4. Partner Project Report: Foliar Strategies to Generate Severe Iron Deficiency Chlorosis (IDC) in Small Plot Research Trials.

Project duration

- March 1, 2021 to Jan 31, 2022

Collaborators

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- PESAI (Nirmal Hari)
- Murphy et al. (Keith Murphy)
- Manitoba Agriculture (Dennis Lange)

Objectives

- To evaluate a method of forcing symptoms of iron deficiency chlorosis (IDC) in soybean research trials across the Interlake and Red River Valley regions on Manitoba.
- In addition, to directly compare the yield response of IDC resistant and IDC susceptible cultivars (grouped) in response to the applied forcing treatment. IDC is a major stress factor that severely reduces the yield potential of soybeans grown upon calcareous, high pH soils in Manitoba.

Results

1. Background: Application of soil amendments/foliar treatments to generate symptoms of IDC in research trials

Over the 2021 growing season three identical small plot research trials were established in the Interlake and Red River Valley regions of Manitoba at Arborg (PESAI), Balmoral (Solum Valley Biosciences) and Ste. Agathe (Murphy et al.) upon soils with a medium to high risk for developing symptoms of IDC (Fig. 4.1). At each location one of six herbicide tolerant soybean varieties were planted. Three varieties were selected based on previous scores (IDC resistant) in the MPSG soybean IDC nursery, and an additional three varieties included based on scores (IDC susceptible) in the same nursery.

In addition to selecting trial locations with the potential to generate symptoms of IDC, these trial locations were also paired with traditional MPSG variety evaluation trials in 2021. Following seeding operations and emergence, site visits (Kevin Baron) were made to each site to apply a mixture of nitrate, bicarbonate and table salt to the trial area to “force” symptoms of IDC. Similar solutions have been used to generate severe symptoms of IDC in growth room screens of soybean germplasm (Baron, 2021). Field researchers also manipulate soil nitrate level to increase IDC when screening soybean germplasm or performing agronomic assessments of soil of iron (Fe) chelate fertilizers (Wiersma 2010). Throughout June and July 2021 all three research sites were monitored for development of IDC symptoms, ratings collected, trials photographed and drone missions executed to generate a time-course series of images tracking the growth of individual soybean plots. Beyond final site visits in late July, trials were managed and harvested in a similar manner to adjacent MPSG variety evaluation trials.

