

ABSTRACT

GOLD, NATALIE LYNN Understanding Soybean Drought Responses in Variable Field Conditions (Under the direction of Dr. Anna M. Locke and Dr. Amanda A. Cardoso).

Frequent and unpredictable drought poses a significant threat to global soybean production. Farmers that rely on rainfed production systems are seeing significant loss in soybean yield due to drought. Multiple drought tolerance mechanisms have been described in different soybean genotypes, but there is limited information about how particular drought tolerance mechanisms operate under the wide range of stress conditions that occur in the field. The objective of this study was to explore the drought response patterns of three soybean genotypes, selected based on prior literature, ancestry, and preliminary data, to determine under what environmental conditions the expected physiological responses occur. This study was conducted over two years at the Sandhills Research Station in Jackson Springs, North Carolina. Soybean genotypes PI 471938, N11-10295, and N93-110-6 were grown under irrigated and rainfed conditions, and these differential irrigation treatments were imposed from developmental stages R1 (beginning flowering) through R5 (beginning seed). Plant physiological responses, including leaf gas exchange and chlorophyll fluorescence, leaf water potential, osmotic potential, root depth, leaf area index, and nitrogen concentration were measured 6-7 times each year during the treatment period. Canopy biomass and nitrogen concentration were measured at developmental stage R6 (full seed), and yield and seed composition were measured at maturity. Soil moisture was measured in two depths for every plot.

Several possible drought tolerance mechanisms were observed, and these were often not as expected from prior literature. Drought responses varied throughout the treatment period, indicating that the degree and/or duration of stress heavily influenced the response mechanisms observed in each genotype. All three genotypes exhibited osmotic adjustment in leaves at some

point, but these times varied among the three genotypes. Osmotic adjustment helped maintain significantly higher turgor pressure in PI 471938. Photosynthesis, stomatal conductance, and photosystem II operating efficiency were more sensitive to stress in N11-10295 than in N93-110-6 and PI 471938. N11-10295 and PI 471938 maintained leaf nitrogen content better in drought conditions than N93-110-6. Leaf area index differed among genotypes under drought conditions. There was no evidence that any genotype had greater root depth. Notably, no genotype had more limited transpiration under high vapor pressure deficit. Thus, although each genotype may have some traits conferring drought tolerance, N93-110-6 and N11-10295 derived from well-studied PI 416937 may not have inherited their drought response mechanisms from that parent.

Overall, this research shows that predicted soybean drought tolerance mechanisms may only be apparent under specific conditions, which emphasizes the importance of complementing in-depth controlled environment studies with field experiments. This information can help guide the optimization of phenotyping in breeding programs and highlights critical and varying stress thresholds for different physiological responses.

© Copyright 2025 by Natalie Gold

All Rights Reserved

Understanding Soybean Drought Responses in Variable Field Conditions

by
Natalie Lynn Gold

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Crop Science

Raleigh, North Carolina
2025

APPROVED BY:

Anna M. Locke
Committee Co-Chair

Amanda A. Cardoso
Committee Co-Chair

Rachel A. Vann

DEDICATION

To my amazing husband, Ben. I love you to the moon and past the stars.

BIOGRAPHY

Natalie Gold was born on February 7th, 1997, in Nazareth, Pennsylvania where she grew a love for the outdoors. With her passion for the environment, she pursued a Bachelor of Science degree in Environmental Science with a minor in Plant Biology and Applied Ecology at North Carolina State University. During her time at NC State, her passion shifted to plant biology research due to working in the Sheth Lab, studying how climate change affected the Scalet Monkey flower. After graduating in 2019, Natalie started working for the United States Department of Agriculture, Agricultural Research Services, Soybean and Nitrogen Fixation Research Unit, in Raleigh NC. She was a biological science technician in the Locke Lab, where she helped research genotype × environmental interactions among soybean to help identify mechanisms that determine stress tolerance and seed composition. In January of 2023, Natalie started pursuing a Master of Science degree in Crop Science at NC State, under Dr. Locke, researching drought tolerance mechanism in soybean.

ACKNOWLEDGMENTS

I would like to thank Dr. Anna Locke for taking me on as a master student. Thank you for believing in me and helping me achieve my dream of getting a master's degree. It has been an honor to work with you these past four years, you truly are a wonderful boss, mentor, and friend. I would like to thank Jeremy Martin and all the staff at the Sandhills Research Station for helping with my experiment. Especially Denise Bufmeyer, you made my day every time I walked into your office. I would like to thank the USDA-ARS Soybean and Nitrogen Fixation Research Unit for lending me their seeds for my research and to the staff for helping during planting, data collection and harvesting. A huge thank you to Jason Pleasant, from collecting root photos in the hot summer heat, to getting stuck in torrential downpours, you always were there to help me no matter what. Thank you! I would like to thank the United Soybean Board for funding my research, I hope this research leads to a more sustainable agricultural future.

Lastly, I would like to express my deepest gratitude and thanks to my wonderful husband, Ben Eve. I always tell you I couldn't have done this without you, and you always say, "of course you could have", but I honestly could not have done this without you by my side. Thank you for always being there for me and for all the love, support, and patience you have given me. How lucky am I to have you in my life.

TABLE OF CONTENTS

LIST OF TABLES	vii
LIST OF FIGURES	xi
Chapter 1: Literature Review	1
1.1 History of Soybean (Glycine max l.)	1
1.2 Seed Production in the United States and Worldwide	1
1.3 Climate Change and Drought.....	2
1.4 Drought Affecting Soybean Reproduction, Seed Composition and Development.....	3
1.5 Drought Tolerance Mechanisms	6
1.5.1 Osmotic Adjustment	6
1.5.2 Root Growth and Development	8
1.5.3 Stomatal Function	11
1.5.4 Leaf Area Index	14
1.6 Conclusion	16
1.7 References	17
Chapter 2: Exploration of Drought Tolerance Mechanism in N93-110-6, N11-10295, and PI 471938	28
2.1 Abstract	28
2.2 Introduction.....	29
2.3 Material and Methods	30
2.3.1 Genotypes	30
2.3.2 Field Study.....	30

2.4 Data Collection	32
2.4.1 Root Growth	32
2.4.2 Developmental Stage	33
2.4.3 Yield	33
2.4.4 100-Seed Weight	33
2.4.5 Seed Oil and Protein Content.....	33
2.4.6 Biomass.....	33
2.4.7 Soil Moisture.....	34
2.4.8 Weather Data	34
2.4.9 Leaf Carbon and Nitrogen Content.....	35
2.4.10 Leaf Water, Turgor, and Osmotic Potential	35
2.4.11 Leaf Area Index	36
2.4.12 LI-COR 6800	36
2.4.13 Statistical Analysis.....	36
2.5 Results:	38
2.6 Discussion Experiment 2023 and 2024:	48
2.7 Conclusion	66
2.8 References	68

LIST OF TABLES

Table 2.1	Soybean pedigree for genotypes N11-10295, N93-110-6, and PI 471938	103
Table 2.2	2023 Daily total rain, maximum air temperature, and average air temperature data collected from NC ECONet Jack station.....	103
Table 2.3	2024 Daily total rain, maximum air temperature, and average air temperature data collected from NC ECONet Jack station	106
Table 2.4	Type III analysis of variance results table with Satterthwaite's method for 2023 LI-COR 6800 data collection	109
Table 2.5	Type III analysis of variance results table with Satterthwaite's method for 2024 LI-COR 6800 data collection	110
Table 2.6	Type III analysis of variance results table with Satterthwaite's method for 2023 leaf water potential, turgor pressure, and osmotic potential.....	112
Table 2.7	Type III analysis of variance results table with Satterthwaite's method for 2024 leaf water potential, turgor pressure, and osmotic potential.....	113
Table 2.8	Type III analysis of variance table with Satterthwaite's method for 2023 VPD data... ..	114
Table 2.9	Type III analysis of variance table with Satterthwaite's method for 2024 VPD data... ..	114
Table 2.10	2023 Type II analysis of variance results with Satterthwaite's method for g_{sw} response to soil moisture at 30.5 cm depth with PI 471938 removed from analysis	115
Table 2.11	2023 Type II analysis of variance results with Satterthwaite's method for g_{sw} response to soil moisture at 61 cm depth with PI 471938 removed from analysis	115
Table 2.12	2024 Type II analysis of variance results with Satterthwaite's method for 2023 g_{sw} response to soil moisture at 30.5 cm depth	115
Table 2.13	2024 Type II analysis of variance results with Satterthwaite's method for 2023 g_{sw} response to soil moisture at 61 cm depth.....	115
Table 2.14	Type III analysis of variance results table with Satterthwaite's method for 2023 root depth	116

Table 2.15	Type III analysis of variance results table with Satterthwaite's method for 2024 root depth	116
Table 2.16	Type III analysis of variance results with Satterthwaite's method for 2023 LAI... ..	117
Table 2.17	Type III analysis of variance results with Satterthwaite's method for 2024 LAI... ..	117
Table 2.18	Type II analysis of variance results table with Satterthwaite's method for 2023 biomass	118
Table 2.19	Type II analysis of variance results table with Satterthwaite's method for 2024 biomass	118
Table 2.20	Type II analysis of variance results table with Satterthwaite's method for 2023 biomass canopy nitrogen and carbon concentrations	119
Table 2.21	Type II analysis of variance results table with Satterthwaite's method for 2024 biomass canopy nitrogen and carbon concentrations	119
Table 2.22	Type III analysis of variance table with Satterthwaite's method for 2023 leaf carbon and nitrogen concentration	120
Table 2.23	Type III analysis of variance table with Satterthwaite's method for 2024 leaf carbon and nitrogen concentration	121
Table 2.24	Type II analysis of variance table with Satterthwaite's method for 2023 total canopy nitrogen g m^{-2}	122
Table 2.25	Type II analysis of variance results table with Satterthwaite's method for 2024 total canopy nitrogen g m^{-2}	122
Table 2.26	Type III analysis of variance table with Satterwhite's method for 2024 leaf nitrogen g cm^2	123
Table 2.27	Type III analysis of variance table with Satterwhite's method for 2024 photosynthetic nitrogen use efficiency	123
Table 2.28	Type II analysis of variance results with Satterthwaite's method for 2023 yield (kg ha^{-1}).....	124
Table 2.29	Type II analysis of variance results with Satterthwaite's method for 2024 yield (kg ha^{-1}).....	124
Table 2.30	Type II analysis of variance table with Satterthwaite's method for 2023 100 seed weight (g)	124

Table 2.31	Type II analysis of variance table with Satterthwaite's method 2024 100 seed weight (g)	124
Table 2.32	Type II analysis of variance results table with Satterthwaite's method for 2023 oil and protein NIR analysis	125
Table 2.33	Type II analysis of variance results table with Satterthwaite's method for 2024 oil and protein NIR analysis	125
Table 2.34	2023 WUE, yield, biomass, water potential, VPD contrasts between irrigated and rainfed treatments with a significant p value < 0.05	126
Table 2.35	2024 Seed weight contrasts between irrigated and rainfed treatments with a with a significant p value < 0.05	126
Table 2.36	2024 VPD contrasts between irrigated and rainfed treatments with a significant p value < 0.05	126
Table 2.37	2023 Root depth contrasts among dates with a significant p value < 0.05	126
Table 2.38	2024 Minirhizotron root depth contrasts among dates with a significant p value < 0.05	127
Table 2.39	2024 % nitrogen biomass, seed protein, seed oil, 100 seed weight, biomass carbon g m^{-2} , and yield contrasts among genotypes with a significant p value < 0.05	128
Table 2.40	2023 Seed weight contrast between irrigated and rainfed treatments among genotypes with a significant p value < 0.05	129
Table 2.41	2023 Total canopy nitrogen contrasts between treatments and among genotypes. None of the contrasts were significant even though the ANOVA interaction is significant	129
Table 2.42	2024 Biomass (g m^{-2}) contrasts between irrigated and rainfed treatments among genotypes. None of the contrasts were significant even though the ANOVA interaction is significant	129
Table 2.43	2023 Water potential contrast with a significant interaction among genotypes and dates with a significant p value < 0.05	130
Table 2.44	2024 G_{sw} and soil moisture contrast with a significant interaction among genotypes and soil moisture depths with a significant p value < 0.05	130
Table 2.45	2023 Leaf area index (LAI), Leaf % Nitrogen and % Carbon content pairwise comparison between irrigated and rainfed among dates with a significant p value < 0.05	130

Table 2.46	2024 Assimilation, Φ PSII, and root depth contrasts between irrigated and rainfed treatments among dates with a significant p value < 0.05.....	131
Table 2.47	2023 A contrast between irrigated and rainfed treatments among genotype and date with a significant p value < 0.05	132
Table 2.48	2023 g_{sw} contrast between irrigated and rainfed treatments among genotype and date with a significant p value < 0.05	132
Table 2.49	2024 g_{sw} contrast between irrigated and rainfed treatments among genotypes and date with a significant p value < 0.05.....	133
Table 2.50	2023 Osmotic Potential contrast between irrigated and rainfed treatments among genotype and date with a significant p value < 0.05	133
Table 2.51	2023 Φ PSII contrast between irrigated and rainfed treatments among genotype and date with a significant p value < 0.05.....	134
Table 2.52	2024 Water potential, turgor potential, an osmotic potential contrast between irrigated and rainfed treatments among genotypes and dates with a significant p value < 0.05	135
Table 2.53	2024 WUE pairwise comparison between irrigated and rainfed treatments among genotypes and dates for water use efficiency significant p value < 0.05	136
Table 2.54	2024 LAI pairwise comparison between irrigated and rainfed treatments among genotypes and dates with a significant p value < 0.05	136
Table 2.55	2024 Leaf % nitrogen content pairwise comparison between irrigated and rainfed treatments among genotypes and dates with a significant p value < 0.05.....	137
Table 2.56	2024 Leaf nitrogen $g\ cm^{-2}$ content pairwise comparison between irrigated and rainfed treatments among genotypes and dates with a significant p value < 0.05 .	137
Table 2.57	2024 Photosynthetic nitrogen use efficiency (PNUE) $\mu mol\ CO_2\ mol^{-1}\ s^{-1}$ pairwise comparison between irrigated and rainfed treatments among genotypes and dates with a significant p value < 0.05	137
Table 2.58	Estimated marginal means for 2023 Biomass, yield, 100 seed weight, protein and oil concentrations based on genotype and treatment excluding plots affected by nematode	138
Table 2.59	Estimated marginal means for 2024 biomass, yield, 100 seed weight, protein and oil concentrations based on genotype and treatment	138

Table 2.60	2023 Developmental stages for rainfed plots during drought treatment	139
Table 2.61	2023 Developmental stages for irrigated plots during drought treatment	139
Table 2.62	2024 Developmental stages for rainfed plots during drought treatment	139
Table 2.63	2024 Developmental stages for rainfed plots during drought treatment	139

LIST OF FIGURES

- Figure 2.1 Total monthly precipitation from May 2023 through August 2023, data used from NC ECONet Jack Station 74
- Figure 2.2 Total monthly precipitation from May 2024 through August 2024, data used from NC ECONet Jack Station 74
- Figure 2.3 Average maximum air temperature from May 2023 through August 2023, data used from NC ECONet Jack Station 75
- Figure 2.4 Average maximum air temperature from May 2024 through August 2024, data used from NC ECONet Jack Station 75
- Figure 2.5 Daily soil moisture means 30.5 and 61 cm from soil surface between treatments from May 2023 through August 2023. Differential irrigation started on July 13th and ended on August 16th with whole field irrigation starting again on August 23rd 76
- Figure 2.6 Daily soil moisture genotype means 30.5 cm from soil surface between treatments from May 2023 through August 2023. Differential irrigation started on July 13th and ended on August 16th with whole field irrigation starting again on August 23rd 77
- Figure 2.7 Daily soil moisture genotype means 61 cm from soil surface between treatments from May 2023 through August 2023. Differential irrigation started on July 13th and ended on August 16th with whole field irrigation starting again on August 23rd 77
- Figure 2.8 Daily soil moisture means 30.5 and 61 cm from soil surface between treatments from May 2024 through August 2024. Differential irrigation started on July 1st and ended on August 27th with whole field irrigation starting again August 27th 78
- Figure 2.9 Daily soil moisture genotype means 30.5 from soil surface between treatments from May 2024 through August 2024. Differential irrigation started on July 1st and ended on August 27th with whole field irrigation starting again on August 27th 79
- Figure 2.10 Daily soil moisture genotype means 61 cm from soil surface between treatments from May 2024 through August 2024. Differential irrigation started on July 1st and ended on August 27th with whole field irrigation starting again on August 27th 79
- Figure 2.11 Sandhills 2023 field map. Planted May 4th, 2023, with 22 ranges of 23 feet long, 15 feet rows with 8-foot alley. Each number plot consisted of 4 rows; the yellow plots are genotype N93-110-6, N11-10295, and PI 471938. Similar genotypes were planted side by side to create 8 row wide plots for the 3 selected genotypes 80
- Figure 2.12 Sandhills 2024 field map. Planted April 30th, 2024, with 22 ranges of 23 feet long, 15 feet rows with 8-foot alley. Each number plot consisted of 4 rows; the yellow plots are genotype N93-110-6, N11-10295, and PI 471938. Similar genotypes were planted side by side to create 8 row wide plots for the 3 selected genotypes 81

Figure 2.13 Sandhill NCDA & Agronomic Division Nematode Report for 2023 field location.....	82
Figure 2.14 Figures are from Arnaud et al (2019) which we based our experiments minirhizotron on. Figures show how images are collected in the field and how the tubes are placed in the soybean rows	83
Figure 2.15 2023 assimilation (A) was affected by drought with a significant three-way interaction among treatment, genotype, and date	84
Figure 2.16 2024 assimilation (A) was affected by drought with a significant interaction among treatment and date	84
Figure 2.17 2023 stomatal conductance (g_{sw}) was affected by drought with a significant interaction among treatment, genotype, and date	85
Figure 2.18 2024 stomatal conductance (g_{sw}) was affected by drought with a significant interaction among treatment, genotype, and date	85
Figure 2.19 2023 Φ PSII was affected by drought with a significant interaction among treatment, genotype, and date	86
Figure 2.20 2024 Φ PSII was affected by drought with a significant interaction among treatment and date.....	86
Figure 2.21 2023 iWUE was affected by drought with only a significant treatment effect	87
Figure 2.22 2024 iWUE was affected by drought with a significant interaction among treatment, genotype, and date	87
Figure 2.23 2023 Water potential was affected by drought with only a significant treatment effect	88
Figure 2.24 2024 Water potential was affected by drought with a significant interaction among treatment, genotype, and date	88
Figure 2.25 2023 Osmotic potential was affected by drought with a significant interaction among treatment, genotype, and date. On 7/20/2023 sampling day N93-110-6 was removed from graph due to psychrometers malfunctioning	89
Figure 2.26 2024 Osmotic potential was affected by drought with a significant interaction among treatment, genotype, and date	89
Figure 2.27 2023 Turgor pressure was not significant	90

Figure 2.28 2024 Turgor pressure was affected by drought with a significant interaction among treatment, genotype, and date	90
Figure 2.29 2023 Stomatal conductance (g_{sw}) was affected by drought and had a significant Treatment by VPD interaction	91
Figure 2.30 2024 Stomatal conductance (g_{sw}) was affected by drought and had a significant Treatment by VPD interaction	91
Figure 2.31 2023 Root depth only had a significant date effect	92
Figure 2.32 2024 Root depth was affected by drought with a treatment by date interaction	92
Figure 2.33 2023 Leaf area index (LAI) was affected by drought with a significant treatment by date interaction	93
Figure 2.34 2024 Leaf area index (LAI) was affected by drought with a significant treatment, genotype, and date interaction.....	93
Figure 2.35 2023 % Nitrogen was affected by drought with a significant interaction between treatment and date	94
Figure 2.36 2024 % Nitrogen was affected by drought with a significant interaction between treatment, genotype, and date.....	94
Figure 2.37 2023 Total Canopy Nitrogen was affected by drought with a significant interaction among treatment and genotype. There were not significant pairwise comparison ..	95
Figure 2.38 2024 Total Canopy Nitrogen as affected by drought with a significant interaction among treatments and genotypes. There were not significant pairwise comparisons	95
Figure 2.39 2023 Biomass was affected by drought with a significant treatment affect.....	96
Figure 2.40 2024 Biomass was affected by drought with a significant interaction among treatments and genotypes. No pairwise comparisons significant.....	96
Figure 2.41 2023 Yield was affected by drought with a significant treatment affect.....	97
Figure 2.42 2024 Yield was not affected by drought but had a significant genotype affect	97
Figure 2.43 2023 Seed weight was affected by drought with a significant treatment by genotype interaction	98

Figure 2.44 2024 Seed weight had a genotype and treatment affect	99
Figure 2.45 2023 Seed oil and protein was not significant in ANOVA	100
Figure 2.46 2024 Oil and protein had a genotype affect	101
Figure 2.47 2024 Leaf nitrogen (g/cm ²) was affected by drought with a significant treatment, genotype, and date interaction.....	102
Figure 2.48 2024 Photosynthetic nitrogen use efficiency was affected by drought with a significant treatment, genotype, date interaction.....	102

CHAPTER 1: Literature Review

1.1 History of Soybean (*Glycine max* L.)

Soybean (*Glycine max* (L.) Merr.), is part of the legume family. The specific location where soybeans were originally domesticated is still debated, however, there is strong evidence that shows the Chinese Yellow River Basin as the origin of domestication 6000-9000 years ago (Sedivy et al., 2017). By the first century A.D., soybean farming and domestication started to make their way into European farming practices (Qui & Chang, 2010). Samuel Bowen introduced soybean to the United States in 1765, when he planted seeds in Georgia (K.-I. Chen et al., 2012). It was not until the 1870's that farmers began growing soybeans around the country for livestock feed (Specht et al., 2015).

World War II halted trading products with other countries, and at the time the US imported half of the edible fats and oils, resulting in consumers to look into soybean oil uses (Hart, 2017). Soybean farming and production then took off in America, from 1940's to the 1950's production grew from around 5,000 million hectares to 10 million hectares, peaking at 29,000 hectares in 1979 (Mohan Jainn DennissVJohnson Editors, 2019). Soybeans have become one of the main crops produced here today.

1.2 Soybean Production in the United States and Worldwide

In the 1960's worldwide soybean production was 20 to 30 million tons, whereas in 2020 production increased to 350 million tons (Ritchie & Roser, 2021). About 77% of global soybean production is processed into 20% oil and 80% meal, the meal being used primarily livestock production and the oil for human consumption and use (Ritchie & Roser, 2021). That 20%, is made into soy sauce, tofu, soy milk, and other consumer products for consumption and into commercial biofuels.

The United States, Brazil, and Argentina are the largest soybean-producing countries in the world. Lindsey & Dahlman. (2023), reported in 2021 that the United States planted 87.2 million acres of soybean, with an average yield of 51.4 bushels per acre. During the combined calendar year 2020/2021, the overall soybean production was 4.44 billion bushels, making total crop value \$45.7 billion in 2020 (Soybean Highlights from 2022, 2022.). Combined, the US and Brazil account for 69% of global soybean production (Ritchie & Roser, 2021) In the United States, soybean is the second-largest crop grown for yield and used as a rotation crop. Soybeans have the capability to fix nitrogen through a symbiotic process, called nitrogen fixation. Farmers take advantage of this symbiotic process because soybean generally do not require nitrogen fertilizer like other non-legume agricultural crops (Hart, 2017).

1.3 Climate Change and Drought

In 2009, researchers suggested soybean production needs to increase 140% by 2050 to sustain projected increase in population and demand (Bruinsma, 2009). However, since 2009, temperatures have been warmer, and drought has been frequent. Since record keeping began, 2022 was the sixth-warmest year worldwide (Lindsey & Dahlman, 2023). Warmer temperatures increase evaporation rates, leading to an increase in evaporation of surface water and drier soils, this drying leads to soil cracking, salinization and degradation (An et al., 2018). Cooler regions of the world that usually experience mild or moderate drought, now are experiencing severe or extreme drought due to the cooler regions increase in temperature. Increased global temperatures have altered precipitation patterns globally. These droughts affect 45% of agricultural lands worldwide (H. Chen & Jiang, 2010). Soybean are grown in tropical, subtropical, and temperate regions. These regions face unstable weather conditions and selecting stress-resistant soybean

genotypes are required to meet food security demands (Xiong et al., 2021) (K. J. Kunert et al., 2016).

The United States Drought Monitor (USDM) states that precipitation and temperature are the drivers of drought (Kuwayama et al., 2019). Many agricultural lands vary in drought status due to factors affecting water availability besides rainfall. Warmer winter temperatures have been seen recently in regions where snowpack melts are critical to agricultural production during the spring and summer. These interactions between temperature and rainfall predict how plants respond to their environments (Zipper et al., 2016). In the United States, the Southeast portion of the country sees a high variability in drought (Kuwayama et al., 2019). Droughts will continue to cause production issues worldwide for high-value crops (Kuwayama et al., 2019). Drought reduces global soybean yield by more than 50% annually (Wijewardana et al., 2019). This loss in yield can cause devastating effects on the economy, affecting farming profit, job security, and food security.

1.4 Drought Affecting Soybean Reproduction, Seed Composition, and Development

A soybean seed is mostly made up of protein and oil; seed protein ranges from 34- 57% of total seed weight, and oil ranges from 8.3-28% of total seed weight (Wilson, 2004). The main storage of protein in soybeans are glycinin and β -conglycinin (Medic et al., 2014). Soybean seed composition also has saturated fatty acids (approximately 4% palmitic acid and 22% stearic acid) and unsaturated fatty acids (approximately 22% oleic acid, 54% linoleic acid, and 10% linolenic acid) (Wilson, 2004). Most of the carbohydrates in seeds are insoluble polysaccharides like pectin, cellulose, hemicellulose, and starch. Soluble carbohydrates include monosaccharides of glucose and fructose and disaccharides and oligosaccharides (Lozovaya et al., 2005). Genotypes,

management practices, and environmental conditions play a role in the quality of the seeds, and drought is one of the major abiotic stressors.

