

# Increasing soybean yield and protein under periodic moisture stress by prolonging nitrogen fixation

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## Introduction:

- The productivity of short season soybean cultivars in the Canadian Prairies is challenged by variable precipitation and climate change.
- Nitrogen (N) fixation in soybean is sensitive to moisture stress. Precipitation during reproductive stages is needed for high nitrogen fixation, seed protein, and seed yield (Cober et al. 2023).
- Prolonging nitrogen fixation under periodic moisture stress has been shown to be an effective mechanism in long season soybean cultivars (Sinclair et al. 2010).
- Drip irrigation is a novel method to provide supplemental water to screen soybean cultivars under field conditions.
- <sup>15</sup>N natural abundance (Unkovich et al. 2008) and relative ureide nitrogen (Goos et al. 2015) are methods that show potential as screening tools for soybean cultivars with prolonged nitrogen fixation under periodic moisture stress.

## Objective:

Evaluate a novel field method to screen short season soybean cultivars to test for prolonged nitrogen fixation under ambient and irrigated conditions in Manitoba.

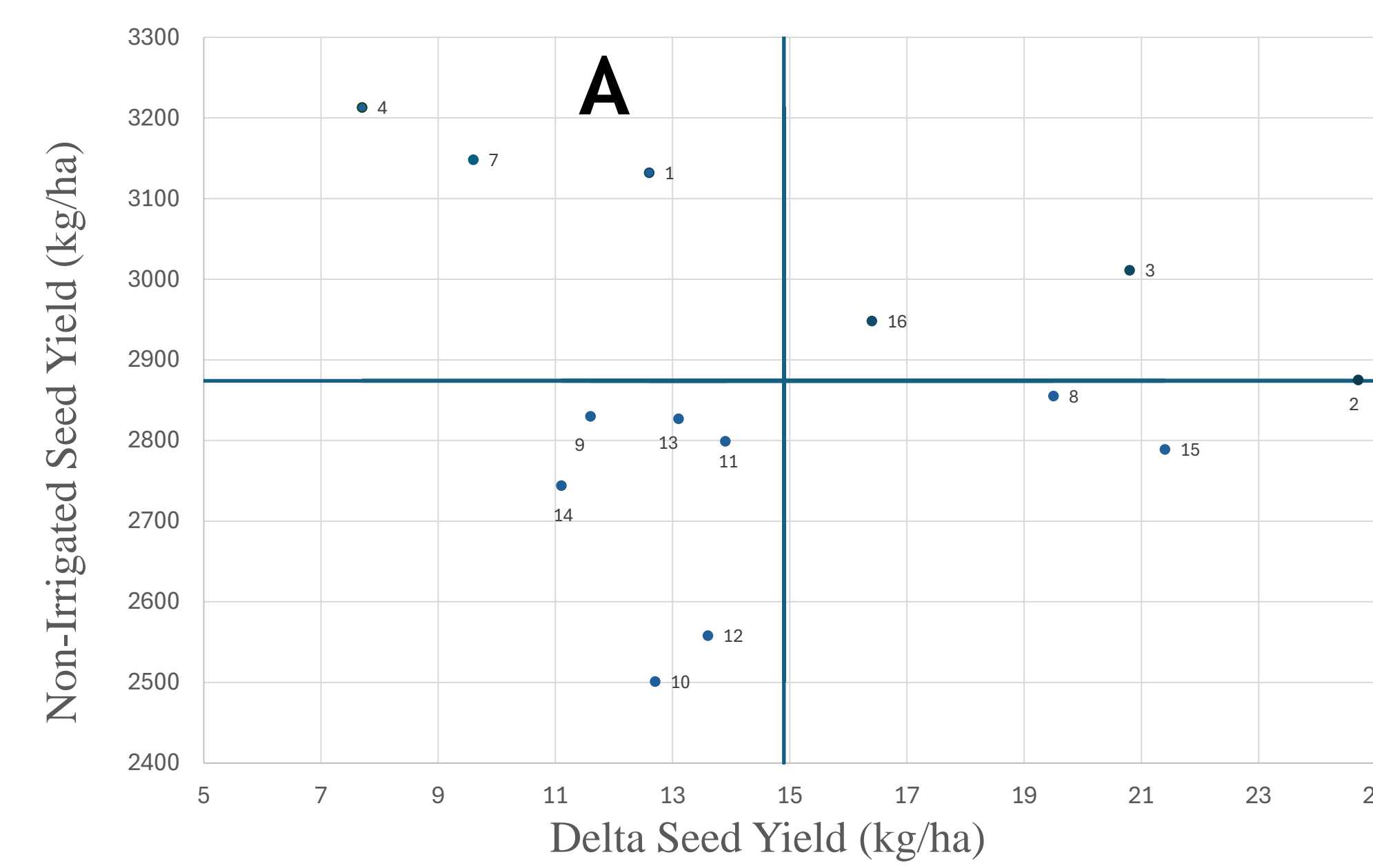
## Materials and Methods

**Experimental design:** A split plot field experiment was conducted at the University of Manitoba Carman Research Farm in 2022 and 2023. The main plot factor was with and without