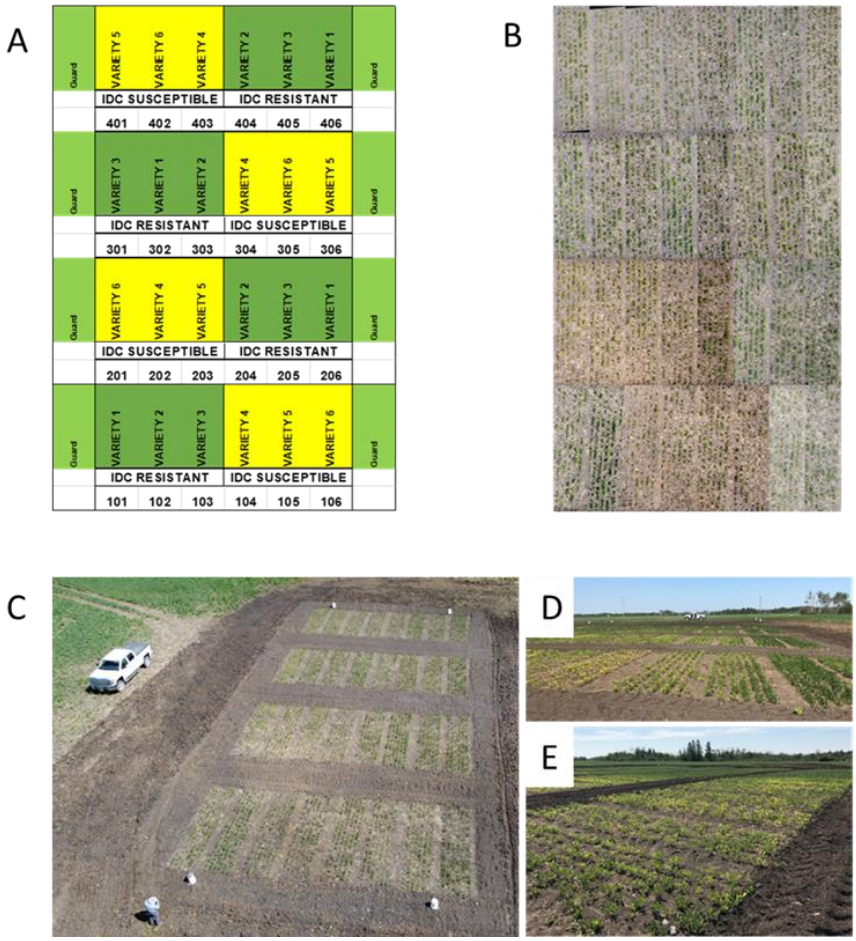

Fig. 4.1. Experimental design (A, B) and aerial drone image (C) (Arborg site) depicting the layout of the small plot trials assessing IDC resistant versus IDC susceptible soybean varieties. At each location, trials consisted of 24 plots totals with 6 varieties of soybean per replicate/block. Within each block 3 IDC resistant varieties (grouped green) were planted beside 3 IDC susceptible varieties (grouped yellow) and surrounded by guard plots. (D,E) Across all three locations IDC symptoms were most severe in an MPSG variety trial situated adjacent to the small plot IDC trial assessing forcing treatments.

2. Yield Assessment of IDC Resistant vs IDC Susceptible Varieties

At all three trial sites (Arborg, Ste. Agathe, Balmoral), soybean plots were taken to harvest with trial means ranging from 14.9 bu/ac to 21.2 bu/ac (Fig. 4.2). These values are significantly below the 10-year provincial average yield for soybean (~ 35 bu/acre, MASC) in Manitoba and reflect the influence that lack of precipitation and drought conditions exerted on the yield of several crops across much of Manitoba. Moreover, comparable MPSG soybean variety trials harvested at these same sites (Ste. Agathe, Balmoral) and evaluating > 40 varieties of herbicide tolerant soybeans generated trials means that did not exceed 25 bu/acre (2021 MPSG Variety Guide).

Although symptoms of IDC were identified, rated and documented at two of three sites over the 2021 growing season, it is important to emphasize that significant differences in the performance of the six varieties (IDC resistant n=3, IDC susceptible n=3) or significance

differences between IDC groupings could be attributed to factors other than IDC stress. (e.g. drought stress). It is more likely that drought stress (as opposed to IDC stress) was a more prominent external environmental factor influencing the yield potential and performance of varieties across this set of small plot research trials.

Lack of precipitation and drought conditions presented challenges for IDC ratings at all three locations, in addition to comparable IDC screening sites in Manitoba, North Dakota and Minnesota. In general, extended periods of cool, wet soil conditions early in the season would lead to persistent and prolonged IDC stress. These environmental conditions would also be ideal for a direct yield comparison of IDC resistant versus IDC susceptible germplasm.

Taken together, yield results from all three locations in 2021 indicate that in the absence of adequate precipitation to keep high risk soils (calcareous, high pH) saturated and cool, the risk of developing severe symptoms IDC is diminished and the yield gap between IDC susceptible and IDC germplasm will be diminished. Nonetheless, in spite of the prolonged moisture deficit that extended across the 2021 growing season, at two of three sites (Arborg, Balmoral) it was possible to discern IDC susceptible and IDC resistant germplasm based on visual chlorosis scores (VCS). In addition, for the most severely affected plots, differences in canopy coverage and leaf greenness were quantified.

3. Visual Chlorosis Scores (VCSs) and Drone Imagery to Track Soybean Growth and Monitor Small Plot Trials for Symptoms of IDC or Drought Stress

Following seeding operations subtle differences in IDC symptoms could be identified through visual chlorosis scores (VCS) (1 = green, tolerant 5 = yellow, chlorotic) at both the Arborg (Table 4.1) and Balmoral locations (not shown). However, these symptoms were relatively mild and transient. No visual symptoms of IDC were recorded at the Ste. Agathe site through June and July of 2021. With successive visits to sites in Arborg and Balmoral, initial symptoms of IDC diminished and similar observations were noted for adjacent MPSG variety trials (Fig. 4.3).

