# Symbiotic Fungal Endophytes that Confer Tolerance for Plant Growth in Saline and Dry Soils

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# Outline

## Background

- ✓ Symbiosis
- ✓ Fungal endophytes
- ✓ Saline and alkaline soils
- ✓ Symbiosis fungal biotechnology

## Research strategies and objectives

## Preliminary results

## Plan for the future

## ➢ Symbiosis in fungi

- 1879 Anton de Bary: ‘the living together of unlike organisms
- Symbiotic relationships between plants and fungi are extremely common in nature
- Symbiotic fungi contribute to and may be responsible for the adaptation of plants to environmental stresses

## ➢ Fungal endophytes in plants

- There are several types of endosymbiotic fungi that grow within roots and shoots

- Certain fungal endophytes permit adaptation and survival of plants to high salt stress habitats

# Table 1 Symbiotic criteria used to characterize fungal endophytic classes

| Criteria | Class1 | Class2 | Class3 | Class4 |
|---|---|---|---|---|
| **Host Range** | Restricted | Unrestricted | Unrestricted | Unrestricted |
| **Colonization** | Shoot | Shoot & root | Shoot | root |
| **Transmission** | Vertical & Horizontal | Vertical & horizontal | Vertical | horizontal |
| **Fitness benefits*** | NHA | NHA and HA | NHA | NHA |

**Nonhabitat-adapted (NHA) benefits such as drought tolerance and growth enhancement are common among endophytes regardless of the habitat of origin. Habitat-adapted (HA) benefits result from habitat-specific selective pressure such as pH, temperature and salinity.*

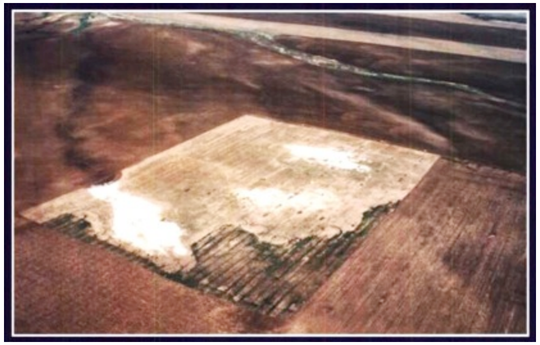
R.J.Rodridguez et al. 2009. Fungal endophytes: diversity and functional roles. New Phytologist

# Saline soils

- Widespread, Saskatchewan and worldwide
  → causes include natural conditions and irrigation
- ~ 10 % of agricultural soils are salinized to some extent
  → reduced crop yields
- Salinity is a particularly intractable stress because *it cannot be remediated by supplementation*
  - Salinity reduces water uptake, which is required for germination and growth
  - Potash tailings (>90% NaCl) are an excellent model system

Summit Creek, SK
*White in this picture is* calcium sulfate

Chaplin Lake, SK:
*White in this picture* is sodium sulfate



*Salinity management for sustainable irrigation* (2000), Daniel Hillel
*White in this picture* is a mixture of salts

