生，但由本研究發現，第一期作插秧時各菌株浸根接種之急性萎凋率高，0.3-55.4%，且筆者等早先於 1998 年第一期作曾以彰化縣大村所採集且分離自淡黃化型病株之菌株做淡黃化型白葉枯病之病原學研究，在 2 月 25 日插秧接種時適值寒流，當日平均氣溫為 15.7°C (13.3~20.6°C)，接種以後 10 天之每日均溫在 16.6°C (13.9~21°C)，結果 5 個菌株接種區都產生 9.7-31.5%之急性萎凋率。其餘未枯死稻株於其中 3 株分離自淡黃化型病徵之菌株接種區，至抽穗時亦都出現淡黃化病株。故知無論在第一期作的較低溫 (圖 2) 或第二期作的較高溫 (圖 3) 情況下進行接種，均會出現 kresek 型和淡黃化型病徵，據此推論高溫並非此二病徵型病害產生的必要條件。此結果與謝氏報告於 16°C 接種水稻嘉農秈 11 號之葉和根部，均未產生 kresek 病害之結果不同 (Hsieh 1978)。另由實驗中兩期作於插秧時剪葉接種結果，均未產生 kresek 病株，亦與謝氏實驗結果由 21 天株齡水稻嘉農秈 11 號之剪葉接種，均會產生 kresek 病徵者不同 (Hsieh 1978)。

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The Reaction of Taichung Sen No. 10 Rice Variety at Different Growth Stages to Pale-yellow Isolates of *Xanthomonas oryzae* pv. *oryzae*¹

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Abstract

Hsieh, L. J., Y. C. Chang, and T. F. Hsieh. 2006. The reaction of Taichung Sen No. 10 rice variety at different growth stages to pale-yellow isolates of *Xanthomonas oryzae* pv. *oryzae*. J. Taiwan Agric. Res. 55:201-211.

The objectives of this study were to evaluate the susceptibility of Taichung sen 10 rice cultivar to the isolates of *Xanthomonas oryzae* pv. *oryzae*, the causal agent of bacterial leaf blight of rice, at the seedling, tillering and booting stages. The rice plants were inoculated with bacterial suspension by root-dipping and leaf-clipping methods at the seedling stage, with leaf-clipping and spraying methods at the tillering and booting stages. Totally five isolates of *X. oryzae* pv. *oryzae* were used for inoculation, of which 2 isolates were isolated from plants with leaf blight symptoms and 3 isolates from plants with pale-yellow-leaf symptoms. The experiments were conducted in two continuous crops of 2002 at the experimental field of Taiwan Agricultural Research Institute. Results showed that the seedlings inoculated by the root-dipping method before transplanting in both crops appeared kresek symptoms (death of whole seedlings), except seedlings inoculated with XM 30 isolate in the 2nd crop. Meanwhile, the seedlings inoculated by leaf-clipping method showed leaf blight symptoms in both crops. However, all bacterial isolates didn't induce pale-yellow-leaf symptoms of inoculated rice plants by both inoculation methods. Disease survey was done at the heading stage after inoculation at the three different growth stages of rice in two crops. Leaf blight symptom was not observed when seedlings inoculated by both methods before transplanting. However, leaf-blight symptoms were observed on the inoculated plants when inoculations were conducted at tillering and booting stages by both methods and in both two crops. Meanwhile, all of the 3 pale-yellow-leaf isolates induced pale-yellow-leaf symptoms on inoculated rice plants at the heading stage when inoculations were done at transplanting and tillering stages by both methods in both crops, except inoculation at the seedling stage by leaf-clipping method in the first crop. In addition, the pale-yellow-leaf symptoms were not found on rice plants inoculated at the booting stage by both methods. Besides, none of the leaf-blight isolates caused pale-yellow-leaf symptoms by all tested methods at all growth stages. These results indicated that the virulence of different types of pathova of the bacterium was different, and symptoms of pale-yellow-leaf were only induced by infection at the earlier stages, such as at the seedling and tillering stages. Both kresek and pale-yellow-leaf symptoms were due to the systemic infection with bacterial pathogen. The kresek symptoms

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were observed at the seedling stage, whereas the pale-yellow-leaf symptoms on mature plants. In addition, both kresek and pale-yellow-leaf symptoms occurred under lower temperature at the first crop in Taiwan. This indicated that high temperature was not the necessary for induction of these two types of disease symptoms on rice plants.

Key words: Rice, Growth stages, Bacterial leaf blight of rice, Pale-yellow-leaf symptom, Kresek symptom, Temperature determination.

