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Studies on Tolerance and Rate-reducing Bacterial Pustule of Soybean Cultivars/Lines

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ABSTRACT

Bacterial pustule caused by *Xanthomonas campestris* pv. *glycines* (Nakano) Dye, was investigated from all soybean growing regions in Thailand and sixteen isolates of causal pathogen were collected. Morphological, physiological and biochemical characteristics of the causal organism were also studied, after that they were kept in paraffin oil for further studies.

Approximately 68 soybean cultivars/lines of 35-45 days old were tested for their bacterial pustule reaction at Kampangsan and Suwan Farm locations. All plots were inoculated 2 times. Yield and seed size or seed weight were compared under heavily artificial infection. The result revealed that 20 soybean cultivars/lines were resistant, 9 susceptible while the rest 39 were intermediate. Yield and seed size of the susceptible cultivars/lines differ significantly from those of the resistant ones. Difference in bacterial pustule infection between resistant and susceptible cultivars/lines was 53.79% The average seed yield reduction in the susceptible cultivars/lines was 37.04% and 4.35% was attributed to seed size reduction, while that of resistant ones was 30.32% and 7.92% respectively. This study would be further conducted in the next 2 years to reach the ultimate goal of the project.

INTRODUCTION

Bacterial pustule caused by *Xanthomonas campestris* pv. *glycines* (Nakano) Dye is one of the most important diseases of soybean in Thailand and some cultivated areas in the world. In Thailand, the occurrence of bacterial pustule was reported firstly by Sngawongse (1972). Symptoms and severity in the field were briefly explained and the disease was considered to be of rare occurrence at that time, but now it has become a serious problem since ten years ago. It is prevalent throughout the year and is known to reduce yield as high as 11 to 15 percent (Hartwig and Johnson, 1953; Laviolette et al., 1970). During the favorable condition of bacterial pustule, yield losses from this pathogen were up to 40 percent (Wolf, 1924), while Prathuangwong (1983) has investigated and reported the yield loss of this disease to be 20.7 to 34.9 percent in some local cultivars. The local cultivars commonly grown in Thailand such as SJ2, SJ4 and SJ5 were found to be heavily infected with this disease in every growing seasons, The disease is favored by warm, hot and humid conditions. In Thailand, it is more prevalent in rainy season, and daily rains, cloudy and hot weather before raining provides an ideal environment for the development of bacterial pustule. This climate would be particularly interested because heavier infection of the disease frequently occurs in the central than in the northern part of the country. The night seems to be cool in the north and the pathogen does

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not prefer that conditions. It is probably one of the reasons bacterial pustule has existed in central region more than other areas in Thailand.

Bacterial pustule has been relegated to minor importance through host resistance largely derived from a resistant parent Clemson Non Shatter or CNS. Hartwig and Lehman (1951), and Feaster (1951) showed that resistance to bacterial pustule was conditioned by a recessive single major gene. They believed that differences in degree of susceptibility among soybean varieties indicated that the phenotype of the dominant allele was influenced by modifying genes. The resistance of CNS has been used effectively in developing improved resistant cultivars adapted to the southern United States. Even though more resistant cultivars have been released and have greatly reduced the incidence of the disease, there is still considerable effort for incorporating resistance into other cultivars. In Thailand, according to the author, local cultivars are heavily infected by this disease, so breeding programs for disease resistance are still being conducted and resistant cultivars have not been released yet. It seems that bacterial pustule is more important and questionable in Thailand.

The purposes of our studies were: 1) to collect the isolates of bacterial pustule pathogen from all soybean-growing cultivated regions in Thailand and to determine some characteristics of causal bacterium. 2) to determine the effect of bacterial pustule on the yield of soybean cultivars and germplasm lines. 3) to search for resistance or tolerance among the soybean cultivars and germplasm lines introduced in the country by artificial inoculations with the isolate of the pathogen mentioned above. After tolerance in some of the pustule resistant lines was found, those which were believed to be indigenous to Thailand and other countries, were chosen for the investigation in order to see if the result pattern was different from that reported elsewhere.

MATERIALS AND METHODS

The studies included the collection of causal bacterium and its biological studies, and seed multiplication of the soybean cultivars and germplasm lines which were introduced in the country.

Sample collection, Isolation and Purification

The surveillance and collection of bacterial pustule were made from various soybean growing locations around Thailand. Sixteen isolates (isolate number 001-016) were obtained from these locations. The pathogen was isolated from the infected leaves by conventional agar plating method. The marginal portion of the lesion were cut into small pieces of 1-2 mm^2. They were placed in the distilled water for 1 min., in 0.1% $HgCl_2$ solution for another 1 min., 80% ethyl alcohol for 10 sec. and distilled water for 1 min., respectively (Schadd, 1980). The pieces were then transferred into 10 ml. distilled water and thoroughly ground with glass rod and let them stand for 10-15 min. Two dilutions, 1:20 and 1:200, were made with distilled water. Those were streaked on peptone agar plate previously prepared and incubated at room temperature (approximately 30°C) for 24-48 hr. Single colonies with yellow, glistening and greasy smooth surface and cutive margin was chosen. These colonies were restreaked on the same media for 2 times for purification. The pathogenicity of each isolate was confirmed by fulfilling Koch's Postulate. The isolates proved to be the causal bacteria were grown on potato peptone agar slants after being covered with sterilized liquid paraffin and maintained for further investigation.

Studies on bacteriological characteristics

Cultural characteristics:- Bacterial cells grown on peptone agar for 24 hrs. at room temperature was diluted with sterilized distilled water. One loopful of the suspension were streaked on potato peptone agar plates and incubated

at room temperature. The shape, size and colour of colonies were observed. The 24 hr culture old that grew on peptone agar slants were checked on their morphology, and Gram reaction of bacteria.