drip tape irrigation. The subplot factor was 16 short season soybean lines including a nodulating and non-nodulating check. Fourteen lines were derived from backcrosses with long season lines with prolonged nitrogen fixation traits that were then screened for their ability to mature in Canadian environments. Soybean was seeded at a population of 55 plants/m<sup>2</sup> on 40 cm row spacing. Sub plot size was 5 x 1.6m.

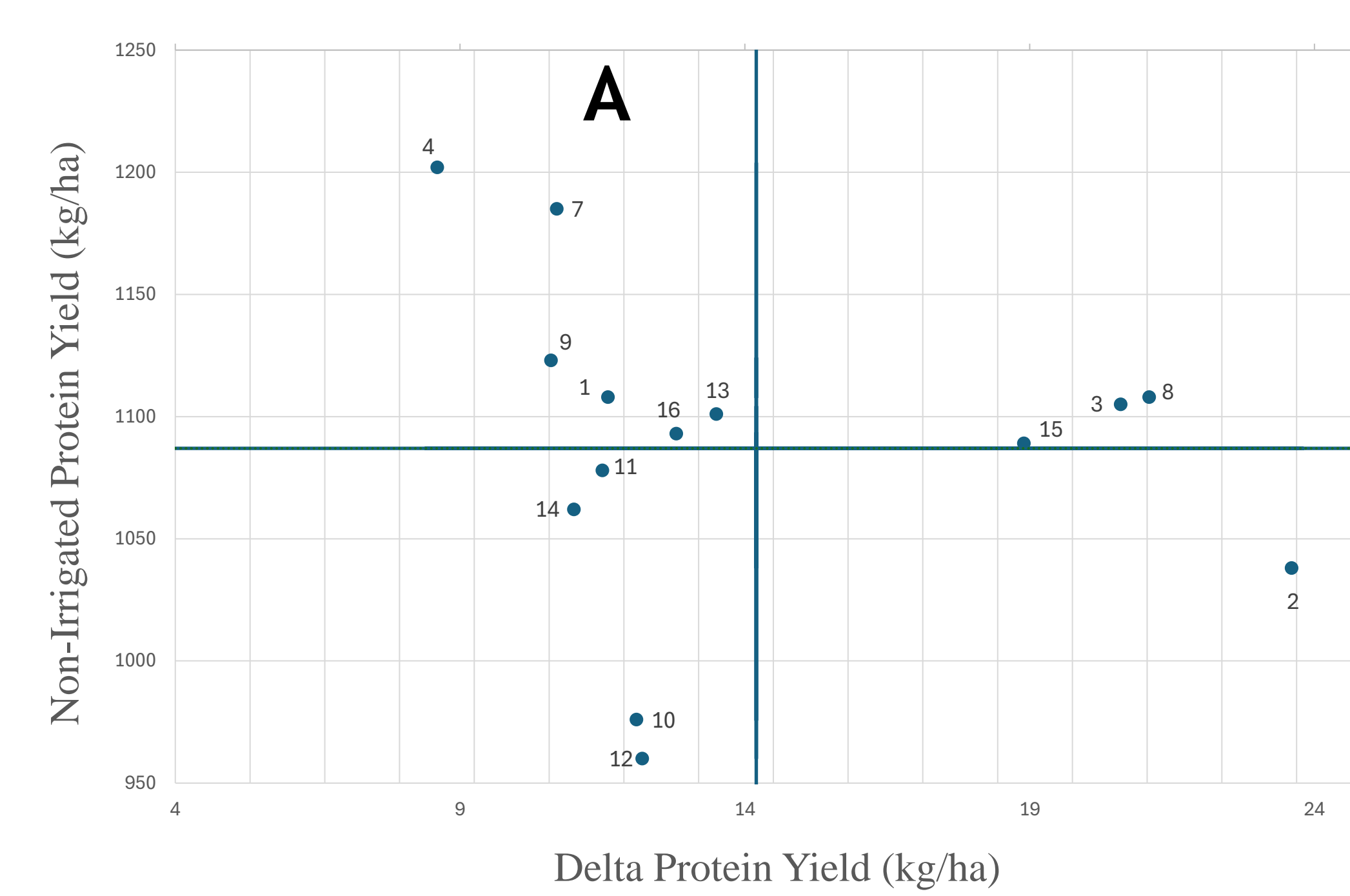
**Irrigation:** Sub-surface drip tape was installed 10 cm deep in between the two central soybean rows prior to planting soybean (Figure 5). Irrigation was applied at 2.5 mm per day starting at second trifoliolate (V2) when ambient conditions limited soil moisture.