## ➢ Symbiosis fungal biotechnology

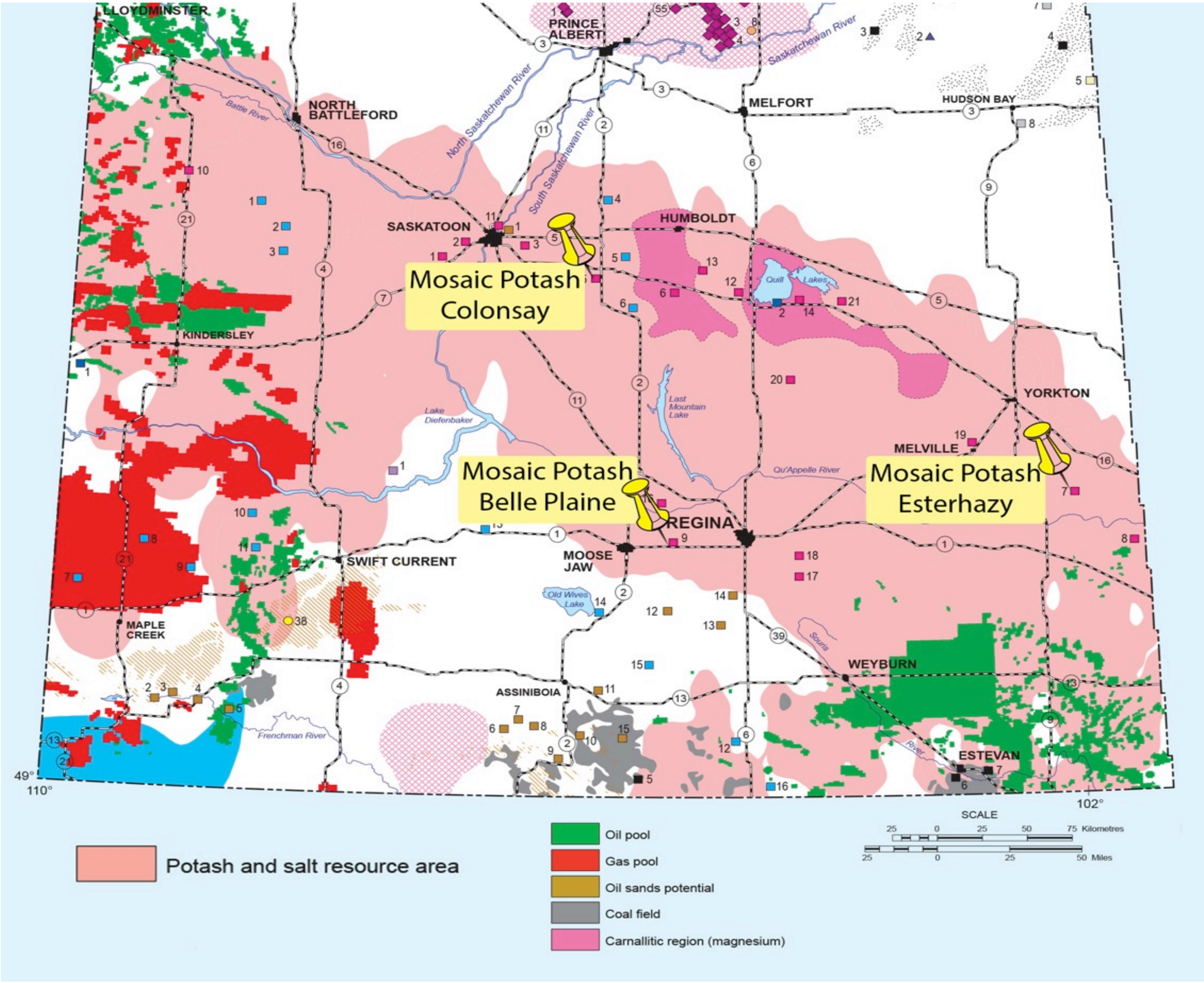
- Potential biotech application of facultative fungal ‘symbiogenesis’ is now being realized
- A symbiogenic strategy for reducing the effects of biotic stress in agriculture
- Symbiotic technology may be helpful in mitigating impacts of climate change in crops and expanding agriculture production onto marginal lands

# Survey for local Saskatchewan adaptive strains

- **May 2012 collection**
- **9 sites; 90 collections**
- **~ 450 endophyte strains**