Morphological characteristics:- One millilitre of sterilized distilled water was added into 48 hour old peptone agar slants and left for 30 min. to allow the flagella bacteria swarming into water. Two ml. of this bacterial suspension was poured into sterilized test tubes. The fixative, 2.5% glutaraldehyde (glutaraldehyde: bacterial suspension = 1:10) was added to fix the bacteria and set it still at room temperature for 30 min. for keeping of bacterial cell in the natural form. The suspension was dropped on the grid with membrane, left for approximately 2 min., and then blotted water with filter paper from under grid. The sterilized distilled water was dropped on the grid again, left it for 1 min., and blotter water with filter paper. This was examined under an electron microscope.

Physiological and biochemical characteristics:- The study based on the ability of each isolate to grow on culture medium coutaining the test chemical. Each isolate was inoculated on various differential media for nutritional physiology test. Investigation on nitrate reduction, action on tryptone or tryptophane, gelatin liquefaction, starch hydrolysis, Voges-Proskauer (VP) reaction, methyl red (MR) test and utilization of carbon hydrates such as glucose, arabinose, galactose, fructose, sucrose and manose were carried out. The media and reagents used in each test were nitrate broth which consisted of sulfanilic acid and dimethyl napthalamine for nitrate reduction test. If the pathogen could reduce nitrate into nitrite, the broth turned to pink or red. The ability of pathogen to reduce nitrite into ammonia was also checked by adding Nessler's solution into the broth and then, yellow or brownish-yellow colour would be indicated. Tryptone broth and Kovac's solution for action on tryptone or tryptophane were used to observe indole production by the pathogen. Nutrient gelatin for gelatin liquefaction test. Potassium iodine was reagent tested for starch hydrolysis. VP medium, napthol and potassium hydroxide were employed in VP test where acetyl methyl carbinal produced by pathogen could be noticed. The nutrient broth consisted of glucose was tested for pH characteristic by adding methyl red into the broth (MR test). After incubation period, pink or red colour of the broth meant acid production while yellow meant basic production by the pathogen. Nutrient broth in test tube consisting of different kinds of sugar or carbohydrate such as glucose etc, mentioned above and also Durham tube were tested for carbon utilization. Bromthymal blue and the space in Durham tube were used to indicated pH change and gas production.

Effect of bacterial pustule on disease severity and yield of soybean cultivars/germplasm lines

Seventy-three and seventy-four soybean cultivars and germplasm lines were planted in the field trials both at Kamphangsan (KPS) and Suwan Farm (SF). Split plot in randomized complete block design (RCBD) with inoculated (inoc.) and uninoculated plant (uninoc.) as the main plot and soybean cultivars/lines as the sub-plot. Two replications were planned. Planting methods were as following: plot size with 2 row at 5 x 0.5 m, space between row with 0.5 m, space between plant with 0.125 m, path with 2 m end of each plot, planted 2-3 seeds per hill with jab and 1 plant/hill was left after emergence, number of plants with 60 plants per plot after thinning. Weed control, fertilizer application and insect pest control are based on the local standard management. Furrow irrigation was applied daily at 7-9 days for 3 hr. throughout the experiment.

Preparation of inocula for field trial:- Sixteen isolates of the pathogen obtained from various growing areas in Thailand, were cultured on Kelmen's tetrazolium agar or TZC agar (Friedman, 1964). Only dark red colonies on TZC agar which provided as high virulence, and hence one isolate was used for the investigation. The multiplication of virulent inoculum was needed before testing the artificial inoculation in the large acale of field trial which was prepared by adding 5 ml sterilized distilled water into stock culture to make bacterial suspension, then 0.1 ml of the suspension was transferred with pipette into potato peptone agar plate, after that the "L" glass rod was employed for spreading the suspension over the plate by rotating agar plate. After incubation for 36-40 hours, one colony of the pathogen was transferred into 5 ml potato peptone broth and shaked for 24 hours. This suspension was poured into 500 ml potato peptone broth and shaked again for 36-40 hours to increase inoculum. Optical density 0.26 corresponded approximately to 1 x 10^7cells/ml.

The preparation of plants in the test plots before inoculation:- The plants were predisposed for infection by water-spraying or sprinkle irrigation one day before inoculation. Humid condition was maintained for 1-2 hours after inoculation. Continuous water-spraying were conducted every 2 days for 3 consecutive times to provide humid conditions for successful infection.

The preparation of inoculum and the method of inoculation:- When the required bacterial concentration of 1 x 10^7 cells/ml was obtained, 0.03 percent of 600 mesh carborundum (0.3 g of carborundum: one litre of bacterial suspension) was added and shaked or stirred to evenly distribute carborundum. The mixture was transferred into knapsack sprayer with pressure of 10-12 lb/inch^2. Approximately 10 ml of bacterial suspension was sprayed on one plant. The inoculation began when the plant became 35 days old. The plants were inoculated two time at 10 day interval. All rows were inoculate between 11.00 AM and/or 1.00 PM on clouded and/or bright day. (Fig.1)

The checking method and data on disease development :-After 10 days of the last inoculation, the nineth real leaf from below of the infected plants was sampled at every other plant from each replicate, except for the first and last plant of the row. The number of spots on 5 x 1 cm^{2}area of the leaves were counted through windows in 4 x 7 cm^2 areas of pre-punched card or stencil (Fig.2). In addition, seed size and yield were determined for each plot. As to the yield assay, the procedure was similar to that of infected leaves, that was, the yield were harvested from every other plant from each replicate, except for the first and last plants of the row. Analysis of variance was applied to data pertaining to number of spot/leaf, seed size and also seed yield/area.

