Partner Project Reports

Project Reports for Partner-led Projects were submitted to PESAI by the Lead Partner listed. The information contained in the report was not verified.

Soil-Based Methods to Screen Soybean Plants for Resistance to Iron Deficiency Chlorosis (IDC) and Seedling Vigor on Calcareous Manitoba Soils

Project Duration
March 1, 2020 to Jan 31, 2021

Objectives
The objective of this project is to develop an improved growth chamber or greenhouse method to enable rapid screening of soybean seedlings for resistance to iron deficiency chlorosis (IDC). IDC is a major stress factor impacting the yield potential and profitability of soybean production in the Interlake and Eastman regions of Manitoba.

Collaborators
Kevin Baron (Solum Valley Biosciences), Craig Riddell (Riddell Seed Co.), Rick Rutherford (Rutherford Farms Ltd.)
Email contact: Kevin.Baron@solumvalley.com

KEY FINDINGS:

- Successfully developed a growth chamber screening methodology to evaluate the IDC tolerance of soybean germplasm with soils sourced directly from commercial fields in the South Interlake region of Manitoba
- Adapted the "Cone-tainer™" tube and tray system used by soybean researchers in the United States to induce symptoms of IDC resulting in significant changes in visual chlorosis score (VCS), relative chlorophyll levels, and dry weight of soybean seedlings within a 4-5 week time frame.
- Evaluated several low-cost sensors and imaging techniques to monitor plant growth and stress tolerance in a quantitative and non-destructive manner.

Results

Identify, Source and Characterize Regional Soils Prone to Iron Deficiency Chlorosis (IDC)
During the 2019 growing season communication with local agronomists and growers led to identification of commercial soybean fields in the R.M. of Woodlands and Rockwood that displayed severe symptoms of iron deficiency chlorosis (Figure 1). Careful selection of soils would increase the likelihood IDC symptoms could be reliably induced with little or no external influence. In the fall of 2019 prior to freeze up multiple buckets of soil (0-6") were sampled from each of these fields, sample locations geo-referenced and soils collected for subsequent use in growth chamber tests.

In addition to collecting two IDC prone soils from the South Interlake region, a professional potting mix (Sunshine Mix 4 Aggregate Plus), and an agricultural soil from the R.M. of North Cypress-Langford (Carberry: non-IDC reference soil) were submited to AgVise Laboratories for complete analysis. Based on direct comparison of carbonate and soluble salt levels against the AgVise IDC risk assessment table (Table 1), Stonewall and Marquette soils were assessed as very high and high, respectively, for risk of developing IDC symptoms (Table 2).