Chaplin Lake Jun 2012

Focus on endophytes *from Mosaic tailings management area sites at Colonsay and Belle Plaine*, collected in May 2012






http://www.economy.gov.sk.ca/mineralresourcemap

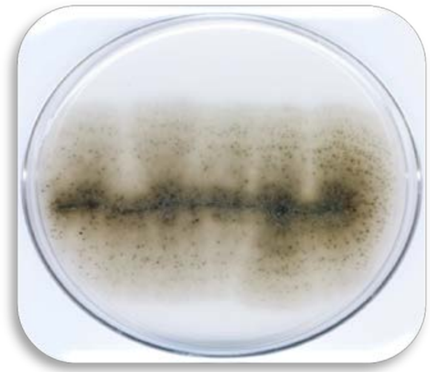
# Methodology

**Plant collected from saline site**

**Surface sterilized and cut into pieces**

**Plated on potato dextrose agar**

**Single strain isolated**



**Grown for spore harvest**

**Spores harvested suspended in water**

**Spore suspension applied to charcoal granules**

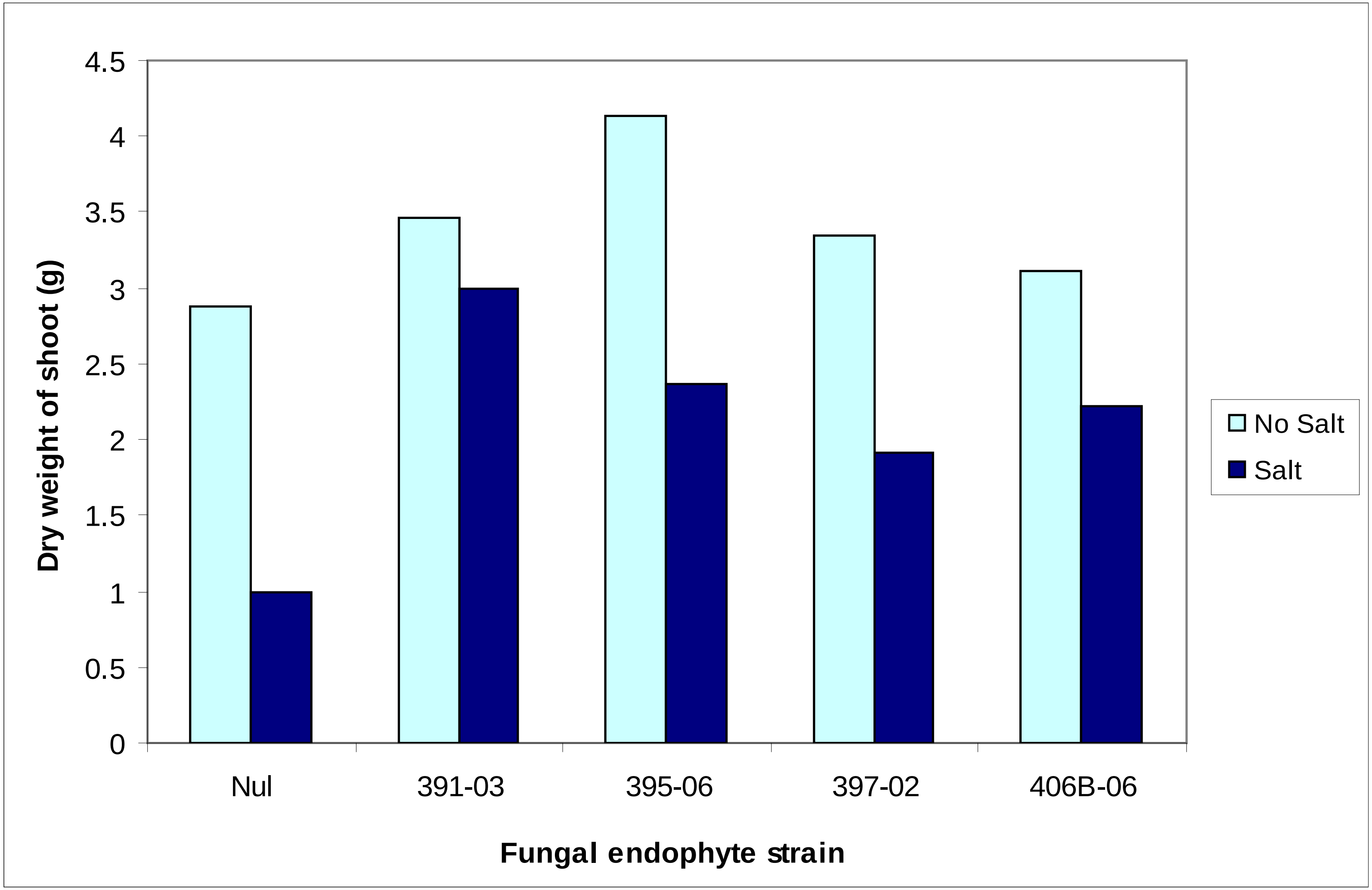
**Charcoal tested for good fungal growth**