Drought not only affects soybean plant health and growth, but it also affects soybean seed composition, which in turn affects the economic value. The value of soybean depends on the concentration of protein and oil (Nakagawa et al., 2018; Rotundo & Westgate, 2009). The overall goal for soybean breeders is to find genotypes that are high-yielding and high in protein content, improving value to both farmers and end-users. However, with drought as an environmental stressor, farmers are seeing variable seed oil and protein composition even with high-yielding genotypes. The quality of a soybean is essential in determining germination rate, vigor, and the likelihood of a seed emerging during the following planting season, as well as its value to end users.

Drought stress affects every stage of a soybean's growth cycle, from germination to seed fill; drought could impact the yield quality for that growing season (Dietz et al., 2021). Seeds that grow under drought stress conditions can result in lesser germination quality and a decrease in seed vigor. Seed weight and storage reserves are essential for seed germination and development, and when those elements are lacking, it can cause a decrease in the seed germination rate (Wijewardana et al., 2019). Heatherly et al. (1993), reported in a field drought experiment those cultivars exposed to drought stress has an inferior quality to control cultivars, with the standard germination < 80%. Drought stress on plants can lead to a lesser quality of F1 generation seeds. In Wijewardana et al. (2019), a drought in the reproductive stage in the maternal plants caused a decrease in maximum seed germination and germination rate in F1 generations. Drought causing a decrease in germination in the F1 generation seed could lead to less yield to support soybean production when farmers plant.

The timing and intensity of the drought are very important factors to consider when estimating the effects of yield loss and altered seed composition (Dornbos et al., 1989). Researchers have found that soybeans are less susceptible to yield loss when drought stress occurs during the vegetative stage (VE, VC, V1) but are more susceptible to yield loss during the reproductive stages (R4-R6) (Xiong et al., 2021; Sloane et al., 1990; Wijewardana et al., 2019). Drought in the reproductive stages affects soybean pod and seed number, along with individual seed weight, yield, and seed number, all of which is positively correlated to soil moisture (Wijewardana et al., 2019). Drought occurring during R4-R6 can cause a decrease in protein and oil content of seeds and a decrease in seed weight. Dornbos et al. (1992), research found when drought occurred there was decrease in protein content by 4.4% and oil decreased by 2.9%. This was similar to Rotundo et al. (2009), meta-analysis in which drought stress occurring during R5-R8 stages saw a decrease in oil content of 35%.

Nakagawa et al. (2018), greenhouse pot experiment saw a decrease in seed oil content during the later stages of seed fill (R6) in drought stress genotypes compared to the control. Oil content decreases less than protein content during stages R5-R8 (Rotundo & Westgate, 2009). A decrease in lipid can be seen as a correlation to the rate of photosynthesis, lipid biosynthesis is dependent on the supply of photosynthate from plant tissues (Baud & Lepiniec, 2010). Drought stress decreases stomatal conductance and photosynthetic carbon assimilation, which is strongly correlated to seed quality, yield, and weight (Du et al., 2020). Photosynthesis supplies the plant with carbohydrates. During the seed-filling stages carbohydrate mobilization and transport of proteins rely on the source-sink ratio to off-load assimilates from leaf to seed (Mertz-Henning et al., 2018). Some soybean varieties have drought tolerance mechanism that help sustain

photosynthesis under drought stress conditions, leading these genotypes to maintain yield and seed quality under drought stress conditions.

1.5 Drought Tolerance Mechanism:

1.5.1 Osmotic Adjustment

To tolerate drought-stress conditions, plants have developed drought tolerance mechanisms that help the plant survive under stress. One of the many mechanisms that soybeans and other plants use in drought-stressed environments is osmotic adjustment. Osmotic adjustment is a net increase of solutes within the cells, that reduces osmotic potential (becoming more negative), drawing water into the cell to maintain turgor pressure (Sanders & Arndt, 2012). Osmotic adjustment is a response to water deficiency or salinity (Flower D. J. & Ludlow M. M., 1983; Munns, 1988; Turner & Jones, 1980).

The organic solutes that play a role in osmotic adjustment are glycine, proline, and sugars (H. Chen & Jiang, 2010) Inorganic ions also help in osmotic adjustment (Blum, 2017). Different factors influence how much osmotic adjustment occurs in plants and how effective it is. The rate of stress development and degree of stress effects the response osmotic adjustment (Turner, 2018; Turner & Jones, 1980). If a plant experiences fast dehydration, osmotic adjustment will not occur, and it is an adaptive process that occurs over a few days when triggered by drought and slow dehydration (Blum, 2017a; Flower D. J. & Ludlow M. M., 1983; Turner & Jones, 1980). This was seen in an experiment with water stress on pigeon pea leaves (Flower D. J. & Ludlow M. M., 1983). Plants that exhibited rapid onset of drought stress had less osmotic adjustment than plants that had a slow onset of drought stress, leading to more osmotic adjustment (Flower D. J. & Ludlow M. M., 1983). Experimental design and environment can also affect the responses from osmotic adjustment and osmotic adjustment itself. In Tangpremsri et al (1995),

high and low lines of osmotically adjusting sorghum were compared in greenhouse vs. field experiments to see if osmotic adjustment changed the plants response to soil water stress. In both environments osmotic adjustment was higher in the high osmotic adjusting line by 0.1 to 2.5 MPa, but the high osmotic adjusting line only had higher grain number in the field, not in the greenhouse (Tangpremsri et al., 1995). In a greenhouse, because of restricted pot volumes, extreme water stress develops more rapidly than in the field, causing osmotic adjustment not to respond as effectively due to the sudden onset of water stress (Tangpremsri et al., 1995; Blum, 2017; Flower D. J. & Ludlow M. M., 1983). Environmental factors, in either field and/or greenhouse experiments, have effect on the rate of soil drying, temperature fluctuations, and light (Turner & Jones, 1980).

Not all crop plants and genotypes within a crop species respond to water stress with osmotic adjustment in the same way (Turner & Jones, 1980; J. Zhang et al., 1999). Sloane et al. (1990), compared two different soybean genotypes, PI 416937 and Forrest, in a field drought experiment and found that PI 416937 experienced a higher osmotic adjustment than genotype Forrest. Genotype PI 416937 maintained higher pressure potential, and higher relative water content (RWC) when compared to Forrest (Sloane et al., 1990). By studying the responses of different genotypes within a species, researchers could pinpoint how drought responses differ among genotypes and what physiological mechanisms underlie drought tolerance. Understanding physiological mechanisms in response to drought stress will help lead to a better understanding of genotype selection (Dietz et al., 2021). These genotypes could then be used in breeding for drought tolerance. Traits that breeders and researchers look for in drought tolerant soybeans include water use efficiency, deep rooting, sustained nitrogen fixation, and sustained photosynthetic rates (Mutava et al., 2015).

Osmotic adjustment has many benefits in sustaining the survival of crops when experiencing drought stress conditions. Research shows one of main benefits to these responses is an impact on crop yield in water stress conditions. In a review, Blum, (2017b) compiled 26 published studies that investigated if osmotic adjustment had any influence on yield under drought stress conditions. Of the 26 papers, 24 saw a positive link between osmotic adjustment and yield; the species in these studies were barley, wheat, maize, grain, sorghum, chickpea, pea, pigeon pea, soybean, canola, mustard, castor bean, and sunflower. Gonzalez et al. (1999), found that osmotic adjustment had a positive correlation with yield and stomatal conductance across the eight barley genotypes grown in a field rain shelter under drought stress conditions. Of the barley lines with the highest osmotic adjustment, there was little difference in stomatal conductance as water stress increased (Gonzalez et al., 1999).

In research experiments, osmotic adjustment has been seen to improve overall plant water status by facilitating continued access to deep soil water (Moinuddin & Khanna-Chopra, 2004; Morgan & Condon Ab, 1986; Subbarao et al., 2000). In a potted wheat field drought experiment, high osmotic adjustment lines of wheat maintained higher turgor and yield compared to low osmotic adjustment wheat lines (Morgan & Condon Ab, 1986). Morgan & Condon Ab. (1986), concluded with these findings that turgor maintenance and or osmoregulation were associated with the difference in water uses due to the plant's ability to extract water at soil depth between 25 and 150 cm.

1.5.2 Root Growth and Development

Root exploration of the soil has been related as a drought tolerance mechanism, due to the ability to access deeper soil reservoirs under drought stress conditions (Cortes & Sinclair, 1986) Roots play a major role in maintaining physiological function by supplying a crop with sufficient

water supply. Roots are the first plant organ to perceive soil moisture status, and thus the root response to soil drying is often a key determinant of survival and yield (Demicheli et al., 2023a; Shoaib et al., 2022). Drought tolerant genotypes may change their root architecture depending on water availability, investing in deeper root growth for increased water and nutrient access compared to genotypes that are drought sensitive (Shoaib et al., 2022).

In Hoogenboom et al. (1987), a field experiment using a rhizotron system investigated daily root growth rates of soybean under drought stress conditions. With supplemental irrigation, soybeans produced roots in shallower soils, but in rainfed soybeans, roots were seen in deeper soil layers during drought. When a crop has sufficient soil water, resources are not expended for additional root growth; however, when drought stress occurs, a plant will supply nutrients and energy to grow deeper root architecture (Hu & Xiong, 2014). By accessing deeper soil moisture during drought conditions, soybeans can maintain physiological functions and stabilize yield output.

The depth of a root is significant in determining nutrient supply and yield. A study found that soybean genotypes with greater root depth had a higher yield output compared to soybean genotypes that did not have deeper roots (Barber & Bouldin, 1984). In Seversike et al. (2014a), drought resistant wild soybean genotype PI 468917 was compared to cultivated soybean cultivar Hutcheson in hydroponic greenhouse systems, to evaluate inherited differences in root morphology between genotypes. In PI 468917, greater root length and a more fibrous root system was noted compared to Hutcheson in a 23°C solution and some greater lengths in a 28°C solution. It was also found that root developmental differences between the two genotypes were present in early developmental stages and became less pronounced in later developmental stages. This difference in early root development between genotypes could allow breeders to select for

early rooting traits, to establish more soil contact area before potential drought stress can occur later in development.

Root length density and root surface area can also play a role in soybean drought responses. Having a greater root length density would allow more water absorption due to having a greater root surface area. In Dayoub et al. (2021), soybean grown under drought conditions in growth chambers developed 10% more root surface area and 12% more length per unit of root dry weight compared to non-water stressed conditions. There was also a 5% increase in root diameter under water stress compared to soybean in non-stressed conditions.

Studies have shown that having a greater root length density and deep rooting capabilities can lead to a greater yield under drought conditions. However, other studies have shown smaller root development can also conserve water. A soybean having a low root length density and/or surface area could use water more slowly in drought conditions, allowing for more water use later in severe conditions (Gao et al., 2020; He et al., 2017). In Gao et al. (2020), comparing older vs new soybean cultivars under drought conditions, root length density and root surface area were different at the same soil depths. Soybean cultivars with a lower root length density and root surface area at the same soil depths had higher yield and higher water use efficiency (Gao et al., 2020) Similarly, He et al., (2017), found that smaller root size led to soybean plants conserving water in water stressed environments.

Depending on the soil type and location, root elongation as a drought tolerance mechanism may not help some growers. In Sinclair et al. (2010), a simulation analysis modeled soybean growth to quantify changes in yield in modifications of rooting development. It was found that compared to the Midwest, the Southern part of the United States would derive more yield benefit from the deeper rooting. The simulation also investigated early stomatal closure

with the onset of soil drying. If roots can no longer find soil water sources, stomatal closure can reduce the soil to leaf gradient in water potential, which reduces the plant's water demand.

1.5.3 Stomatal Function

The stomata of a plant play a critical role in leaf gas exchange, regulating the diffusion of atmospheric CO₂ into the intercellular air spaces. Stomata also play an important role in limiting water loss under high evaporative demand and/or low soil moisture availability, which may avoid a lethal loss of turgor pressure. The stomata evolved around 400 million years ago, as plants moved into terrestrial environments and evolved with changing atmospheric conditions (Brodribb & Mcadam, 2011; Raven, 2002). The evolution of stomata is believed to be a response in optimizing greater CO₂ intake due to the increase in atmospheric CO₂ to the amount of water loss during photosynthesis (Raven, 1995, 2002). The ratio of water lost relative to photosynthetic carbon assimilation, also known as water use efficiency (WUE), is related to the amount of CO₂ in the leaf substomatal (Brodribb et al., 2009). This is controlled by guard cells surrounding the stomata.

Guard cells regulate stomatal aperture through changes in guard cell turgor pressure (Brodribb & Mcadam, 2011). The opening of the stomata is driven by many factors, one being the rate of photosynthesis. When a signal reaches the guard cells, an influx of ions creates a high osmotic pressure, driving water intake and higher turgor pressure. This turgor pressure pushes the guard cells away from each other to open the stomatal pore, allowing CO₂ to enter and water to transpire. When these ions leave the guard cells, turgor pressure is lost, reducing the stomatal aperture and decreasing stomata conductance; in turn, this reduces leaf water loss, which can help maintain higher water potential in the plant (Bharath et al., 2021; Brodribb & McAdam, 2011; Brodribb & McAdam, 2017). Regulation of the stomata aperture is seen as a drought

tolerance mechanism that vascular plants have developed to tolerate drought conditions and delay dehydration (Raven, 2002). Outside of guard cells, osmotic adjustment can help maintain water uptake and turgor pressure in drought conditions, which also facilitates continued stomata opening (Turner & Jones, 1980). Maintaining cell turgor and volume through osmotic adjustment during drought stress can help maintain a higher photosynthetic rate (González et al., 1999). This was observed by Gunasekera et al. (1994), in a growth chamber drought stress experiment with wild and cultivated barley genotypes; they observed that osmotic adjustment was correlated with greater stomatal conductance, and the wild genotypes maintained higher photosynthesis at low leaf water potential. The greater the osmotic adjustment under stress, the greater the cell volume was maintained at low leaf water potential.

When a plant senses drought, it can close its stomata to conserve soil moisture. Early stomata closing can be seen as a drought tolerance mechanism in soybeans and other crops. A plant closing their stomata early during the onset of drought can reduce the amount of soil water used, lessening the severity of the drought later in the growing season (Sinclair, 2017; Sinclair et al., 2010). In Sinclair et al. (2010) a simulation model of early stomatal closure in soybeans during the onset of soil drying showed to provide significant effects on yield in both dry and wet conditions across the United States. The probability of soybean yield increase was greater than or equal to 0.79. Closing of the stomata has been seen in soybean genotypes, creating a higher water use efficiency, maximizing soil water use over long periods of time (Demicheli et al., 2023a). In addition to hydraulically mediated signals, the closing of the stomata in response to soil drying is partially due to a phytohormone called abscisic acid, also known as ABA. Control of stomatal conductance under drought conditions and ABA responses under drought conditions

can be a promising trait for soybean breeders in developing drought tolerant soybeans (Manavalan et al., 2009).

Abscisic acid is a hormone that plays multiple roles in a plant's growth and development, and it is an essential hormone triggered by abiotic and biotic stress like drought. When the roots of a plant sense the onset of drought in the soil, ABA synthesis is triggered in the roots and transported to the shoot via xylem (Bharath et al., 2021; Brodribb & McAdam, 2017; Davies & Zhang, 1991; F. Liu et al., 2005) and there also have been studies that show leaves are a site for ABA biosynthesis under drought stress conditions (Cardoso et al., 2020; Manzi et al., 2015; McAdam et al., 2016; F. P. Zhang et al., 2018). When ABA accumulates in the leaves, it activates guard cell specific ion channels, initiating the efflux of ions in the guard cells, causing a loss in turgor pressure, closing the stomata (Brodribb & McAdam, 2017; Yoshida et al., 2006). ABA initiating the closing of the stomata limits transportation rates to maintain or increase whole plant water potential; this conservation of water use protects the plant from reaching desiccation and lethal water potential levels under drought stress conditions (Gilbert et al., 2011; F. Liu et al., 2005; Seversike et al., 2014b). F. Liu et al. (2005), investigated stomatal control and water use efficiency of soybeans under drought stress conditions; as the soil was drying, soybean stomatal conductance decreased, causing a decrease in transpiration rates, thus increasing the soybean water use efficiency in mild soil water deficits. Decreased root water potential coincided with an increase in xylem ABA concentration, indicating that soybeans had the ability to produce ABA in the root tips in response to soil drying, closing the stomatal aperture and increasing whole plant WUE (F. Liu et al., 2005). Zegada-Lizarazu & Monti, (2019) found when studying giant reeds in soil water deficit conditions, in shallow drought

treated roots, ABA concentration increase was positively correlated with foliar ABA concentration, water use efficiency, and leaf water potential.

1.5.4 Leaf Area Index

Crop canopy coverage is an important physiological factor in crop ecology for suppressing weed competition and maximizing yield. Maximizing the canopy coverage of a soybean crop can increase net canopy photosynthesis, but it has consequences as a drought response. The leaf area index (LAI) is an important measurement characteristic that describes the canopy structure of the plant, as the one-sided leaf area per unit ground area. The LAI of a plant is affected by management variables like row spacing, and less controlled variables like leaf shape, arrangement, and environment (Pearce et al., 1965).

Drought can significantly reduce soybean leaf area development due to limited water availability for photosynthesis and biomass; however, lower LAI can help conserve water in drought situations (Sinclair et al., 2007). Slowing leaf area development in soybean during the early stages of development could limit water use early on, allowing the plant to access more soil water later in the season, during a drought (Sinclair et al., 2010). Sinclair et al. (2010) showed in a model simulation that in the 25% of scenarios, there was a benefit to slowing leaf area development in the beginning of the season, with a predicted yield gain range of up to 20 g m⁻². Gao et al. (2020), investigated soybean canopy architecture, water uptake, and root traits and found that of eight soybean genotypes, Zhonghuang 30 and Jindou 19 had the least water use and the lowest leaf area. This reduction in leaf area could reduce water demand and improve yield in drought conditions. Having slow leaf area development or a decreased leaf area could be investigated in the field as a drought tolerance mechanism for water conservation.

On the other hand, selecting soybean genotypes that exhibit a greater LAI could impact yield and biomass output due to a higher canopy coverage intercepting a greater percentage of light. In a study by Wells. (1991), there was a relationship between the light intercepted by the soybean canopy and LAI. This leaf area interception created a higher canopy photosynthesis. Having a higher canopy photosynthetic rate due to a greater LAI, a greater biomass accumulation and yield could be achieved. Soybeans that have a greater LAI have the capacity for a larger area of solar energy interception, and this maximized interception leads a plant to have a higher rate of net photosynthesis and more accumulation of assimilates (Kumudini et al., 2001; X. Liu et al., 2005a; Wells, 1991). X. Liu et al. (2005) researched if soybean LAI and dry matter accumulation influence yield, and found that higher accumulation of dry matter, LAI, and LAD during the reproduction stage of soybeans was associated with high yielding genotypes in both early and late maturity groups. Differences in yield can be seen between different soybean cultivars and maturity groups due to differences in whole canopy photosynthesis. Wells et al. (1982), conducted an experiment consisting of four soybean cultivars of four maturity groups and found that a difference in yield among the cultivars were partially due to canopy photosynthetic capacity. Soybean development and the timing of maximum LAI can play a role in maximizing yield potential (Kumudini et al., 2001; X. Liu et al., 2005a; Wells, 1991). The accumulation of dry matter during soybean flowering is a key factor in achieving high yield (Koutroubas et al., 1998). Having a higher dry matter accumulation before seed fill stage generally requires higher LAI, allowing for a larger interception of solar energy to increase assimilate accumulation (Kumudini et al., 2001). Kumudini et al., (2001) found that greater LAI in newer soybean cultivars during the seed fill stage was associated with a 30% increase in yield compared to older cultivars.

1.6 Conclusion

This review highlights how drought caused by climate change threatens soybean production worldwide and how necessary it is to find soybean genotypes which exhibit favorable drought response patterns. Soybeans may hold multiple drought response mechanisms, or none, and it is important to identify which responses a genotype may or may not have, and under what conditions they are favorable. Utilizing multiple tools to measure different plant parameters can allow for thorough investigation into drought response. Identifying these key mechanisms is one of the ways breeders are developing soybean genotypes that can withstand longer periods of drought and still produce a high yield and maintain oil and protein content. There has been extensive research on drought tolerance mechanism in soybeans in both greenhouse and field experiments, but the question remains, what is the most efficient way to improve drought tolerance?

1.7 References

- An, N., Tang, C. S., Xu, S. K., Gong, X. P., Shi, B., & Inyang, H. I. (2018). Effects of soil characteristics on moisture evaporation. *Engineering Geology*, *239*, 126–135.
<https://doi.org/10.1016/j.enggeo.2018.03.028>
- Barber, S. A., & Bouldin, D. R. (1984). *Roots, Nutrient and Water Influx, and Plant Growth*. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America.
- Baud, S., & Lepiniec, L. (2010). Physiological and developmental regulation of seed oil production. In *Progress in Lipid Research* (Vol. 49, Issue 3, pp. 235–249).
<https://doi.org/10.1016/j.plipres.2010.01.001>
- Bharath, P., Gahir, S., & Raghavendra, A. S. (2021). Abscisic Acid-Induced Stomatal Closure: An Important Component of Plant Defense Against Abiotic and Biotic Stress. In *Frontiers in Plant Science* (Vol. 12). Frontiers Media S.A. <https://doi.org/10.3389/fpls.2021.615114>
- Blum, A. (2017a). Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. In *Plant Cell and Environment* (Vol. 40, Issue 1, pp. 4–10). Blackwell Publishing Ltd. <https://doi.org/10.1111/pce.12800>
- Blum, A. (2017b). Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. In *Plant Cell and Environment* (Vol. 40, Issue 1, pp. 4–10). Blackwell Publishing Ltd. <https://doi.org/10.1111/pce.12800>
- Brodribb, T. J., & McAdam, S. A. M. (2011). Passive Origins of Stomatal Control in Vascular Plants. *Source: Science*, *331*(6017), 582–585. <https://doi.org/10.1126/science.1197175>
- Brodribb, T. J., & McAdam, S. A. M. (2017). Evolution of the stomatal regulation of plant water content. *Plant Physiology*, *174*(2), 639–649. <https://doi.org/10.1104/pp.17.00078>

- Brodribb, T. J., McAdam, S. A. M., Jordan, G. J., & Feild, T. S. (2009). Evolution of stomatal responsiveness to CO₂ and optimization of water-use efficiency among land plants. *New Phytologist*, 183(3), 839–847. <https://doi.org/10.1111/j.1469-8137.2009.02844.x>
- Bruinsma, J. (2009). The resource outlook to 2050: By how much do land water and crop yield need to increase by 2050? FAO expert meeting on “How to feed the world in 2050” (33 pp), Rome, Italy, 24-26 June 2009.
- Chen, H., & Jiang, J. G. (2010). Osmotic adjustment and plant adaptation to environmental changes related to drought and salinity. In *Environmental Reviews* (Vol. 18, Issue 1, pp. 309–319). National Research Council of Canada. <https://doi.org/10.1139/A10-014>
- Chen, K.-I., Erh, M.-H., Su, N.-W., Liu, W.-H., Chou, C.-C., & Cheng, K.-C. (2012). Soyfoods and soybean products: from traditional use to modern applications. *Applied Microbiology and Biotechnology*, 96(1), 9–22. <https://doi.org/10.1007/s00253-012-4330-7>
- Cortes, P. M., & Sinclair, T. R. (1986). Water Relations of Field-Grown Soybean under Drought 1. *Crop Science*, 26(5), 993–998.
<https://doi.org/10.2135/cropsci1986.0011183x002600050031x>
- Davies, W. 1, & Zhang, L. (1991). ROOT SIGNALS AND THE REGULATION OF GROWTH AND DEVELOPMENT OF PLANTS IN DRYING SOIL. In *Annu. Rev. Plant Physiol. Plant Mol. Bioi* (Vol. 42). www.annualreviews.org
- Dayoub, E., Lamichhane, J. R., Schoving, C., Debaeke, P., & Maury, P. (2021). *Early-Stage Phenotyping of Root Traits Provides Insights into the Drought Tolerance Level of Soybean Cultivars*. <https://doi.org/10.3390/agronomy>
- Demicheli, J., Sabljic, I., Beguy, G., Ploschuk, E., Sahrawy, M., Serrato, A. J., & Pagano, E. A. (2023). Improving drought tolerance in soybean by classical breeding leads to physiological

- adjustments of photosynthesis and stomata functioning. *Plant Stress*, *10*.
<https://doi.org/10.1016/j.stress.2023.100275>
- Dietz, K. J., Zörb, C., & Geilfus, C. M. (2021). Drought and crop yield. In *Plant Biology* (Vol. 23, Issue 6, pp. 881–893). John Wiley and Sons Inc. <https://doi.org/10.1111/plb.13304>
- Dornbos, D. L., & Mullen, R. E. (n.d.). *Soybean Seed Protein and Oil Contents and Fatty Acid Composition Adjustments by Drought and Temperature*.
- Dornbos, D. L., Mullen, R. E., & Shibles, R. E. (1989). Drought stress effects during seed fill on soybean seed germination and vigor. *Crop Science*, *29*(2), 476–480.
<https://doi.org/10.2135/cropsci1989.0011183X002900020047x>
- Du, Y., Zhao, Q., Chen, L., Yao, X., Zhang, H., Wu, J., & Xie, F. (2020). Effect of drought stress during soybean r2–r6 growth stages on sucrose metabolism in leaf and seed. *International Journal of Molecular Sciences*, *21*(2). <https://doi.org/10.3390/ijms21020618>
- Flower D. J., & Ludlow M. M. (1983). Contribution of osmotic adjustment to dehydration tolerance of water-stressed pigeonpea (*Canjanus cajan* (L.) millsp.) leaves. *Plant, Cell & Environment*, *9*(1), 33–40. <https://doi.org/10.1111/1365-3040.ep11589349>
- Gao, X. B., Guo, C., Li, F. M., Li, M., & He, J. (2020). High soybean yield and drought adaptation being associated with canopy architecture, water uptake, and root traits. *Agronomy*, *10*(4). <https://doi.org/10.3390/AGRONOMY10040608>
- Gilbert, M. E., Zwieniecki, M. A., & Holbrook, N. M. (2011). Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought. *Journal of Experimental Botany*, *62*(8), 2875–2887. <https://doi.org/10.1093/jxb/erq461>

- Gonzalez, A., Ân, I. M., & Ayerbe, L. (1999). *Barley yield in water-stress conditions. The influence of precocity, osmotic adjustment and stomatal conductance.*
- Gunasekera, D., Santakumari, M., Glinka, Z., & Berkowitz, G. A. (1994). *plan ience Wild and cultivated barley genotypes demonstrate varying ability to acclimate to plant water deficits.*
- Hart, C. (2017). *The Economic Evolution of the Soybean Industry* (pp. 1–9).
https://doi.org/10.1007/978-3-319-64198-0_1
- He, J., Du, Y. L., Wang, T., Turner, N. C., Yang, R. P., Jin, Y., Xi, Y., Zhang, C., Cui, T., Fang, X. W., & Li, F. M. (2017). Conserved water use improves the yield performance of soybean (*Glycine max* (L.) Merr.) under drought. *Agricultural Water Management*, 179, 236–245.
<https://doi.org/10.1016/j.agwat.2016.07.008>
- Heatherly, L. G. (1993). Drought Stress and Irrigation Effects on Germination of Harvested Soybean Seed. *Crop Science*, 33(4), 777–781.
<https://doi.org/10.2135/cropsci1993.0011183x003300040029x>
- Hoogenboom, G., Huck, M. G., & Peterson, C. M. (1987). Root Growth Rate of Soybean as Affected by Drought Stress 1 . *Agronomy Journal*, 79(4), 607–614.
<https://doi.org/10.2134/agronj1987.00021962007900040004x>
- Hu, H., & Xiong, L. (2014). Genetic engineering and breeding of drought-resistant crops. In *Annual Review of Plant Biology* (Vol. 65, pp. 715–741). Annual Reviews Inc.
<https://doi.org/10.1146/annurev-arplant-050213-040000>
- Koutroubas, S. D., Papakosta, D. K., & Gagianas, A. A. (1998). The importance of early dry matter and nitrogen accumulation in soybean yield. In *European Journal of Agronomy* (Vol. 9).