RESULTS AND DISCUSSION

Collection of bacterial pustule isolations

Sixteen isolates of pustule bacteria were collected from various soybean growing regions in Thailand and were kept in paraffin oil for further studies. Those isolates were as followings:

ISOLATE NO.	SOURCE OF SPECIMENS
001	CMU, Chiang Mai
002	Suwan Farm
003	Sawankhalok, Sukhothai
004	Si Satchanalai, Sukhothai
005	Chainat
006	Mae-Tang, Chiang Mai
007	Hangdone, Chiang Mai
008*	KPS, Nakorn Pathom
009	Phitsanulok
010	Loei
011	Uttaradit
012	Pra Phutthabat, Saraburi
013	Chai Badan, Lopburi

014	Sukhothai
015	Chanthaburi
016	Chachoengsao

*The only one isolate used for inoculum at both locations KPS and SF.

Some characteristic of each bacterial pustule isolate

Those sixteen isolates were rod-shaped, 0.23-0.93 by 0.57-2.59 um. (Fig.3) Colonies on solid media are round, yellow, convex with smooth and greasy surface and entire margin. Acid but no gas was produced from various sugars. The organisms grew profusely of culture broths and liquefied gelatin medium. Litmus milk turned blue with curd formation and protonization were observed. Starch hydrolysis was positive, while Indole test was negative. Production of ammonia and hydrogen sulfide was positive. All isolates were pathogenic on soybean producing typical symptoms. The results of those studies confirmed that the causal pathogen of bacterial pustule was *Xanthomonas campestris* pv. *glycines* (Nakano) Dye (1978)

Effect of bacterial pustule on soybean crops

Bacterial pustule occurred on all of the inoculated soybean cultivars/lines. (Fig.4-6). The mean of disease severity for both locations KPS and SF was similar regardless the control, that was 14.64 spots/leaf at KPS and 13.40 spots/leaf at SF for disease severity in each location. When the mean of control or uninoculated plants was checked, however it was a little bit different. Uninoculated plants at KPS showed the average lesions of 9.36 spost/leaf while those at SF 0.51 spots/leaf, Hence the difference between the mean of inoculated and uninoculated plants at both locations was 5.28 spots/leaf and 12.89 spots/leaf after reduction from the corresponding control at KPS and SF, respectively. Apart from these, the infected severity at SF seemed to be higher than at KPS.

Of the soybean bacterial pustule symptom tested at both locations, 3 were observed to be highly resistant (HR), 17 resistant (R), 21 moderately resistant (MR), 9 moderately susceptible (MS), and 9 susceptible (S). Table 1 shows the names of these cultivars/lines. Mostly each soybean lines/cultivars exhibited similar reaction to bacterial pustule at both locations, except for the other 18 cultivars/lines. Those cultivars/lines IAC 6, AGS 8P, AGS 172,TG x 724-01D, Taichung N (15 kr), AGS 168, Wakashima (yellow seedling), and TG x 604-01D, displayed resistance at KPS but susceptibility at SF. The rest of cultivars/lines which showed different reactions to bacterial pustule were AGS 164, M90,TG x 342-375D,AGS162, Sansai, SB 60, Papillon, OCB, Davis, and Jupiter. They were resistant at SF but susceptible at KPS. These variations in symptom expression may be due to environmental effects, such as soil and atmospheric moisture, diurnal period, cloudiness, soil fertility, time at inoculation and cultural practices. Moreover the efficacy of pathogen inoculum to reach the proper receptor site would be other key roles of symptom variations. Therefore, to furtherly pursue this study, the technique of handling and application need to be verified in the future.

Table 1 The effect of *Xanthomonas campestris* pv. *glycines* to disease severity of soybean cultivars/lines

Cultivars/lines	Sources	Spot/leaf				Disease reaction	
		KPS		SF			
		inoc.	uninoc.	inoc.	uninoc.	KPS	SF
William-79	USA	10.78	4.48	-	-	R	-
P 759207	-	14.75	4.11	16.19	1.22	MR	MS
Clark-63	USA	10.10	7.81	13.81	0.08	R	MR
IAC 6		10.05	7.37	20.40	0.98	R	S
Tunia	-	7.00	6.28	-	-	HR	-
AGS 18P	AVRDC	10.64	5.95	-	-	R	-
AGS 209	AVRDC	11.83	7.02	13.72	0.00	R	MR
AGS 8P	AVRDC	10.69	7.00	20.61	0.73	R	S
AGS 172	AVRDC	9.30	6.62	20.17	2.56	R	S
AGS 129	AVRDC	11.14	6.31	4.77	0.00	R	HR
G 5463	AVRDC	7.17	0.00	5.72	0.00	HR	HR
TG x 573-104 C	-	8.62	7.41	11.65	0.27	R	R
TG x 724-01 D	-	10.29	8.46	18.98	1.36	R	S
TG x 330-054 D	-	8.33	5.75	6.41	0.00	HR	HR
TG x 297-192 C	-	10.15	7.57	6.88	0.00	R	HR
Taichung N (15 kr)*	THAI	7.65	6.43	19.26	1.01	HR	S
G 8587 (15 kr)*	THAI	10.38	8.14	15.24	1.99	R	MS
S.J. 4 (15 kr)*	THAI	12.85	4.82	14.38	0.22	R	MR
Taichung N (30 kr)*	THAI	7.32	5.86	12.53	0.27	HR	R
KS 419	AVRDC	7.95	6.47	12.59	0.11	HR	R
AGS 184	AVRDC	15.97	14.97	13.18	0.37	MS	R
AGS 227	AVRDC	13.61	11.44	12.20	0.00	MR	R
AGS 164	AVRDC	16.17	7.59	7.54	0.00	MS	HR
AGS 215	AVRDC	17.94	12.32	17.05	0.46	MS	MS
AGS 168	AVRDC	12.03	7.23	18.70	1.62	R	S
AGS 133	AVRDC	16.06	11.68	22.58	0.00	MS	S
AGS 208	AVRDC	12.67	7.37	22.45	0.31	MR	S
AGS 154	AVRDC	15.55	10.28	12.30	0.00	MS	R
AGS 66	AVRDC	14.30	13.74	6.77	0.00	MR	HR
AGS 8	AVRDC	12.48	9.27	3.72	0.66	R	MR
AGS 200	AVRDC	12.93	11.08	10.45	0.46	R	R
AGS 8180 (M 26P-1)	AVRDC	14.32	13.14	12.24	1.50	MR	R
AGS 58	AVRDC	9.93	9.68	12.54	0.36	R	R
AGS 160	AVRDC	12.64	7.58	13.39	0.00	R	R
S.J. 2	THAI	17.94	9.83	14.43	0.68	MS	MR
S.J. 4	THAI	16.98	11.81	9.85	0.52	MS	R
S.J. 5	THAI	15.40	11.52	12.14	0.36	MS	R
IAC 73-5115	-	15.05	10.75	11.04	0.00	MS	R
M 90	-	20.94	8.72	2.10	0.00	S	HR