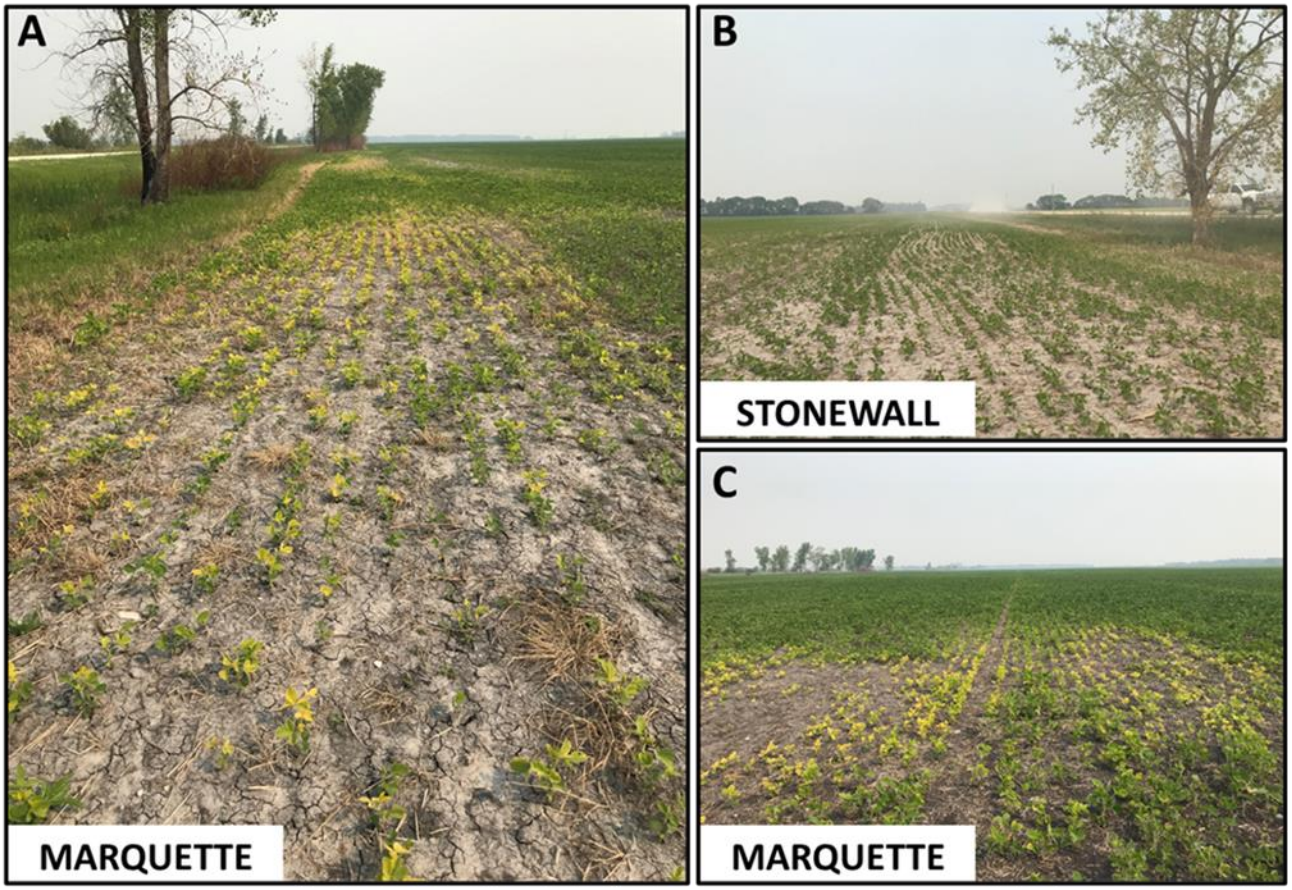

Figure 1. *Field symptoms of iron deficiency chlorosis (IDC) in the Rural Municipalities of Rockwood and Woodlands. These specific fields were identified, geo-referenced, and sampled in 2019. Soils were submitted to AgVise Laboratories for analysis.*

TABLE 1. FIELD RISK OF IDC BASED ON CARBONATE AND SOLUBLE SALT LEVELS

SOLUBLE SALTS	CARBONATE LEVEL (%)		
(mmhos/cm)	0 to 2.5	2.6 to 5.0	> 5.0
0 to 0.25	Low	Low	Moderate
0.26 to 0.50	Low	Moderate	High
0.50 to 1.0	Moderate	High	Very High
> 1.0	High	Very High	Extreme

*Adapted with permission from AgVise Laboratories

TABLE 2. CHARACTERIZATION OF SOILS SOURCED FOR GROWTH ROOM IDC ASSAYS								
LOCATION	SOLUBLE SALTS (mmhos/cm)	CARBONATES (%)	pH	Nitrate	O.M. (%)	SAND (%)	SILT (%)	CLAY (%)
STONEWALL (R.M. of Rockwood)	0.61 Very High	7.5 Very High	8.1	170 lb/ac	3.8	54	24	19
MARQUETTE (R.M. of Woodlands)	0.44 High	10.7 High	8.2	36 lb/ac	3.6	38	39	23
CARBERRY (R.M. of North Cypress-Langford)	0.25 Low	0.8 Low	6.7	48 lb/ac	5.0	72	17	11
GREENHOUSE MEDIA (Sunshine Mix 4 Aggregate Plus)	1.31	0.8	6.2	180 lb/ac	> 20% PEAT	-	-	-

Furthermore, the Stonewall soil was high in nitrate levels (170 lbs N/acre), another factor known to increase the severity of IDC symptoms in soybeans (Wiersma, 2010). In contrast, the chemical properties and nutrient levels of the professional grow mix and Carberry soil (low pH, low carbonates) indicate these reference soils were at lower risk for developing IDC symptoms (Table 2). Collectively, these results demonstrate that prior to growth chamber tests both Marquette and Stonewall soils had high risk to generate IDC symptoms with little external influence.