Table 4.1. IDC ratings according to visual chlorosis scores (1-5 rating scale) - 2021 Arborg (1 = resistant, green ;5= yellow, chlorotic).

Variety	IDC Group	Visual Chlorosis Score VCS (1-5) Jun 14 2021	Visual Chlorosis Score VCS (1-5) Jun 30 2021
IDC RES Variety 1	RESISTANT	1.6 d	1.7 b
IDC RES Variety 2	RESISTANT	1.7 cd	1.7 b
IDC RES Variety 3	RESISTANT	1.8 c	1.7 b
IDC SUS Variety 4	SUSCEPTIBLE	2.1 ab	1.8 a
IDC SUS Variety 5	SUSCEPTIBLE	2.0 b	1.8 a
IDC SUS Variety 6	SUSCEPTIBLE	2.3 a	1.9 a
CV		6.9	3.5
LSD (0.05)		0.20	0.10
Sign. Diff		YES	YES

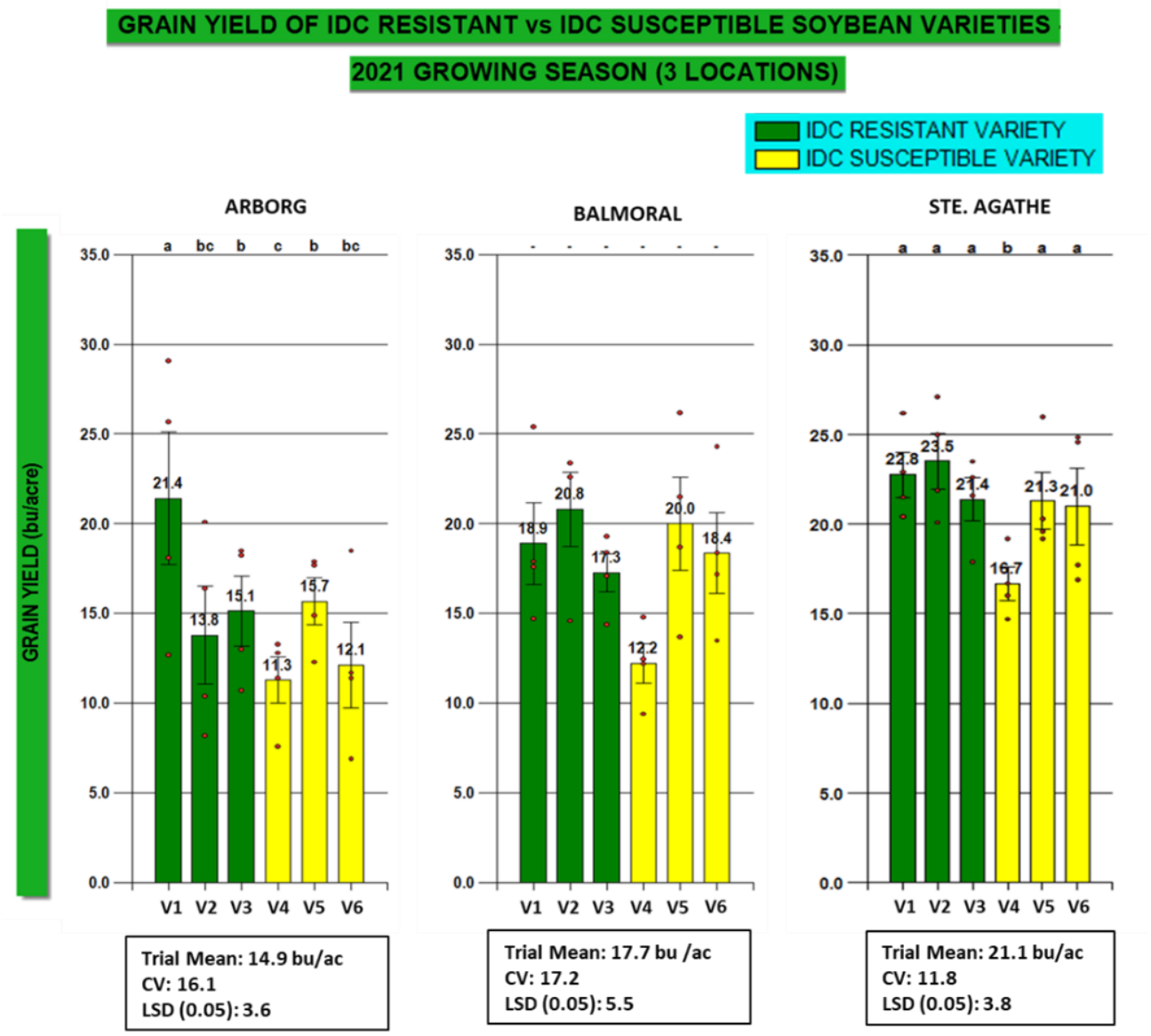

Fig. 4.2. Grain yield of individual IDC-resistant and IDC-susceptible cultivars at Arborg, Balmoral and Ste. Agathe research trial sites over the 2021 growing season. At both Arborg and Ste. Agathe sites, significant differences in yield of individual lines were reported. One specific variety (V4 – IDC susceptible) was consistently ranked as lowest yielding at all three sites. However, this ranking did not appear to related to notable differences in IDC scores across locations.

Collectively, the variety-specific symptoms of IDC and VCSs noted at both Arborg and Balmoral locations provided support that varieties initially selected based on past performance in the IDC nursery would respond appropriately and demonstrate an injury/yield contrast if moderate to severe symptoms of IDC persisted at either location. However, the rapid recovery of all IDC susceptible lines at both locations highlighted that in order for significant yield loss to occur, IDC symptoms must persist from the first trifoliate stage through to the V5/V6 stage of development.

In addition to rating individual plots for visual chlorosis scores (VCSs), trials at each site were further monitored (3x) with drone mapping missions using a DJI Mavic Mini. Mapping missions were then imported into software (Plot Phenix) that enables individual plots to be identified and assessed for quantitative parameters such % canopy coverage, leaf greenness (G/R ratio), or stand counts (Fig. 4.4).

Fig. 4.3. Ground level images of IDC susceptible (foreground) and IDC resistant (background) varieties at the 2021 Arborg site on June 30, 2021 (top) and July 21, 2021 (bottom). These images capture the relatively low IDC pressure/symptoms early in the season, in addition to the recovery and canopy development of plots corresponding to canopy coverage and leaf greenness (G/R) measurements presented in following sections.