**Measurements:** Above ground soybean biomass from 5 plants per plot was harvested at pod-fill (R5). Stems and petioles were separated and used to measure relative ureide nitrogen (RUN) as a measure of nitrogen fixation (Goos et al. 2015). Soybean grain samples were harvested using a plot combine. Seed protein was measured using NIR. Train samples were then ground to measure nitrogen fixation using the <sup>15</sup>N natural abundance method using a mass spectrophotometer to estimate percent nitrogen derived from the atmosphere (Ndfa) (Unkovich et al. 2008). When calculating Ndfa, the non-nodulating soybean line was used as the reference crop.

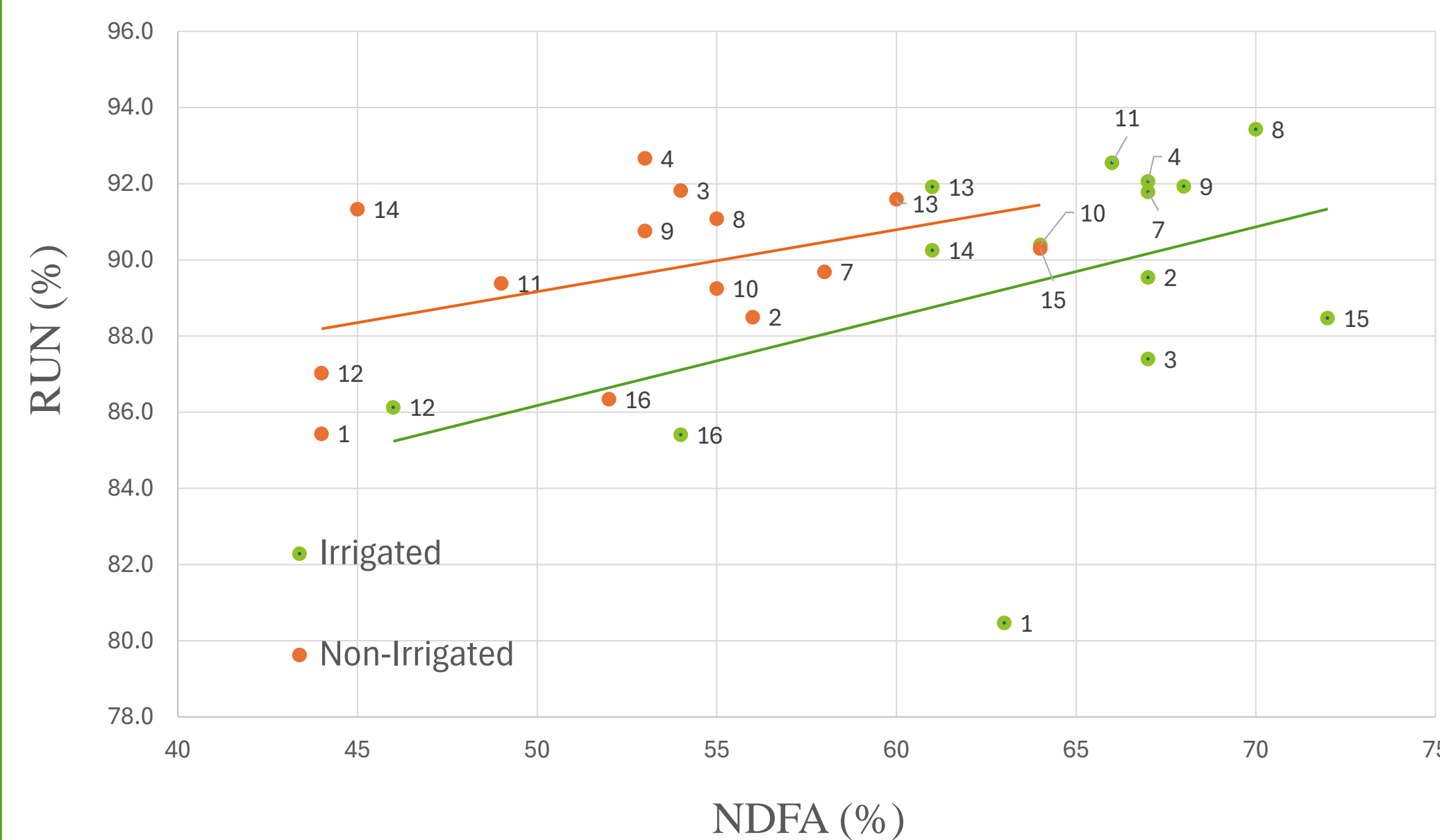
**Data analysis:** Delta seed yield and delta protein yield were calculated using equations in Figure 4. Means for soybean lines were compared using least significant difference (LSD) within each year (Table 1). Bi-plots of means from both experiment years were used to compare individual soybean lines to the average of all lines using the criteria of delta seed yield and delta protein yield (Figure 1-2).