**Charcoal granules placed with germination seeds**

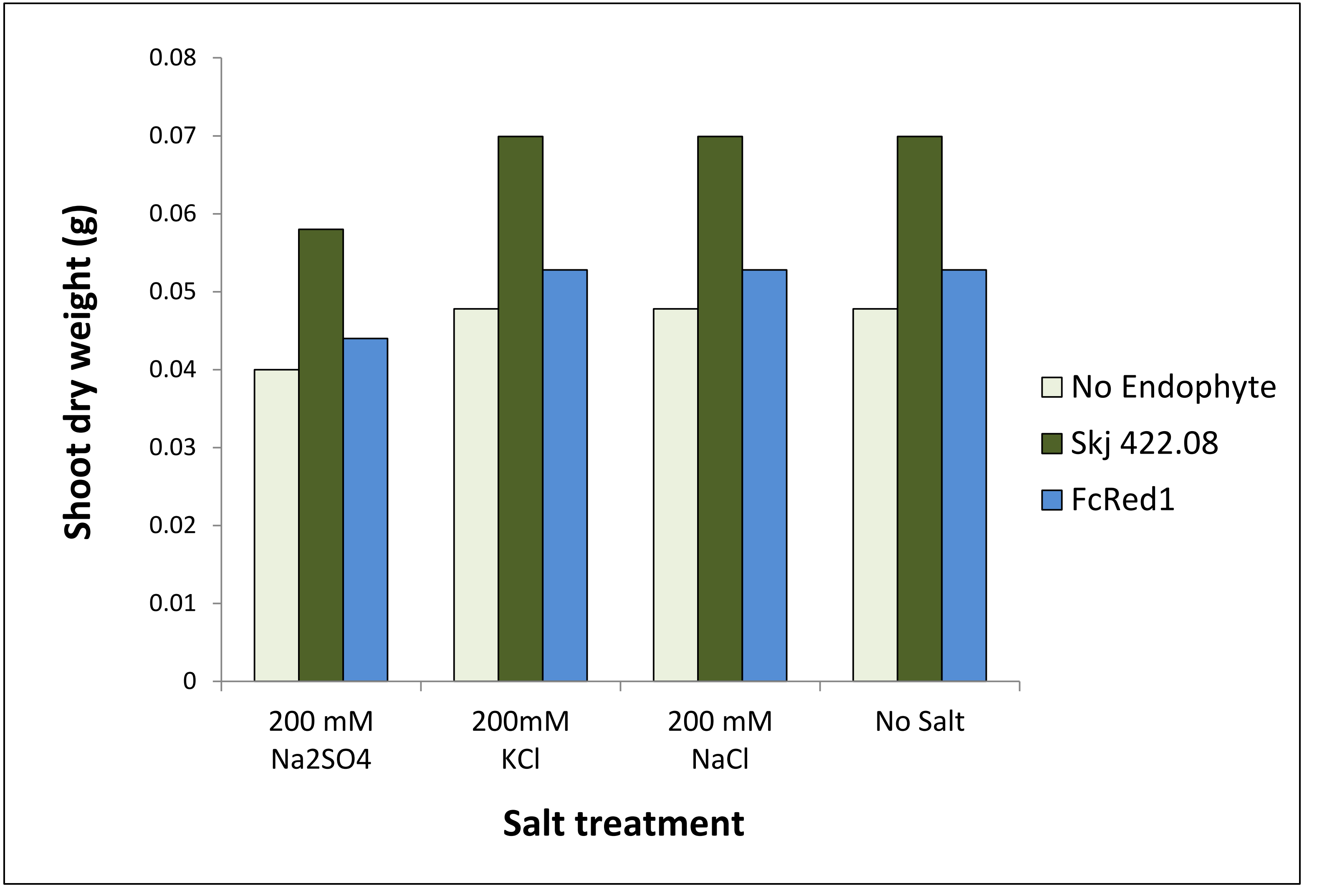
**Colonized plant grown under variety of conditions according to experiment design**

