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Ice-Nucleation Activity of *Pseudomonas syringae* pv. *syringae* in Relation to Frost Injury of Wheat

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Ice-nucleation activity (INA) of four *Pseudomonas syringae* pv. *syringae* isolates from wheat (*Triticum aestivum* L), along with an isolate from basil, was determined by the droplet test at –5°C. The basil isolate showed low pathogenicity to wheat, though the five isolates gave positive (INA) reaction. Wheat seedlings (cvs. Sakha 69 and Giza 146) exposed to frost at –5°C before inoculation is obviously increased susceptibility to infection.

Wheat seedlings inoculated with a bacterial suspension (10^8 cfu/ml) expressed greater sensitivity to frost compared to checks sprayed with water. The frost damage was observed to be increased as the exposure to freezing was increased, though wounding and misting were among the most important factors. It could be concluded that a direct relationship exists between the inoculum concentration and the sensitivity to frost.

key words: Ice-nucleation activity, *Pseudomonas syringae* pv. *syringae* and *Triticum aestivum* L.

Bacterial blight, caused by *Pseudomonas syringae* pv.*syringae* van Hall (Dye *et al.*, 1980) is a foliar disease which spreads sporadically in some wheat-growing areas (Otta, 1974; Sellam and Wilcoxon, 1976; Scharen *et al.*, 1976; Shane and Baumer, 1987 and El-Sadek *et al.*, 1992). Meanwhile, *P. syringae* pv.*syringae* has been reported as an epiphytic bacterium on wheat (Shane and Baumer, 1987) as well as on other hosts (Lindow *et al.*, 1978).

The bacterium was considered to be one of the most common ice-nuclei on the surface of most plant species at relatively high freezing temperature, hence they are important in limiting supercooling of plant tissue at -2 to -5°C. (Lindow *et al.*, 1978, 1982). Azad and Schaad, 1988 indicated that the existence of both *Xanthomonas campestris* pv. *translucens* and *Pseudomonas syringae* pv. *syringae*, being pathogenic to wheat and ice-nucleating as well, were interrelated to sensitivity of wheat plants to frost injury.

In El-Minia area, the disease was reported for the first time during the growing seasons 1988 and 1989 (El-Sadek *et al.*, 1992). Field observations carried out during the course of this study indicated that the infection is confined to the lower leaves and usually occurred during the booting stage. Frost is a regular event at El-Minia governorate because of certain geographical and weather conditions.

Therefore, this work was undertaken to investigate the effect of frost on disease severity and the effect of the causal bacterium on the amount of damage induced by frost.

Materials and Methods

Bacterial isolates:

Four isolates (PW_2; PW_3; PW_5 and PW_8) of *Pseudomonas syringae* pv. *syringae* from wheat and one isolate (PB_3) from basil were revived from stored cultures collection at the Plant Pathology Dept., Minia Univ., Minia, Egypt. These cultures were isolated from the corresponding hosts and typified by El-Sadek *et al.* (1991) and (1992). The five isolates were checked for their pathogenicity and ice-nucleation activity (INA).

Pathogenicity tests:

Wheat cultivars Sakha 69 and Giza 164 obtained from Ministry of Agric., Egypt, were used in pathogenicity trial.

Wheat plants were grown for 15 days in plastic pots (15 cm in diam) in the greenhouse at a temperature 22±2°C for 16 hr photoperiod. The plants were atomized by the bacterial suspension (10^8) to runoff or rubbed gently with the mixture of 400-mesh carborandum. Control plants were sprayed by water. Inoculated plants were bagged by polyethylene for 24 hr and then placed under greenhouse conditions. Five pots, with two seedlings each, were used for each treatment. Disease severity was determined, 10 days after inoculation. The disease rating as described by El-Sadek *et al.* (1992) was used according to the following: 0= No symptoms, 1= Infection is less than 5% of the leaf surface, 2= 6-10% of the leaf surface is infected, 3= Coalesced lesion covered 11-25% of the leaf, 4= 26-50% of the leaf is blighted and 5= 51-100% of the leaf is blighted.