- Kumudini, S., Hume, D. J., & Chu, G. (2001). Genetic improvement in short season soybeans: I. Dry matter accumulation, partitioning, and leaf area duration. *Crop Science*, 41(2), 391–398. <https://doi.org/10.2135/cropsci2001.412391x>
- Kunert, K. J., Vorster, B. J., Fenta, B. A., Kibido, T., Dionisio, G., & Foyer, C. H. (2016). Drought stress responses in soybean roots and nodules. In *Frontiers in Plant Science* (Vol. 7, Issue JULY2016). Frontiers Research Foundation. <https://doi.org/10.3389/fpls.2016.01015>
- Kuwayama, Y., Thompson, A., Bernknopf, R., Zaitchik, B., & Vail, P. (2019). Estimating the impact of drought on agriculture using the U.S. Drought Monitor. In *American Journal of Agricultural Economics* (Vol. 101, Issue 1, pp. 193–210). Oxford University Press. <https://doi.org/10.1093/ajae/aay037>
- Lindsey, R., & Dahlman, L. (2023). *Climate Change: Global Temperature* | NOAA Climate.gov. <https://www.climate.gov/news-features/understanding-climate/climate-change-global-temperature>
- Liu, F., Andersen, M. N., Jacobsen, S. E., & Jensen, C. R. (2005). Stomatal control and water use efficiency of soybean (*Glycine max* L. Merr.) during progressive soil drying. *Environmental and Experimental Botany*, 54(1), 33–40. <https://doi.org/10.1016/j.envexpbot.2004.05.002>
- Liu, X., Jin, J., Herbert, S. J., Zhang, Q., & Wang, G. (2005a). Yield components, dry matter, LAI and LAD of soybeans in Northeast China. *Field Crops Research*, 93(1), 85–93. <https://doi.org/10.1016/j.fcr.2004.09.005>
- Liu, X., Jin, J., Herbert, S. J., Zhang, Q., & Wang, G. (2005b). Yield components, dry matter, LAI and LAD of soybeans in Northeast China. *Field Crops Research*, 93(1), 85–93. <https://doi.org/10.1016/j.fcr.2004.09.005>

- Lozovaya, V. V., Lygin, A. V., Ulanov, A. V., Nelson, R. L., Daydé, J., & Widholm, J. M. (2005). Effect of temperature and soil moisture status during seed development on soybean seed isoflavone concentration and composition. *Crop Science*, 45(5), 1934–1940.
<https://doi.org/10.2135/cropsci2004.0567>
- Manavalan, L. P., Guttikonda, S. K., Phan Tran, L. S., & Nguyen, H. T. (2009). Physiological and molecular approaches to improve drought resistance in soybean. In *Plant and Cell Physiology* (Vol. 50, Issue 7, pp. 1260–1276). <https://doi.org/10.1093/pcp/pcp082>
- Medic, J., Atkinson, C., & Hurburgh, C. R. (2014). Current knowledge in soybean composition. In *JAOCS, Journal of the American Oil Chemists' Society* (Vol. 91, Issue 3, pp. 363–384). Springer Verlag. <https://doi.org/10.1007/s11746-013-2407-9>
- Mertz-Henning, L. M., Ferreira, L. C., Henning, F. A., Mandarino, J. M. G., Santos, E. D., Oliveira, M. C. N. D., Nepomuceno, A. L., Farias, J. R. B., & Neumaier, N. (2018). Effect of water deficit-induced at vegetative and reproductive stages on protein and oil content in soybean grains. *Agronomy*, 8(1). <https://doi.org/10.3390/agronomy8010003>
- Mohan Jainn DennissVJohnson Editors, S. (2019). *Advances in Plant Breeding Strategies: Legumes Volume 7*.
- Moinuddin, & Khanna-Chopra, R. (2004). Osmotic adjustment in chickpea in relation to seed yield and yield parameters. *Crop Science*, 44(2), 449–455.
<https://doi.org/10.2135/cropsci2004.4490>
- Morgan, J. M., & Condon Ab. (1986). Water Use, Grain Yield, and Osmoregulation in Wheat*. In *Aust. J. Plant Physiol* (Vol. 13).
- Munns, R. (1988). Why Measure Osmotic Adjustment? In *Aust. J. Plant Physiol* (Vol. 15).

- Mutava, R. N., Prince, S. J. K., Syed, N. H., Song, L., Valliyodan, B., Chen, W., & Nguyen, H. T. (2015). Understanding abiotic stress tolerance mechanisms in soybean: A comparative evaluation of soybean response to drought and flooding stress. *Plant Physiology and Biochemistry*, *86*, 109–120. <https://doi.org/10.1016/j.plaphy.2014.11.010>
- Nakagawa, A. C. S., Itoyama, H., Ariyoshi, Y., Ario, N., Tomita, Y., Kondo, Y., Iwaya-Inoue, M., & Ishibashi, Y. (2018). Drought stress during soybean seed filling affects storage compounds through regulation of lipid and protein metabolism. *Acta Physiologiae Plantarum*, *40*(6). <https://doi.org/10.1007/s11738-018-2683-y>
- Pearce, R. B., Brown, R. H., & Blaser, R. E. (1965). Relationships between Leaf Area Index, Light Interception and Net Photosynthesis in Orchard grass 1 . *Crop Science*, *5*(6), 553–556. <https://doi.org/10.2135/cropsci1965.0011183x000500060021x>
- Qui, L.-J., & Chang, R.-Z. (2010). qiu-lijuan-chang-ruzhen-2010-the-origin-and-history-of-soybean. *The Soybean, Botany, Production and Uses*.
- Raven, J. A. (1995). The early evolution of land plants: Aquatic ancestors and atmospheric interactions. *Botanical Journal of Scotland*, *47*(2), 151–175. <https://doi.org/10.1080/03746609508684827>
- Raven, J. A. (2002). Selection pressures on stomatal evolution. In *New Phytologist* (Vol. 153, Issue 3, pp. 371–386). <https://doi.org/10.1046/j.0028-646X.2001.00334.x>
- Ritchie, H., & Roser, M. (2021). *Soy - Our World in Data*. <https://ourworldindata.org/soy>
- Rotundo, J. L., & Westgate, M. E. (2009). Meta-analysis of environmental effects on soybean seed composition. *Field Crops Research*, *110*(2), 147–156. <https://doi.org/10.1016/j.fcr.2008.07.012>

- Sanders, G. J., & Arndt, S. K. (2012). Osmotic adjustment under drought conditions. In *Plant Responses to Drought Stress: From Morphological to Molecular Features* (Vol. 9783642326530, pp. 199–229). Springer-Verlag Berlin Heidelberg.
https://doi.org/10.1007/978-3-642-32653-0_8
- Sedivy, E. J., Wu, F., & Hanzawa, Y. (2017). Soybean domestication: the origin, genetic architecture and molecular bases. In *New Phytologist* (Vol. 214, Issue 2, pp. 539–553). Blackwell Publishing Ltd. <https://doi.org/10.1111/nph.14418>
- Seversike, T. M., Sermons, S. M., Sinclair, T. R., Carter, T. E., & Rufty, T. W. (2014a). Physiological properties of a drought-resistant wild soybean genotype: Transpiration control with soil drying and expression of root morphology. *Plant and Soil*, 374(1–2), 359–370.
<https://doi.org/10.1007/s11104-013-1757-2>
- Seversike, T. M., Sermons, S. M., Sinclair, T. R., Carter, T. E., & Rufty, T. W. (2014b). Physiological properties of a drought-resistant wild soybean genotype: Transpiration control with soil drying and expression of root morphology. *Plant and Soil*, 374(1–2), 359–370.
<https://doi.org/10.1007/s11104-013-1757-2>
- Shoaib, M., Banerjee, B. P., Hayden, M., & Kant, S. (2022). Roots' Drought Adaptive Traits in Crop Improvement. In *Plants* (Vol. 11, Issue 17). MDPI.
<https://doi.org/10.3390/plants11172256>
- Sinclair, T. R. (2017). *SPRINGER BRIEFS IN ENVIRONMENTAL SCIENCE Water-Conservation Traits to Increase Crop Yields in Water-dee cit Environments Case Studies*.
<http://www.springer.com/series/8868>

- Sinclair, T. R., Messina, C. D., Beatty, A., & Samples, M. (2010). Assessment across the united states of the benefits of altered soybean drought traits. *Agronomy Journal*, *102*(2), 475–482. <https://doi.org/10.2134/agronj2009.0195>
- Sinclair, T. R., Purcell, L. C., King, C. A., Sneller, C. H., Chen, P., & Vadez, V. (2007). Drought tolerance and yield increase of soybean resulting from improved symbiotic N₂ fixation. *Field Crops Research*, *101*(1), 68–71. <https://doi.org/10.1016/j.fcr.2006.09.010>
- Sloane, R. J., Patterson, R. P., & Carter, T. E. (1990). Field Drought Tolerance of a Soybean Plant Introduction. *Crop Science*, *30*(1), 118–123. <https://doi.org/10.2135/cropsci1990.0011183x003000010027x>
- Soybean Highlights from 2022* |. (n.d.). Retrieved April 9, 2023, from <http://soystats.com/2019-highlights/>
- Specht, J. E., Diers, B. W., Nelson, R. L., de Toledo, J. F. F., Torrion, J. A., & Grassini, P. (2015). Soybean. In *Yield Gains in Major U.S. Field Crops* (pp. 311–355). Wiley Blackwell. <https://doi.org/10.2135/cssaspecpub33.c12>
- Subbarao, G. V., Nam, N. H., Singh Chauhan, Y., & Johansen, C. (2000). • JOURNAL OF • PLANT PHYSIOLOGY Osmotic adjustment, water relations and carbohydrate remobilization in pigeonpea under water deficits. In *J. Plant Physiol* (Vol. 157).
- Tangpremsri T., Fukai S. & Fisher K.S (1995) Growth and yield of sorghum lines extracted from a population for differences in osmotic adjustment. *Crop and Pasture Science* *46*, 61-74.
- Turner, N. C. (2018). Turgor maintenance by osmotic adjustment: 40 years of progress. In *Journal of Experimental Botany* (Vol. 69, Issue 13, pp. 3223–3233). Oxford University Press. <https://doi.org/10.1093/jxb/ery181>

- Turner, N. C., & Jones, M. M. (1980). *Turgor Maintenance by Osmotic Adjustments: A Review and Evaluation*. <https://www.researchgate.net/publication/284419782>
- Wells, R. (1991). Soybean Growth Response to Plant Density: Relationships among Canopy Photosynthesis, Leaf Area, and Light Interception. *Crop Science*, *31*(3), 755–761. <https://doi.org/10.2135/cropsci1991.0011183x003100030044x>
- Wells, R., Schulze, L. L., Ashley, D. A., Boerma, H. R., & Brown, R. H. (1982). Cultivar Differences in Canopy Apparent Photosynthesis and Their Relationship to Seed Yield in Soybeans 1. *Crop Science*, *22*(4), 886–890. <https://doi.org/10.2135/cropsci1982.0011183x002200040044x>
- Wijewardana, C., Alsajri, F. A., Irby, J. T., Krutz, L. J., Golden, B., Henry, W. B., Gao, W., & Reddy, K. R. (2019). Physiological assessment of water deficit in soybean using midday leaf water potential and spectral features. *Journal of Plant Interactions*, *14*(1), 533–543. <https://doi.org/10.1080/17429145.2019.1662499>
- Wilson, R. (2004). Improvement, Production, and Uses wilson. In Roger. H. Boerma & James. E. Specht (Eds.), *Soybeans: Improvement, Production, and Uses*. (3rd ed., pp. 621–669). American Society or Agronomy.
- Xiong, R., Liu, S., Considine, M. J., Siddique, K. H. M., Lam, H. M., & Chen, Y. (2021). Root system architecture, physiological and transcriptional traits of soybean (*Glycine max* L.) in response to water deficit: A review. *Physiologia Plantarum*, *172*(2), 405–418. <https://doi.org/10.1111/ppl.13201>
- Yoshida, R., Umezawa, T., Mizoguchi, T., Takahashi, S., Takahashi, F., & Shinozaki, K. (2006). The regulatory domain of SRK2E/OST1/SnRK2.6 interacts with ABI1 and integrates abscisic acid (ABA) and osmotic stress signals controlling stomatal closure in Arabidopsis.

Journal of Biological Chemistry, 281(8), 5310–5318.

<https://doi.org/10.1074/jbc.M509820200>

Zegada-Lizarazu, W., & Monti, A. (2019). Deep root growth, ABA adjustments and root water uptake response to soil water deficit in giant reed. *Annals of Botany*, 124(4), 605–615.

<https://doi.org/10.1093/aob/mcz001>

Zhang, J., Nguyen¹, H. T., & Blum², A. (1999). Genetic analysis of osmotic adjustment in crop plants. In *Journal of Experimental Botany* (Vol. 50, Issue 332).

<https://academic.oup.com/jxb/article/50/332/291/540841>

Zipper, S. C., Qiu, J., & Kucharik, C. J. (2016). Drought effects on US maize and soybean production: Spatiotemporal patterns and historical changes. *Environmental Research Letters*, 11(9).

<https://doi.org/10.1088/1748-9326/11/9/094021>

Chapter 2: Exploration of Drought Tolerance Mechanism in N93-110-6, N11-10295, and PI 471938

2.1 Abstract

Drought has become an increasing concern for soybean growers across the United States and the world due to consistent damage in yield production. Identifying drought tolerance mechanisms in soybeans can allow breeders to breed for more drought tolerant cultivars that can withstand more unpredictable droughts. This study was conducted over the course of two growing seasons (2023 and 2024) evaluating three soybean genotypes, N93-110-6, N11-10295, and PI 471938 and their response to drought, to determine if these genotypes show the predicted drought responses and if so, under what specific conditions.

This field study occurred at the Sandhills Research Station in Jackson Springs North Carolina due to having sandy soil which is ideal for inducing drought conditions. Our results show osmotic adjustment in all three genotypes and a variation in stomatal conductance, photosynthesis, and Φ PSII in response to drought. N11-10295, and PI 471938 have drought tolerance nitrogen fixation and N93-110-6 drought sensitive nitrogen fixation. Leaf area index differed among genotypes under drought conditions. N93-110-6 use drought tolerance mechanism to maintain high yield and oil and protein content. Root depth growth and any one genotype exhibiting limited transpiration under high vapor pressure deficit was not different among genotypes under drought stress conditions.

By advancing our understanding how these three genotypes respond under drought stress, this research contributes to the ongoing goal developing more drought tolerant soybean genotypes and how to conduct future drought research.

2.2 Introduction

Drought is a major constraint on soybean production in the United States and across the world (Sinclair et al., 2008). Drought can induce physiological stress in soybeans limiting the amount of yield and this stress can also have an adverse effect on seed yield quality (Medic et al., 2014; Wijewardana et al., 2019). Drought's impact on soybeans can depend on the growing region, span and severity of drought, and soybean genotype (Purcell & Specht, 2016). To reduce yield loss to drought, researchers are working to develop drought tolerant soybeans that reduce the impact of drought stress on yield.

Three different soybean genotypes PI 471938 (V), N11-10295 (VII), and N93-1106 (VI), were selected based on data from field trials in 2021 and 2022 (Ramanathan et al., unpublished data). During those previous research trials, these three genotypes exhibited some form of drought tolerance that merited further investigation. PI 471938 had high osmotic adjustment occurring in 2021 and intermediate osmotic adjustment in 2022, and previously published research also found that this genotype has improved nitrogen fixation under drought stress conditions (Devi and Sinclair, 2013; Riar et al., 2018). N93-110-6 exhibited osmotic adjustment occurring in 2021 under mild drought conditions; however, under severe drought in 2022 there was no osmotic adjustment occurring suggesting they may be a tipping point in osmotic adjustment. N11-10295 maintained turgor even though the genotype did not exhibit osmotic adjustment, suggesting that N11-10295 is accessing soil water better to maintain turgor.

The objective of this study is to further explore the drought tolerance potential of PI 471938, N11-10295, and N93-110-6 and their response to drought stress and try to determine if and how those drought responses are related to seed composition. We investigated specific drought responses that have previously been observed in soybean. The specific questions we are

asking are: do specific genotypes utilize osmotic adjustment to maintain turgor pressure under drought conditions, do these genotypes exhibit limited transpiration at high vapor pressure deficit (VPD) to conserve soil moisture early in drought, does drought conditions affect root depth among genotypes, does stomatal conductance respond differently among genotypes, and how is photosynthesis affected under drought conditions, do these genotypes have different nitrogen responses to drought, does LAI differ among genotypes under drought, and do these genotypes maintain yield and seed composition under drought?

2.3 Materials and Methods

2.3.1 Genotypes

In 2023, nine genotypes were planted and in 2024 five genotypes were planted, however the study was focused on genotypes, PI 471938, N11-10295, and N93-110-6 (Table 2.1). Seeds for 2023 and 2024 trials were used from the USDA-ARS Soybean and Nitrogen Fixation Research Unit located in Raleigh, North Carolina.

2.3.2 Field Study

2023 and 2024 field studies were conducted at the Sandhills Research Station in Jackson Springs, Montgomery County, North Carolina. The station is operated by a partnership between North Carolina State University and North Carolina Department of Agriculture and Consumer Services. Sandhills Research Station was selected for this study because of the deep sandy, high-porosity soil (Candor sand, loamy) (Soil Survey., 2024) and the resulting ability to induce drought in the absence of irrigation. These two experiments were identical in planting, irrigation, data collection, harvesting, and data analysis. Before planting, nematicide was sprayed on the field for 2023 due to having soybeans planted the year before at a rate of 37.35 L ha⁻¹. Seeds were planted on May 4th, 2023, in field 4A (35°11'02"N 79°40'46"W) and for 2024, seeds were

planted April 30th, 2024, in B5 (35°11'07"N 79°41'28"W) with a an Almaco four-row planter, planting ½ - 1 inches deep with a 38-inch row spacing. For 2023, the plots were designed in a randomized complete split block design consisting of 9 genotypes across 4 replications of 2 different treatments (Figure 2.11). For 2024, the plots were designed in a randomized complete split block design consisting of 5 genotypes across 4 replications of 2 different treatments (Figure 2.12). Less genotypes were planted in 2024 to have more filler rows due to the field location having heavy deer presence. The field for 2024 had a 12-row filler buffer on the South side and an 8-plot filler buffer on the West side of the filed. In both experiments genotypes PI 471938, N11-10295, and N93-110-6 were planted side by side making a 8 rows wide plots, with the other genotypes 4 rows wide per plot. Each plot row was 15-feet long with 4 row borders on each size of the plots, with 8ft wide alleys. In 2023, a single row in plots, 6, 7, 10, 14, 19, and 23 needed to be hand replanted on May 24th, 2023, due to planter malfunction. In 2023, a 3ft tall electrical deer fencing was installed on May 10th, 2023, and in 2024 a 7ft tall electrical deer fencing was installed on May 1st, 2024. The deer fencing was installed around the perimeter of the field to help decrease deer pressure. Irrigation lines were placed in the fields to induce drought on two of the replicates and irrigate the other two replicates. On June 27th, 2023, plots developed yellow leaves and became stunted in growth. Soil sample testing through the North Carolina Department of Agriculture and Consumer Services confirmed it to be nematode damage (Figure 2.13). In early July of 2024, leaves developed some wrinkling due to a pest disease, the field was sprayed with Prevathon/Bifenthrin on July 24th to help with white flies and kudzu bugs. On July 13th, 2023, rainfed and irrigated treatments were initiated, watering only on the irrigated fields replicates Tuesday and Friday mornings. For 2024, rainfed and irrigated treatment started on July 1st, 2024, and only irrigated on Mondays and Thursdays. Wet/dry treatment stopped on

August 16th, 2023, and whole field irrigation started again on August 23rd. On August 27th, 2024, wet/dry treatment stopped and whole field irrigation started that same day. In 2023, early maturing varieties (MG V) were hand harvested in late October, with the remaining genotypes harvested on November 14th and for experiment 2024 early maturing varieties were hand harvested middle of October and the reminding field harvested on October 25.

2.4 Data Collection

2.4.1 Root Growth

For genotypes PI 471938, N11-10295, and N93-110-6, root growth was measured using a minirhizotron system as described by Arnaud et al, (2019). Briefly, the acrylic tubes were inserted into the soil after removing a core with a hydraulic soil probe (Figure 2.14), early in developmental stage VI in 2023 and development stage VE in 2024 to minimize damage to the plants. In each plot, two tubes were installed in the same non-border row, at least 90 cm from the plot ends and at least 200 cm apart. Tubes were installed non-perpendicular to the soil surface, figures 2.11 and 2.12 show the plot location of the tubes for each experiment. Root data for 2023 was collected from June 7th through August 14^{th, 2023}, once a week. In 2024 root data was collected from May 22nd through August 21st, 2024. Measurements were taken at the minirhizotrons 50 cm, 75 cm, 100 cm, 125 cm, 150 cm depths. Three 1.875” photos are taken per depth, creating a 360-degree view of the soil profile. The camera used for the minirhizotron was a Depstech industrial endoscope DS500. Root images were visually analyzed for the presence of roots.

2.4.2 Developmental Stage

Soybean developmental stage was recorded once a week from day of planting to day of harvesting (Table 2.60, 2.61, 2.62, 2.63) Descriptions of the developmental stages were based on the Purdue Extension 2008 Corn and Soybean Field guide as protocol for the lab. During these recordings, any stunting in growth, pests, or leaf yellowing was noted.

2.4.3 Yield

For 2023 and 2024 early maturity plots (MGV) were hand harvested in October and threshed with an Almaco belt thresher, while the remaining plots were harvested with a small-plot combine (ALMACO) on November 14th 2023 and October 25th 2024. Only one of the middle rows of the 8 row plots was harvested per plot. Seeds were dumped into 3 x 1 7/8 x 5 7/8" paper bags. These seeds were then weighed for yield by kg ha⁻¹.

2.4.4 100-Seed Weight

Seed weight was measured by counting 100 random seeds from the harvest bag and weighing them on a scale in grams.

2.4.5 Seed oil and Protein Content

Seeds from the harvest bag were placed into a flexible 1-400mL dish then analyzed by the DA 7250 At-line NIR instrument for dry weight percentage of protein and oil concentration.

2.4.6 Biomass

One row was selected from plots to be hand harvested at a meter in length and dried in a drying barn for two weeks. This row had no leaf samples taken from in through the experiment. The dried sample were then weighed and then ground down into fine material using a Wiley grinder. The samples were then homogenized and three 2ml Eppendorf tubes were filled from each biomass plot for carbon and nitrogen analysis using a ThermoScientific FlashSmart Flash

2000 NCS. Biomass was harvested on August 24th, 2023 and September 6th, 2024, during R6 developmental stage.

2.4.7 Soil Moisture

Two types of soil moisture sensors, HOBOnet T11 soil moisture/temp sensors and EC5 soil moisture smart sensors, were installed in plots that had genotypes PI 471938, N11-10295, and N93-110-6. The sensors were placed in the middle of the 8 row plots at a depth of 61 cm and another other at 30.5. 2023 sensors were installed on May 8th, 2023, and May 2nd, 2024, for 2024. Soil moisture content was recorded every 15 minutes from the first week of planting, to harvesting. On June 14th, 2023, a station tractor damaged the RX station data logger that recorded sensor data in plots 63, 67 71, 82, 83, 87, 90, and 94. These sensors were able to start collecting data again on August 10th, 2023. On July 7th, 2023, sensors in plot 30 and plot 78 were replaced due to sensor malfunctions. For 2024, on May 31st, 2024, a sensor was installed in plot 50. Plot 62 24-inch depth never reconnected to the RX station after initial install day and plot 63 30.5 cm sensor malfunctioned on June 24th and stopped recording data. Plot 15 sensor malfunctioned on June 27th, 2024, and could not readout data.

