

The rhizobacterium *Pseudomonas aeruginosa* 23₁₋₁ protects watermelon against gummy stem blight caused by *Didymella bryoniae*

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Screening for disease-reducing rhizobacteria:

- Screening of a total of 190 bacterial strains from roots of watermelon in four provinces in the Mekong Delta, Vietnam.
- Testing in dual culture experiments for antibiosis.
- Reduction of plant infection by four of the tested strains.
- Detailed investigation of isolate *Pseudomonas aeruginosa* 23₁₋₁.
- Application of bacteria by seed soaking and soil drenching.

Mechanisms of protection against *D. bryoniae* investigated by:

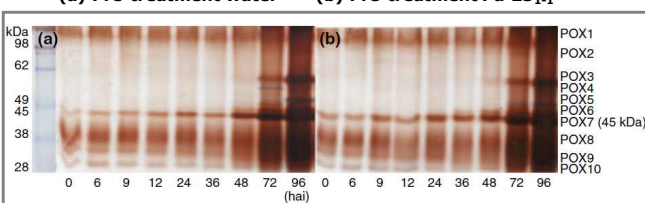
- Microscopy
- Peroxidase isoforms
- Bacterial population in plants
- H₂O₂-accumulation

Microscopy of the infection course of *D. bryoniae* in cv. 232-0125/B (susceptible)

Pct. appressoria:	32 hai		44 hai	
	<i>Pa</i> 23 ₁₋₁	Water	<i>Pa</i> 23 ₁₋₁	Water
causing penetration	7.0	4.0 ^{NS}	41.0	39.5 ^{NS}
with successful penetration and tissue colonization	5.0	0.0 ^{***}	34.4	58.4 ^{***}
with accumulation of H ₂ O ₂	43.5	17.5 ^{NS}	30.5	11.5 [*]
with failed penetration and accumulation of H ₂ O ₂	39.2	14.2 ^{NS}	10.2	7.1 ^{NS}

Acidic peroxidase isozymes (native-PAGE) in watermelon cv. PI 189225 (resistant) after inoculation with *D. bryoniae*

(a) Pre-treatment water (b) Pre-treatment *Pa* 23₁₋₁



Symptoms of *D. bryoniae* in watermelon in the field

A: Control without *Pa* 23₁₋₁. **B:** Seed coating + soil drenching with *Pa* 23₁₋₁. **C:** foliar spraying with *Pa* 23₁₋₁. **D:** Seed coating + soil drenching + foliar spraying with *Pa* 23₁₋₁



Leaf symptoms of *D. bryoniae* on watermelon 232-0125/B at 7 dai
Left pot: plant pre-treated with *Pa. aeruginosa* 23₁₋₁.
Right pot: plant pre-treated with water

Reduction of gummy stem blight in two watermelon lines under greenhouse conditions. *Ps. aeruginosa* 23₁₋₁ applied by seed soaking and soil drenching.

Treatment	Pct. disease	Pct. disease reduction
PI 189225 (resistant)	7 dai	
Bacterial pre-treatment	16.0	69.7
Water (control)	52.8	-
LSD _{.95}	24.9	-
<i>P</i> -value	0.0139	-
232-0125/B (susceptible)	4 dai	
Bacterial pre-treatment	13.5	49.0
Water (control)	26.6	-
LSD _{.95}	9.0	-
<i>P</i> -value	0.0024	-

Conclusion

- The bacterial strain *Pseudomonas aeruginosa* 23₁₋₁ can reduce gummy stem blight in watermelon under greenhouse and field conditions
- Protection involves both antibiosis and induced resistance
- *Pa* 23₁₋₁ colonises the stem of treated plants endophytically, but only first true leaves when the plant is attacked by *D. bryoniae*
- Reference: Nga et al. (2010): Journal of Applied Microbiology. 109: 567-582.

Population of *Ps. aeruginosa* 23₁₋₁ in stems and leaves of cv. 232-0125/B Bacteria were applied by seed soaking and soil drenching (7 day-old seedlings) Inoculation on the first true leaf when this was fully developed (15 day-old plants)

Bacterial population (log ₁₀ cfu/g tissue)	Days after soil drenching			Days after inoculation ^A		
	1	3	5	1 (9)	2 (10)	3 (11)
HYPOCOTYL						
Not inoculated	2.3	3.2	2.6	1.7	2.3	3.1
Inoculated with <i>D. bryoniae</i>	na	na	na	1.4	3.4	4.9
<i>P</i> -value	-	-	-	<0.0001	<0.0001	<0.0001
FIRST TRUE LEAF						
Not inoculated	nd	nd	nd	0.0	0.0	0.0
Inoculated with <i>D. bryoniae</i>	nd	nd	nd	1.2	4.5	6.4
<i>P</i> -value	-	-	-	<0.0001	<0.0001	<0.0001

^A Numbers in brackets indicate days after soil drenching with bacteria
na: not applicable; nd: not determined

Pct. infected leaves and watermelon yield under field conditions in Vietnam in the season September to November, 2008

Treatment	Days after sowing				Yield (t/ha)
	19	33	47	61	
Seed soaking/soil drenching <i>Pa</i> 23 ₁₋₁	0.0 ^{***}	12.3 ^{**}	6.4 ^{***}	59.8 ^{***}	14.7 ^{***}
Foliar spraying <i>Pa</i> 23 ₁₋₁	3.5 ^{NS}	18.5 ^{NS}	15.5 ^{***}	74.7 ^{***}	11.6 ^{***}
Seed soaking/soil drenching/foliar spraying <i>Pa</i> 23 ₁₋₁	0.0 ^{***}	5.8 ^{***}	5.0 ^{***}	41.0 ^{***}	20.6 ^{***}
Control without <i>Pa</i> 23 ₁₋₁	4.4	17.8	23.1	85.1	8.0