Growth Chamber Screening for Iron Deficiency Chlorosis (IDC)

Prior to test runs of the IDC assay in the fall of 2020, plant growth facilities were established at Riddell Seed Co. in Warren,MB. Plastic Ray-Leach “Cone-tainer™” cells used in the greenhouse nursery industry were also purchased. Several soybean researchers in the US (Lee et al. 2008; Goos, 2019) employ this equipment as a cost-effective and space-efficient alternative to hydroponic systems to screen germplasm (See Figure 2 and Materials and Methods section). Two regionally adapted soybean varieties (Mahony, Redvers) sourced from Riddell Seed Co. and Rutherford Farms Ltd, were seeded into pots/cells containing sieved and mixed Stonewall, Marquette or Carberry soils (Figure 2). Over a 4-5 week period seedlings were monitored for appearance of IDC symptoms and evaluated for visual chlorosis score (VSCs). Experiments ended with individual soybean seedlings harvested for dry matter assessments at the V2-V3 stage of development. Direct comparison of soybean growth across non-IDC (Carberry) and high IDC risk soils (Marquette, Stonewall) revealed significant differences in visual chlorosis score (VCS) and seedling dry matter (DM) (Table 3).

Hand-Held Sensors to Obtain Quantitative and Non-Destructive Data on Soybean Growth and Stress Tolerance

In addition to assessing soybean varieties for symptoms of IDC using the subjective rating system of visual chlorosis scores (VCSs) (1= green and tolerant, 5 = chlorotic and susceptible), the current study evaluated a chlorophyll meter (AtLEAF) (Zhu et al. 2012), a FLIR thermal imaging camera (Prashar and Jones, 2014), and iOS Canopeo app (Patrignani & Ochsner, 2015) to obtain quantitative information regarding the performance of individual soybean seedlings in response to stress (Figure 4; Table 3). These low cost cameras and sensors were evaluated as a means to obtain non-destructive and quantitative information on growth and stress tolerance of seedlings without harvesting plants. These tools will continue to be evaluated in the context of designing screens that can be applied to individual plants in a controlled environment through to field research trial scenario. Significant differences in relative chlorophyll content of unifoliate and trifoliate leaves (Figure 3; Table 3) corresponded with observed changes in visual chlorosis score assigned to whole seedlings (Table 3).

TABLE 3. EFFECT OF SOIL TYPE AND IDC SOLUTION ON DRY MATTER PRODUCTION, VISUAL IDC SCORE, CHLOROPHYLL CONTENT LEAF TEMPERATURE AND CANOPY COVERAGE OF SOYBEAN VARIETIES

VARIETY	SOIL TYPE	VISUAL CHLOROSIS SCORE (VCSs)* (1-5 Rating)	AtLEAF LEAF GREENNESS Unifoliate Leaf	AtLEAF LEAF GREENNESS 1st Trifoliate Leaf	Leaf Temperature (Celsius)	Green Pixel Percentage (GPP) 29 DAP	SEEDLING DRY MATTER (DM) g/plant
Mahony	Carberry	1.0 c	37.0 a	35.8 a	28.8 d	3.64 a	0.52 a
Mahony	Marquette	2.8 b	33.7 b	30.2 b	31.3 ab	2.30 bc	0.39 c
Mahony	Stonewall	3.3 ab	31.6 c	30.7 b	33.7 c	1.95 bc	0.29 d
Redvers	Carberry	1.3 c	37.3 a	38.7 a	33.1 ab	2.56 b	0.42 ab
Redvers	Marquette	3.5 a	35.2 b	27.9 b	32.5 b	2.40 b	0.40 bc
Redvers	Stonewall	3.3 ab	34.2 b	30.9 b	34.1 a	1.79 c	0.29 d
CV %		19.34	7.41	14.06	4.49	18.8	1.84
LSD (0.05)		0.4	2.1	3.7	1.1	0.80	0.06
Sign. Diff		Yes	Yes	Yes	Yes	Yes	Yes