2.4.8 Weather Data

Daily average relative humidity (%), minimum relative humidity (%), maximum relative humidity (%), total precipitation, minimum and maximum air temperature (F), average air temperature (F), maximum photosynthetically active radiation (micromole/m²s), and average photosynthetically active radiation (W/m²) data was recorded from the North Carolina State Climate Office Cardinal Sandhills Research Station (ECONet).

2.4.9 Leaf Carbon and Nitrogen Content

During the wet/dry treatment, 4 2-cm diameter leaf disks were taken from a single plant's upper most fully expanded leaf and placed into small 2.5 x 4.25-coin envelopes. For 2023 samples were taken on July 18th, 20th, 26th and August 2nd, 9th, 16th. Samples in 2024 were taken on July 3rd, 9th, 17th and August 1st, 23rd, 27th. Leaf envelopes were placed into ovens at 15 degrees Celsius to dry. Leaf samples were placed into 2ml Eppendorf tubes and ground with a Geno / Grinder 2000 SPEX CertiPrep at a rate of 400 strokes/min for 30 seconds. These samples were then weighed and analyzed using a ThermoScientific FlashSmart Flash 2000 NCS.

2.4.10 Leaf Water, Turgor, and Osmotic Potential

Four 2cm disks were taken from the upper most fully expanded leaf from a plant in the middle of the plot and sealed into Wescor C-30 sample chambers within 5 seconds, and then the chambers were placed into a cooler. After returning to the lab, the sample chambers were allowed to acclimate to room temperature for 2-3 hours, and then water potential was measured with a water potential datalogger (PSYPRO, Wescor, Inc.). The psychrometers were then placed into liquid nitrogen for a minute to rupture the cell, then laid on a countertop to return to room temperature, which was around 20- 22 degrees Celsius. After coming to room temperature, the psychrometers were run again, recording the voltage, temperature, and osmotic adjustment. Samples were collected in 2023 on July 18th, 20th, 26th and August 2nd, 9th, 16th and samples for 2024 were taken on July 3rd, 9th, 17th and August 1st, 23rd, 27th.

2.4.11 Leaf Area Index

Leaf Area Index was recorded on July 24th, 31st, and August 7th, 17th, and 23rd in 2023 and on July 2nd, 15th, 22nd, and August 15th, 20th, 26th in 2024 using the LI-COR LAI-2200C Plant Canopy Analyzer. Data collection followed the LAI 6800 manual protocol for row crops. 8

data points were taken between rows on a diagonal transect from the beginning to the end of the row. Each plot had 24 below data points with 3 above ground data points. K-readings were taken at the beginning of each treatment rep and if any cloud diffusion depending on sky conditions. LAI data was run through LI-COR FV2200 software creating individual plot level leaf area index.

2.4.12 LI-COR 6800

A LICOR LI-6800 portable photosynthesis system was used to collect assimilation (A), stomatal conductance (g_{sw}), Φ PSII. Assimilation and stomatal conductance were used to calculate water use efficiency (WUE). Samples were collected in 2023 on July 18th, 20th, 26th and August 2nd, 9th, 16th. Samples in 2024 were taken on July 3rd, 9th, 17th and August 1st, 23rd, 27th. All samples were collected from the upper most fully expanded leaf from a middle row.

2.4.13 Statistical Analysis

Data analysis was carried out using R software (R Core Team, 2021). A three-way analysis of variance (ANOVA) was used to determine if there were statistical differences between the means of independent groups (treatment, genotype, date, genotype \times treatment, genotype \times date, treatment \times date, and genotype \times treatment \times date interaction). If the three-way interaction was not significant but the two-way interactions were among the three factors, then we considered them as a statically significant three-way interaction. lme4 package was used to determine significance of factors and the emmeans package for pairwise comparison. Emtrends was used to compare genotype slopes in soil moisture affecting g_{sw} analysis. A p value < 0.05 was used for significance, however for exploratory research purposes p values < 0.1 were considered for analysis. Due to the nematode damage throughout the field, plots with visual signs of yellowing and stunted growth damage were excluded from the analysis for non-skewed data

for 2023. WUE was calculated as A/g_{sw} . When g_{sw} was very low ($< xx$), WUE values became extremely high values ($> xx$) and were omitted from the analysis as outliers. While checking data for quality before statistical analysis, it was apparent that several psychrometers in 2023 had malfunctioned, producing readings that were not biologically sensible (e.g., turgor pressure < -0.1). These values were considered unreliable and omitted from further analysis. Soil moisture that have malfunction producing reading that were not sensible were omitted from analysis. Strip and rep were included in model to see if they were significant.

2.5 Results

2023 Weather and Field Conditions

From July through August 2023, Sandhills Research Station received 15.77 cm of rain. The average maximum air temperature was 32.95 °C during July and August. The average maximum temperature across 15 years of weather data collected by NOAA (U.S. Climate Normals, 2024) at Jackson Springs, NC was 32.11 °C and an average precipitation of 11.63 cm in July. The average monthly maximum temperature for August over the past 15 years is 31.2 °C and an average precipitation of 11.46 cm (NOAA). With this higher than average temperature and lower than average rain for 2023, it created very hot/dry conditions in the field. The average soil moisture for irrigated plots in July was 0.0932 m³ m⁻³ at 30.5 cm and 0.09705 m³ m⁻³ at 24 inches (Figure 2.5). The average soil moisture in August at 30.5 cm was 0.1210 m³ m⁻³ and 0.1350 m³ m⁻³ at 61 cm (Figure 2.5). The average soil moisture for rainfed plots in July at 30.5 cm was 0.0570 m³ m⁻³ and 0.0705 m³ m⁻³ for 61 cm. The average soil moisture in August for rainfed plots were 0.0514 m³ m⁻³ at 30.5 cm and 0.0692 m³ m⁻³ at 61 cm. Some soil sensors lost connection due to a mechanical incident on June 14th; due to manufacturer repair time, data collection was not resumed until August 10th. Sensors that were affected were in plots 63, 67, 71, 82, 83, 87, 90, and 94.

During 2023, nematode infection affected plant height and function; first symptoms were noted on June 27th, 2023. Soil samples were collected on June 29th and tested by the NC Department of Agriculture, which confirmed that the population of nematodes was high enough to cause severe stress (Figure 2.13). Visually apparent symptoms of nematodes were recorded weekly and used to exclude those plots from the statical analysis to control for biased data. Plots that had no visual signs or symptoms could have experienced nematode damage without

exhibiting obvious symptoms; these plots were not excluded from the analysis but may still impact the results.

2024 Weather and Field Conditions

From July 1st through August 27th, the research station received 30.9118 cm of rain with an average max air temperature of 31.44 °C. A majority of the rain fell in August, (9.14 cm) due to a tropical storm and heavy storms during the beginning of the month. Following heavy rain, measurements were paused until visible signs of water limitation returned in rainfed plots. These signs included manual examination of the top 10 cm of soil for moisture presence and leaf wilting. In July the average soil moisture at 30.5 cm in irrigated plots was 0.20193494 m³ m⁻³ and for 61 cm was 0.24683042 m³ m⁻³, while the average soil moisture at 30.5 cm in rainfed plots was 0.06555812 m³ m⁻³ and 0.08229428 m³ m⁻³ for 61 cm (Figure 2.8) In August the average soil moisture in irrigated plots at 30.5 cm was 0.19822470 m³ m⁻³ and at 61 cm was 0.29357827 m³ m⁻³. For the rainfed plots soil moisture at 30.5 cm was 0.08670577 m³ m⁻³ and at 61 cm was 0.09679829 m³ m⁻³ (Figure 2.8). Some soil moisture sensors malfunctioned during the experiment and could not be repaired, in plots 15 and 3 at both depths, plot 62 at depth 61 cm, and plots 63 and 11 at depth 30.5 cm.

Unlike 2023, the weather conditions in 2024 did not create as severe of a drought as 2023 had. However, weather conditions in 2024 still created enough drought in the rainfed plots that there was a noticeable visual difference among treatments along with biological differences. It was noticed during the beginning of July that the upper most leaves on all plots were having leaf curling and damage. The cause was found to be white flies and/or kudzu bug damage. To help with pests, Prevathon 1169.8 ml ha⁻¹, Bifenthrin 438.8 ml ha⁻¹ and Micro 581 4675 ml ha⁻¹ was sprayed on July 24th.

2023 Leaf gas exchange and chlorophyll fluorescence

Assimilation (A) had a statistically significant two-way interaction of genotype \times treatment ($p = 0.01158$) and treatment \times date ($p = 0.04405$) (Table 2.4) (Figure.2.15). Due to these two interactions, a pairwise comparisons were made between treatments, within genotype and date (Table 2.47). A was significantly reduced in N11-10295 from July 18th through August 16th. N11-10295 was the first genotype to have a significant difference in assimilation rate, on July 18th ($p < 0.0001$). A was reduced in rainfed plots ($p < 0.05$) in N93-110-6 and PI 471938 on July 20th, 26th and August 9th, 16th. On August 2nd, only irrigated N11-10295 is statistically significant ($p < 0.0001$).

Stomatal conductance (g_{sw}) had a statistically significant two-way interaction of genotype \times treatment ($p = 0.02829$) and treatment \times date ($p = 0.01356$) (Table 2.4) (Figure 2.17). Due to these two interactions, treatments were compared within genotype and date (Table 2.48). Drought reduced g_{sw} in N11-10295 on July 18th, 20th, 26th and August 2nd, 9th, 16th. Rainfed PI 471938 g_{sw} was also reduced by drought, on July 18th, 20th and August 16th with p values < 0.05 but was not different ($p > 0.05$) on July 26th, August 2nd and 9th. Drought reduced g_{sw} in N93-110-6 on July 20th, 26th and August 16th ($p < 0.05$) but was not affected on July 18th, August 2nd and 9th.

Φ PSII was affected by the genotype \times treatment \times date interaction ($p < 0.05$) (Table 2.4). Like assimilation and stomatal conductance, Φ PSII for rainfed N11-10295 was reduced due to drought conditions on all sampling days (Table 2.51) (Figure 2.19). Drought lowered Φ PSII in PI 471938 on July 26th, August 9th, and August 16th and in N93-1106 on July 20th, July 26th, August 2nd, and August 16th.

Water use efficiency (WUE) did not have statistically significant interaction effects; however, the irrigated plots had lower water use efficiency than rainfed plots, with an estimate of the difference of $-44.67997 \mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$ and a p value of 0.0478 (Table 2.4 and 2.34) (Figure 2.21).

2024 Leaf gas exchange and chlorophyll fluorescence

A was affected by drought having a two-way interaction between treatment and date that was statistically significant (p value < 0.05) (Table 2.5) (Figure 2.16). Irrigated plots had a greater assimilation rate than rainfed plots on July 9th, 17th, and August 23rd and 27th. Rep was statically significant ($p = 0.01379$) (Table 2.46).

Stomatal conductance (g_{sw}) had statistically significant interactions of genotype \times date ($p = 0.0161$) and treatment \times date ($p < 0.0001$) (Table 2.5) (Figure 2.18). Due to these two interactions, treatments were compared within genotypes and dates (Table 2.49) Rep was statically significant ($p = 0.0001$). Drought reduced g_{sw} in N11-10295 on July 3rd 9th, 17th and August 23rd and 27th (p -value < 0.05). Drought reduced g_{sw} ($p < 0.05$) in N93-110-6 on July 9th, 17th and August 27th. Drought reduced g_{sw} in PI 471938 was statistically significant on July 9th, 17th and August 28th.

Water use efficiency (WUE) had statically significant two-way interactions of genotype \times date ($p = 0.0478$), treatment \times date ($p < 0.0001$), and treatment \times genotype ($p = 0.0090$) (Table 2.5), and rep was significant ($p = 0.00299$). Due to these significant interactions, treatments were compared within genotype and date (Table 2.53) (Figure 2.22). Drought increased WUE in N11-10295 on July 3rd, 9th, 17th, and August 23rd, and 27th. Drought increase WUE in N93-110-6 on July 3rd, 9th, and August 23rd. PI 471938 was statically significant on July 3rd, 9th, and August 23rd with rainfed plots having a greater WUE than PI 471938 irrigated plots.

Φ PSII had statically significant two-way interactions of treatment \times date ($p < 0.0001$) and a significant genotype effect (p value = 0.03132) (Table 2.5) (Figure 2.20). For the treatment \times date interaction, drought reduced Φ PSII rates in rainfed plots on July 9th, 17th and August 23rd and 27th (Table 2.46) Among genotypes, PI 471938 was statistically significant (p value < 0.05), with a greater Φ PSII (0.0172 1-Fs/Fm) than N11-10295 and a greater Φ PSII (0.0157 1-Fs/Fm) than N93-110-6.

2023 Leaf water potential

Water potential was different between irrigated and rainfed treatments (p value = 0.0009270) (Table 2.6). Rainfed plots had 0.5206 MPa lower water potential than the irrigated treatment (Table 2.34) (Figure 2.23).

The Genotype \times Treatment \times Date interaction was significant ($p < 0.05$) for osmotic potential (Table 2.6), and treatments were compared within genotypes and dates (Table 2.50) (Figure 2.25). Drought reduced osmotic potential in irrigated genotypes N93-110-6 and N11-10295 later in the drought treatment. Drought reduced osmotic potential in N11-10295 ($p < 0.05$) from July 20th, July 26, and August 9th but not on July 18th, and August 2nd, and August 16th. Rainfed N93-110-6 had a more negative osmotic potential than irrigated N93-110-6 ($p < 0.05$) only on July 26th and August 9th. Drought only reduced osmotic potential for PI 471938 on July 18th ($p < 0.05$). Turgor pressure was not significantly different among genotypes or treatments (Table 2.6) (Figure 2.27).

2024 Psychrometer

Water potential was affected by drought and had significant interactions between genotype \times treatment ($p = 0.01699$) and treatment \times date ($p < 0.0001$) (Table 2.7). Because of the two interactions, treatments were compared within genotypes and dates (Table 2.52) (Figure

2.24). Rainfed N11-10295 had a more negative water potential on July 9th and 17th and August 23rd and 27th (p value < 0.05). Rainfed N93-110-6 had a significantly lower water potential than irrigated N93-110-6 on July 9th and 17th and August 27th (p value < 0.05). Water potential in rainfed PI 471938 was lower than irrigated PI 471938 on July 9th, 17th, and August 27th (p value < 0.05).

Turgor pressure has a statistically significant three-way interaction with genotype \times treatment \times date (p value < 0.05) (Table 2.7), so treatment was compared within genotype and treatment, and genotypes were compared within treatments and dates (Table 2.52) (Figure 2.28). Drought reduced turgor pressure in N11-10295 turgor pressure on August 23rd and 27th, while drought reduced turgor pressure in N93-110-6 turgor pressure on only July 17th. Drought reduced turgor pressure in PI 471938 on July 17th and August 23rd and 27th. Under rainfed conditions N93-110-6 had a greater turgor pressure (0.397 kPa) but under irrigated conditions PI 471938 had a greater turgor pressure (0.656 kPa).

Osmotic potential was affected by drought and has a statistically significant interaction of genotype \times treatment ($p = 0.056394$) and treatment \times date ($p < 0.0001$) (Table 2.7). Due to genotype and treatment having an interaction along with date and treatment, treatments were compared within genotypes and dates (Table 2.52) (Figure 2.26). Rainfed N11-10295 and N93-110-6 had a lower osmotic potential on July 9th and 17th. Osmotic potential in PI 471938 was not affected by drought conditions.

2023 Stomatal sensitivity to VPD

G_{sw} had a VPD \times Treatment interaction ($p < 0.0001$) (Table 2.8) Genotypes do not have a different response to VPD and treatment. Rainfed plots had a greater slope of 0.3848 mol m⁻² s⁻¹ kPa⁻¹ than irrigated slope (Table 2.34) (Figure 2.29).

2024 Stomatal sensitivity to VPD

G_{sw} had a VPD \times Treatment interaction ($p < 0.0001$) (Table 2.9). Genotypes do not have a different response to VPD and treatment. Rainfed plots had a greater slope of $0.04176 \text{ m}^{-2} \text{ s}^{-1} \text{ kPa}^{-1}$ than irrigated slope (Table 2.36) (Figure 2.30).

2023 Stomatal sensitivity to soil moisture

Only genotypes N93-110-6 and N11-10295 were used in this analysis due to missing sensor data for PI 471938. Soil moisture at 30.5 cm or 61 cm did not affect stomatal conductance differently between genotypes ($p > 0.05$) (Table 2.10, 2.11)

2024 Stomatal sensitivity to soil moisture

Soil moisture at 30.5 cm affected stomatal conductance differently among genotypes ($p < 0.0001$) (Table 2.12, 2.13). G_{sw} in N11-10295 and PI 471938 was more sensitive to soil moisture at 30.5 cm than N93-110-6. G_{sw} was also affected by soil moisture at 61 cm differently among genotypes ($p = 0.01149$). G_{sw} was the most sensitive to soil moisture at 61 cm in PI 471938 (Table 2.44).

2023 Root depth

Root depth only varied with date, not with genotype or treatment or any interactions ($p < 0.0001$) (Table 2.14). From July 7th through the last sampling day, August 10th root depth increased among all genotypes regardless of treatment (Table 2.37) (Figure 2.31).

2024 Root depth

Root depth had a significant interaction between treatments and dates ($p = 0.0085$) (Table 2.15). Root depth was greater in rainfed plots than in irrigated plots, although irrigation was not withheld from rainfed plots until July 1st (Table 2.38, 2.46) (Figure 2.32).

2023 Leaf Area Index

Rainfed plots had lower leaf area index (LAI) due to leaf senescence caused by extended, severe drought. LAI had a significant treatment \times date interaction (p value = 0.033668) (Table 2.16). Treatments were compared within dates (Table 2.45) (Figure 2.33). This decrease in LAI in rainfed conditions over time follow the decrease in precipitation over the weeks of the experiment. On August 7th irrigated plots' LAI was higher (0.8808 m³ m³) than rainfed plots.

2024 Leaf Area Index

LAI in 2024 was similar to 2023 data with a significant treatment \times date interaction (p value < 0.0001) and genotype \times treatment interaction (p -value 0.094) (Table 2.17). Although this was slightly above the 0.05 threshold, for exploratory research purposes, treatments were compared within genotype (Table 2.54) (Figure 2.34). Drought reduced LAI in N11-10295 on July 22nd, 31st, and August 15th and 26th. Drought reduced LAI in N39-110-6 on July 31st and August 15th and 20th. Drought reduced LAI in PI 471938 on July 22nd, July 31st, and August 15th and 20th. PI 471938 had a greater LAI than N11-10295 and N93-110-6 under both rainfed and irrigated conditions. No genotypes and treatment were statistically significant on July 2nd and July 15th.

2023 Harvest data

Leaf % carbon and nitrogen content both had significant treatment \times date interactions (p value < 0.005) (Table 2.22). A two way pairwise comparison was analyzed for both (Table 2.45) (Figure 2.35). Drought reduced leaf nitrogen concentration on July 20th and on July 26th. Drought reduced leaf carbon concentration only on July 26th.

Total canopy nitrogen had a p -value 0.074 (Table 2.24) for treatment \times genotype. Although this was slightly above the 0.05 threshold, for exploratory research purposes,

treatments were compared within genotype (Table 2.41) (Figure 2.37). However, none of the treatment effects were significant within genotypes.

Drought significantly reduced biomass ($p = 0.04935$), with irrigated plots having 266.0 g m⁻² greater biomass than rainfed plots (Table 2.18, 2.34, 2.58) (Figure 2.39). Drought did not affect biomass differently among genotypes ($p > 0.05$) (Table 2.18).

Treatment was significant for the yield in ANOVA ($p = 0.007197$; Table 2.28), but the treatment effect did not vary among genotypes. Irrigated genotypes produced 806 kg ha⁻¹ and rainfed produced 234 kg ha⁻¹ (Table 2.34) (Figure 2.41). Drought reduced yield by 572 kg ha⁻¹ (Table 2.58).

There was a significant genotype \times treatment interaction ($p = 0.05988$) for the seed weight among genotypes and treatments (Table 2.30). Rainfed N11-10295 had 1.8667 kg ha⁻¹ more weight than irrigated plots (Table 2.40, 2.58) (Figure 2.43). Seed oil and protein content were not different among genotypes and treatments (Table 2.32, 2.58) (Figure 2.45).

2024 Harvest data

In the leaf % nitrogen concentration analysis, genotype \times date and treatment \times date was significant (Table 2.23). A pairwise comparison of treatment, genotype, and date was analyzed (Table 2.55) (Figure 2.36). Leaf nitrogen concentration for PI 471938 was greater in rainfed plots than irrigated on July 17th, and August 23rd and August 27th. Both N11-10295 and N93-110-6 %N concentration in rainfed plots was only greater than irrigated on August 27th.

Total canopy nitrogen had a significant genotype \times treatment interaction ($p = 0.0231$) (Table 2.25). The random effect of rep had a significant p value of 0.0005. Treatments were compared within genotypes. A two way pairwise comparison was analyzed (Table 2.42) (Figure 2.38). Pairwise data was not significant.

Leaf nitrogen g/cm² content has a statically significant three-way interaction among genotype, treatment, and date (p value < 0.05) (Table 2.26). Irrigated genotypes N93-110-6 and PI 471938 had a greater Nitrogen per leaf area than their rainfed companions on August 23rd and irrigated N11-10295 was statically significant on August 27th ($p = 0.0006$) with irrigated plots having a greater nitrogen per leaf area than rainfed plots (Table 2.56) (Figure 2.47).

Photosynthetic nitrogen-use efficiency (PNUE) had a significant treatment \times date and genotype \times date interactions (p value < 0.05) (Table 2.27). Genotypes were compared within treatments and dates (Table 2.57) (Figure 2.48). Drought increased PNUE in N11-10295 and PI 471938 on July 9th 17th and August 23rd and 27th, while drought increased PNUE in N93-110-6 on July 9th 17th and August 27th.

Drought affected biomass differently among genotypes (p value = 0.02135) (Table 2.19, 2.59), but pairwise tests did not indicate which genotype(s) responded differently from others (Table 2.42) (Figure 2.40).

Yield varied among genotypes ($p = 0.0004$) (Table 2.29). N11-10295 had a greater yield over genotype N93-110-6 and PI 471938. N93-110-6 was greater in yield than PI 471938 (Table 2.39) (Figure 2.42). Drought did not affect yield in 2024. Seed weight had a significant genotype and treatment effect (Table 2.35, 2.39, 2.59) (Figure 2.44). Seed protein was not affected by drought; however, there was a significant genotype effect ($p = 0.0027$) (Table 2.33). Seed protein differed among genotypes with $p < 0.0001$, with N93-110-6 having higher protein concentration than PI 471938 (Table 2.39) (Figure 2.46). Seed Oil also differed among genotype (Table 2.33), and PI 471938 had higher seed oil than N93-110-6 and N11-10295 (Table 2.39) (Figure 2.46).

2.6 Discussion Experiment 2023 and 2024:

The objective of this study is to explore whether genotypes PI 471938, N11-10295, and N93-11-06 show predicted drought response patterns in the field, and if so, under what specific sets of environmental conditions. This research aims to enhance our understanding of how drought affects PI 471938, N11-10295, and N93-11-06 to contribute to improving soybean productivity under drought conditions. Analyzing the data from 2023, there is a clear indication that drought significantly affected the rainfed genotypes compared to the irrigated genotypes. From July through August drought conditions became extreme in rainfed plots, but drought stress was seen in irrigated plots as well. This was due to high temperatures and low precipitation throughout data collection. For 2024, drought significantly affected rainfed genotypes. Compared to the year prior, 2024 rainfed treatment experienced a more moderate drought than in 2023, this was due to heavy precipitation throughout the end of July and beginning of August, but weather conditions began to dry during the middle and end of August.

2023 and 2024 G_{sw} and A

Stomatal conductance was affected by drought differently among genotypes throughout the experiment. N93-110-6 was the only genotype to make it 7 days past the initial irrigation withholding with no stomatal closure, as PI 471938 and N11-10295 both saw a decrease in g_{sw} 5 after drought initiation. Because of this reduction in g_{sw} for N93-110-6 rainfed plots, A was also affected by drought. N93-110-6 experienced a delay in drought affecting A until July 20th, which is also 7 days after drought treatment started. Rainfed N93-110-6 A was decreased on July 20th, 26th and August 9th and 16th. Although there was not a significant difference in A between irrigated and rainfed plots on August 2nd this is likely due to irrigated plots also experiencing stress during a very hot period with no precipitation, rather than stress resilience in the rainfed

plots. Like A , Φ PSII had a delayed treatment response in N93-110-6 relative to the other genotype N11-10295, and like g_{sw} and A , Φ PSII was not different between treatments on August 9th, when irrigated plots also appeared to experience stress. Despite measuring differences in g_{sw} and A , we could not measure a change in WUE. We speculate that because variation in measurements of both A and g_{sw} compounded in calculations of WUE, differences would have to be very large to be statistically resolved.

The delay in g_{sw} , A , and Φ PSII reduction suggests that N93-110-6 has a greater tolerance to drought stress than the other genotypes, particularly compared with N11-10295. N93-110-6's parental lines are Young and PI 416937. Past research shows that PI 416937's slow wilting phenotype is related to its ability to limit transpiration trait at high VPD (Fletcher et al., 2007). Having reduced transpiration rates in drought conditions would allow soybeans to conserve soil water early in the season, helping to maximize yield under drought conditions (Sinclair et al., 2010).