Table 1 (continued)

Cultivars/line	Sources	Spot/leaf					
		KPS		SF		Disease reaction	
		inoc.	uninoc.	inoc.	uninoc.	KPS	SF
SH 1274	-	13.73	8.24	12.92	0.41	MR	R
ICAL 109	-	14.10	10.18	9.92	1.01	MR	R
ICAL 129	-	12.68	10.57	13.19	0.00	R	R
SB 02	AUSTRALIA	14.08	11.63	9.85	0.98	MR	R
EGSY 1917	-	16.54	6.48	-	-	MS	-
UFV 1	BRAZIL	12.35	10.50	7.01	0.56	R	HR
OCB	-	15.59	6.68	4.86	0.00	MS	HR
DAVIS	USA	15.52	7.58	7.56	0.00	MS	HR
Jupiter	USA	14.63	6.59	6.27	0.00	MS	HR
Bossier	USA	13.53	7.37	6.21	0.00	MR	HR
Improved Pelican	USA	13.97	13.07	12.79	0.79	MR	R
Wakashima	-	14.06	6.31	19.99	0.11	MR	S
Wakashima (yellow seedling)	THAI	11.53	8.36	24.13	0.88	R	S
BM 98 (white flower)*	THAI	11.24	9.15	6.05	0.25	R	HR
Taichung N*	THAI	16.18	10.49	24.61	0.19	MS	S
Sansai x S.J. 2 (white flower)*	THAI	17.06	12.98	21.23	1.41.	MS	S
S.J. 2 (30 kr)*	THAI	13.72	8.88	12.14	0.10	MR	R
TG x 713-06 D	-	18.32	9.58	20.59	2.14	MS	S
TG x 604-01 D	-	13.22	10.47	18.50	1.30	R	S
TG × 536-100 C	-	17.91	9.75	9.75	1.13	MS	R
TG × 604-027 D	-	16.94	9.45	13.63	0.83	MS	MR
TG × 342-356 D	-	15.52	9.61	12.20	0.00	MS	R
TG × 342-375D	-	14.56	12.80	7.10	0.00	MS	HR
G 2507	AVRDC	24.03	13.22	19.49	0.00	S	S
AGS 222	AVRDC	19.06	18.30	20.89	1.00	S	S
AGS 162	AVRDC	21.64	11.94	13.33	0.22	S	R
AGS 146	AVRDC	20.23	13.12	—	—	S	—
Sansai	-	27.80	11.67	9.41	0.00	S	R
ISRAIRAI 44 A/173	-	25.79	11.63	14.51	1.07	S	MR
TG × 307-047 D	-	16.87	15.45	11.80	0.22	MS	R
TG × 711-01 D	-	20.23	12.21	13.62	1.93	S	MR
Wakashima mutant 10*	THAI	28.35	13.33	21.95	0.30	S	S
SB 60*	THAI	30.42	9.78	8.88	0.20	S	R
Papillon	-	17.77	12.44	8.95	0.00	MS	R
G 7856	AVRDC	-	—	13.89	1.12	—	MR
TN# 4	-	-	—	11.05	0.07	—	R
7138	-	-	—	13.37	0.33	—	R
(Vegetative soybean)	AVRDC	-	—	25.08	0.19	—	S
MEANS	-	14.64	9.36	13.6	0.51		

Table 1 (continued)

Resistant evaluation:-	
Highly Resistant (HR)	= Disease severity similar to or less than 8.4 spots/leaf
Resistant (R)	= Disease severity showed the lesion between 8.15-13.4 spots/leaf
Moderately Resistant (MS)	= Disease severity distinctly more than 13.4 spots/leaf, but distinctly less than 15.4 spots/leaf
Moderately Susceptible (MS)	= Disease severity more than 15.4 spots/leaf, but less 18.4 spots/leaf
Susceptible (S)	= Disease severity similar to or greater than 18.5 spots/leaf

*Soybean lines received radiation which were bred by Atomic Energy Dept., Kasetsart Univ.