*= Visual chlorosis score (1 = green leaves, tolerant) (5 = severe chlorosis/yellowing, stunted)

DAP = Days After Planting

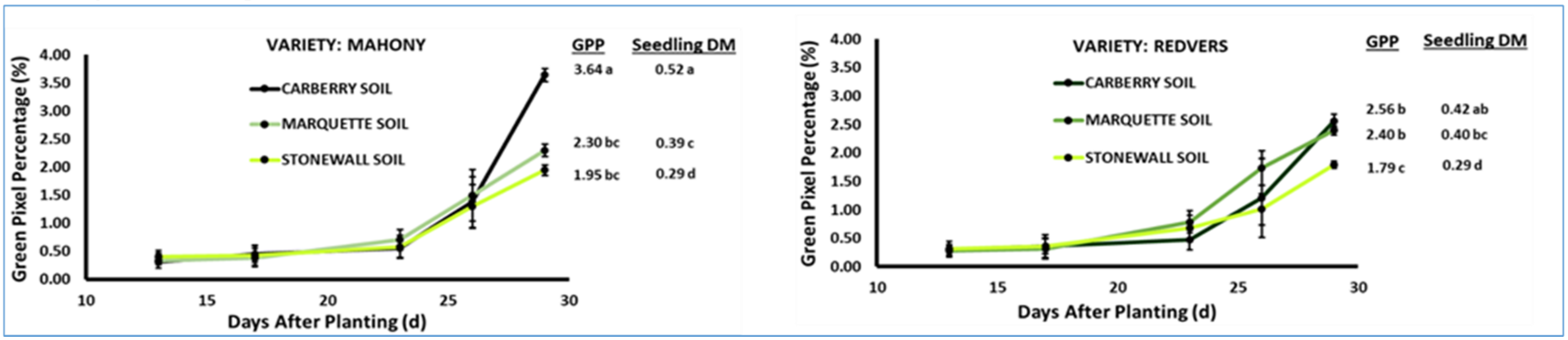

Figure 5. *Soybean seedlings of two varieties (Mahony, Redvers) were grown within Carberry, Marquette and Stonewall soils (See Figure 2). Following emergence, individual seedlings (n=12 per soil tray) were imaged every 3-6 days until seedlings were harvested for dry matter assessments. The Canopeo App (See Figure 4) was used to estimate % green pixel percentage (GPP) for each seedling. This method has previously been applied to indirectly estimate the biomass of sorghum seedlings without the need to harvest plants (Chung et al. 2017). The relationship between progression of green pixel percentage (GPP = estimate of biomass) and actual seedling dry matter (DM) are displayed side by side on graphs above.*

