Enhancing Soybean Sprout Food Safety Through Seed Disinfection Treatments

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Introduction

Soybean sprouts, a traditional vegetable in Asia, are gaining popularity in the U.S. Soybean sprouts are generally prepared by soaking, thus sprouting conditions could favor propagation of various microorganisms including plant pathogenic fungi. Sprout consumption has been associated with numerous outbreaks of foodborne illness and, in some cases, the pathogens are known to be seed-borne. To improve sprout safety seed disinfection treatments have been used on other kinds of sprouts. However, there is little information about seedborne pathogen incidence on soybean seeds for sprout industry in Virginia.

Goal and Objectives

Project goal: Identify seedborne pathogens and evaluate different decontamination technologies for soybean sprout seeds.

1. Isolate and identify seed borne fungi from MFS-561, a commercial soybean sprout variety.
2. Evaluate the effect of several seed decontamination treatments on seedborne pathogens before germination.
3. Determine the effect of decontamination seed treatments on soybean sprout quality traits.

Materials

MFS-561, a sprout commercial variety, from three different growing regions: Southern Virginia, Eastern Virginia and Northeastern North Carolina in 2015.

Methods

Objective 1*

- Seeds: treat with sodium hypochlorite 2% for 1 min and sterile water for 1 min
- Mold test: 10 seeds on PDA plates at room temperature for 5 days (2 replications)
- Fungi isolation and DNA extraction
  - Fungi growing on seeds will be isolated and DNA will be extracted
  - ITS region of fungi DNA will be amplified and sequenced (primers ITS1f and ITS4r)
  - Microorganism genetic resources will be used for fungus specie identification

*ongoing research

Objective 2

- Seed treatments:
  60 °C hot water, 2 min
  50 °C dry heat, 1-hour
  5% acetic acid, 2 min
  5% lactic acid, 10 min
  2% sodium hypochlorite, 1 min
  2% sodium hypochlorite, 10 min
  2% calcium hypochlorite, 10 min
  Sterile water, 1 min (control)
- Mold test: 10 seeds/plate
  - 3 reps of each treatment from the three different regions
  - Response variable: percentage of mold incidence

Objective 3

- Seed treatments:
  5% acetic acid, 2 min
  60 °C hot water, 2 min
  2% calcium hypochlorite, 10 min
  Sterile water, 1 min (control)
- Sprout trait evaluation: 350 seeds will be grown in a bean sprouter machine during 5 days, for each treatment from 2 regions (3 replications)
- Sprout traits:
  Hypocotyl length (cm)
  High-, mid- and low- quality sprout percentage (%)
  Sprout yield (g/g of seeds)

Statistical Analysis:

- Analysis of variance (ANOVA) to evaluate treatment effects.
- Fungi incidence was transformed using the root square to meet ANOVA assumptions.
- Tukey's HSD test to identify significant differences among locations and treatments at P=0.05.
- Statistical analysis was calculated using JMP 11.0 by the SAS institute Inc.

Results

- **Decontamination seed treatments**

  - Fungi incidence among treatments and seed growing regions were significant different (Table 1). Southern Virginia seeds had higher fungi incidence than seeds from Northeastern North Carolina, while fungi incidence in Eastern North Carolina was not significantly different from the other two growing regions (Fig.1).

Table 1. ANOVA of the root square of fungi incidence.

Source	DF	Sum of Squares	F Ratio	Prob > F
Seed treatments (ST)	7	573.42	23.03	<.0001*
Seed growing regions (SGR)	2	31.62	4.44	0.014*
ST*SGR	14	80.55	1.62	0.088