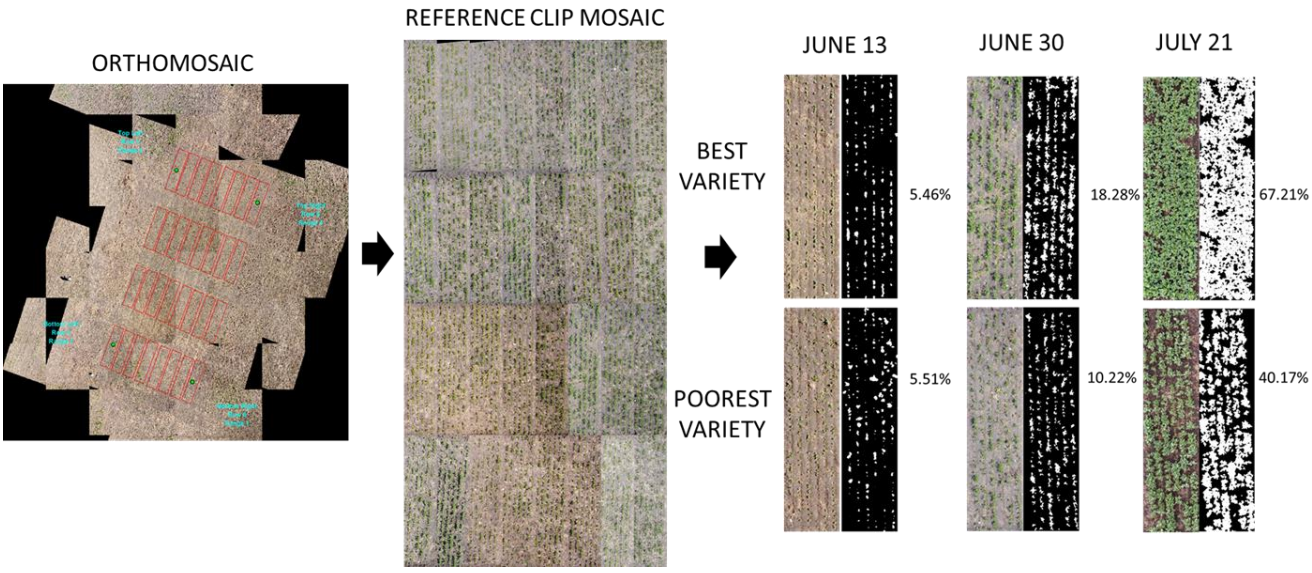

Fig. 4.4. Schematic representation of drone mapping workflow at the Arborg site in 2021 season. Individual mapping missions are gridded and processed into a reference clip mosaic that identifies each individual plot. Each plot is then evaluated for quantitative measurements related to canopy coverage, leaf greenness, stand count, etc.

Traditional visual chlorosis scores (VCS) are subjective based upon the person conducting ratings, and focus largely on the degree of chlorosis/yellowing observed in new vegetative growth. Monitoring canopy coverage scores represents an alternative means to assess the growth and biomass accumulation (or growth inhibition) of soybean varieties in response to IDC or related stresses such as drought, salinity, or waterlogging tolerance. This strategy is being explored and applied to the current project as several public and private soybean breeding programs are currently developing aerial imaging approaches to evaluate and rate soybean germplasm (Dobbels and Lorenz, 2019).

Focusing on software outputs from the Arborg site only, significant differences in canopy coverage (Table 4.2) and G/R ratio (leaf greenness) (Table 4.3) were detected amongst the six varieties evaluated. Note that with successive visits the canopy coverage score for an individual variety continues to increase reflecting the accumulation of biomass and canopy closure noted in pictures (Fig. 4.3). At several time points, canopy coverage and G/R scores of specific IDC resistant varieties exceed those of select IDC susceptible lines.

Overall, time-course assessments of canopy coverage scores are intended to quantify the growth and biomass accumulation of IDC resistant varieties that occurs when IDC susceptible varieties display symptoms of stress leading to growth inhibition. Moving forward N49 Genetics will continue to assess drone imagery and canopy coverage scores as means to monitor the growth, stress tolerance and recovery of soybean varieties in response to IDC and related stresses such as drought, salinity or waterlogging.

Table 4.2. Time-course progression of canopy coverage scores (% cover) - Arborg 2021

Variety	IDC Group	Canopy Coverage Jun 14 2021	Canopy Coverage Jun 30 2021	Canopy Coverage July 21 2021
IDC RES Variety 1	RESISTANT	5.8	16.9 a	61.0 a
IDC RES Variety 2	RESISTANT	5.5	11.4 bc	50.9 bc
IDC RES Variety 3	RESISTANT	4.3	12.0 b	51.0 bc
IDC SUS Variety 4	SUSCEPTIBLE	3.5	10.4 bc	54.4 b
IDC SUS Variety 5	SUSCEPTIBLE	4.0	9.5 cd	47.9 c
IDC SUS Variety 6	SUSCEPTIBLE	4.0	12.0 d	54.8 b
CV		25.3	4.5	6.1
LSD (0.05)		1.7	2.8	4.9
Sign. Diff		NO	YES	YES

Table 4.3. Time-course progression of soybean G/R ratio scores (leaf greenness) - Arborg 2021