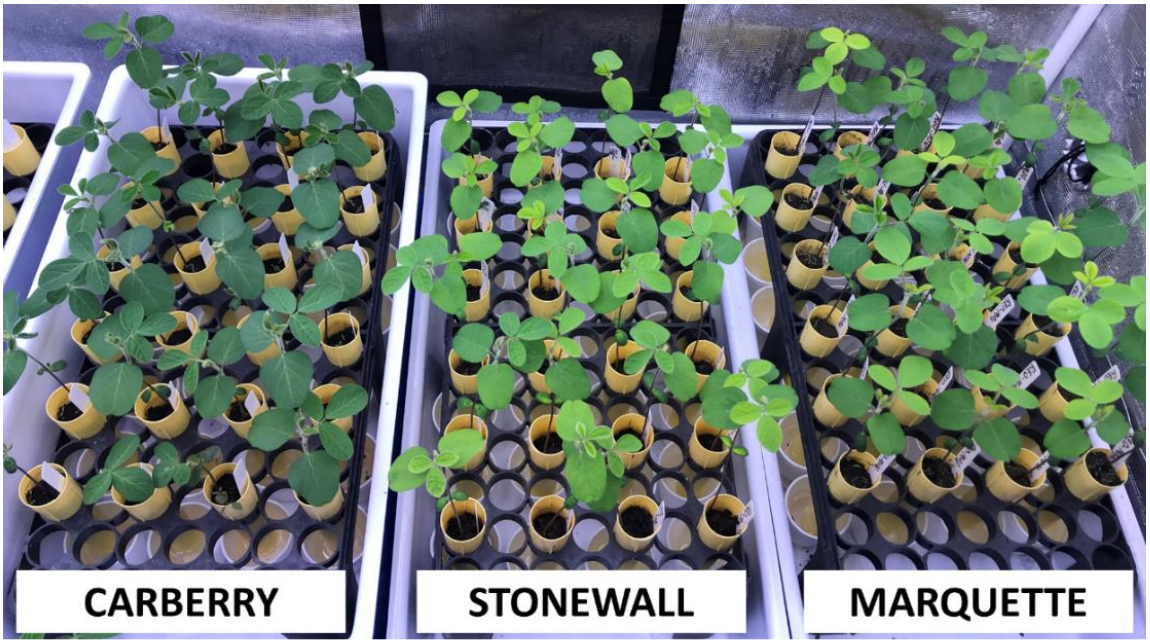

Figure 2. *Soybean seedlings growing within Ray-Leach "Cone-tainer ™" system, a space- efficient tube and tray system used in nursery greenhouse production. White flow trays contain cups filled daily with IDC sub-irrigation solution to induce IDC symptoms. Each white flow tray contains 24 seedlings (n=12, Mahony; n=12 Redvers). Middle (Stonewall soil) and far right (Marquette soil) trays contain two high IDC risk soils (Table 2) showing visible symptoms of chlorosis across Mahony and Redvers cultivars. In contrast, the same two varieties grown in the low IDC risk soil from Carberry (far left) and also receiving the sub-irrigation solution to induce IDC symptoms remain green and do not display visible symptoms of IDC.*

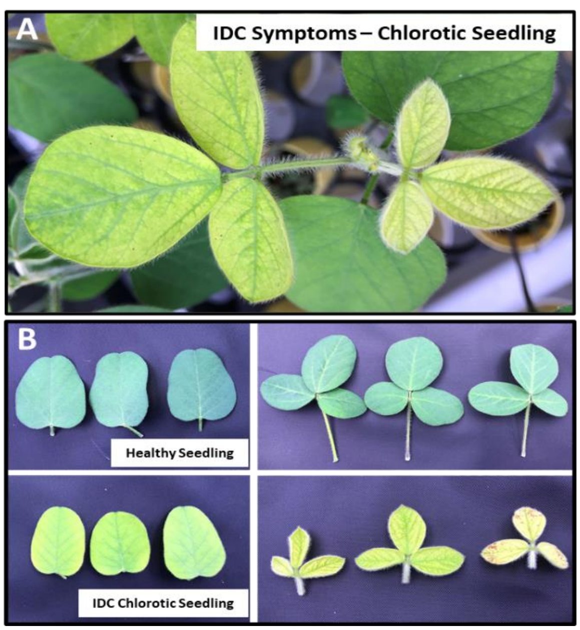

Figure 3. *Plant level symptoms of iron deficiency chlorosis (IDC) in growth room tests. (A) Soybean seedling displaying symptoms of iron deficiency chlorosis. (B) Top panels - Unifoliate leaves (left panel) and trifoliate leaves (right) from seedlings grown in Carberry soil appear healthy and green. Bottom panels – Unifoliate (left panel) and trifoliate leaves (right) with visible reductions in leaf greenness or chlorosis in Stonewall soil.*