# 34 d → 12 g/L (200 mM) NaCl added at 15 d

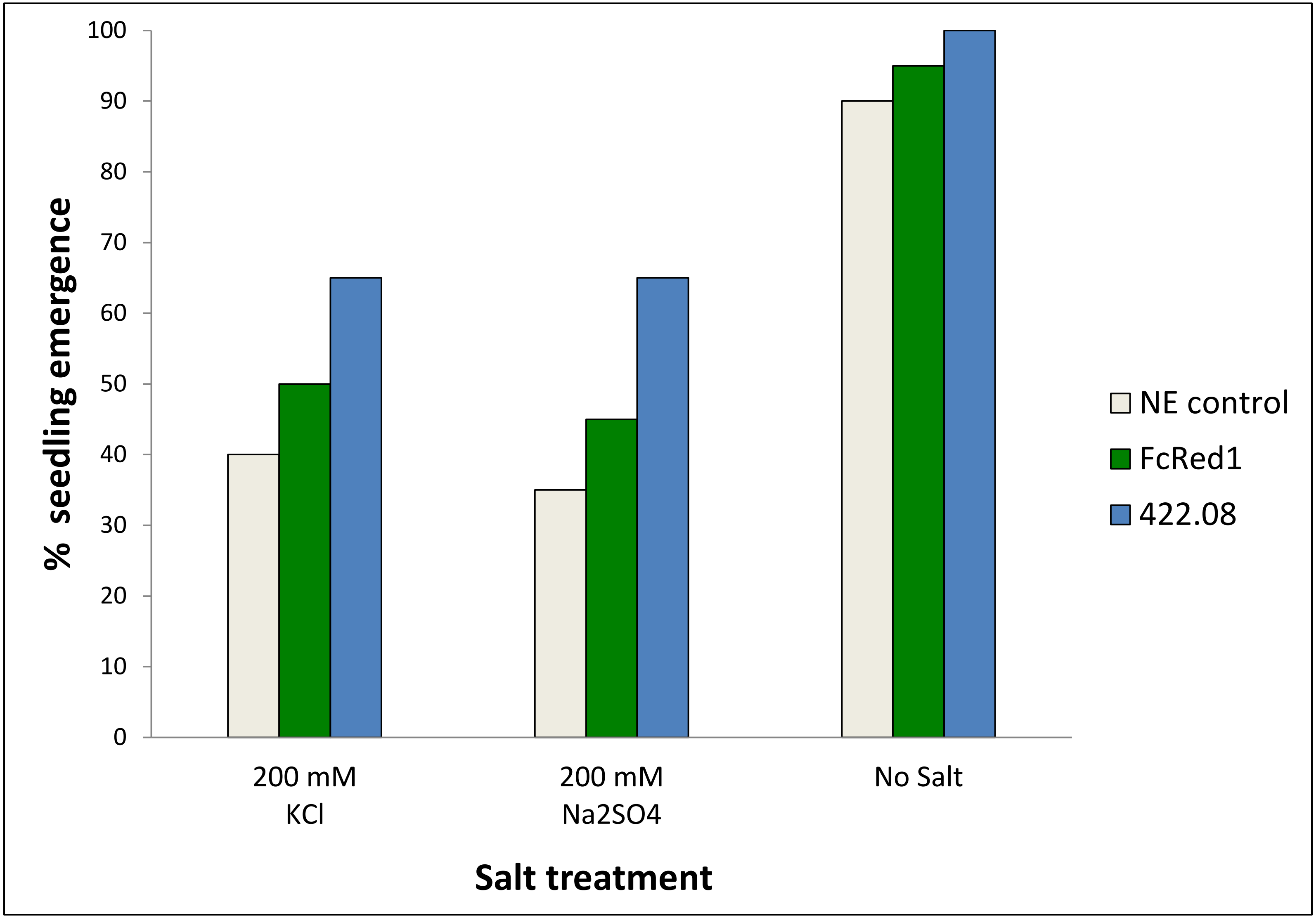
# Dry shoot biomass of wheat



# Comparative effect of different salts stress on shoot dry weight in nonsymbiotic and symbiotic wheat



# Effect of fungal endophytes on seedling emergence



# SkJ422.08 → drought stress

Grown from seed 7 weeks →
abandoned 25 days


7jan2013

# For the best of our 450 strains

- Molecular identification → patenting
- Fungicide sensitivity?
- Fertilizer compatibility?
- Compatibility with other bio-fertilizer organisms
- Winter survival?
- Dispersal?

# Liz

# Fungal endophytes from saline environments in Saskatchewan confer salt and drought tolerance to crop plants

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## Abstract

Fungal endophytes are filamentous fungi that live as plant symbionts. When isolated from stressful environments they have been shown to have the ability to colonize a variety of host plants and confer resistance to the stress to them(1,2). We have isolated fungal endophytes from plants grown in saline sites and tested their ability to confer salt tolerance to wheat and tomato plants. Our preliminary results suggest that many of them are indeed capable of conferring salt and even drought tolerance. We hope to confirm this with further research, and to identify and characterize one or more endophyte strains that can be used to grow plants on saline soils in the field.

## Introduction

Fungal endophytes appear to be ubiquitous in plants growing in natural soils. S Redman and RJ Rodriguez have outlined functional classes for endophytes, and have done pioneering work showing that class II fungal endophytes, which colonize the entire plant and are compatible with monocots and dicots, confer tolerance to a variety of environmental stresses (1). Once