After the analysis of variance was applied to data pertaining to disease severity presented in Table 1, it appeared that the difference between inoculated and uninoculated soybean cultivars/lines was significant at the 95% level of probability at both locations. The statistical significant difference of disease severity among these cultivars/lines was also significant. (Table 4)

It is interesting to note that some of the U.S. varieties like William-79, Clark-63, Bossier and Improved•Pelican having derived from CNS were also resistant to the Thai isolate of the pathogen. This suggests that there are no difference in the pathogenic strains of the pustule organiam between Thailand and U.S.A and the high yielding pustule resistant U.S. varieties can directly be adapted for cultivation if otherwise found suitable, or utilized as resistant donors. The resistance reaction of the reportedly, susceptible cultivars Davis and Jupiter in the test at KPS might be due to difference in environmental factors as mentioned above. The number of other resistant cultivars listed in Table 1 should be determined if resistance in them is also a monogenic recessive character as in the CNS (Hartwig and Lehman, 1951). In further varietal improvement programmes now underway, involving high yielding varieties, it would be desirable to prevent erosion of their pustule resistance. At the same time resistance should be incorporated in recommended susceptible cultivars/lines.

Among the susceptible soybean cultivars/lines liked AGS 133, SJ.2, Taichung N, G 2507, AGS 222 and Wakishima mutant 10 that presented in Table 1, it should be given some consideration for the author to develop the sick plot technique used as the maintainance of the pathogen for inoculating of the tested clutivars.

The data in Table 2 showed the yields and 100 seed weight of inoculated and uninoculated soybean cultivars/lines and their percentage of decreasing. Although the disease development on the plants was not that heavy, the mean yield and seed size were significantly different from the corresponding means of the control. From the data presented in Table 2, it showed that the degree of bacterial pustule infection in this study reduced the seed size and yield of tested soybean lines/cultivars. The percentage of decreased seed size and yield of each inoculated cultivars/lines was also significant (Table 4). The mean of decreased seed size was 7.64 percent and the mean of reduced yield was 32.6 percent. Apart from these, yield and seed size appear to be stimulated and the yield reduction due to bacterial pustule was associated with decreased seed size and seed number. The average seed yield reduction for all inoculated soybean lines/cultivars was 187.53 kg/rai while the control or uninoculated plants was 279.20 kg/rai and decreased seed sized was 10.72 g while the control was 11.60 g. This indicated that bacterial pustule, which begins to increase when plants are in full bloom, causes abortion of seed and pods.

In Table 3, the differences between the mean values for disease severity and 100 seed weight of the resistant and susceptible cultivars/lines were significant at 95% level of probability. The mean disease severity between resistance and susceptibility was 11.018 spots/leaf vs. 23.851 spots/leaf which was regardless for moderate reaction. The difference between the mean value for 100 seeds was 11.39 g vs. 9.77 g. In all comparison, the mean disease severity and 100 seed weight of resistance were higher than susceptibility. In the same Table 3, the mean seed yield of resistance was also higher than susceptibility, but after the analysis of variance was applied, it appeared that the difference between resistance and susceptibility was not significant at the 95% level of probability. However, the yield difference between resistance and susceptibility was associated with a difference in mean pustule number of spo leaf.

Table 2 The effect of *Xanthomonas campestris* pv. *glycines* to yield and seed size of soybean

Cultivars/lines	Disease reaction	Seed size (gm)		Reduced seed size (%)	yield (kg/rai)		Reduced yield (%)
		inoc.	uninoc.		inoc.	uninoc.	
William-79	R	12.74	14.70	13.33	123.34	228.29	45.97
P 759207	MR	9.36	10.34	9.48	142.29	255.68	44.35
Clark-63	R	13.52	14.01	3.48	180.01	182.54	1.38
IAC 6	R	9.95	11.65	14.59	135.64	251.07	45.98
Tunia	HR	11.19	16.38	31.64	210.54	348.47	39.58
AGS 18P	R	11.51	12.47	7.73	136.89	248.08	51.81
AGS 209	R	13.25	14.96	11.43	137.29	204.73	32.94
AGS 8 P	R	13.72	14.15	3.05	237.03	312.51	24.15
AGS 172	R	12.06	13.23	8.85	152.69	312.49	51.14
AGS 129	R	13.65	13.88	1.66	163.04	186.41	12.54
TG x 573-104 C	R	7.86	8.59	8.52	133.84	185.36	27.79
TG x 724-01 D	R	8.02	9.25	13.23	186.39	235.06	20.71
TG x 330-054 D	HR	9.09	9.40	3.35	145.66	150.81	3.42
TG x 297-192 C	R	6.51	7.15	8.88	118.19	185.63	36.33
Taichung N (15 kr)	HR	11.42	11.86	3.71	404.78	437.67	7.51
G 8587 (15 kr)	R	10.97	11.28	2.73	225.67	376.53	40.07
S.J. 4 (15 kr)	R	13.94	13.95	0.03	278.08	454.60	38.83
Taichung N (30 kr)	HR	12.59	13.09	3.84	566.10	632.65	10.52
KS 419	HR	15.17	15.88	4.44	134.55	280.07	51.96

Talbe 2 (continued)