**Figure 1:** Fourteen experimental soybean lines were evaluated for prolonged nitrogen fixation using a biplot of delta seed yield and the non-irrigated treatment yield when averaged over two years (2022, 2023) near Carman, MB. Lines were sorted into quadrants by comparing their performance relative to the group mean (blue lines). Soybean lines in biplot quadrant A displayed desirable combinations of low delta seed yield and high non-irrigated yield



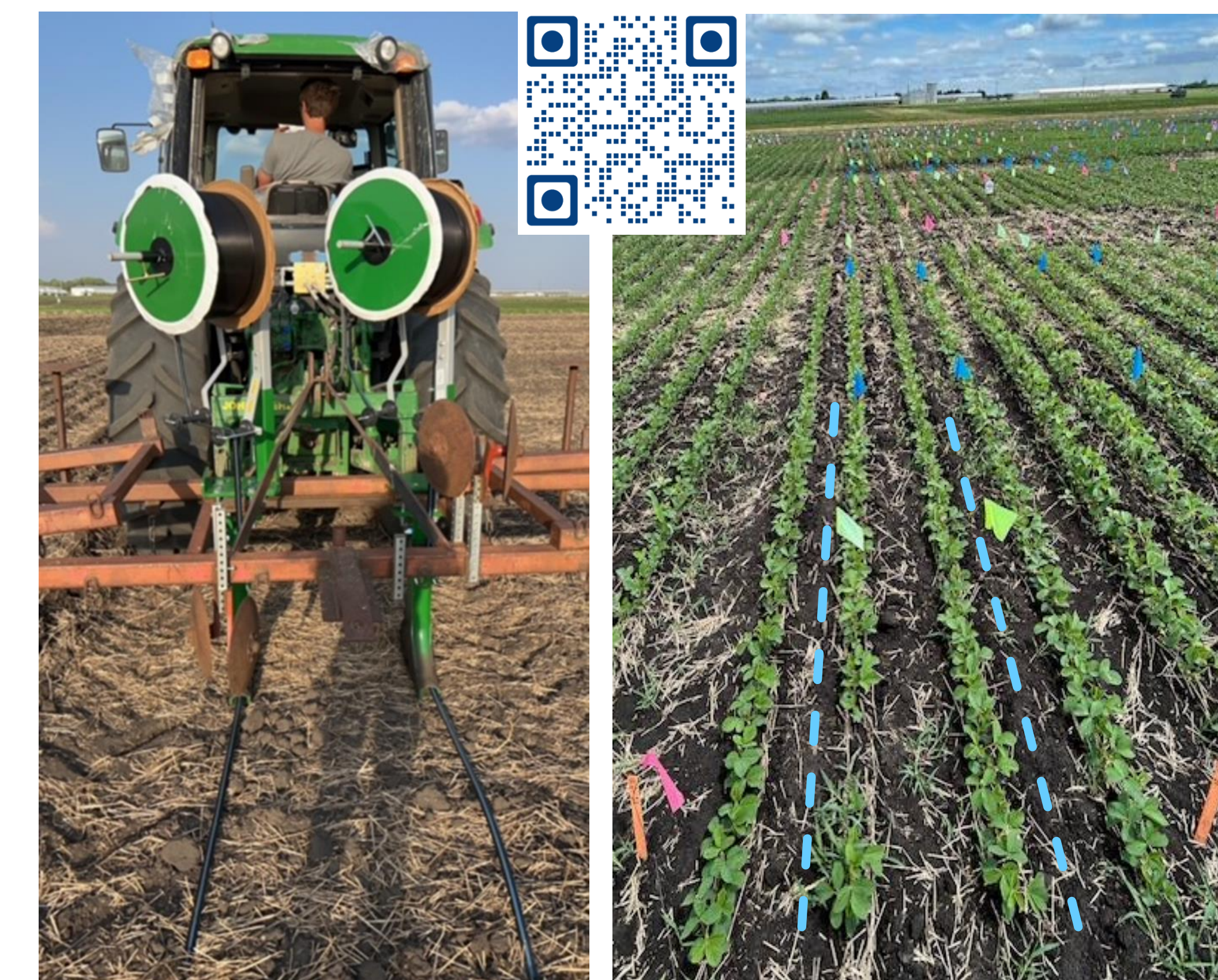
**Figure 2:** Fourteen experimental soybean lines were evaluated for prolonged nitrogen fixation using a biplot of delta protein yield and the non-irrigated treatment protein yield when averaged over two years (2022, 2023) near Carman, MB. Lines were sorted into quadrants by comparing their performance relative to the group mean (blue lines). Soybean lines in biplot quadrant A displayed desirable combinations of low delta protein yield and high non-irrigated yield



**Figure 3:** Percent nitrogen derived from the atmosphere (Ndfa) and relative ureide nitrogen (RUN) when averaged over two years (2022, 2023) as measures of nitrogen fixation in 14 experimental soybean lines were responsive to the drip tape irrigation treatment.

**Table 1:** Selection criteria for soybean performance when evaluating prolonged nitrogen fixation of soybean lines included seed yield, protein concentration, and seed protein yield for irrigated (I) and non-irrigated (N) treatments in 2022 and 2023 near Carman, MB. Nitrogen fixation was evaluated using percent nitrogen derived from the atmosphere (Ndfa) and relative ureide nitrogen (RUN). Nitrogen fixation of experimental short season soybean lines were compared to paired nodulating and non-nodulating check lines when calculating Ndfa.