Ice-nucleation activity (INA):

INA activity of the isolates was determined using the droplet freezing procedure of Lindow *et al.* (1982). The bacterial cells of all five isolates were suspended in 0.1M phosphate buffer, pH 7.0 (10^8 cfu/ml), and serially diluted to 10^3 and then tested for INA at -5°C. Twenty aliquots of 10-μl of each isolate were applied to parafilmcoated aluminum foil boats. The aluminum boats were floated in a beaker containing ethanol: water (1:1 v/v). The number of droplets frozen within 3 min were counted and recorded. Each trial was repeated 3 times.

Effect of cold temperature at -5°C on frost damage and disease severity:

Seedlings of wheat cultivars Sakha 69 and Giza 164 (2-week-old) were atomized by the bacterial suspension as previously described. Control plants were sprayed by water alone. Inoculated plants were placed in an upright, frostless freezer at -5°C for 5, 10, 15 and 25 minutes. After cold treatment, plants were covered by two layers of wetted cheesecloth for at least 18 hr and checked for frost damage. Five pots contained five plants were used for each treatment and two leaves were assayed for each plant. Damage was expressed as percentage of leaf area with water soaking typical to frost injury (Azad and Schaad, 1988).

For disease severity the procedure described before was followed and the plants were rated 10 days after inoculation (El-Sadek *et al.*, 1992).

Effect of incubation period before cold treatment on frost damage:

Seedlings of wheat cultivars were inoculated by atomizing the bacterial suspensions as described before. Control plants were treated by water. A group of inoculated plants were kept under wet conditions for 8 hr in 3 successive days by using wetted cheesecloth, and the group was kept dry. All treated plants were placed in an upright frostless freezer at -5°C for 15 minutes. Five pots, each contained two plants, for each treatment and two plant leaves were examined. All treated plants were assessed for frost damage results were expressed as percentage of leaf area affected.

Effect of the method of inoculation on frost damage.

Seedling of wheat plants (cv. Sakha 69) were inoculated by atomization and carborandum-rubb methods. Control plants were treated by water only. Inoculated plants were exposed to low temperature at -5°C for 15 minutes. Plants were assessed for frost damage and the percentage of affected leaf areas was recorded. Five pots each contained two plants, were used for each treatment and two leaves were assessed for each plant.

Determination of population of P. syringae pv. syringae on wheat:

Wheat seedlings (2-week-old) of cvs. Sakha 69 and Giza 164, were inoculated with 10^8cfu/ml of isolate PW_3 of *P. syringae pv. syringae* as described before. Five pots each contained two plants were used for each treatment. Inoculated plants were placed in the greenhouse at 25±2°C for at least 12 hr covered with continuously wetted cheesecloth. Two leaves were then cut from each plant after 1, 3, 5, 10 and 15 days and each leaf sample was cut into 2-cm-long segments. Segments of each leaf were placed in a test tube containing 5 ml of sterile saline plus 0.01% Tween 80 and were shaken with reciprocal shaker for 5 minutes and spun for 1 min. Appropriate tenfold of serial dilutions of the wash water and 0.1 ml pipetted onto each of three plates of KB medium (Azad and Schaad, 1988). Colonies of each plate were counted and recorded 3 days after incubation.

Results

Table (1) shows the pathogenicity of the five isolates tested of *P. syringae pv. syringae* to the wheat cultivars tested and *in vitro* ice-nucleation activity of the bacteria. The bacterial isolates showed marked ice-nucleation activity and the number of frozen droplets at -5C° ranged between 29 and 48 out of sixty droplets, in three trials.

The results recorded that the disease severity varied according to the cultivar tested. However, the isolates PW_5 and PW_2 were the most pathogenic to both cultivars. The basil isolate of (BP_3) gave the lowest disease severity on Sakha 69 while it was not pathogenic to cv. Giza 164 of wheat.