Cultivars/Lines	Disease Reaction	Seed size (gm)		Reduced seed size	yield (kg/rai)		Reduced yield (%)
		inoc.	uninoc.	(%)	inoc.	uninoc.	
AGS 184	MS	12.45	13.11	5.00	212.44	303.41	29.98
AGS 227	MR	12.91	12.96	0.42	156.85	248.28	36.83
AGS 164	MS	12.87	13.68	5.96	271.12	279.66	3.05
AGS 215	MS	13.90	14.48	4.91	197.64	261.99	24.56
AGS 168	R	14.45	14.96	3.46	269.86	283.91	4.95
AGS 133	MS	14.41	14.87	3.06	171.95	270.12	36.34
AGS 208	MR	14.10	14.66	3.79	223.72	363.54	38.46
AGS 154	MS	10.06	14.66	31.34	148.62	194.43	23.56
AGS 66	MR	11.22	11.39	1.48	130.02	232.84	44.16
AGS 8	R	12.07	12.12	0.43	181.83	382.91	52.51
AGS 200	R	9.49	9.68	2.01	107.79	184.27	41.51
AGS 8180 (M26P-1)	MR	5.68	6.40	11.20	168.10	351.25	52.14
AGS 58	R	15.16	16.90	10.31	187.62	318.92	41.17
AGS 160	R	9.96	11.24	11.34	206.46	369.57	44.13
S.J. 2	MS	10.09	10.22	1.30	152.62	270.10	43.49
S.J. 4	MS	10.89	12.35	11.79	128.34	149.32	14.05
S.J. 5	MS	11.90	12.44	4.34	180.68	219.66	17.75
IAC 73-5115	MS	12.50	12.61	0.88	213.00	301.91	29.45
M 90	S	7.70	8.35	7.75	148.50	182.95	18.83
SH 1274	MR	10.79	11.22	3.83	150.51	169.22	11.06
ICAL 109	MR	7.20	8.26	12.86	162.60	332.93	51.16
ICAL 129	R	10.09	11.12	9.23	178.87	199.81	10.48
SB 02	MR	5.42	6.98	22.30	126.96	134.69	5.74
UFV 1	R	11.73	13.81	15.03	158.71	262.21	39.47
OCB	MS	17.15	18.84	8.96	105.62	209.88	49.67
Davis	MS	11.33	12.09	6.24	144.14	182.49	21.01
Jupiter	MS	7.89	8.45	6.64	125.62	291.21	56.86
Bossier	MR	9.91	11.65	14.95	127.23	268.75	52.65
Improved Pelican	MR	10.16	11.83	14.11	222.86	428.71	48.02
Wakashima	MR	13.17	14.20	7.26	180.59	360.54	49.91
Wakashima (yellow seedling)	R	11.85	12.76	7.18	244.84	418.96	41.56
BM 98 (white flower)	R	9.46	9.98	5.18	261.39	301.82	13.40
Taichung N	MS	13.79	14.25	3.27	239.49	510.63	53.10
Sansai x S.J.2 (white flower)	MS	7.01	7.98	12.15	162.96	400.08	59.27
S.J.2 (30 kr)	MR	10.12	12.54	19.35	365.27	431.99	15.44
TG x 713-06 D	MS	10.54	11.28	6.53	175.00	194.17	9.87
TG x 604-01D	R	7.60	8.04	5.44	244.01	261.69	6.76
TG x 536-100 C	MS	7.33	7.94	7.71	112.37	145.67	22.86

Table 2 (continued)

Cultivars/lines	Disease Reaction	Seed size (gm)		Reduced seed size	yield (kg/rai)		Reduced yield (%)
		inoc.	uninoc.	(%)	inoc.	uninoc.	
TG x 604-027 D	MS	8.53	8.85	3.57	198.04	248.54	20.32
TG x 342-356 D	MS	6.78	6.86	1.11	141.26	145.06	2.62
TG x 342-375 D	MS	6.82	7.52	9.40	110.77	184.29	39.89
AGS 222	S	11.51	12.58	8.46	243.30	260.21	6.50
AGS 162	S	14.38	14.75	2.52	326.58	657.16	50.30
AGS 146	S	12.41	12.45	0.31	93.48	155.57	39.91
Sansai	S	7.24	7.47	3.10	163.40	255.68	36.09
ISRAIRAI 44A/173	S	8.94	8.38	6.22	183.17	360.82	49.32
TG x 307-047 D	MS	7.68	7.82	1.82	140.93	203.02	30.58
TG x 711-01 D	S	10.69	11.00	2.79	187.88	202.87	7.39
Wakashima mutant # 10	S	9.00	10.77	16.15	155.65	286.23	45.62
SB 60	S	6.06	6.35	4.62	160.71	279.36	42.47
Papillon	MS	9.93	11.17	11.10	102.93	198.65	48.19
EGSY 1917	MS	10.49	11.46	8.48	188.91	249.50	24.28
Means		10.72	11.60		187.53	279.20	

Table 3 Mean disease severity, seed size and seed yield for resistant and susceptible selections.

Characteristic study	Disease Reaction			
	R	MR	MS	S
Disease severity (spots/leaf)	11.018^{b} (3.120)	13.305^{ab} (5.816)	16.388^{ab} (9.374)	23.851^{a} (5.002)
Grams/100 seeds	11.398^{a} (12.379)	10.007^{ab} (11.074)	10.656^{ab} (11.493)	9.775^{b} (10.239)
Yield (kg/rai)	203.970^{a} (292.754)	179.753^{a} (296.705)	164.751^{a} (246.084)	184.744^{a} (293.432)

- The figures in parenthesis are the average of uninoculated plants or control treatments
- Means in each column followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.

The AOV analysis of the results from each experiment in Table 4 revealed that the inoculated and uninoculated soybeans were different in the virulence of infection as well as the number of lesion spots/leaf with C.V. for inoculated and uninoculated soybeans of 3.63 and 44.13% respectively. There was also the difference in seed size between both of them with C.V. of 17.89% and 9.28% for the inoculated and uninoculated soybeans, respectively. However, no difference was noticed between the yield of the 2 kinds and C.V. was quite high. The reason might due to the naturally infecting bacterial pustule that was rather high during the investigation thus, causing no difference between artificial and natural infection.

Table 4 Analysis of variance of *Xanthomonas campestris* pv. *glycines* affected on spot/leaf, seed size and seed yield of soybean Cultivars /lines.