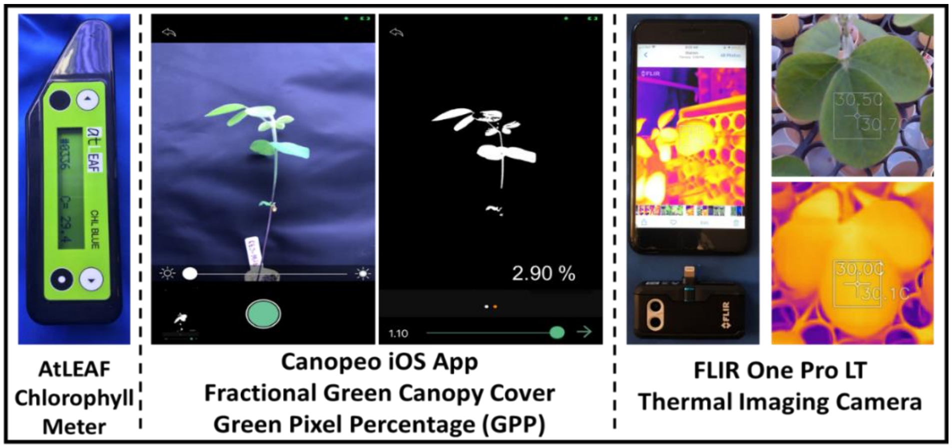

Figure 4. *A handheld chlorophyll meter (AtLEAF), iPhone app (Canopeo), and thermal imaging camera (FLIR One Pro LT) were utilized to obtain quantitative information regarding leaf greenness/relative chlorophyll content, green pixel percentage (biomass estimate), or leaf temperature of soybean seedlings exposed to IDC stress. These tools are being assessed as a complement to subjective visual chlorosis scores (VSC) often used to rate IDC in soybeans.*

IDC Symptoms Across Iron Efficient (EFF) and Iron Inefficient (INF) Soybean Plant Introductions.

Beyond the two cultivars evaluated in the current study, several well-characterized IDC resistant and susceptible soybean lines have been obtained from germplasm stock centres. The reaction of select iron efficient (EFF) and iron-inefficient lines (INF) sown in Stonewall soil with and without IDC solution applied are shown in Figure 6 below.