* significant difference among treatments at the level P≤0.05 level

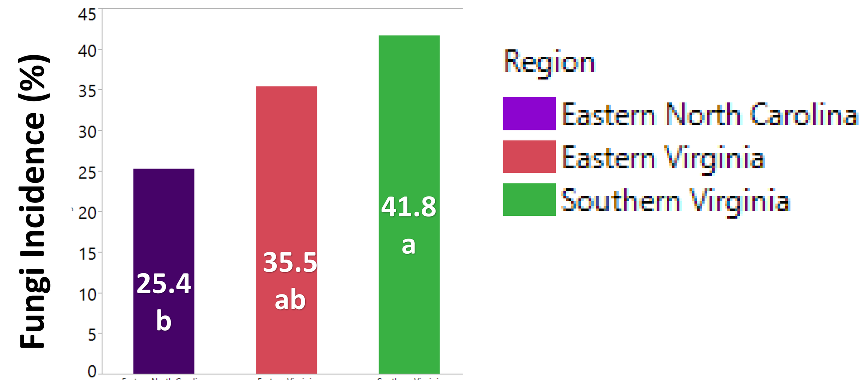

Figure 1. Average Fungi incidence by region.

- Untreated seeds had a fungi incidence of 59% across the three regions. The most effective treatment was hot water, which reduced significantly the fungi incidence to 4%, followed by calcium hypochlorite and acetic acid with 14.7% and 16.7% of fungi incidence, respectively (Fig. 2). The effectiveness of calcium hypochlorite, acetic acid and hot water, was easily observed on the PDA plates (Fig.3).

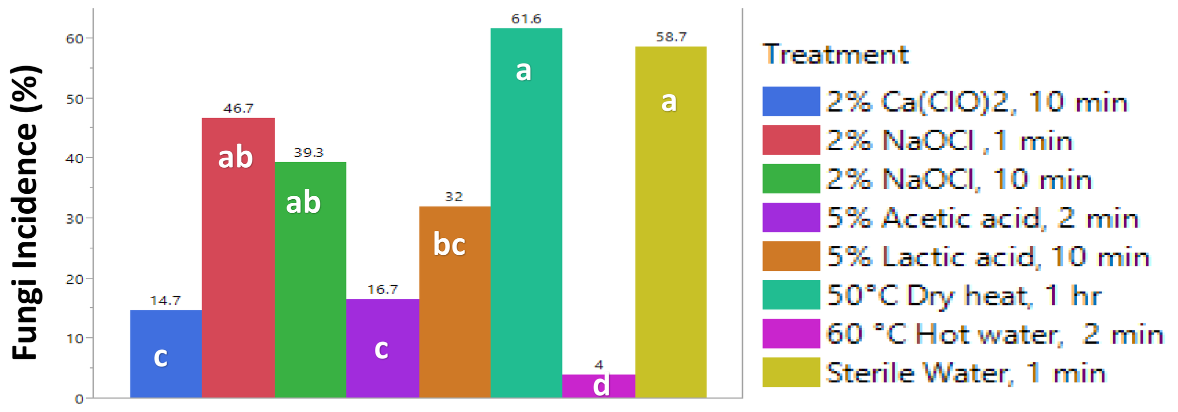

Figure 2. Average fungi incidence by treatment across three regions.