SOURCG OF VARIATIONS	SPOTS/LEAF AT KPS		SPOTS/LEAF AT SF		SEED SIZE		YIELD(KG/RAI)	
	DF	MS	DF	MS	DF	MS	DF	MS
REPLICATIONS	1	7.2277**	1	0.0001^{ns}	1	3.4896^{ns}	1	13714.9828^{ns}
INOCULATIONS	1	2037.6504*	1	11955.8628*	1	73.8716**	1	457313.4812^{ns}
ERROR	1	0.1896	1	17.9850	1	3.9941	1	62142.7676
CULTIVARS	72	49.8127**	71	35.4530*	70	115.0714**	70	28768.3655**
INOCULATION x CULTIVARS	72	16.0529^{ns}	71	26.8721^{ns}70	5.3642**		70	7301.5106*
ERROR	144	28.0851	142	25.7772	140	1.074	140	5211.2124
TOTAL	291		287		283		283	
C.V. (INOCULATION)	3.63%		60.97%		17.89%		107.20%	
C.V. (CULTIVARS)	44.13%		72.99%		9.28%		31.04%	

** = SIGNIFICANT AT 99% LEVEL
* = SIGNIFICANT AT 95% LEVEL
ns = NON SIGNIFICANT AT 95% LEVEL

Apart from these, the trial was conducted in very large area of approximately 3 rai up, therefore, it was not able to keep the environmental factors in the best control. The inappropriate harvesting practice which a lot of man labour was employed, was also different wihch would be the other key role for non-significance of yield between inoculated and uninoculated plants, thus, affecting the standard efficiency of harvesting, and in return causing high C.V. as in Table 4.

After introducing several soybean varieties into Thailand, attempts are now being made to breed varieties for better yield and suitability to different agro-climatic condition. Some of the parents involved in breeding programes are resistant to bacterial pustule. If no selection pressure for pustule resistance is applied in the segregating populations obtained from crossing, it is possible that the new varieties developed will prove susceptibly. Such an erosion of resistance can nullify the advantages of higher yields expected from such crosses. This will be an avoidable yet substancial loss.

SUMMARY

Sixteen isolates of pustule bacteria (*Xanthomonas campestris* pv. *glycines*) were collected

from all soybean cultivated areas in Thailand and kept under paraffin oil for further studies.

Among 68-77 soybean cultivars/lines tested for resistance and susceptibility for bacterial pustule at Kampangsan and Suwan Farm locations, 59 cultivars/lines showed similar reaction at both locations while the other 18 cultivars/lines showed different reactions between two locations. Among 59 cultivars/lines, 20 were proved to be resistant and susceptible regardless moderate reaction. The differences between resistant and susceptible cultivars/lines for disease severity and seed size was significant at 95% level of probability. Under KPS condition susceptibility showed 53.79% higher disease severity and 10.4% less yield than resistance. The susceptible lines/cultivars also produced seed 16.58% smaller than resistant lines/cultivars.

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Fig.1 The artificial inoculation of causal bacteria to soybean plants was made through knap sack sprayer with the pressure of 10-12 lb/inch^2

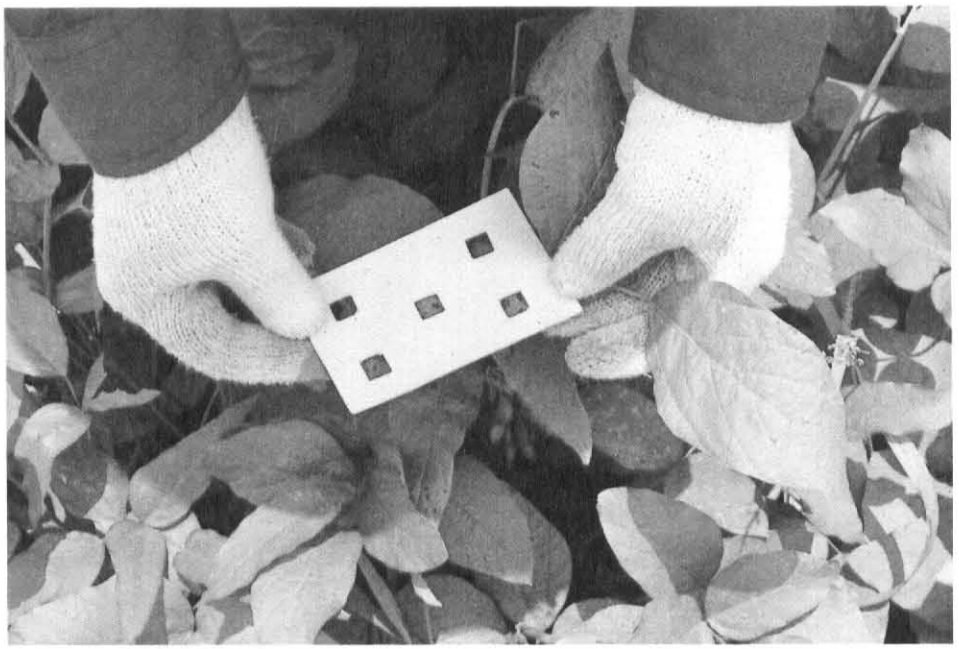

Fig.2 The number of lesion spots on each leaf were counted on 5 x 1 cm^2, through windows in 4 x 7 cm^2 areas of stencil.