Variety	IDC Group	G/R Ratio Jun 14 2021	G/R Ratio Jun 30 2021	G/R Ratio July 21 2021
IDC RES Variety 1	RESISTANT	0.98	1.16 a	1.24 a
IDC RES Variety 2	RESISTANT	1.00	1.15 bc	1.20 ab
IDC RES Variety 3	RESISTANT	0.98	1.16 a	1.18 bc
IDC SUS Variety 4	SUSCEPTIBLE	0.97	1.12 bc	1.21 ab
IDC SUS Variety 5	SUSCEPTIBLE	0.95	1.10 cd	1.19 b
IDC SUS Variety 6	SUSCEPTIBLE	0.96	1.08 d	1.16 c
CV		2.6	4.5	6.1
LSD (0.05)		0.04	2.8	4.9
Sign. Diff		NO	YES	YES

Project findings

The current project sought to assess alternative methods of evaluating iron deficiency chlorosis (IDC) in soybean field research trials and quantify the yield impacts of severe IDC on resistant and susceptible soybean varieties currently grow in Manitoba. However, the overall lack of precipitation at sites for the 2021 growing season limited upper end yield potential, and also hindered the development of moderate to severe levels of IDC stress at these same sites.

Based upon the transient, mild symptoms of IDC stress noted at both Arborg and Balmoral field locations following seeding operations and spring rains, sites selected for the study were conducive to developing severe IDC symptoms. Moreover, the subset of varieties further displayed the intended differential in visible symptoms of chlorosis.

These observations suggest that to reliably screen soybean germplasm for IDC year over year there may be merits to execute related agronomic studies or variety evaluations on high risk soils in conjunction with irrigation infrastructure. Maintaining cool, wet and saturated soil conditions for extending periods in the spring (May to June) may be necessary to consistently generate severe IDC pressure. N49 Genetics is developing such irrigation capacity for the 2022 season. The outcomes of this field project further indicate that downstream IDC screening activities may concentrate on phenotyping germplasm in controlled environments versus field environments. Future efforts aimed at evaluating the

yield performance of IDC resistant versus IDC susceptible germplasm may also require alternative experimental designs (e.g higher replication, spatial analysis of yield data) to obtain high quality yield data.

References

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Wiersma JV (2010) Nitrate-Induced Iron Deficiency in Soybean Varieties with Varying Iron-Stress Responses. Agron. J. 102:1738-1744.

Dobbels A, Lorenz A (2019) Soybean iron deficiency chlorosis high-throughput phenotyping using and unmanned aircraft system. Plant Methods. 15:97

Acknowledgments

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Materials and methods

Experimental design: Randomized complete block design with treatments arranged in a factorial split-plot design.

Replications: 4; Treatments: six varieties (n=6) assigned to an IDC group (n=2).

Six regionally adapted Round-up Ready soybean cultivars were selected in coordination with MB Provincial Pulse Specialist (Dennis Lange) based on past performance of varieties in the regional IDC rating nursery. Within one week of planting operations, an initial visit was made to apply a combination of granular fertilizer (calcium nitrate, urea) with a spin spreader in addition to foliar treatments (bicarbonate, table salt) with a back pack sprayer and hand boom. Solutions were made based on the amount of product that could practically be dissolved and dispensed.

Data collection

Following the initial application of fertilizer and soil amendments, each site was visited on 2-3 week intervals to photograph sites, rate plots for visual chlorosis scores (VCSs), and execute drone mapping missions.

Efforts were also made to observe and monitor adjacent MPSG variety evaluation trials planted and managed on these same sites, but not receiving supplemental soil amendments/fertilizer to induce symptoms of IDC.

Agronomic management

Trials were managed for weed control, pests, etc. in a manner similar to adjacent MPSG variety trials. Trials were harvested by the respective contract research organ and raw yield data relayed to N49 Genetics.

Seeding date: Arborg: May 27, 2021; Balmoral: May 17, 2021; Ste. Agathe: May 14, 2021

Harvesting date: Arborg: Oct 08, 2021; Balmoral: Sept 25, 2021; Ste. Agathe: Sept 23, 2021.