In N11-10295, drought reduced g_{sw} early in the experiment, only 5 days after drought treatment was initiated, and g_{sw} for rainfed N11-10295 was lower than in irrigated plots on all sampling days. This decrease in g_{sw} due to drought led to lower A early in the drought treatment. This genotype was the first to have a reduction in photosynthesis and in Φ PSII, 5 days after drought initiation, making it the most sensitive to drought in 2023. In Sinclair et al. 2010, early stomata closure in the beginning stages of soil drying could allow the soybean plant to save water to use later in more severe drought stages, but at a potential loss of growth (Sinclair et al., 2010). N11-10295's parents are N01-11298 x N04-9646, and PI 416937 is 12.5% of its lineage. Parent N01-11298 is a slow wilting genotype similar to PI 416937; however, g_{sw} , A , and Φ PSII

data suggests that N11-10295 did not inherit the drought tolerance traits that would be expected from its ancestry.

Drought reduced g_{sw} in PI 471938 early in the experiment, 5 days after drought initiation. Rainfed PI 471938 g_{sw} was lower on July 20th and August 16th. Due to severe drought affecting both irrigated and rainfed plots, g_{sw} were not significantly different between the treatments from July 26th through August 9th. The research station received 0.762 cm of rain on August 10th. Despite being minimal, this precipitation with irrigation was enough for g_{sw} in irrigated PI 471938 plots to become higher than rainfed plots again. Even though g_{sw} was reduced earlier than *A* in rainfed conditions for PI 471938, it suggests that WUE may have improved, even though we could not resolve a $G \times T$ interaction due to the amplified variation in the WUE data. Looking at the average daily soil moisture content for PI 471938 under drought conditions, we see soil moisture at 30.5 cm in PI 471938 plots was greater than other genotypes, but it declined faster than N11-10295. At 61 cm for PI 471938 there was a steady decline in soil moisture. PI 471938 Φ_{PSII} had an even longer delay physiological response to drought compared to the other genotypes. Φ_{PSII} was not affected by drought until 13 days after drought initiation. This is a more delayed response than *A*, suggesting that the electron transport chain is more tolerant to drought than *A*. *A* likely became CO_2 -limited by reduced g_{sw} before there was damage to the molecular machinery of the light reactions. On all but one measurement day, PI 471938 had a higher g_{sw} rate than N11-10295 and N93-110-6 in irrigated conditions. The g_{sw} , *A*, Φ_{PSII} , WUE data suggests PI 471938 is a more drought tolerant genotype than the other two in this experiment but does not limited transpiration at high VPD more so than the other genotypes. In a field experiment with PI 471938, Sadok et al. (2012) did not find a significant restriction in g_{sw} at a threshold VPD for maximum transpiration rate.

Drought affected g_{sw} during the 2024 experiment, similar to 2023, A and Φ PSII did not respond differently to drought among the three genotypes. A in rainfed plots were affected by drought throughout the experiment on July 9th, 17th and August 23rd and 27th. Treatments did not differ in A two days after the treatment began, indicating that soil moisture in the rainfed plots had not fallen below a critical threshold, and 10.06 cm of rain between July 17th and August 1st also prevented a significant reduction in A in rainfed plots. Φ PSII responded similarly to A . These results, alongside weather data, indicate that drought conditions were more moderate in 2024 than in 2023, and the plants were not as severely stressed. In these more moderately stressful conditions, the genotypes' responses to drought did not differ.

For N93-110-6, drought did not affect g_{sw} until eight days after treatment initiation. This delay in g_{sw} reduction is similar to this genotypes' response in 2023. After 19.6 cm of rain in early- through mid-August, g_{sw} was again maintained in rainfed plots until August 27th. N93-110-6 had an early improvement in WUE on July 3rd, but not as much as genotypes PI 471938. On August 1st irrigated N93-110-6 plots had a greater WUE over rainfed. Demicheli et al. (2023), saw that drought-tolerant soybeans had a high WUE due to stomatal closure. Looking at the soil moisture data for rainfed plots N93-110-6, soil moisture at 30.5 and 61 cm in N93-110-2 rainfed plots at 30.5 and 61 cm decreased slowly from July 1st to July 9th, when g_{sw} decreased. This genotype maintained soil moisture at 30.5 cm longer than N11-10295 and maintained higher soil moisture at 61 cm than N11-10295 and PI 471938.

For N11-10295 drought caused g_{sw} to be reduced only two days after drought treatment was initiated. Similar to 2023's data, N11-10295 was the most sensitive genotype for g_{sw} . After rain events later in July and early August, it was the only genotype that did not exhibit recovery on the subsequent measurement date. WUE for N11-10295 was lower on July 3rd compared to

the other two genotypes. After rain events N11-10295 had a very high WUE compared to N93-110-6 and PI 471938. Although g_{sw} was drought-sensitive, this higher WUE may provide N11-10295 with some drought resilience. In rainfed N11-10295 plots, soil moisture at 30.5 cm decreased more rapidly from July 1st to the 9th, compared to N93-110-6 and PI 471938, but N11-10295 maintained higher soil moisture content at 61 cm than PI 471938.

For PI 471938, drought did not affect g_{sw} until eight days after drought treatment was initiated on July 1st. This delay in g_{sw} reduction was not similar to PI 471938's 2023 g_{sw} having a delay five days after irrigation was withheld. We can see this delay in g_{sw} dropping again due to drought on August 27th, which was nine days after the field received 19.5834 cm of rain from August 3rd through August 18th. Changes in g_{sw} were not reflected in A or Φ_{PSII} ; as a consequence, PI 471938 had the highest improvement in WUE early in the rainfed treatment. PI 471938 due to rain permitting the recovery of g_{sw} . WUE in PI 471938 was also not significantly different on August 27th, due to irrigated plots being affected by drought conditions. This genotype maintained a higher soil moisture at 30.5 cm than N11-10295 and N93-110-6 but had the lowest soil moisture at 61 cm.

There are other factors that could have control stomatal conductance in our research study. Water potential and ABA can have major effects on g_s . ABA is a drought stress hormone that reduces the stomatal aperture and regulates leaf water status (Brodribb et al., 2020; Brodribb & McAdam, 2017). ABA is synthesized in the root tips and transported to the shoot, and there also have been studies that show leaves are a site for ABA biosynthesis under drought stress conditions (Cardoso et al., 2020; Manzi et al., 2015; McAdam et al., 2016; F. P. Zhang et al., 2018). Stomatal closure in this experiment may be, in part, an ABA response under drought stress conditions. Stomatal sensitivity to ABA or the sensitivity of ABA production to soil

moisture could vary among genotypes. Water potential is another factor that can cause stomata to closure under drought stress conditions. Water potential can be lowered to maintain turgor pressure, keeping stomata open with turgor pressure.

2023 and 2024 Osmotic, Water, and Turgor Potential

From July 13th through August 16th the research station received 1.8034 cm of rain, and rainfed plots in figure 2.5 had a lower soil moisture content than irrigated plots at both depths. Osmotic potential was lower in rainfed plots, and the change varied among genotypes.

Only 5 days irrigation was withheld, rainfed PI 471938 had a more negative osmotic potential than irrigated PI 471938, by -0.4525 MPa. Rainfed PI 471938 was the first to respond to drought stress conditions with osmotic adjustment, on July 18th, followed by N11-10295 and then N93-110-6. G_{sw} was lower on July 18th; however, other factors of plant function could have been partially closing stomatal aperture. Water potential was lowered, and turgor pressure was maintained, stomata were partially closed causing a reduction in PI 471938 g_{sw} . With this reduction in g_{sw} , PI 471938's photosynthesis was reduced on July 18th. PI 471938 did not exhibit osmotic adjustment at later sampling dates which may have indicated that both treatments were experiencing stress in the extreme drought conditions, or that the plants stopped osmotic adjustment as conditions worsened.

In 2024 drought did not reduce PI 471938's osmotic potential on any measurement dates in 2024. A growth chamber experiment found that PI 471938's relative drought tolerance was not due to greater osmotic adjustment during drought (Bagherzadi et al., 2017). In contrast, in a previous experiment (Ramanathan et al., unpublished) we observed high osmotic adjustment in PI 471938 under moderate drought, and intermediate osmotic adjustment under severe drought conditions. The variation in osmotic potential responses among studies and between years in this

experiment suggest that PI 471938 may need very specific conditions to initiate osmotic adjustment as a drought tolerance mechanism.

In the absence of osmotic adjustment in PI 471938 in 2024, turgor pressure was reduced, beginning 16 days after withholding irrigation. G_{sw} was lower on July 9th, so partial stomatal closure likely helped maintain water potential, at the cost of lower photosynthesis. After multiple heavy rain events later in 2024, water potential was no longer lower in the rainfed plots.

In 2023, drought reduced osmotic potential in N11-10295 on most dates. On days that N11-10295 had lower osmotic potential, g_{sw} was also reduced, water potential and turgor was maintained. As N11-10295 became more stressed, it exhibited more osmotic adjustment. A small rain event on August 10th and 14th could help explain the lack of osmotic adjustment on August 16th. On August 2nd osmotic potential was not significantly different between treatments, which could be related to either osmotic adjustment in irrigated both stressed plots, or related to the average maximum air temperature becoming lower from August 1st through August 4th which would reduce transpiration demand.

In 2024, drought caused N11-10295 to reduce osmotic potential on July 9th and 17th. Later in the experiment, osmotic potential in N11-10295 was not different between treatments, probably due to the field receiving significant precipitation later in July and early August. In 2023, N11-10295 lowered osmotic potential only 7 days after drought initiation and continued to osmotically adjust as drought stress continued. Water potential was also reduced on July 9th and 17th and August 23rd and 27th. With osmotic potential lowering along with water potential, turgor pressure was maintained on July 9th and 17th. It was not until August 23rd and 27th that turgor pressure was reduced. N11-10295 was able to maintain turgor pressure in its leaves on July 9th and 17th due to osmotic adjustment. On July 9th when water potential was lowered, g_{sw} was also

lowered, turgor pressure was not lowered which helped keep partial stomatal closure. As rainfed N11-10295 received rain, it was able to recover, increasing g_{sw} due to greater turgor recovery causing water potential not to lower. N11-10295 has PI 416937 as an antecedent, a slow wilting genotype that has been shown to maintain higher osmotic adjustment and low water potential (Sloane et al., 1990). N11-10295 could have inherited PI 416937's drought tolerance characteristic of maintaining turgor pressure from the slow wilting through osmotic adjustment.

In 2023, rainfed N93-110-6 osmotic potential was affected by drought on July 26th and August 9th. N93-110-6 initiates osmotic adjustment as a drought tolerance mechanism 13 days after drought initiation. A severe drought was happening in the rainfed plots, driving the genotypes to have a more negative osmotic potential to move the solutes into cells to drive water intake from the soil (Turner, 2017, 2018). Water potential was lower in rainfed plots but no $G \times T$ interaction was significant, and turgor was not affected. On the dates that osmotic potential was significant for N93-110-6 g_{sw} were lower and had a lower water potential. N93-110-6 could be osmotically adjusting later as drought becomes more severe due to being very drought tolerant.

We saw in the g_{sw} , and A data that there was a delay to drought. g_{sw} and A did not see a reduction until July 20th and during this time water potential was lower and turgor pressure was maintained. It was then we saw osmotic adjustment occurring on July 26th. Similar to PI 471938 and N11-10295, on August 16th we no longer see osmotic potential significant in N93-110-6 due to irrigated plots potentially osmotically adjusting and/or the small rain event happening on August 10th and 14th. Similar, August 2nd osmotic potential was not significant, this could have been due to irrigated plots osmotically adjusting or due to the average max air temperature becoming lower from August 1st through August 4th.

In 2024 drought affected N93-110-6's osmotic potential due to the genotype osmotically adjusting on July 9th and 17th. There was a delay of 8 days after drought initiation until the genotype osmotically adjusted in response to drought. N93-110-6 had a faster response in initiating osmotic adjustment in 2024 compared to 2023, where we did not see the genotype osmotically adjusting until 13 days after drought initiation. The difference in response time between the two years could be due to 2024 being a moderate drought and 2023 under a severe drought. N93-110-6's osmotic potential was not significant after July 17th due to the field received 10.06 cm of rain, between July 18th to August 1st. This amount of rain does not require the plant to lower osmotic potential. From the August 1st sampling day to the 23rd sampling day the field received 23.22 cm of rain which again caused the plants not to use osmotic adjustment to maintain turgor due to ample soil water.

In 2023, water potential was only significant among treatment, but in 2024 we saw water potential had a genotype treatment interaction. With that we can see under moderate drought stress all three genotypes did not lower water potential until 8 days after drought initiation. In 2024, N93-110-6 responded sooner with osmotic adjustment to drought than in 2023. PI 471938 was osmotically adjusting early on in 2023, but under moderate drought conditions in 2024 it was not seed osmotically adjusting. N11-10295 behaved very similar in both years under two different drought conditions.

2023 and 2024 VPD

VPD is one of the driver's stomatal closures (McAdam & Brodribb, 2015) and keeping the stomata open is important in maintaining photosynthesis to achieve high yield under drought conditions. We investigated if one or more genotypes have "limited transpiration at high VPD" (vapor pressure deficit) to help increase soil moisture under drought conditions and have a greater stomatal conductance longer or high. In 2023 and 2024 there is no evidence that shows a genotype has more limited transpiration at high VPD than the other genotypes. The significant treatment \times VPD leaf interaction shows that drought makes genotypes more sensitive to VPD. Sadok et al. 2012, observed in their greenhouse study that PI 471938 did not exhibit limited transpiration rate with an increasing VPD and did not maintain high leaf water potential as VPD increased due to lack of stomatal closure. This was also observed in Bagherzadi et al. (2017), with rising VPD PI 471938 respiration rates continued to increase. Our data supports the same conclusion as the research states above that PI 471938 did not exhibit limited transpiration rates under high VPD. Soybeans that have a low transpiration rate can see a reduction in water with a reduction in water use due to having higher efficiencies without affecting yield (K. Kunert & Vorster, 2020). Doing so will maintain soil moisture, allowing soybeans to function longer under drought conditions due to soil moisture reserves.

2023 and 2024 Total canopy nitrogen, leaf %N, and above ground biomass

Nitrogen fixation is sensitive to soil drying under drought conditions. Drought can cause a decrease in N_2 in aboveground biomass (Purcell & King, 1996). We would expect that a genotype with drought-tolerant nitrogen fixation may have more aboveground nitrogen after drought and might be better able to maintain tissue nitrogen concentration during drought. Although there was a significant genotype treatment interaction for total canopy nitrogen in both

years, pairwise comparisons of treatments for each genotype could not resolve whether any genotype maintained nitrogen concentration in drought better than the others.

In 2024, % N leaf disk concentration was affected by drought across genotypes and dates. PI 471938 had a greater nitrogen concentration in rainfed leaves later in drought conditions. In M. J. Devi & Sinclair. (2013), a drought greenhouse experiment to see the tolerance between genotypes Benning and PI 471938, PI 471938 maintained a relatively high nitrogen fixation activity as soil dried. This greater nitrogen concentration over the course of the drought over other genotypes supports M. J. Devi & Sinclair (2013) data that PI 471938 nitrogen fixation is tolerant under drought conditions. Compared to 2023, we only saw a treatment \times date interaction which could have been due to severe drought conditions. However, nitrogen uptake in a plant is based on multiple factors one of which is soil nitrogen uptake.

N93-110-6 and N11-10295 were sensitive to drought with only rainfed plots only having a greater % nitrogen concentration on August 27th, which was right after a recovery in rain. High sensitivity to of nitrogen fixation in soybeans can result in constraint on nitrogen accumulation and yield potential (M. J. Devi & Sinclair, 2013; Sinclair et al., 1987).

Leaf disks collected throughout the treatment show that drought affected leaf nitrogen concentration on a leaf area basis. Drought did not affect leaf nitrogen among genotypes until August 23rd, when rainfed N93-110-6 was lower than irrigated and rainfed PI 471938 was lower than irrigated plots (Table #). However, g_{sw} was not affected on the 23rd for these two genotypes, even though nitrogen concentration was reduced. N11-10295 maintained leaf nitrogen concentration than the other two genotypes on the last day of sampling, August 27th, with rainfed N11-10295 less than irrigated. G_{sw} was lower for N11-10295 on the 27th along with nitrogen concentration. Among rainfed plots genotype PI 471938 maintained the highest leaf nitrogen

concentration. In a soybean yield simulation by Sinclair et al. (2010), showed the sensitivity of N_2 fixation to drought, caused a loss in yield in all locations throughout the United States. This simulation above showed that out of the five traits simulated in the model, enhancing drought tolerance for nitrogen fixation was the most beneficial for yield (Sinclair et al., 2010). In the context of this study PI 471938 had evidence of nitrogen drought tolerance to soil drying but N11 had a greater tolerance to soil drying with not seeing nitrogen content affected after PI 471938 was (M. J. Devi & Sinclair, 2013).

Looking at 2024's aboveground biomass $g\ m^{-2}$, we see that it follows the same changes as the leaf disk nitrogen and biomass %N with PI 471938 and N11-10295 having that lowest difference in biomass among treatments and N93-110-6 having the greatest difference. Nitrogen fixation under drought in soybeans can affect the amount of biomass production from the plant, which is why we see the same changes in canopy nitrogen and biomass (Sinclair et al., 1987). However, if there is more nitrogen in the biomass, more nitrogen uptake needs to occur, creating a feedback loop which can make the data hard to interpret. For 2023, above ground biomass in rainfed plots were lower. Even though we cannot see a genotype \times treatment interaction, total canopy nitrogen and biomass was affected by drought, with the possibility of drought stressed nitrogen fixation.

Reduction in biomass can also be due to other physiological factors that are being affected by drought like stomata conductance to decrease water loss, reducing normal plant functions, thus reducing growth (Bharath et al., 2021; Brodribb et al., 2020; Brodribb & McAdam, 2017). Stomatal conductance which influences photosynthesis and transpiration can end up correlating with biomass reduction over large amounts of data. In 2023 and 2024, g_{sw} in irrigated plots was greater over rainfed plots as well as assimilation rates. Transpiration is part

of a feedback loop that affects ureide concentration. Low transpiration causes an increase in petiole ureides, this increase can be an indicator of nitrogen fixation during drought (De Silva et al., 1996; M. J. Devi & Sinclair, 2013).

2024 Photosynthetic Nitrogen-Use Efficiency

Photosynthetic nitrogen-use efficiency (PNUE) was affected by drought among genotypes throughout the experiment. All three genotypes maintained PNUE for 8 days until drought caused PNUE to lower on July 9th. All three genotypes saw similar results of lower PNUE except for N93-110-6 on August 23rd. On this day PI 417938 and N11-10295 saw a decrease in PNUE but N93-110-6 PNUE was not affected by drought and g_{sw} was not reduced. This sampling day was after the long stretch of rain, suggesting that N93-110-6 had a delay in drought affecting PNUE after drought occurred. It could also be that this genotype had a greater recovery than the other two genotypes. PI 417938 saw a reduction in PNUE on August 23rd, but g_{sw} was maintained, suggesting that nitrogen concentration in the leaves due to drought, affected PNUE.

2023 and 2024 Minirhizotron

With the minirhizotron, we were testing to see if genotypes had deeper rooting qualities in response to drought conditions. In both years we did not see a difference in genotype affected by drought. In 2024, the data does show rainfed plots had longer roots in May and June, however drought treatment did not start until July 1st, so we cannot say that difference was due to drought. A soybean field experiment by Gray et al. (2016), found soybean root length density was greater under elevated CO₂ but the greater root length density occurred in the shallow to mid-depth dry soils. In Sinclair et al., (2010), a simulation model on drought across the US over 50 years of weather data investigated if rooting rate increased, would it result in a rapid exploration for soil

water. They found that with a faster root growth rate there was an increase in yield < 0.3 depending on growing location within the United States but overall was not a beneficial trait. We did not see either of these results in our research study.

2023 and 2024 Leaf Area Index (LAI)

Drought did not have an affected among genotypes, only a treatment \times date interaction. Drought only reduced LAI on August 7th, which was in the middle of peak severe drought conditions. These results could be due to the severity of the drought during 2023 which affected all plots. Both irrigated and rainfed plots could both be seeing a decrease in LAI, and that is why we only see August 7th significant. The field also received 0.5842 cm of rain from August 4th through the 7th, which could have caused irrigated to have a greater LAI on the 7th just enough to become significant. These results could be caused by the decrease in photosynthesis due to the decrease in g_{sw} in both treatments (Sinclair et al., 2007b). Also, g_{sw} and A are single leaf measurements which might not reflect whole plant function at that time, whereas LAI is measuring whole plant. Biomass also correlates to the LAI data with not differences among genotypes only treatment. Irrigated had a greater biomass $g\ m^{-2}$ than rainfed, even though LAI was only significant on one day, it could be irrigated plots had a greater leaf density.

2024 LAI was affected by drought among genotypes, which is different from 2023. This once again can be due to the severe vs moderate drought conditions. Drought reduced N11-10295 and PI 471938 LAI first on July 22nd, where N93-110-6's LAI did not reduce until July 31st. N93-110-6 could be seeing a delay in drought affecting LAI similar to its g_{sw} rate having a delay in drought. LAI for N11-10295 was not affected by drought on August 20th. The field receive 19.2 cm of precipitation from August 3rd through the 13th. Looking at the estimated marginal means rainfed N11-10295 was 4.28 while irrigated was 5.11, these two treatments were

close in means and that much rain gave rainfed plots just enough to meet irrigated LAI. Even though N93-110-6 was the last genotype to see a reduction in LAI and had the smallest difference in LAI among treatments during the middle and end of August, it had the largest difference in biomass weight between irrigated and rainfed conditions. PI 471938 had the largest difference in LAI among treatments from the middle to the end of August but had the smallest biomass difference among treatments. It could be that PI 471938 is affected by drought for LAI but can maintain a greater leaf density and that is why biomass has the smallest difference among treatments. In Sinclair et al. (2010), a soybean model simulation showed that slowing leaf area development could limit water use early in development. For 2024 LAI was lower in rainfed plots and had a lower soil moisture. Soil moisture gradually reduced under all rainfed genotype along with soil moisture lowering. When LAI became significant on July 22nd soil moisture increased significantly in rainfed due to rain event.

2023 and 2024 Yield, Seed Weight, and Oil and Protein Content

In 2023, drought did not affect yield among genotypes, yield was only affected by treatments. From July 13th through August 16th, 2023, the field location received 1.8034 cm of rain. Looking at figure 2.5, irrigated 30.5 and 61 cm depths were not significantly greater than the rainfed 30.5 and 61 cm depths in moisture content during July and August. Drought conditions and high temperatures became so severe during the 2023 experiment that irrigated plots exhibited symptoms of stress during the end of the experimental treatment period. Severe drought stress can cause stunted growth, reduced flowering, pod abortion (Dietz et al., 2021; Jongdee et al., 1991.) and combined with the nematodes can increase that stress. Decrease in *A* among rainfed genotypes, can lead to a decrease in yield due to lack of assimilates created for seed and pod development. This decrease in *A* can be attributed to a decrease in stomatal

conductance (g_{sw}) (Brodribb & Holbrook, 2003; Brodribb & McAdam, 2017). Drought significantly affected A in rainfed plots over irrigated due to the decrease in stomatal conductance.

2023 drought did affect seed weight (g) we saw a significant difference in weight between treatments with genotype N11-10295. When drought treatment was induced on July 13th, the plants were in developmental stage V10. By July 26th, the field was in developmental stage R2, it was noted that rainfed plots were losing leaves and flowers were dying. With drought occurring during the essential reproductive phase, rainfed N11-10295 had to make up in seed weight instead of yield due to not having enough pods. As the drought treatment progressed, rainfed plots were lacking in pod development due to flower abortion. After sampling was completed, whole field irrigated resumed on August 23rd, plants were in developmental stages R3, R4, R5 due to the severe drought. With whole field watering resuming during the seed fill stages plants were able to recover and enable plants to make up in weight. Drought occurring during R2 and R4 can cause substantial reduction in yield (Wijewardana et al., 2019), with plants having to make up for in other ways. In Smiciklas et al. (1992), soybean drought experiment, stress in R2 and R4 developmental stages, increased seed weight by 7% compared to plants only experiencing drought during R4. Similar, Desclaux et al. (2000), all water deficient genotype Weber soybeans had a higher individual seed weight than well-watered conditions in a soybean drought experiment. Drought did not affect oil or protein in seeds. Due to the severity of the drought, no genotype \times treatment interactions were significant due to both treatments under drought stress conditions.

In 2024, July received 11.3 cm of rain and in August 23.2156 cm of rain. Figure 2.8 shows the variation in soil moisture through the experiment in rainfed and irrigated plots. These

dry conditions followed by precipitation induced a milder drought compared to our 2023 experiment. Yield was significant among genotypes but was not affected by treatment. N11-10295 had a greater yield than PI 471938 and N93-110-6.

In past research found PI 471938 is associated with improved yield under drought stress conditions (Bagherzadi et al., 2017; J. M. Devi et al., 2014; Hufstetler et al., 2007; Sadok et al., 2012). In 2023 and 2024 it was not the highest yielding genotype under drought stress conditions and in 2023 had the largest percent loss. However, Bagherzadi et al. (2017), J. M. Devi et al. (2014), Hufstetler et al. (2007), and Sadok et al. (2012) were all potted chamber and greenhouse experiments, and ours was a field experiment. This could explain why we do not see PI 471938 as the better drought tolerant yielding soybean in 2023. It could also be that drought in these experiments occurred early on in vegetative stages, where ours occurred at the beginning of the reproductive stages. Yield was different for 2024, which could have been due to soil texture and moisture. Our sensors only went to 61 cm, and through our minirhizotron root data, we know roots grew deeper than the 61cm. Plants could have been accessing deeper soil reserves that was unknown to us.