Table 1. Pathogenicity and *in vitro* ice-nucleation activity of *Pseudomonas syringae pv. syringae* isolates

Bacterial isolates	Origin hosts	Disease severity[a] on wheat		INA at -5°C	
		Sakha 69	Giza 164	Response	No of frozen droplets
PW_2	Wheat	2.1b	1.8a	+[b]	48/60[c]
PW_3	Wheat	1.3c	1.2b	+	29/60
PW_5	Wheat	2.7a	1.6a	+	32/60
PW_8	Wheat	1.5c	1.1b	+	38/6
PB_3	Basil	1.2c	0.0c	+	41/60

[a] Disease severity was based on rating scale 0=no symptoms; 5=>50% of leaf area was blighted. Means are average of five replicates of five plants each with two leaves.
[b] + = positive response.
[c] Accumulative results of 3 trials, each twenty droplets were applied.
Means in each column with the same letters are not significantly different according to Duncan's multiple range test.

The effect of cold treatment on disease severity on Sakha 69 wheat seedlings inoculated with the most pathogenic isolates of *P. syringae* pv. *syringae*, are shown in Table (2). Results indicated that cold treatment increased the sensitivity of wheat seedlings to infection by both isolates compared to the untreated ones. The effect is obviously increased by increasing the exposure time of the cold treatment. Significant differences were obtained after 20 and 25 min for the isolate PW_2 and after 25 min. for the isolate PW_5.

Table 2. Effect of cold treatment of wheat at -5°C on disease severity caused by a selected *Pseudomonas syringae* pv. *syringae* isolates

Treatments (min)	Disease severity		
	Water	PW_2 isolate	PW_5 isolate
0	0.0	1.8bc	2.2b
5	0.0	2.0b	2.7b
10	0.0	2.1b	2.7b
15	0.0	2.7b	3.2ab
20	0.0	3.2a	3.4ab
25	0.0	4.0a	3.8a

Mean in each column with the same letter are not significantly different (0.05 level) according to Duncan 's multiple range test.

The percentage of leaf area in (Sakha 69) wheat seedlings that showed frost damage, due to cold treatment as influenced by inoculation with *P. syringae* pv. *syringae*, is shown in Table (3). The isolates used caused significantly greater damage compared to the control treatment when exposed to -5°C for more than 15 min. Longer exposure for 20 and 25 min caused significant differences between the two isolates tested.

Table 3. Effect of cold treatment at -5°C on frost damage in wheat seedlings inoculated with selected *Pseudomonas syringae* pv. *syringae* isolates

Treatment	Leaf area (%) affected after				
	5	10	15	20	25 min.
Water	0a	0a	5c	12c	15c
PW_2	5a	22a	35b	66b	85b
PW_5	10a	25a	45a	87a	100a

Means in each column followed by the same letters are not significantly different (at P≥ 0.05 according to Duncan 's multiple range test.

Results in Table (4) show the effect of the method of inoculation on the degree of damage in wheat seedlings after being exposed to cold treatment. Data showed that the percentage of the damaged leaf area produced by the frost in inoculated plants with *P. syringae* pv. *syringae* using carborandum were significantly higher over those inoculated with the sprayed bacterial suspension(s). The percentages of damage on cv. Giza 164, however, were lower than those obtained for cv. Sakha 69.

Table 4. Effect of inoculation method of wheat seedlings with *Pseudomonas syringae* pv. *syringae* isolates on the amount of frost damage when exposed to cold treatment (-5°C) for 20 min.

Cultivar	Leaf area (%) showed frost damage		
	Method of inoculation	PW_2	PW_5
Sakha 69	Carborandum rubb method	55a	60a
	Atomization	33c	42b
Giza 164	Carborandum rubb method	40b	47b
	Atomization	28c	30c

Means in the same column within the same cultivar followed with the same letter are not significantly different according to Duncan's multiple range test at level 0.05

The effect of time of incubation of wheat seedlings inoculated with *P. syringae* pv. *syringae* isolates and exposed to cold treatment at –5°C for 15 min, on the amount of frost damage is shown in Table (5). It is obvious that as the time of incubation period is increased the average percentage of leaf area showing frost damage increased. Control plants were almost not affected. However the most significant results with both isolates were obtained three days after incubation. Plants exposed to mist conditions during incubation were too much affected with frost damage than those kept under dry conditions.