Line Description	Yield				Seed Protein Concentration				Seed Protein Yield				Ndfa				RUN				
	2022		2023		2022		2023		2022		2023		2022		2023		2022		2023		
	I	N	I	N	I	N	I	N	I	N	I	N	I	N	I	N	I	N	I	N	
	kg/ha				%				kg/ha				%				%				
1	X5731-1-013-3-1-V	3507	3105	3660	3160	37.8	38.2	32.3	33.0	1323	1187	1184	1029	65.6	54.8	60.4	33.3	83.8	83.3	77.2	87.5
2	X5731-1-002-2-2-V	3924	3477	3717	2273	38.4	38.3	32.5	32.7	1509	1333	1207	743	68.4	67.6	64.7	43.5	90.0	86.0	89.1	91.0
3	X5731-1-009-4-1-V	3902	3203	3707	2820	39.4	40.7	33.6	31.9	1539	1303	1244	907	67.7	58.3	67.2	49.2	84.8	92.0	90.0	91.6
4	X5731-1-011-4-5-V	3637	3555	3329	2871	40.2	40.4	35.1	33.7	1463	1436	1167	968	63.6	63.6	70.8	42.6	91.5	94.4	92.6	90.9
7	X5728-1-058-1-1-B	3674	3398	3291	2897	40.2	40.1	35.7	34.8	1477	1363	1175	1006	67.1	51.2	66.0	64.2	92.7	85.7	90.9	93.6
8	X5728-1-003-3-5-B	3701	2943	3394	2768	41.9	42.3	37.1	35.2	1551	1243	1259	973	67.9	60.0	73.1	50.8	92.4	90.4	94.4	91.7
9	X5730-1-005-1-2-B	3088	3104	3315	2556	42.4	42.4	36.3	36.4	1307	1316	1205	930	68.3	57.0	67.9	49.0	90.9	89.4	92.9	92.2
10	X5730-1-005-1-3-B	2591	2287	3142	2716	41.8	41.9	36.3	36.6	1084	958	1139	995	65.9	59.6	61.5	50.1	88.6	87.5	92.2	91.0
11	X6008-1-061-3-3-B	3220	2832	3283	2766	40.1	41.0	34.9	36.0	1292	1160	1145	996	68.0	52.7	64.4	44.7	92.0	88.1	93.1	90.6
12	X6007-1-070-3-3-B	2914	2571	3011	2545	39.4	39.2	34.5	35.9	1147	1007	1040	914	52.9	48.5	39.3	39.0	85.8	81.8	86.4	92.2
13	X6007-1-026-4-3-B	3098	2904	3409	2750	42.1	42.0	36.4	35.6	1303	1223	1242	980	64.3	62.1	57.0	57.6	91.6	90.5	92.3	92.7
14	X6008-1-029-3-1-B	3008	2978	3166	2510	41.7	41.5	35.9	35.5	1253	1236	1135	888	65.4	56.2	56.8	33.1	91.7	90.9	88.8	91.8
15	X6007-1-031-1-2-B	3483	2867	3611	2711	41.6	41.9	34.3	36.1	1449	1199	1238	979	77.3	70.8	67.3	56.4	90.4	91.0	86.5	89.6
16	X6007-1-007-2-4-B	3619	3196	3437	2700	37.6	38.1	33.4	35.9	1360	1219	1147	967	61.6	58.7	47.1	45.6	84.2	83.9	86.6	88.8
17	Nodulating check OT 94-47	2660	2722	2555	2670	38.8	38.7	33.9	33.1	1031	1054	865	886	54.1	47.7	54.5	36.7	88.6	90.7	92.0	92.4
18	Non-nodulating check OT 07-20	2507	2466	2238	2512	35.1	35.4	29.2	32.9	880	873	653	833	0.0	0.0	0.0	0.0	61.9	62.6	53.8	67.3
Mean of Lines 1-16		3383	3030	3391	2717	40.3	40.6	35	35	1361	1227	1181	948	66.0	58.7	61.7	47.1	89.3	88.2	89.5	91.1
LSD for lines 1-16		455	437	455	437	0.7	1.2	0.7	1.2	183	174	183	174	7.4	10.3	17.1	22.8	-	-	-	-



**Figure 5:** Drip tape was installed prior to planting soybeans in a novel field experiment to evaluate prolonged nitrogen fixation. Soybeans were planted on 40 cm spacing with four rows per plot. In irrigated treatments, drip tape was between rows 2 and 3 within each plot (blue dash lines). Use the QR code to view installation of the drip tape.

## Key findings

- Delta seed yield and delta protein yield identified differences between experimental soybean lines when compared to the mean of all lines (Figure 1 and 2).
- Soybean lines in biplot quadrant A displayed desirable combinations of low delta seed yield and high non-irrigated yield (Figure 1 and 2). Soybean lines 1, 4, and 7 appeared in quadrant A using both delta seed yield and delta protein yield as criteria for soybean lines with PNF traits.
- Ndfa as a measure of nitrogen fixation using soybean seed samples identified greater differences between experimental lines and irrigated vs non-irrigated treatments than RUN measured using an earlier development stage (R5) stem and petiole samples (Figure 3).
- This field method shows promise as a screening tool for the development of soybean cultivars with prolonged nitrogen fixation under periodic moisture stress.

## Acknowledgments



## References

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