Genotype and treatment alone were significant for 2024 seed weight. Among genotypes, N93-110-6 had a greater seed weight with an estimated marginal means of 15.2 g, PI 471938 having 14.6 g, and N11-10295 having 12.8 g. N93-110-6 had a delay in g_{sw} which could have kept the plant photosynthesizing longer. Rainfed plots have an estimate of 1.5417 g more than irrigated plots. Drought treatment was induced on July 1st when plants were in developmental stages V10 and R1. The plants continued to experience drought through developmental stage R2. Rain events occurred during developmental stages R2 through R4, with drought occurring again in developmental stages R4 and R5. Rainfed plots could have been filling pods to greater capacity

while in drought conditions, however with the rain events, yield stabilized. After sampling was completed, whole field irrigated resumed on August 27th, plants were in developmental stages R4 and R5 at the time. With whole field watering resuming during the seed fill stages plants were able to recover and enable plants to make up in weight. We did not see a difference in yield, but seed weight was greater. Drought stress occurred during flower and pod development, which could have decreased number of pods. With whole field watering at the end of the experiment, we saw bigger seeds which could have led to no difference in yield.

2024 Seed oil was different genotype alone, we did not see a genotype \times treatment interaction. PI 41938 had a greater oil content than N93-110-6 and N11-10295. Protein was not affected by drought conditions, with only a difference among genotypes. N93-110-6 having a greater protein content than N11-10295 and PI 471938.

The difference in yield data between the two years is due to the two different severities of drought treatment. In 2023, we saw a very drought stressed plants in both treatments due to the severe hot, dry conditions. While in 2024, we saw a more moderate drought with a lot more rainfall throughout the experiment. These two types of droughts created two different types of conditions for the soybeans to grow in with 2023 having all stressed out plants and 2024 having stress and normal function plants.

2.7 Conclusion

Our data supported the hypothesis that specific genotypes utilize osmotic adjustment to maintain turgor pressure under drought conditions with three genotypes in 2023 exhibiting osmotic adjustment to maintain turgor pressure, however in 2024 only N11-10295 and N93-110-6 exhibited osmotic adjustment maintaining turgor pressure. The hypothesis that genotypes varied in response to drought was supported, with PI 471938 and N93-110-6 stomatal conductance, photosynthesis, and Φ PSII having a delay in drought affecting function. The data supports that PI 471938 and N11-10295 have drought tolerance nitrogen fixation and N93-110-6 nitrogen fixation is sensitive to drought. The hypothesis that leaf area index differed among genotypes under drought was supported. The data supports the hypotheses N93-110-6 use drought tolerance mechanism that to maintain high yield and oil and protein content. The hypothesis that these genotypes exhibit limited transpiration at high VPD conserving soil moisture early in drought along with no support that drought conditions affect root growth and length among genotypes was not supported.

As climate change continues to cause severe and unpredictable droughts across agricultural lands, food security will continue to be threatened. This research further shown drought response patterns in N93-110-6, N11-10295, and PI 471938 that hold potential for breeders and farmers to utilize. Studying these genotypes physiological response to drought can tell how quickly or delayed stomatal conductance and/ or photosynthesis is affected and what drought tolerant mechanisms, like osmotic adjustment, are being utilized to maintain yield and survive. This analysis demonstrates the importance of identifying drought tolerant traits that breeders can breed out for in drought tolerant genotypes. Farmers can utilize these findings when they conduct their own phenotyping and under what conditions would it adequate to do so. This

study demonstrates how to further study drought tolerance in soybean. We have seen that field experiments do not always yield similar drought response patterns as seen in chamber and greenhouse experiments. Not all aspects of field experiments can be controlled, but they demonstrate real responses farmers may see when experiencing moderate or severe drought conditions.

2.8 References

- Arnaud, M., Baird, A. J., Morris, P. J., Harris, A., & Huck, J. J. (2019). EnRoot: A narrow-diameter, inexpensive and partially 3D-printable minirhizotron for imaging fine root production. *Plant Methods*, *15*(1). <https://doi.org/10.1186/s13007-019-0489-6>
- Bagherzadi, L., Sinclair, T. R., Zwieniecki, M., Secchi, F., Hoffmann, W., Carter, T. E., & Rufty, T. W. (2017). Assessing water-related plant traits to explain slow-wilting in soybean PI 471938. *Journal of Crop Improvement*, *31*(3), 400–417. <https://doi.org/10.1080/15427528.2017.1309609>
- Bharath, P., Gahir, S., & Raghavendra, A. S. (2021). Abscisic Acid-Induced Stomatal Closure: An Important Component of Plant Defense Against Abiotic and Biotic Stress. In *Frontiers in Plant Science* (Vol. 12). Frontiers Media S.A. <https://doi.org/10.3389/fpls.2021.615114>
- Brodribb, T. J., & Holbrook, N. M. (2003). Stomatal Closure during Leaf Dehydration, Correlation with Other Leaf Physiological Traits. *Physiology*, *132*(4), 2166–2173. <https://doi.org/10.1104/pp..103.023879>
- Brodribb, T. J., & McAdam, S. A. M. (2017). Evolution of the stomatal regulation of plant water content. *Plant Physiology*, *174*(2), 639–649. <https://doi.org/10.1104/pp.17.00078>
- Brodribb, T. J., Sussmilch, F., & McAdam, S. A. M. (2020). From reproduction to production, stomata are the master regulators. In *Plant Journal* (Vol. 101, Issue 4, pp. 756–767). Blackwell Publishing Ltd. <https://doi.org/10.1111/tpj.14561>
- Cardoso, A. A., Gori, A., Da-Silva, C. J., & Brunetti, C. (2020). Abscisic acid biosynthesis and signaling in plants: Key targets to improve water use efficiency and drought tolerance. In *Applied Sciences (Switzerland)* (Vol. 10, Issue 18). MDPI AG. <https://doi.org/10.3390/APP10186322>

- De Silva, M., Purcell, L. C., & King, C. A. (1996). Soybean petiole ureide response to water deficits and decreased transpiration. *Crop Science*, *36*(3), 611–616.
<https://doi.org/10.2135/cropsci1996.0011183X003600030015x>
- Demicheli, J., Sabljic, I., Beguy, G., Ploschuk, E., Sahrawy, M., Serrato, A. J., & Pagano, E. A. (2023). Improving drought tolerance in soybean by classical breeding leads to physiological adjustments of photosynthesis and stomata functioning. *Plant Stress*, *10*.
<https://doi.org/10.1016/j.stress.2023.100275>
- Desclaux, D., Huynh, T. T., & Roumet, P. (2000). Identification of soybean plant characteristics that indicate the timing of drought stress. *Crop Science*, *40*(3), 716–722.
<https://doi.org/10.2135/cropsci2000.403716x>
- Devi, J. M., Sinclair, T. R., Chen, P., & Carter, T. E. (2014). Evaluation of elite southern maturity soybean breeding lines for drought-tolerant traits. *Agronomy Journal*, *106*(6), 1947–1954.
<https://doi.org/10.2134/agronj14.0242>
- Devi, M. J., & Sinclair, T. R. (2013). Nitrogen fixation drought tolerance of the slow-wilting soybean PI 471938. *Crop Science*, *53*(5), 2072–2078.
<https://doi.org/10.2135/cropsci2013.02.0095>
- Dietz, K. J., Zörb, C., & Geilfus, C. M. (2021). Drought and crop yield. In *Plant Biology* (Vol. 23, Issue 6, pp. 881–893). John Wiley and Sons Inc. <https://doi.org/10.1111/plb.13304>
- Fletcher, A. L., Sinclair, T. R., & Allen, L. H. (2007). Transpiration responses to vapor pressure deficit in well watered “slow-wilting” and commercial soybean. *Environmental and Experimental Botany*, *61*(2), 145–151. <https://doi.org/10.1016/j.envexpbot.2007.05.004>
- Gray, S. B., Dermody, O., Klein, S. P., Locke, A. M., McGrath, J. M., Paul, R. E., Rosenthal, D. M., Ruiz-Vera, U. M., Siebers, M. H., Strellner, R., Ainsworth, E. A., Bernacchi, C. J., Long,

- S. P., Ort, D. R., & Leakey, A. D. B. (2016). Intensifying drought eliminates the expected benefits of elevated carbon dioxide for soybean. *Nature Plants*, 2.
<https://doi.org/10.1038/nplants.2016.132>
- Hufstetler, E. V., Boerma, H. R., Carter, T. E., & Earl, H. J. (2007). Genotypic variation for three physiological traits affecting drought tolerance in soybean. *Crop Science*, 47(1), 25–35.
<https://doi.org/10.2135/cropsci2006.04.0243>
- Jongdee, B., Fukai, S., & Cooper, M. (n.d.). *Leaf water potential and osmotic adjustment as physiological traits to improve drought tolerance in rice.*
- Kunert, K., & Vorster, B. J. (2020). In search for drought-tolerant soybean: Is the slow-wilting phenotype more than just a curiosity? *Journal of Experimental Botany*, 71(2), 457–460.
<https://doi.org/10.1093/jxb/erz235>
- Manzi, M., Lado, J., Rodrigo, M. J., Zacariás, L., Arbona, V., & Gómez-Cadenas, A. (2015). Root ABA Accumulation in Long-Term Water-Stressed Plants is Sustained by Hormone Transport from Aerial Organs. *Plant and Cell Physiology*, 56(12), 2457–2466.
<https://doi.org/10.1093/pcp/pcv161>
- McAdam, S. A. M., & Brodribb, T. J. (2015). The evolution of mechanisms driving the stomatal response to vapor pressure deficit. *Plant Physiology*, 167(3), 833–843.
<https://doi.org/10.1104/pp.114.252940>
- McAdam, S. A. M., Manzi, M., Ross, J. J., Brodribb, T. J., & Gómez-Cadenas, A. (2016). Uprooting an abscisic acid paradigm: Shoots are the primary source. *Plant Signaling and Behavior*, 11(6). <https://doi.org/10.1080/15592324.2016.1169359>

- Purcell, L. C., & King, C. A. (1996). Drought and nitrogen source effects on nitrogen nutrition, seed growth, and yield in soybean. *Journal of Plant Nutrition*, 19(6), 969–993.
<https://doi.org/10.1080/01904169609365173>
- Purcell, L. C., & Specht, J. E. (2016). Physiological traits for ameliorating drought stress. In *Soybeans: Improvement, Production, and Uses* (pp. 569–620). Wiley.
<https://doi.org/10.2134/agronmonogr16.3ed.c12>
- R Core Team (2023). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org>.
- Sadok, W., Gilbert, M. E., Raza, M. A. S., & Sinclair, T. R. (2012). Basis of slow-wilting phenotype in soybean PI 471938. *Crop Science*, 52(3), 1261–1269.
<https://doi.org/10.2135/cropsci2011.11.0622>
- Sinclair, T. R., Hammer, G. L., & Van Oosterom, E. J. (2005). Potential yield and water-use efficiency benefits in sorghum from limited maximum transpiration rate. *Functional Plant Biology*, 32(10), 945–952. <https://doi.org/10.1071/FP05047>
- Sinclair, T. R., Messina, C. D., Beatty, A., & Samples, M. (2010). Assessment across the United States of the benefits of altered soybean drought traits. *Agronomy Journal*, 102(2), 475–482.
<https://doi.org/10.2134/agronj2009.0195>
- Sinclair, T. R., Muchow, R. C., Bennett, J. M., & Hammond, L. C. (1987). Relative Sensitivity of Nitrogen and Biomass Accumulation to Drought in Field-Grown Soybean 1. *Agronomy Journal*, 79(6), 986–991. <https://doi.org/10.2134/agronj1987.00021962007900060007x>
- Sinclair, T. R., Purcell, L. C., King, C. A., Sneller, C. H., Chen, P., & Vadez, V. (2007). Drought tolerance and yield increase of soybean resulting from improved symbiotic N₂ fixation. *Field Crops Research*, 101(1), 68–71. <https://doi.org/10.1016/j.fcr.2006.09.010>

- Sinclair, T. R., Zwieniecki, M. A., & Holbrook, N. M. (2008). Low leaf hydraulic conductance associated with drought tolerance in soybean. *Physiologia Plantarum*, 132(4), 446–451.
<https://doi.org/10.1111/j.1399-3054.2007.01028.x>
- Sloane, R. J., Patterson, R. P., & Carter, T. E. (1990). Field Drought Tolerance of a Soybean Plant Introduction. *Crop Science*, 30(1), 118–123.
<https://doi.org/10.2135/cropsci1990.0011183x003000010027x>
- Smiciklas, K. D., Mullen, R. E., Carlson, R. E., & Knapp, A. D. (1992). Soybean Seed Quality Response to Drought Stress and Pod Position. *Agronomy Journal*, 84(2), 166–170.
<https://doi.org/10.2134/agronj1992.00021962008400020008x>
- Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at the following link: <http://websoilsurvey.sc.egov.usda.gov/>. Accessed [07/25/2024].
- Turner, N. C. (2017). Turgor maintenance by osmotic adjustment, an adaptive mechanism for coping with plant water deficits. In *Plant Cell and Environment* (Vol. 40, Issue 1, pp. 1–3). Blackwell Publishing Ltd. <https://doi.org/10.1111/pce.12839>
- Turner, N. C. (2018). Turgor maintenance by osmotic adjustment: 40 years of progress. In *Journal of Experimental Botany* (Vol. 69, Issue 13, pp. 3223–3233). Oxford University Press. <https://doi.org/10.1093/jxb/ery181>
- “U.S. Climate Normals 2020: U.S. Monthly Climate Normals (2006-2020)”. *NOAA National Center of Environmental Information*, Dec.2024,
<https://www.ncei.noaa.gov/access/search/data-search/normals-monthly-2006-2020>
- Wells, R., Schulze, L. L., Ashley, D. A., Boerma, H. R., & Brown, R. H. (1982). Cultivar Differences in Canopy Apparent Photosynthesis and Their Relationship to Seed Yield in

Soybeans 1 . *Crop Science*, 22(4), 886–890.

<https://doi.org/10.2135/cropsci1982.0011183x002200040044x>

Wijewardana, C., Alsajri, F. A., Irby, J. T., Krutz, L. J., Golden, B., Henry, W. B., Gao, W., &

Reddy, K. R. (2019). Physiological assessment of water deficit in soybean using midday leaf water potential and spectral features. *Journal of Plant Interactions*, 14(1), 533–543.

<https://doi.org/10.1080/17429145.2019.1662499>

Zhang, F. P., Susmilch, F., Nichols, D. S., Cardoso, A. A., Brodribb, T. J., & McAdam, S. A. M.

(2018). Leaves, not roots or floral tissue, are the main site of rapid, external pressure-induced ABA biosynthesis in angiosperms. *Journal of Experimental Botany*, 69(5), 1261–

1267. <https://doi.org/10.1093/jxb/erx480>

Figure 2.1 Total monthly precipitation from May 2023 through August 2023, data used from NC ECONet Jack Station

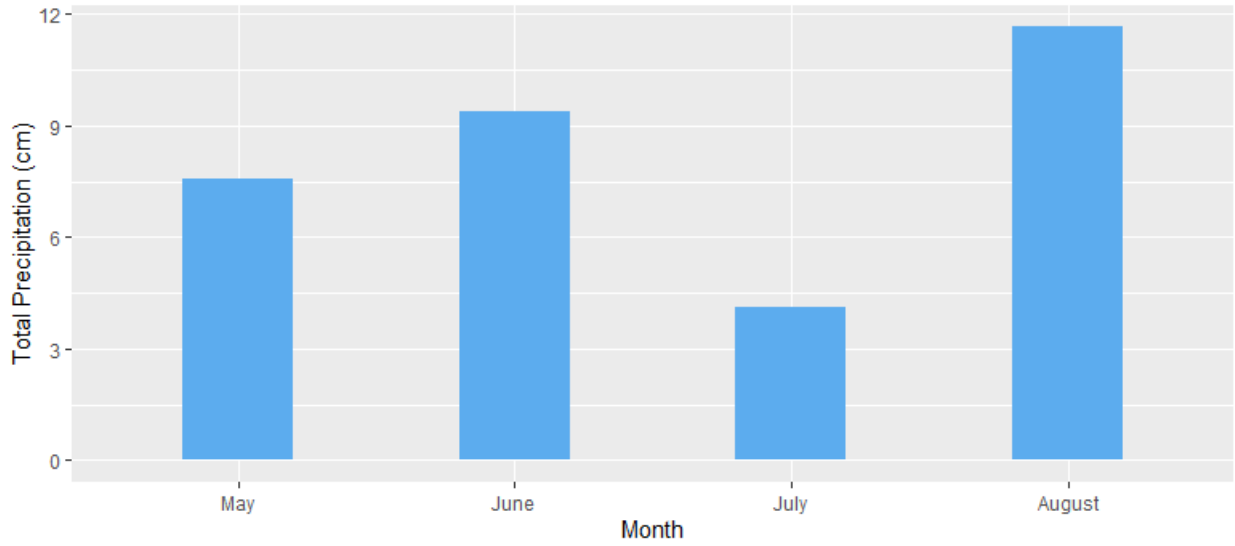


Figure 2.2 Total monthly precipitation from May 2024 through August 2024, data used from NC ECONet Jack Station

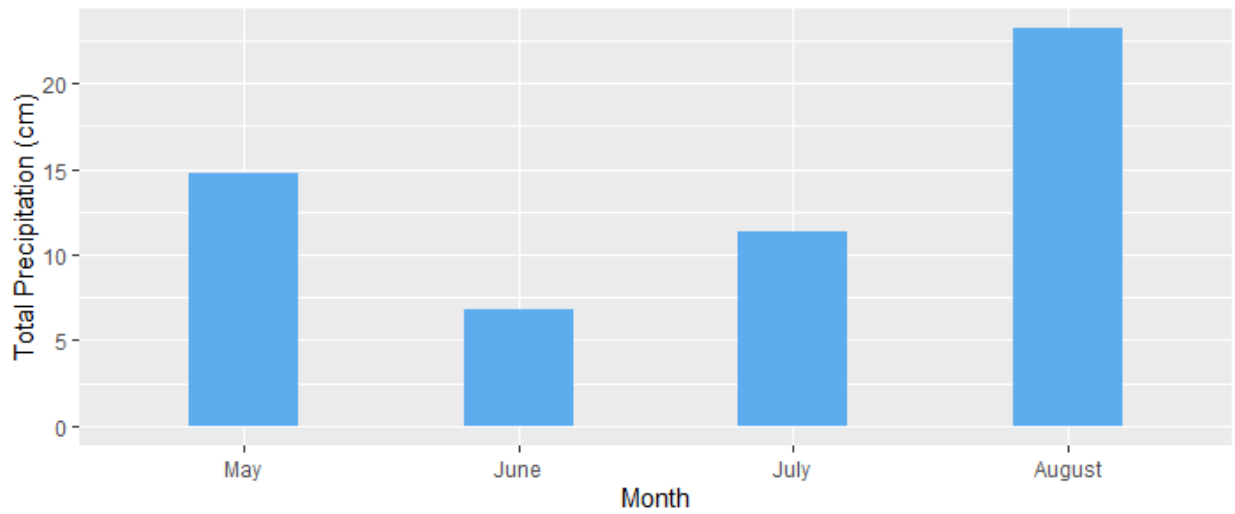


Figure 2.3 Average maximum air temperature from May 2023 through August 2023, data used from NC ECONet Jack Station

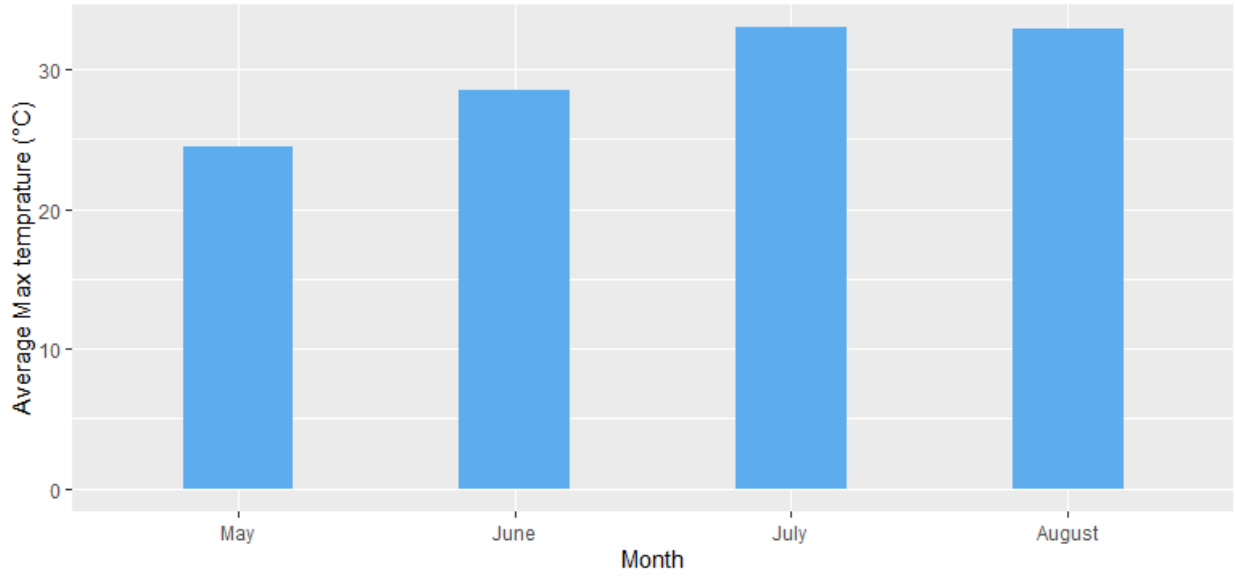


Figure 2.4 Average maximum air temperature from May 2024 through August 2024, data used from NC ECONet Jack Station

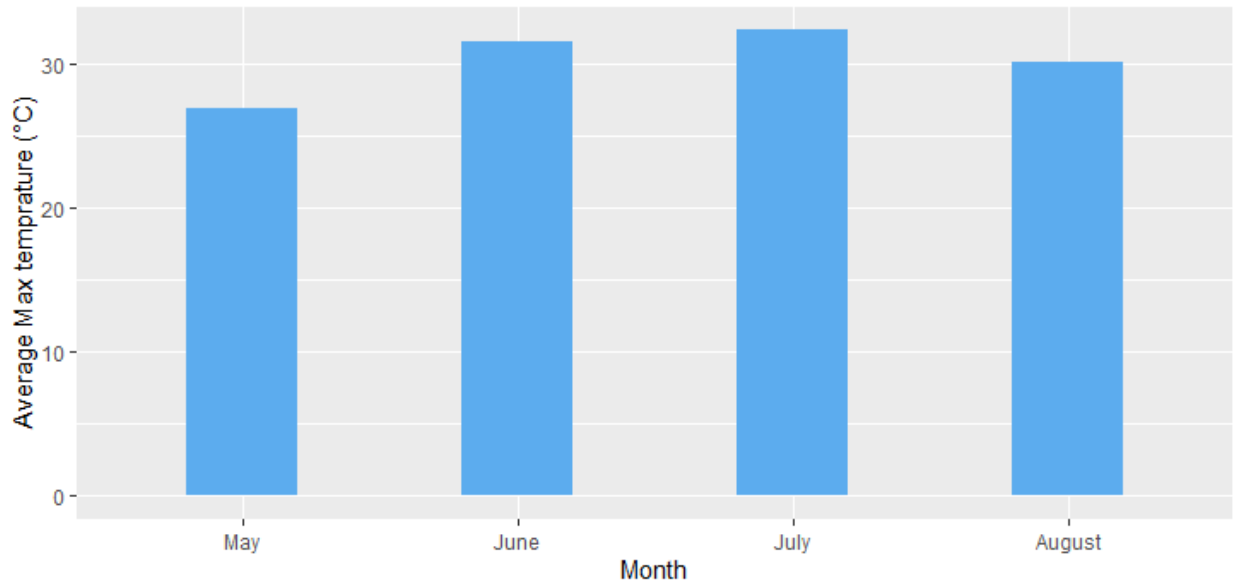


Figure 2.5 Daily soil moisture means 30.5 and 61 cm from soil surface between treatments from May 2023 through August 2023. Differential irrigation started on July 13th and ended on August 16th with whole field irrigation starting again on August 23rd

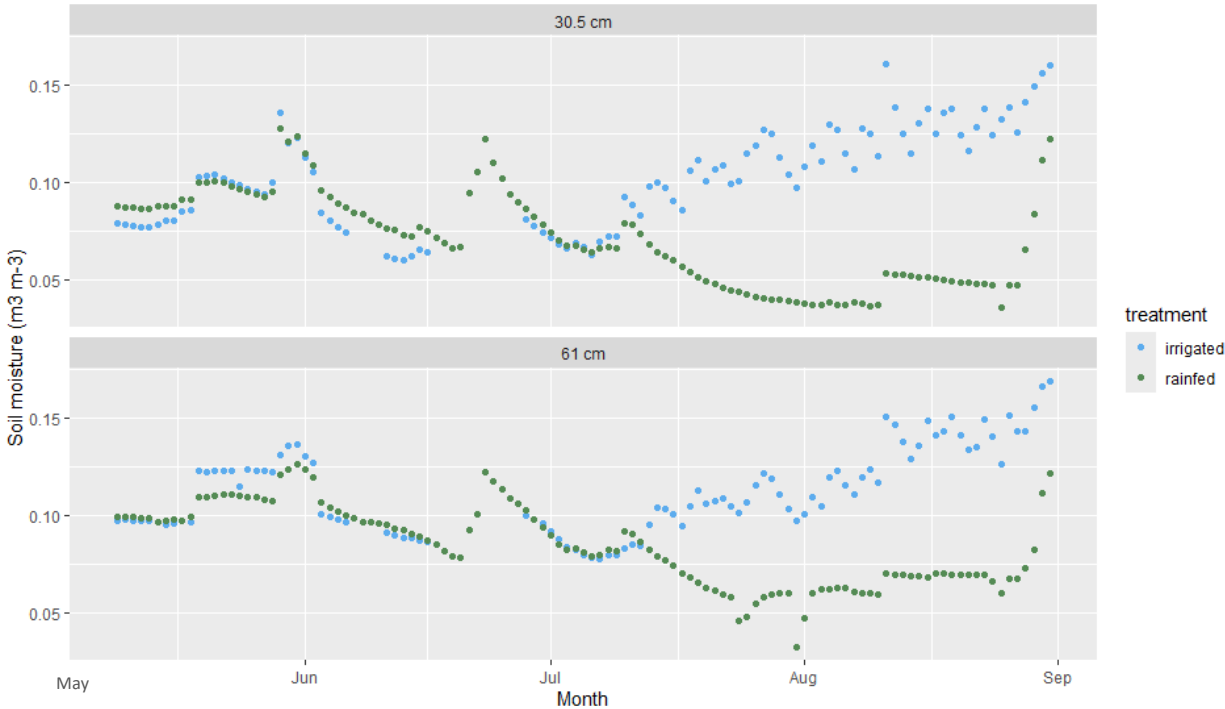


Figure 2.6 Daily soil moisture genotype means 30.5 cm from soil surface between treatments from May 2023 through August 2023. Differential irrigation started on July 13th and ended on August 16th with whole field irrigation starting again on August 23rd