isolated, endophytes shown to confer stress tolerance can be allowed to colonize new host plants in order to help them survive the same type of stress (2). Class II fungal endophytes have been shown to enhance growth and improve nutrient uptake under normal saline conditions, although currently the mechanism(s) is unknown (1).

Saline soil causes reduced plant vigor and lower crop yields. This is a major problem in Saskatchewan, and globally. Saskatchewan has extensive areas and volumes of salinized soils and lakes produced by both natural and anthropogenic processes. These include potash mine tailings, which are high in NaCl. Figure 2 shows the extensive potash deposits in Southern Saskatchewan, which are composed mostly of NaCl with small amounts of KCl and clay, and help explain the large amounts of saline sites Saskatchewan, like Chaplin Lake (see figure 1).

Fig. 1 Chaplin Lake, the second largest naturally saline lake in Canada, Southern Saskatchewan, 2012.

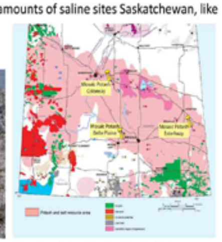

Fig. 2 Potash deposits in Southern Saskatchewan, with Mosaic mining sites. Modified from (3)

## Materials and Methods

In the spring of 2012 we collected 90 plant samples from 9 sites in Southern Saskatchewan, from which we isolated ~ 450 endophyte fungi. This project is being funded by MosaicCo, so strains of fungi from the Belle Plaine and Colonsay potash mine tailings sites have been applied to tomato and wheat plants for testing. These collection sites are very high in NaCl. Colonized plants, as well as no-endophyte controls, have been subjected to salt stress in the form of saline solution applied to double-decker magenta boxes. No salt controls were applied for all strains as well as the no endophyte control. The process of isolation and colonization is outlined in figure 3.