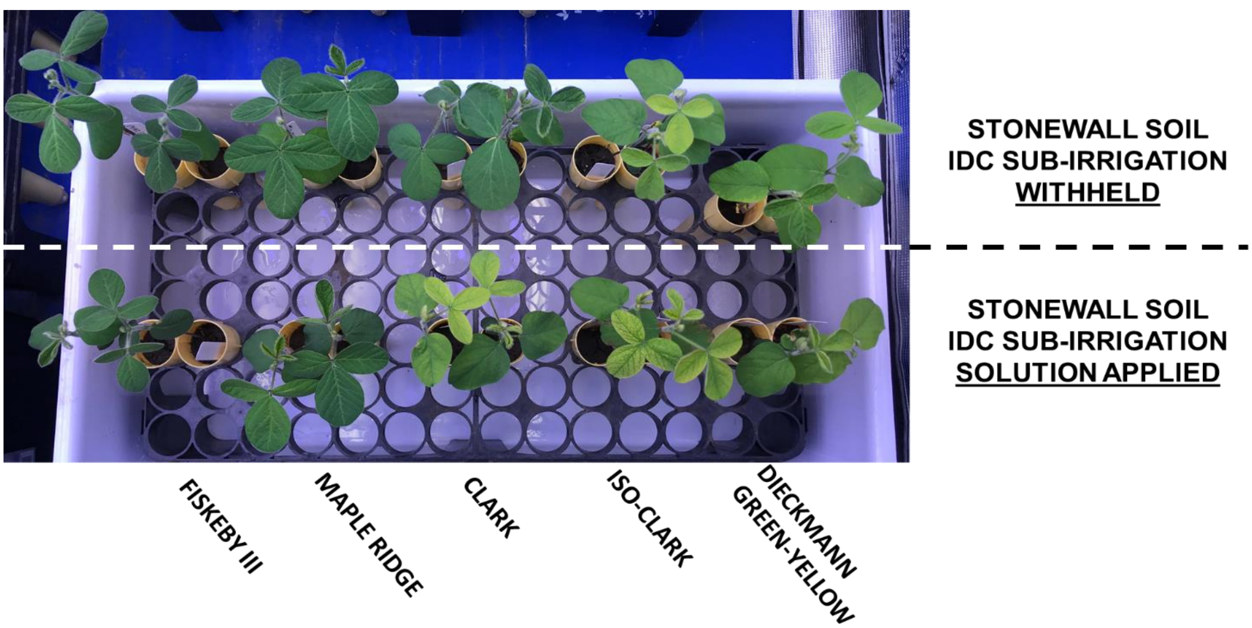

Figure 6. *Plant Introductions (PI) obtained from US and Canadian germplasm stock centres and characterized as being iron-efficient (EFF) or inefficient (INF) were also evaluated in the current study. In the above panel Fiskeby III and Maple Ridge are known EFF lines and do not show symptoms of IDC when grown in Stonewall soil and watered or when IDC sub-irrigation solution are applied. In contrast, Iso-Clark and Dieckmann Green-Yellow, two INF lines, demonstrate symptoms of chlorosis with and without IDC sub irrigation solution applied. Iron-efficient Clark does not display symptoms of chlorosis when grown in Stonewall soil and watered but does display symptoms of IDC when sub-irrigation solution is applied.*

Project Findings

The development of a soil-based method to screen for iron deficiency chlorosis is the first step for several related projects. For external clients, such as commodity organizations or private seed companies, N49 Genetics (Kevin Baron, Craig Riddell, Rick Rutherford) has the capacity to screen soybean germplasm for IDC twelve (12) months a year and outside of a field nursery scenario, or even in advance of the planting season. This is advantageous if agronomists or growers are hesitant to plant new genetics that have not be adequately screened on some of the more challenging soils in Manitoba.

Obtaining reliable and consistent visual chlorosis scores (VCSs) between field environments and across growing seasons is also known to be problematic, and this is intimately linked with spatial variability in soil parameters (carbonates, salts) and the unpredictable nature of weather events (e.g. rainfall, cold temperature) that contribute to IDC. If conditions during the growing season are not conducive to IDC symptoms appearing, this method could be accessed to supplement information generated from varietal screening conducted on an annual basis in field environments.

For N49 Genetics, this soil-based method provides a means to continually select IDC resistant seedlings from breeding populations. Over the 2021 and 2022 seasons, N49 Genetics will establish specialized temperature-controlled facilities that enable soybean seedlings to continuously be screened under conditions (soils, daylength, temperature) that mimic the early season growing conditions in Manitoba and Saskatchewan. Development of a soil-based assay also enables selection of root-related traits (e.g. N_2-fixation, rhizosphere pH, root foraging) that may not be adequately captured in hydroponic systems.

Background and Additional Resources

The equipment & techniques developed in this study were adapted from US researchers seeking to replace hydroponic systems with a more rapid, cost-effective and soil-based method of screening soybean germplasm for salt tolerance (Lee et al. 2008). Locally, MPSG-funded research has also contribued to the development of a hydroponic system at AAFC Morden to screening soybean germplasm for IDC (Hou, 2017). During the course of our study, Jay Goos with North Dakota State University also published a technical report detailing a sand:soil culture method of inducing IDC symptoms using the “Cone-tainer™” system (Goos, 2019). Several aspects of the current methodology have drawn up from previously published work.

References

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Acknowledgments

The financial support of the Prairies East Sustainable Agriculture Initiative (PESAI) towards greenhouse equipment and laboratory costs associated with development of this methodology is greatly appreciated.

Materials and Methods

- High risk IDC soils (Marquette, Woodlands), a non-IDC soil (Carberry) and potting mix were mixed and sieved and all used as growth medium.
- Seeds of two cultivars (Mahony n=12 per tray, Redvers n=12 per tray) were planted into "Cone-tainer™" pots and held within trays. One tray per soil type.
- From planting to harvest of seedlings (< 5 weeks), plants were grown under 14h (day):10(dark) light schedule, 25/20°C day/night temperatures and humidity maintained at 55-75%.
- After emergence of seedlings, each cone-tainer tube was submerged into Styrofoam cups containing a sub-irrigation solution (20mM sodium bicarbonate, 80mM sodium chloride, 10mM calcium nitrate) to induce IDC symptoms.
- Over the course of the experiment seedlings were imaged every 3-6 days for seedling biomass/green pixel percentage (Canopeo app), leaf temperature (FLIR camera) or relative chlorophyll levels (AtLEAF meter).
- Prior to harvest of seedlings for dry weight determinations, seedlings were rated for visual chlorosis scores (VCSs) and seedling height measured.
- Data was analyzed as a randomized complete block design, with cells and trays rotated within the growth room on regular interval.