Table 5. Effect of time of incubation before cold treatment at -5°C for 15 min on frost damage as influenced by *Pseudomonas syringae* pv. *syringae* isolates

Treatment and incubation period (day)	Leaf area (%) showed frost damage		
	Water	PW_2	PW_5
Partially mist condition			
0	0.0	33c	41c
1	0.0	35c	47c
2	0.0	48b	59b
3	5	62a	70a
Dry condition			
1	0.0	0.0d	0.0d
2	0.0	5d	5d
3	0.0	5d	10d

Means in the same column followed with the same letters are not significantly different at level 0.05.

Epiphytic population of *P. syringae* pv. *syringae* on both wheat cultivars (Sakha 69 and Giza 164) inoculated with the spraying method, is presented in Fig 1. The build up of the bacteria on leaves of cv. Sakha 69 was increased more rapidly up to day 10 and then decreased. Generally, the bacterial population was higher on leaves of cv. Sakha 69 than that recorded for cv. Giza 164.

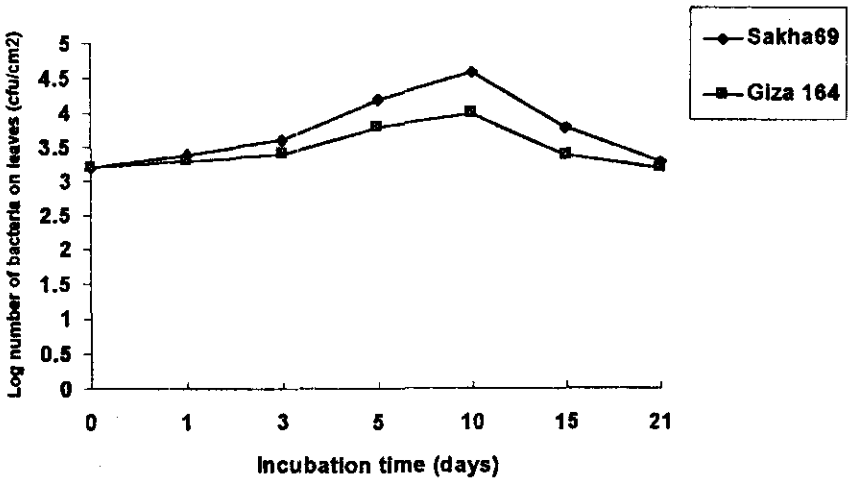

Fig 1. Population of *Pseudomonas syringae* pv. *syringae* isolates on leaves of both cvs. Sakha 69 and Giza 164 wheat. Plants were sprayed with 10^8 cfu/ ml of isolate PW_5 and incubated under mist condition. Each point of the log population was calculated as mean of three readings.

Discussion

Bacterial blight disease of wheat, caused by the bacterium *P. syringae* pv. *syringae* spread sporadically in wheat fields at El-Minia governorate. Field observations indicated that infection is confined usually to the lower leaves, where the humidity is high, and is occurred at the booting stage. The temperature at El-Minia governorate usually drops to zero or below and frost damage is commonly observed on wheat at certain periods. Although the disease might be considered as an economically less important to the wheat crop, it was interesting to investigate its relationship to frost injury and vice-versa.

All isolates of *P. syringae* pv. *syringae* under investigation, either from wheat or basil, were ice-nucleation active at -5°C. However, pathogenicity test showed that the basil isolate was weak on wheat, while PW_2 and PW_5 were the most pathogenic compared with the other two isolates (PW_3 and PW_8).

Cold treatment at -5°C increased the severity of the disease on wheat seedlings of both cvs. Sakha 69 and Giza 164. The effect increased as the exposure time was increased. These results agree with those of Azad and Schaad (1988) when they inoculated wheat seedlings by either *Xanthomonas campestris* pv. *translucens* (the causal of black chaff disease) or *P. syringae* pv. *syringae* and expose the plants to cold treatment at -4°C. However, frost injury might predispose the plants to infection by the bacterium. Many studies showed that *P. syringae* pv. *syringae* could survive as an epiphyte on the surface of wheat leaves (Shane and Baumer, 1987; Azad and Schaad, 1988 and El-Sadek *et al.*, 1992,). The presence of the bacterium prevents supercooling (Gross *et al.*, 1982), therefore increased plant sensitivity to cold temperature. However, the results of this study did confirm this finding. Both wheat cultivars (Sakha 69 and Giza 164) inoculated with the bacterial suspension showed more frost damage. These results could be supported with the previous results obtained by Arny *et al.* (1976), Weaver (1978) and Azad and Schaad (1988).