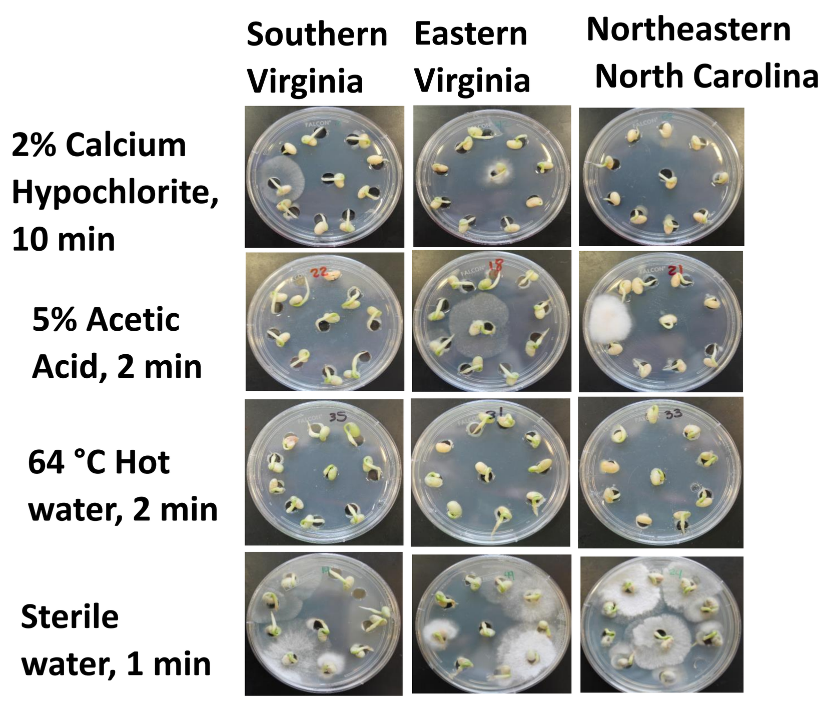

Figure 3. PDA plates after five days of incubation at room temperature of calcium hypochlorite, acetic acid, hot water and sterile water.

- Thus, seed treatments of 5% acetic acid for 2 min, 60°C hot water for 2 min and 2% calcium hypochlorite for 10 min can be considered as potential disinfection treatments for sprout seeds. However, seed treatments could have negative effects on seed germination. For this reason, it is important to evaluate their effect on sprout traits before making a seed treatment recommendation.

- **Effect of decontamination treatments on sprout traits**

  - All sprout quality traits were affected by seed treatments. MQS% was significantly different between two regions, while MQS% and LQS% were affected by the interaction between treatment and region (Table 2).

Table 2. The mean of soybean sprout traits and the effect by regions, seed treatments and their interaction.

Sprout trait	Mean	Treatments (T)	Regions (R)	T x R interaction
HQS%	25.57	0.0001*	0.54	0.49
MQS%	34.64	0.0001*	0.0220*	0.0093*
LQS%	39.78	0.0027*	0.11	0.0284*
Sprout yield	3.5	0.0326*	0.75	0.09
Hypocotyl Length	8.26	0.0102*	0.82	0.26

*significant differences at P≤0.05 level

- Calcium hypochlorite treatment had a positive effect on sprout traits (Table 3). While acetic acid didn't cause any negative effect on sprout traits compared to the control (sterile water for 1 min). The hot water treatment reduced significantly sprout quality by reducing HQS% (8.19%) and increasing MQS% (46.81%), but it didn't affect sprout germination (LQS%).

Table 3. Effect of seed treatments on soybean sprout quality traits.

Level	HQS%	MQS%	LQS%	Sprout Yield	Hypocotyl Length
2% Calcium hypochlorite for 10 min	35.71A	31.57B	32.72B	3.99A	8.90A
Sterile Water for 1 min	29.76A	27.86B	42.38A	3.20B	7.77B
5% Acetic acid for 2 min	28.62A	32.33B	39.05AB	3.37AB	7.72 B
60 °C Hot water for 2 min	8.19B	46.81A	45A	3.28AB	8.67AB

*Different letters represent significant differences at P≤0.05 level

- There were not significant differences on sprout traits among seed growing regions, except for MQS% where Eastern Virginia had significantly higher MQS% of 37.07% than Southern Virginia with 32.21% (Table 4).

Table 4. Effect of the seed growing regions on sprout quality traits.

	HQS%	MQS%	LQS%	Sprout Yield	Hypocothyl Length
Southern Virginia	26.31	32.21B	41.47	3.43	8.24
Eastern Virginia	24.83	37.07A	38.09	3.49	8.29

*Different letters represent significant differences at P≤0.05 level

- In accordance with the results, calcium hypochlorite and acetic acid treatment were the best seed disinfection treatments, because they reduced significantly fungi incidence and improved or keep sprout quality.

Conclusion

An ideal seed treatment would not produce toxic residue, would be harmless to seed and be effective on reducing fungus incidence. Thus, calcium hypochlorite and acetic acid treatments can be recommended as safe and effective seed disinfection treatments without negative effects on seed quality. Further study will evaluate 2016 seeds from these three regions, so year effect will be tested.

Acknowledgment

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