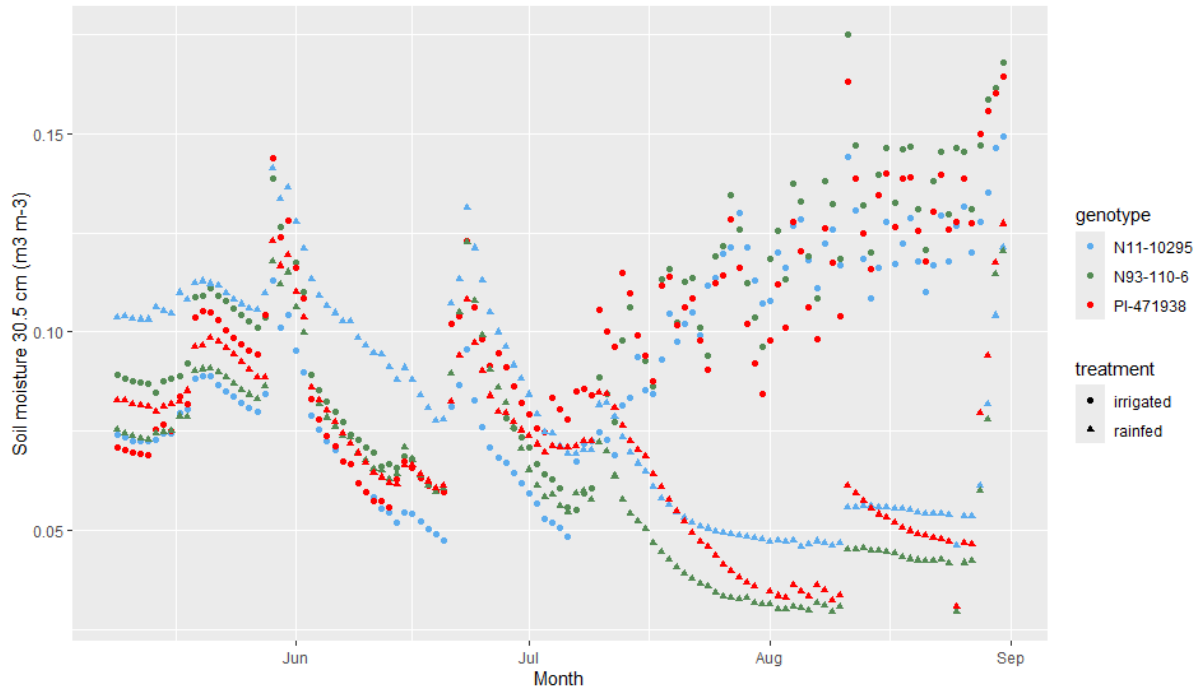


Figure 2.7 Daily soil moisture genotype means 61 cm from soil surface between treatments from May 2023 through August 2023. Differential irrigation started on July 13th and ended on August 16th with whole field irrigation starting again on August 23rd

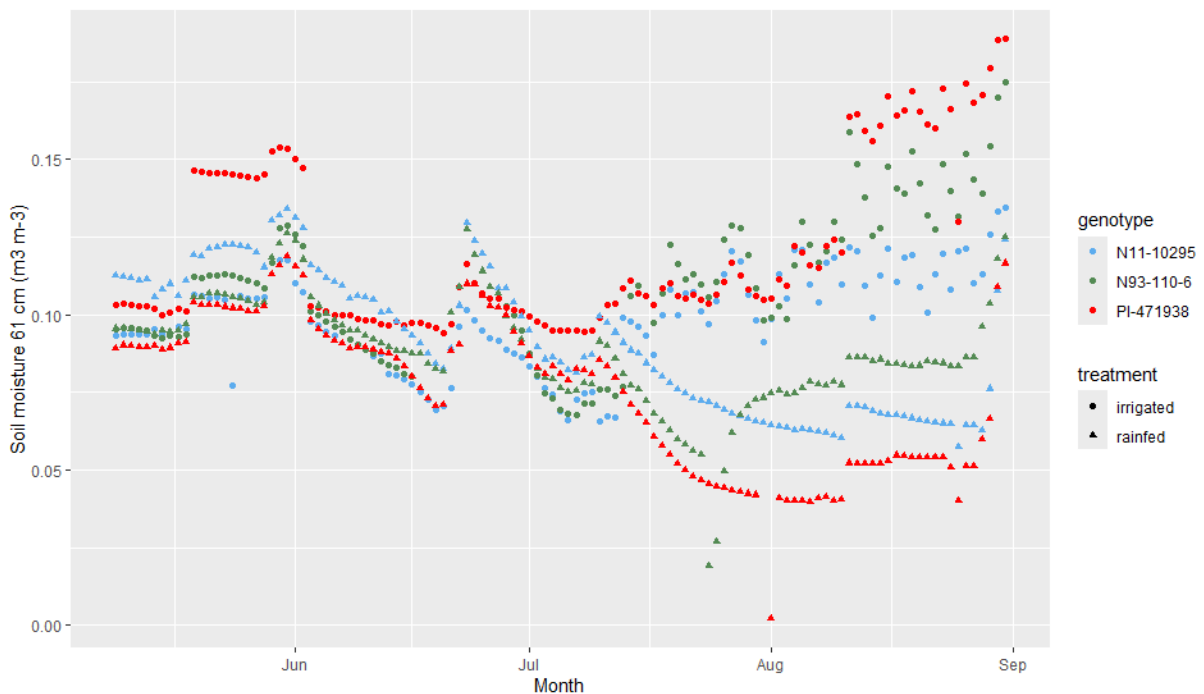


Figure 2.8 Daily soil moisture means 30.5 and 61 cm from soil surface between treatments from May 2024 through August 2024. Differential irrigation started on July 1st and ended on August 27th with whole field irrigation starting again on August 27th

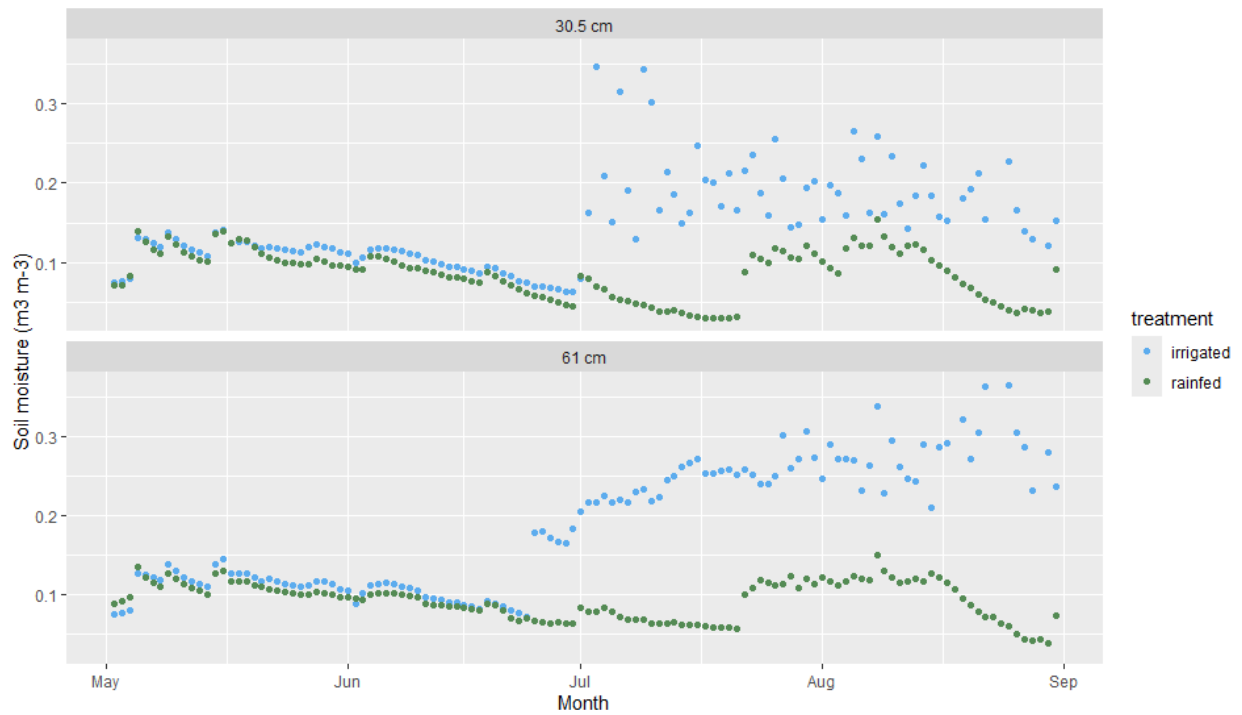


Figure 2.9 Daily soil moisture genotype means 30.5 from soil surface between treatments from May 2024 through August 2024. Differential irrigation started on July 1st and ended on August 27th with whole field irrigation starting again on August 27th

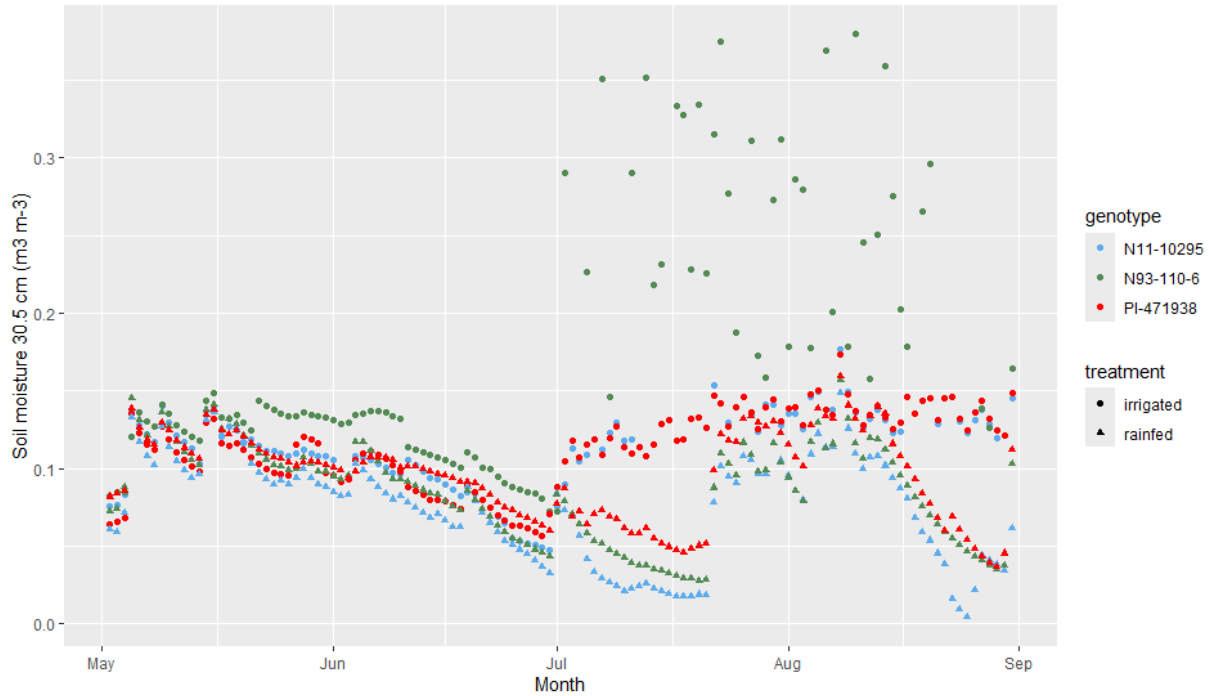


Figure 2.10 Daily soil moisture genotype means 61 cm from soil surface between treatments from May 2024 through August 2024. Differential irrigation started on July 1st and ended on August 27th with whole field irrigation starting again on August 27th

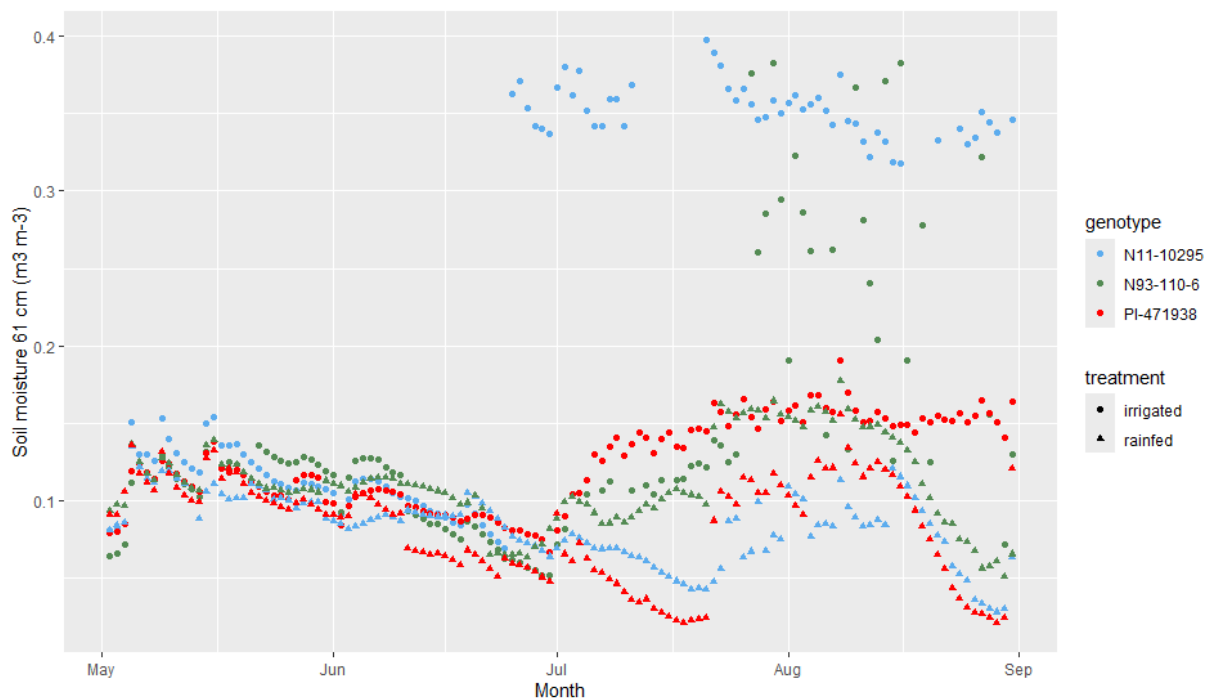
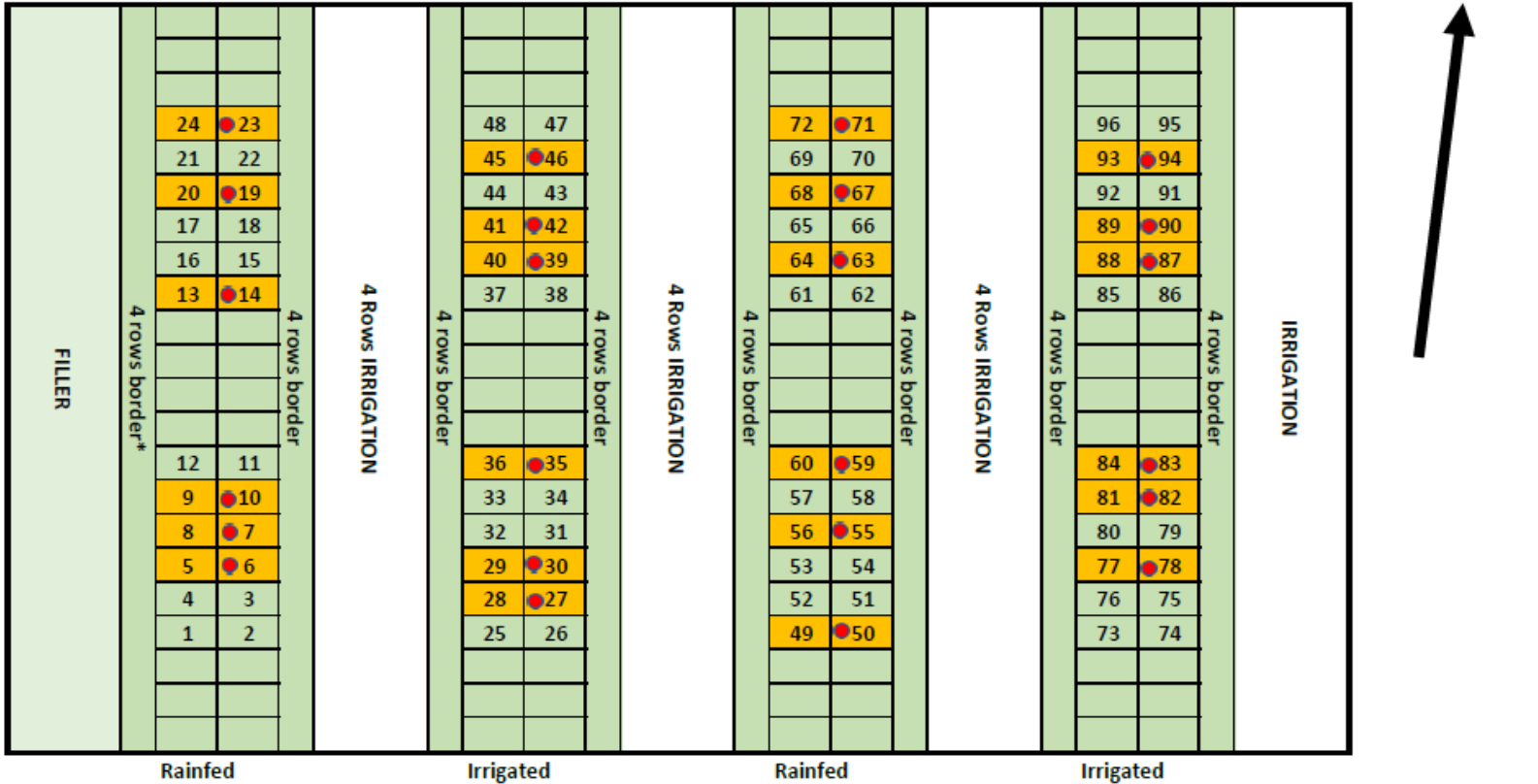


Figure 2.11 Sandhills 2023 field map. Planted May 4th, 2023, with 22 ranges of 23 feet long, 15 feet rows with 8-foot alley. Each number plot consisted of 4 rows; the yellow plots are genotype N93-110-6, N11-10295, and PI 471938. Similar genotypes were planted side by side to create 8 row wide plots for the 3 selected genotypes.

PLANTED 4 MAY 2023

22 ranges of 23 feet long: 15 feet planted + 8 foot alley

265 feet wide





Soil Sensors and tubes: 
 Natalie's genotypes 


Figure 2.12 Sandhills 2024 field map. Planted April 30th, 2024, with 22 ranges of 23 feet long, 15 feet rows with 8-foot alley. Each number plot consisted of 4 rows; the yellow plots are genotype N93-110-6, N11-10295, and PI 471938. Similar genotypes were planted side by side to create 8 row wide plots for the 3 selected genotypes

Planted: April 30th 2024

22 ranges of 23 feet long: 15 feet planted + 8 foot alley



Figure 2.13 NCDA & Agronomic Division Nematode Report for 2023 field location

NCDA&CS Agronomic Division (919) 664-1600 www.ncagr.gov/Divisions/Agronomic-Services				SubmissionID:				Report No. FY24-N000246												
 <p>Research Nematode Report</p>				Client: Natalie Gold 3001 Hillsborough St Raleigh, NC 27607 Sampled County : Montgomery Sampled State : North Carolina				Advisor:												
				Sampled: 08/17/2023 Received: 08/25/2023 Completed 08/29/2023 Farm:				PALS ID: 494083				PALS ID: Nematode Assay Section								
Nematologist's Comments: The soybean cyst nematode population is high enough to be severely stressing the soybeans. If possible, rotation away from soybeans for two years is recommended. Please read Nema Note 2 for additional information (http://www.ncagr.gov/agronomi/pdffiles/nnote2.pdf)																				
sample information			Results and Recommendations: For each species listed, column S = # nematodes per 500 cc soil and column H = hazard index for crop.																	
Total # Samples on Report: 1			Action Code(s)	Nema Notes	CystSoyb		Spiral													
Sample ID	Lab ID	Crop: Last / Current			S	H	S	H	S	H	S	H	S	H	S	H	S	H	S	H
0001	N001227	soybean / soybean	D and E	2-1	410	65-85	120													

Understanding the Research Nematode Report Action Code(s) (if present) A: No expected harm to crop production B: Possible damage; consider chemical treatment C: Chemical treatment recommended D: Use of nematode-resistant variety recommended E: Rotate with nonhost crop(s)			Nema Note(s) (if present) contain information about nematodes detected in the assay as well as possible management options.			Nematode Counts & Hazard Index (potential crop damage) The value in the "H" column is an index of potential harm by the nematode species to the crop listed on the same line: Hazard index: 0-19 20-39 40-59 60-79 80-100 Crop damage: very low low moderate high very high		
---	--	--	---	--	--	---	--	--



Reprogramming of the laboratory-information-management system that makes this report possible is being funded through a grant from the North Carolina Tobacco Trust Fund Commission.

Thank you for using agronomic services to manage nutrients and safeguard environmental quality.
 - Steve Troxler, Commissioner of

Figure 2.14 Figures are from Arnaud et al (2019) which we based our experiments minirhizotron on. Figures show how images are collected in the field and how the tubes are placed in the soybean rows.