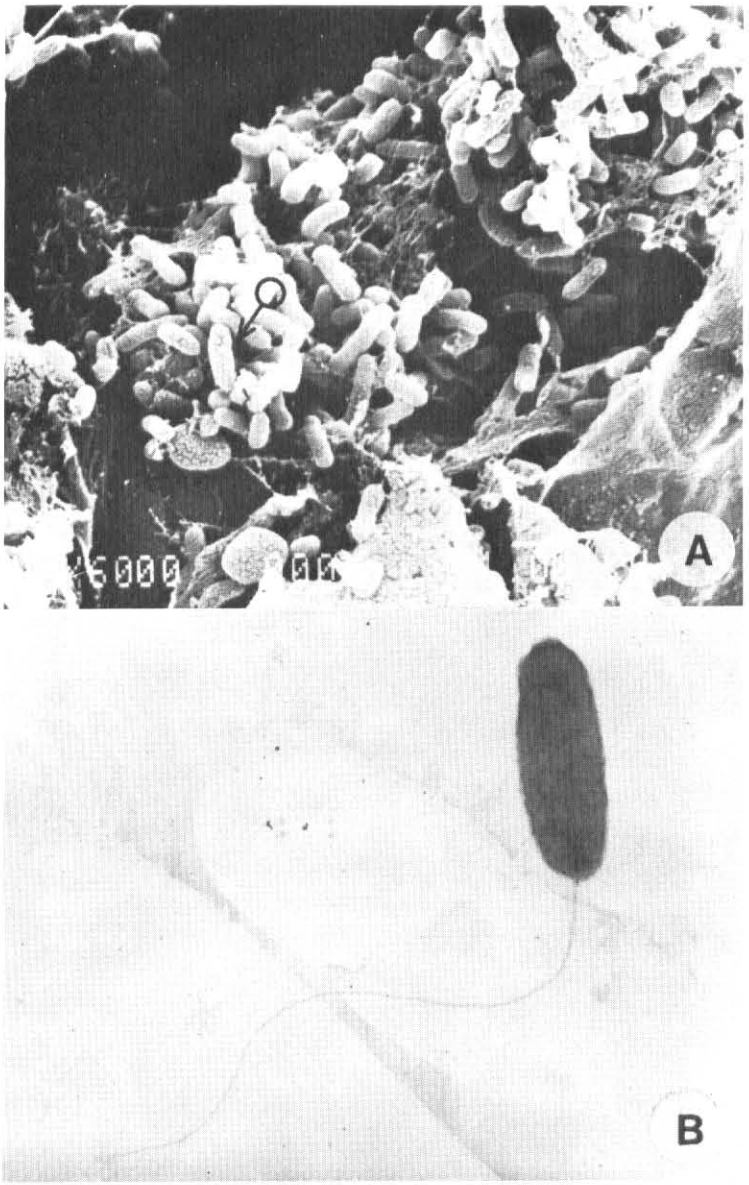

Fig.3 The character of *Xanthomonas campestris* pv. *glycines* caused bacterial pustule of soybean.
A The causal bacteria infected vascular bundle of soybean leaves.
B The character of *X. campestris* pv. *glycines* with monotrichous flagella at the end of each cell.

Fig. 4 Lesion spots of bacterial pustule on in fected soybean leaves.

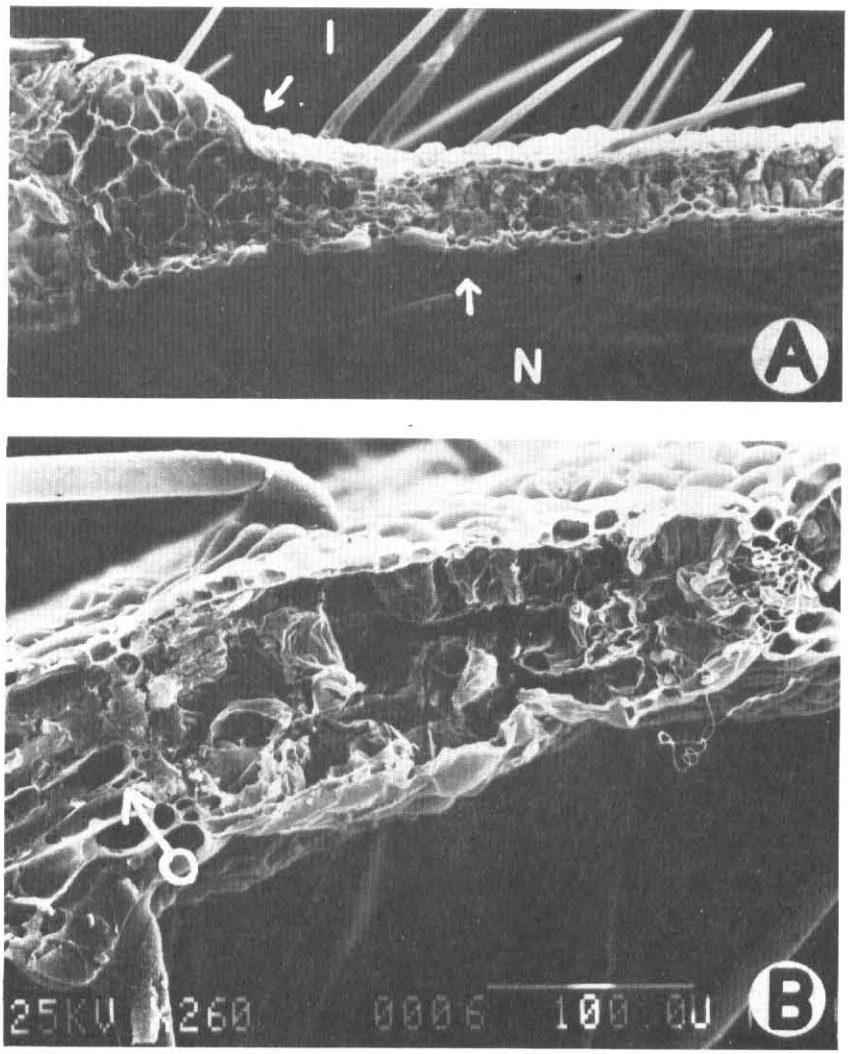

Fig. 5 Infected soybean leaf caused by *Xanthomonas campestris* pv. *glycines* on sideview.

A Infected region with hyperplastic tissue (I) v.s. normal tissue (N)

B The damaged vascular tissue caused by *X campestris* pv. *glycines*

Fig. 6 The heavy infection of bacterial pustule made soybean leaves turned yellow and began deforiating.