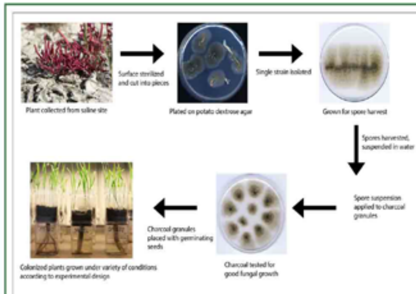


Fig. 3 Diagram of the basic methodology of endophyte collection and experimentation.

Endophytes isolated from the Saskatchewan plants have a variety of colony and spore morphologies. Microscope images of spores have been captured to assist with future taxonomic classification (see figure 4).

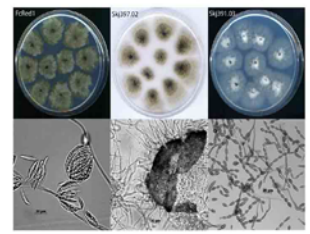


Fig. 4 Endophyte colonies growing from spore-laden charcoal granules, paired with microscope images of the same strains. FcRed1 is a positive control strain, and Skj397.02 and Skj391.03 are strains from saline sites in Saskatchewan.

## Preliminary Results

Some of the endophyte strains have shown promising effects on tomato and wheat plants stressed by salt exposure. Tomato growth under salt stress was noticeably better with the help of promising endophyte strains (see figure 5).


Fig. 5 34-day old tomato plants treated with 200 mM NaCl after 10 days of growth. The control box on the left has no endophyte, FcRed1 is the positive control, and the three remaining boxes are colonized with Saskatchewan saline endophytes.

In a small trial Saskatchewan endophyte Skj 422.08 enhanced wheat shoot biomass even more than the positive control FcRed1 under salinity stress from three different salts, and without salinity stress (figure 6).

The fungal endophyte Skj 422.08 also helped a tomato plant survive for 25 days after the most recent water application, while most other plants died (see figure 8). The ability to confer drought as well as salt tolerance is not surprising, since both salt exposure and drought produce osmotic stress.

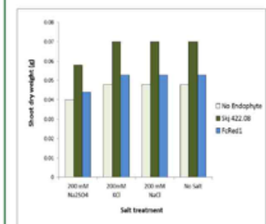


Fig. 6 Dry shoot biomass of colonized (Skj 422.08 and FcRed1) and uncolonized wheat plants grown for 9 days with Hoaglands solution (no salt) and Hoaglands solution modified to 200mM of three different salts.

Fig. 7 Tomato plant colonized by fungal endohypte strain Skj422.08, 25 days after most recent water application.

## Ongoing and Future Research

Preliminary results are being confirmed using methods refined since initial trials. Diatomaceous earth growth medium is allowing us to extract more intact plant roots from magenta boxes than the potting mix used previously. This will allow us to examine endophyte effects on root architecture while improving the accuracy of biomass measurements. We are using Hoaglands plant mineral nutrient solution as a standard in all experiments, modified as necessary with increased salt concentration. We are now applying salt stress starting at germination.

Methods of endophyte delivery to plant will be refined to maximize plant colonization and minimize the number of spores needed per charcoal pellet. Promising endophyte strains will be tested with native and naturalized seeds that could be used in remediation, and will be tested in the field.

## References

1. Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, Kim Y, Redman RS (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME Journal 2: 404-416

2. Woodward C, Hansen L, Beckwith F, Redman RS, Rodriguez RJ (2012) Symbiogenics: An epigenetic approach to mitigating impacts of climate change on plants. Hort Science 47(6): 699-703

3. Saskatchewan Saskatchewan Ministry of the Economy (2012) Mineral Resource Map. http://www.economy.gov.sk.ca/mineralresourcemap

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