Azad and Schaad (1988) found that the highest disease incidence in cold injured wheat plants is directly related to the number of INA bacteria on these plants. A similar trend was reported by Shane and Baumer (1987) and supporting the results of this study which showed that the carborandum-rubb method of inoculation of wheat (cv. Sakha 69) showed more severe cold injury than those inoculated with the spray inoculation. This could be explained by the fact that wounds made by carborandum provided avenues to ingress of the pathogen into the host tissue which promote rapid growth of the pathogen inside the tissues.

In this study, wheat seedlings of cv. Sakha 69 inoculated with 10^8 cfu/ml of PW_2 and PW_5 of the pathogen and incubated for three days under partially mist conditions differed in their response to cold treatment compared with those kept under dry condition. This could be explained by an increase in the growth of *P. syringae* pv. *syringae* A similar results were obtained (Azad and Schaad, 1988). Also, the study suggests that dryness or partially wetting conditions might be

related to the population build up of the pathogen in or on plant tissues. Shane and Baumer (1987) stated that *P. syringae* pv. *syringae* is a weak pathogen that requires moist conditions during incubation for significant infection and this was strongly correlated with the bacterial population.

Results in this study indicated that the bacterial population increased at higher levels on leaves of cv. Sakha 69 than on those of cv. Giza 164. However, cv. Sakha 69 was considered a susceptible cultivar to *P. syringae* pv. *syringae* by El-Sadek *et al.* (1992), while cv. Giza 164 showed more resistance according to this study. This fact, could explain the differences between the population build up of the bacteria on both cultivars.

Marshall (1988) indicated that the amount of freezing damage is dependent on the plants inherent ability to withstand cold temperature, the concentration of INA of bacteria on the leaves, and the exposure time to low temperature. Thus, this study suggested that type of growing cultivar should be considered as a major factor to avoid this problem.

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تكوين البللورات الثلجية بواسطة البكتريا بسيدوموناس سيرنجي سلالة ممرضة سيربجي وعلاقتها بضرر الصقيع في القمح

طه إبراهيم عبد الجواد ، على عبد المنعم البنا ، نبيلة عبد الظاهر عبد العزيز ، صادق أحمد محمد الصادق

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تم اختبار تكوين البللورات الثلجية عند درجة حرارة -٥ °م لأربع عزلات من بسيدوموناس سيرنجي سلالة ممرضة سيرنجي عزلت من القمح وعزلة واحده من نفس البكتريا عزلت من نباتات الريحان . أعطت جميع العزلات المختبرة نتائج إيجابية لهذا الإختبار. بينما أظهرت إختبارات القدرة المرضية أن عزلة الريحان كانت أضعف العزلات الخمس عند إختبارها على بادرات قمح من صنف سخا٦٩ وجيزة١٦٤.

أوضحت النتائج أن تعريض البادرات عمر ١٥ يوم لكل من صنفي القمح لدرجة حرارة منخفضة -٥ °م قبل الحقن بالبكتريا الممرضة أدى الى زيادة قابلية البادرات للإصابة البكتيرية.

أوضحت الدراسة أنة عند حقن بادرات القمح بالمعلق البكتيري ٨١٠ وحدة مكونه للمستعمرة زادت حساسيتها للصقيع مقارنة بالنباتات التي تم معاملتها بالماء فقط. كذلك لوحظ أن ضرر الصقيع زاد بصورة واضحة في البادرات المجروحة كذلك التي حفظت تحت ظروف رطبة عن تلك الغير مجروحة أو التي حفظت تحت ظروف جافة.

ويستخلص من الدراسة أن هناك علاقة مباشرة بين تركيز الحقن بالخلايا البكتيرية وضرر الصقيع في البادرات المعاملة.