Pressure Cycling Technology Sample Preparation System (PCT SPS) Improves Quantification of Pathogen DNA in Plants and Soil

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The Problem

- *Rhizoctonia* spp. cause yield-limiting root diseases in the Pacific Northwest
- *Rhizoctonia* DNA extraction is inconsistent
  - -- in soil and root samples
  - -- at low soil population densities
  - -- due to tough hyphae
  - -- due to formation of survival structures
- Conventional DNA extraction methods cause shearing, heating, cellular debris
- Sample contamination can occur with mortar/pestle homogenization
- Triplicate extracts of samples are required for reliable real-time PCR quantification.

The PCT SPS

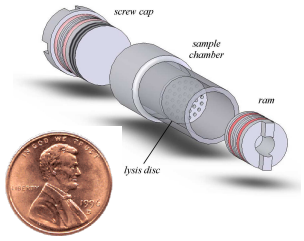
The PCT SPS uses alternating cycles of high and low pressures to induce cell lysis. Cell suspensions or tissues, such root or leaf, or complex matrices, such as soil, are placed in specially designed, single-use processing containers (PULSE Tubes) and are then subjected to alternating cycles of high (up to 35,000 PSI) and ambient pressures in a pressure-generating instrument (Barocycler). Maximum and minimum pressures, the time at each pressure level, and the number of cycles is defined using a programmable logic controller. The reaction chamber of the Barocycler instrument is temperature controlled using a peripheral circulating water bath. Safety features in the PCT System design significantly reduce likelihood of cross contamination during sample extraction [1].

Features of PCT SPS

- From ambient to 35,000 psi
- Precisely controlled variables
  - -- cycle number
  - -- cycle profile
  - -- temperature
  - -- chemistry
  - -- phase changes of ice
- Minimal shearing, heating, debris
- Closed system--reduced contamination
- Universal extraction

Barocycler™ NEP3229

PULSE™ Tube



Improved Pathogen Quantification in Root Samples[2]

Sample	Treatment[a]	Pathogen DNA (ng/g) *R. solani*	Pathogen DNA (ng/g) *R. oryzae*
Bobwhite			
10 mg	PCT	401 ± 122	NA[b]
20 mg	PCT	383 ± 127	NA
20 mg	no PCT	5.9 ± 0.1	NA
TaAB			
10 mg	PCT	942 ± 117	285 ± 60
20 mg	PCT	1689 ± 359	132 ± 20
20 mg	no PCT	1.8 ± 1.4	17 ± 11
TaC			
20 mg	PCT	53 ± 8.9	24 ± 4.7
20 mg	no PCT	5.4 ± 0.04	5.0 ± 1.3

[a] PCT: 35,000 psi 20 sec / ambient 10 sec, 15 cycles
[b] Not applicable; Bobwhite not inoculated with *R. oryzae*.

Enhanced Reproducibility

Sample	*R. solani* S.D.[a] PCT	*R. solani* S.D.[a] BB	*P. abappressorium* S.D.[a] PCT	*P. abappressorium* S.D.[a] BB
T43a	0.48	1.20	0.27	0.06
T5Rd(c)	0.38	1.66	0.72	1.00
T9-06 R3b	0.03	0.53	1.78	0.42
T9-06 R3c	0.32	2.13	0.09	0.43

[a] Standard deviations of the means of triplicate Ct values from real-time PCR reactions, excepting *P. abappressorium* assays of T9-06R3b and R3c (duplicate reactions).

Improved Pathogen Quantification in Soil Samples[2]

Sample	Treatment	*R. solani* DNA (pg/g)	*R. solani* Fold-diff.	*P. abappressorium* DNA (pg/g)	*P. abappressorium* Fold-diff.
T4R3	PCT[a]	551 ± 94	3.6	2.3 ± 0.24	1.0
	BB[b]	152 ± 68		2.3 ± 0.07	
T6R2	PCT	258 ± 36	16	33 ± 10	1.8
	BB	16 ± 10		18 ± 6.7	
T9R3b	PCT	515 ± 4.8	7.3	67 ± 36	12
	BB	71 ± 14		4.8 ± 0.73	
T9 R3c	PCT	432 ± 45	5.6	36 ± 1.2	1.5
	BB	76 ± 13		23 ± 3.4	

[a] PCT: 35,000 psi 20 sec / ambient 10 sec for 15 cycles
[b] Bead-beating (BB): FastPrep120 homogenizer, setting "5", 45 sec

Summary

- Detectable amounts of *Rhizoctonia* DNA were obtained from wheat roots that were previously recalcitrant to homogenization.
- In 80% of the root samples, pathogen DNA was extracted in amounts high enough to be quantified using real-time PCR.
- The PCT SPS improved the extraction of *Rhizoctonia* DNA from agricultural soils up to 30-fold.
- PCT SPS samples were free from contamination that can occur during conventional extraction procedures.
- PCT SPS confers a significant advantage in screening of germplasm for pathogen tolerance.

[1] Schumacher, RT *et al.* (2002). Am. Laboratory 34, 38-43.

[2] Okubara, PA *et al.* (2007) Can. J. Plant Pathol., in manuscript.