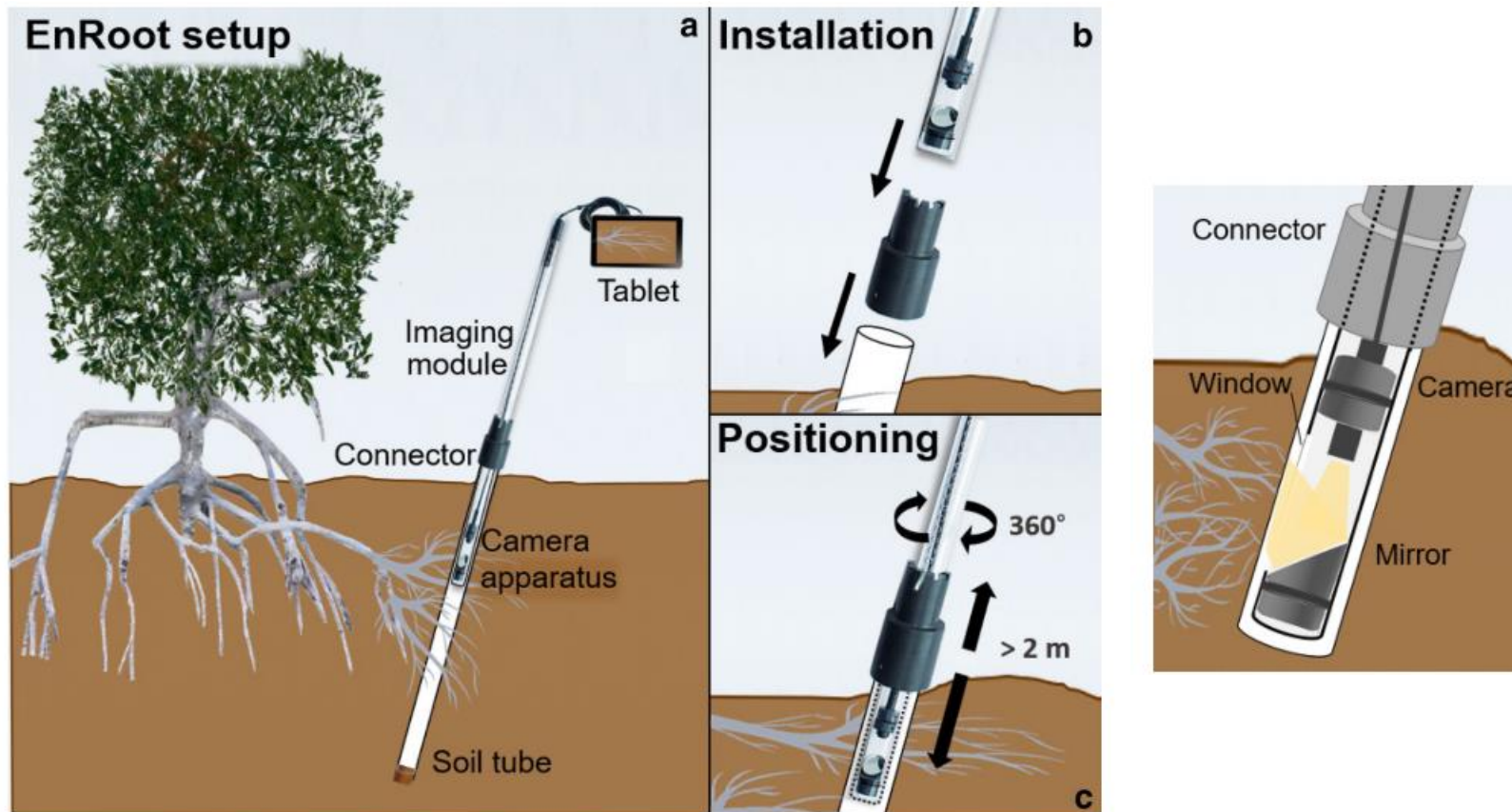


Figure 2.15 2023 assimilation (A) was affected by drought with a significant three-way interaction among treatment, genotype, and date

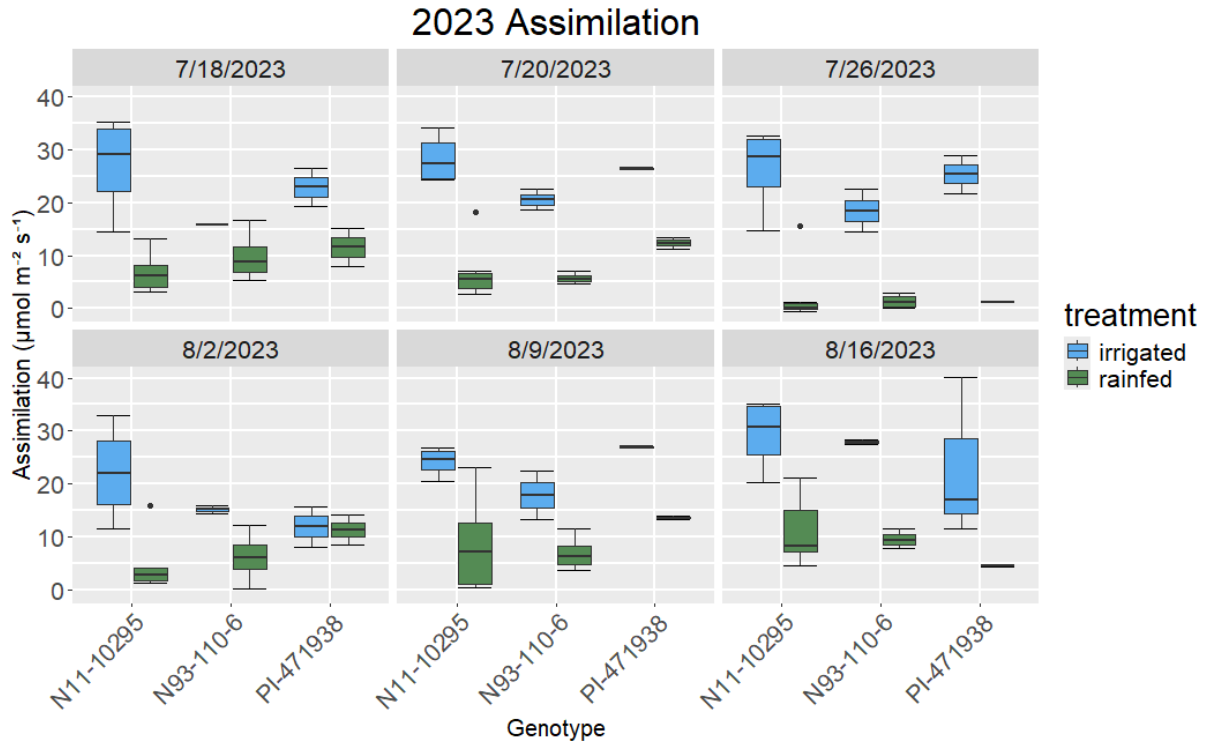


Figure 2.16 2024 assimilation (A) was affected by drought with a significant interaction among treatment and date

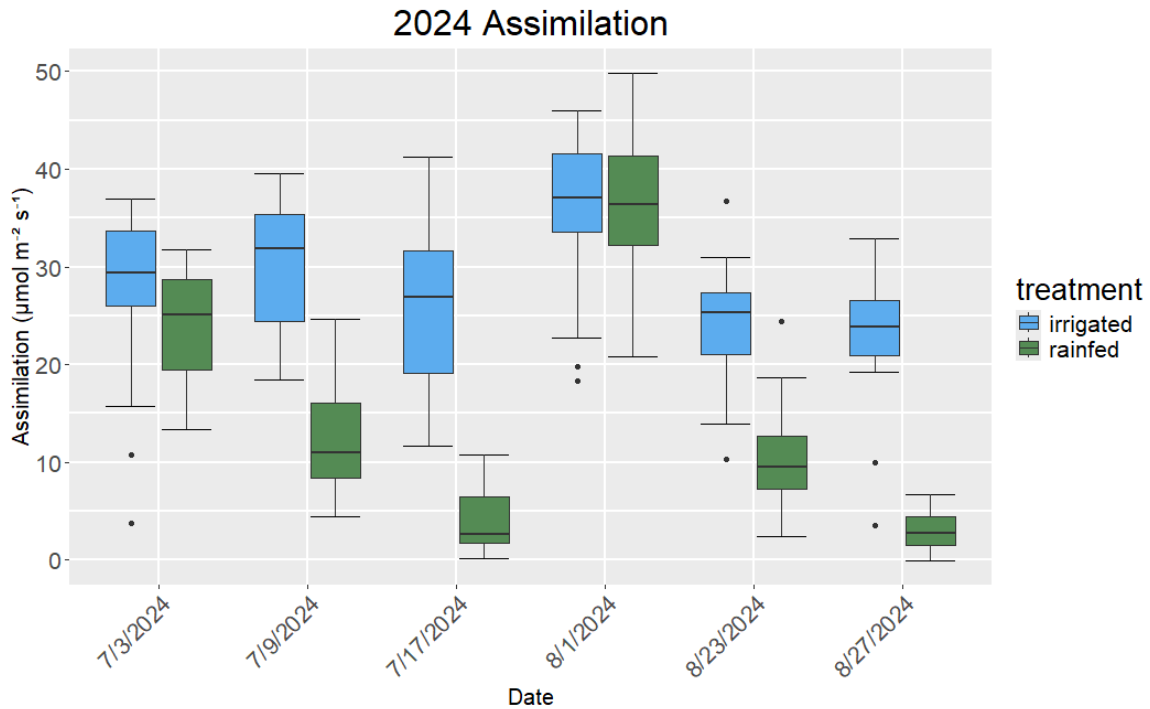


Figure 2.17 2023 stomatal conductance (g_{sw}) was affected by drought with a significant interaction among treatment, genotype, and date

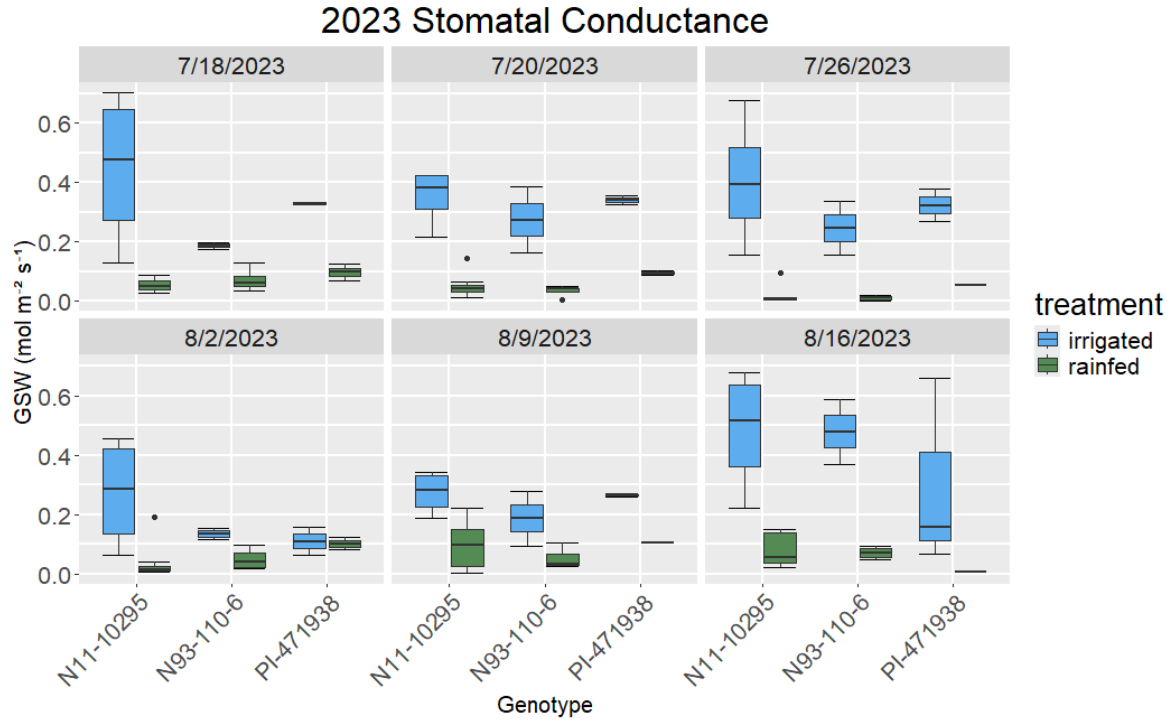


Figure 2.18 2024 stomatal conductance (g_{sw}) was affected by drought with a significant interaction among treatment, genotype, and date

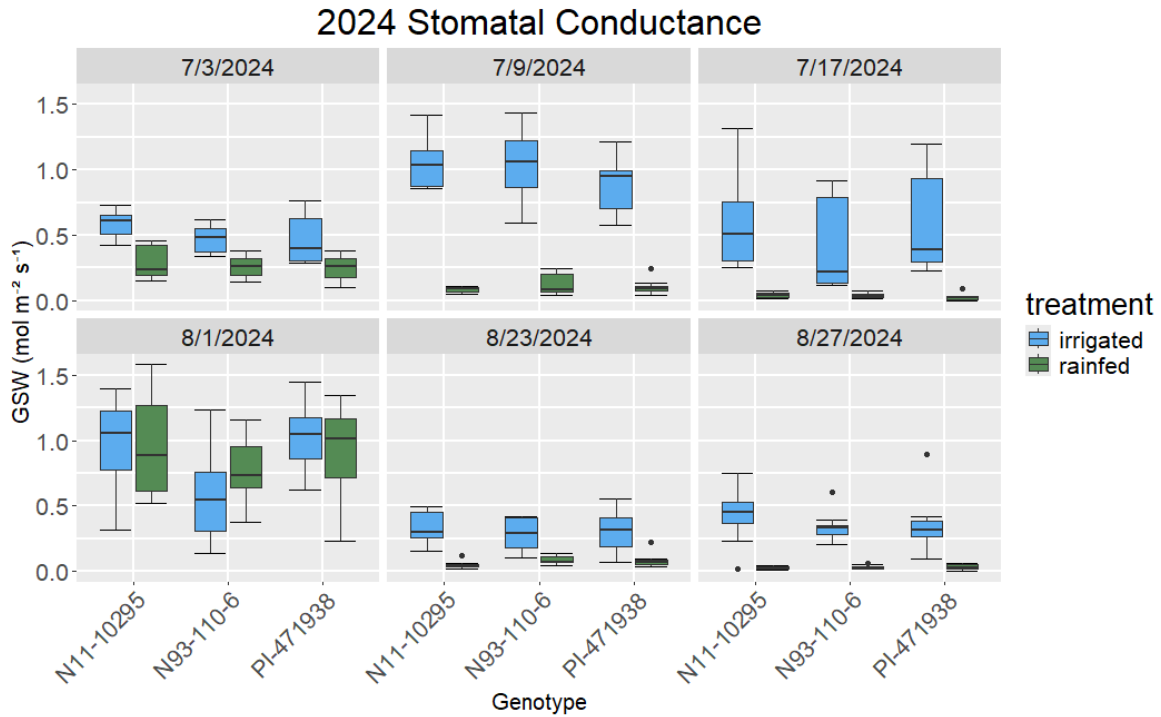


Figure 2.19 2023 Φ PSII was affected by drought with a significant interaction among treatment, genotype, and date

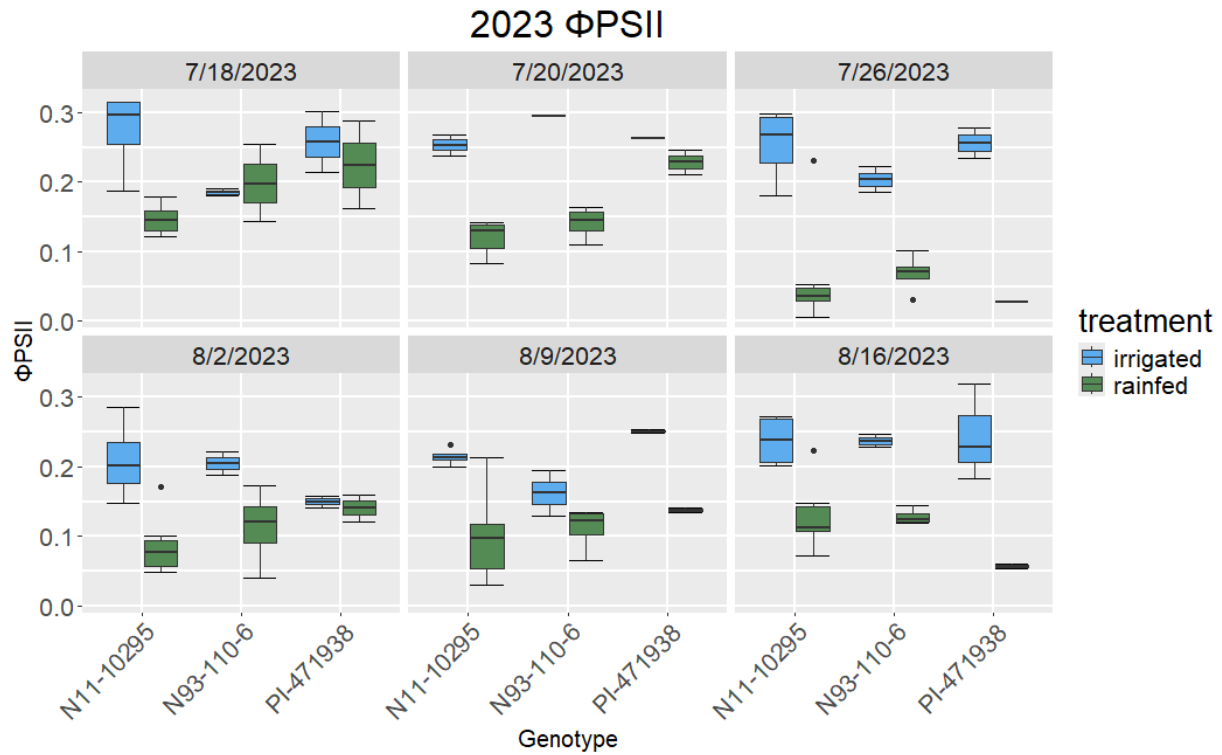


Figure 2.20 2024 Φ PSII was affected by drought with a significant interaction among treatment and date

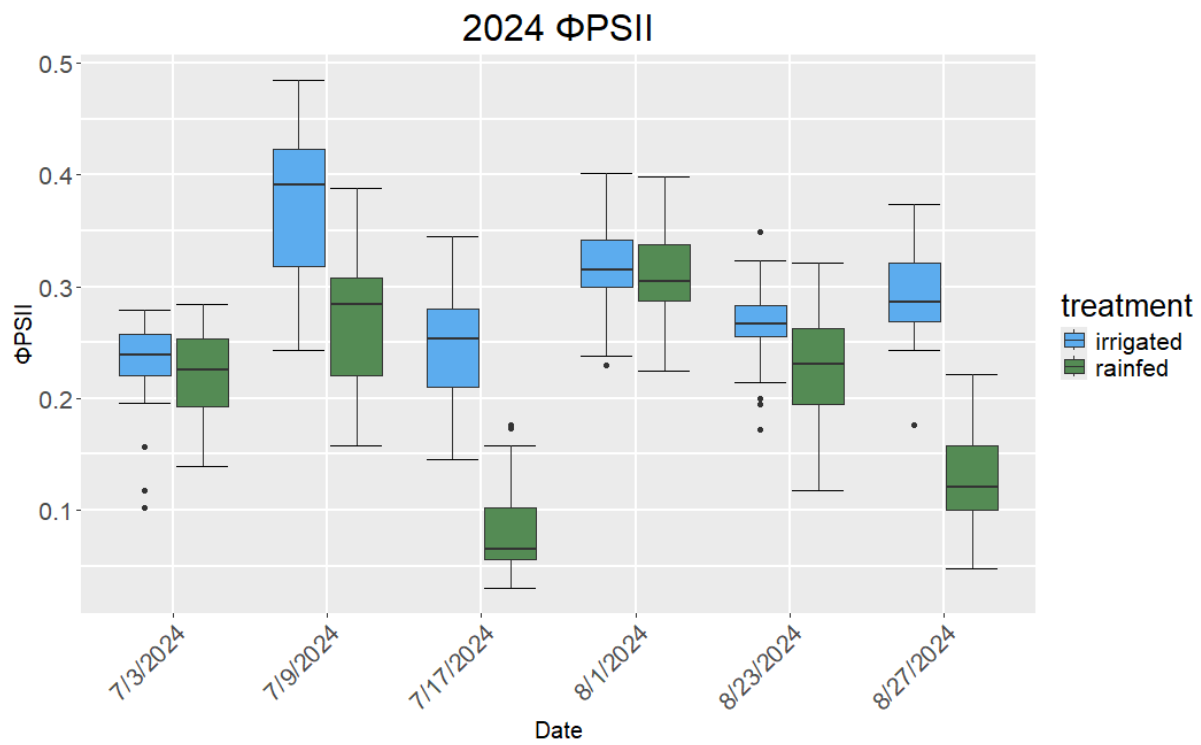


Figure 2.21 2023 iWUE was affected by drought with only a significant treatment effect

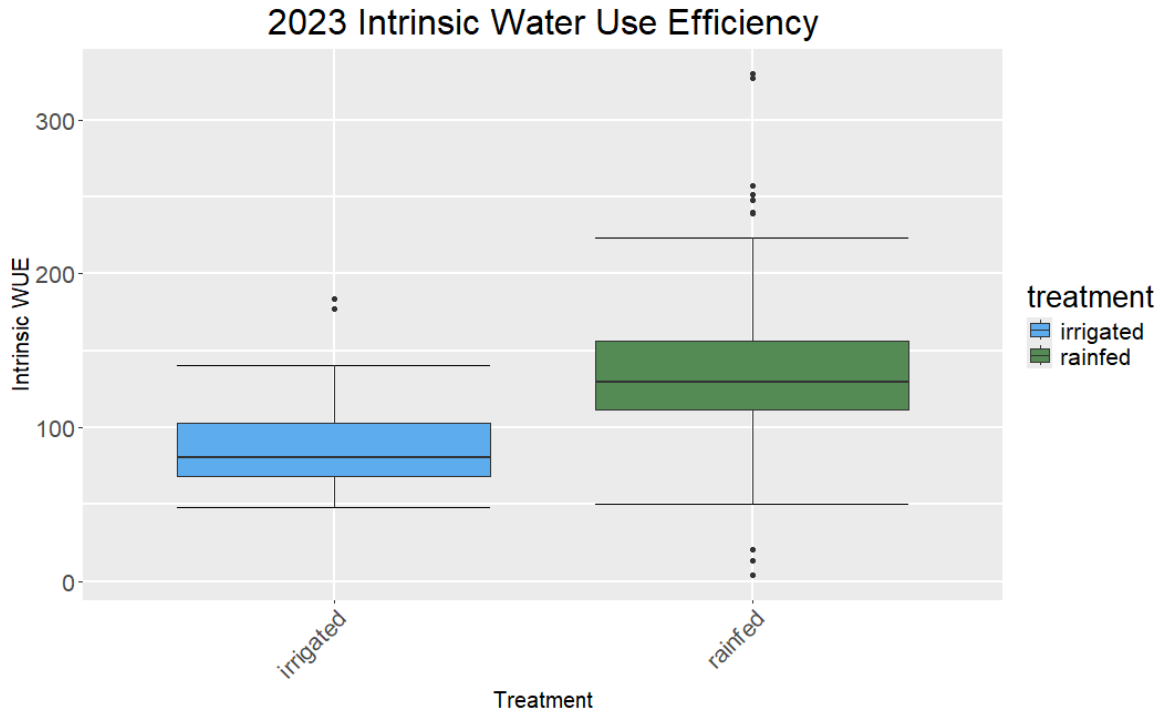


Figure 2.22 2024 iWUE was affected by drought with a significant interaction among treatment, genotype, and date

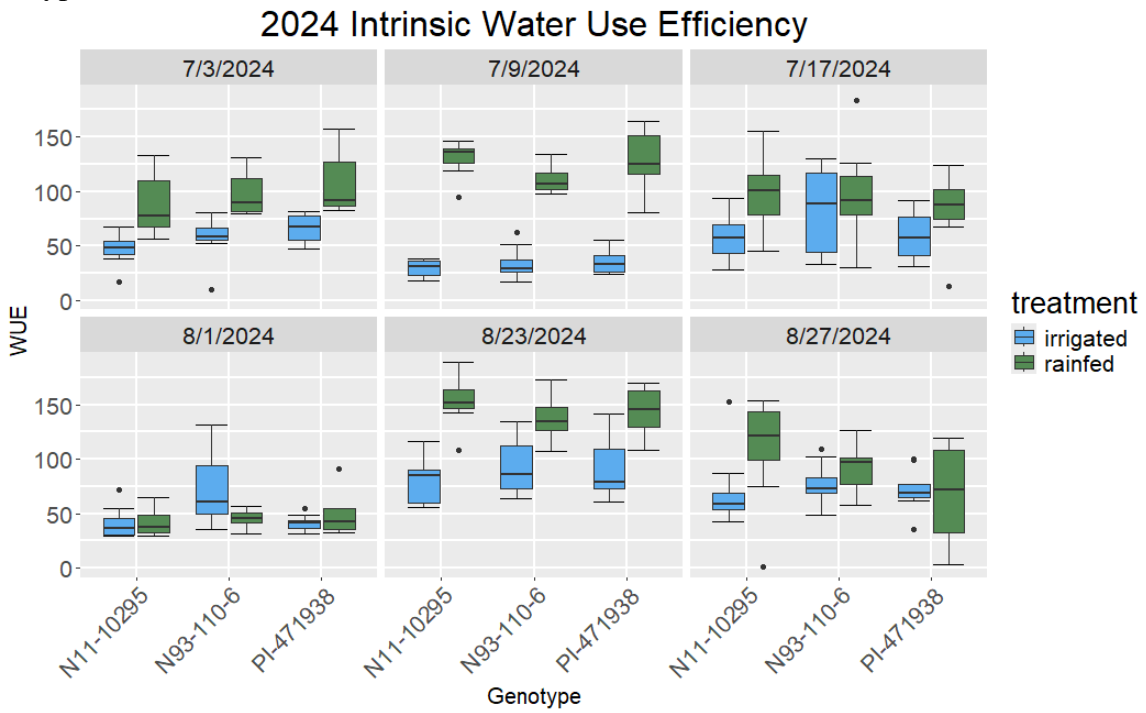


Figure 2.23 2023 Water potential was affected by drought with only a significant treatment effect

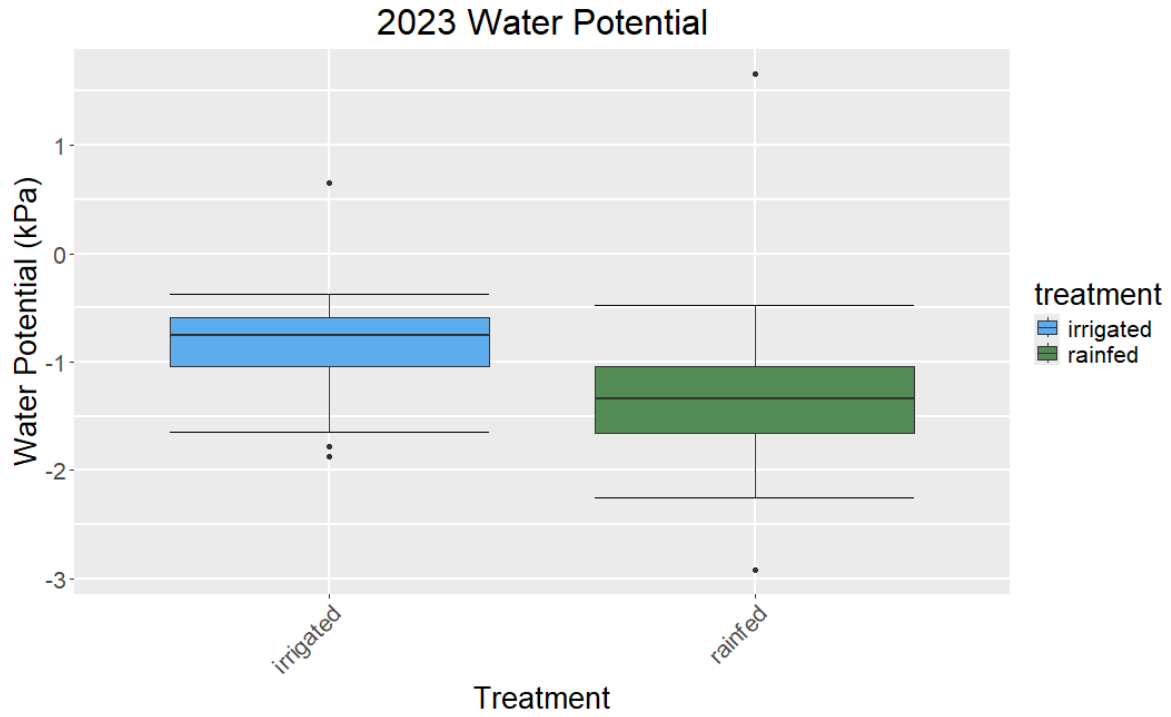


Figure 2.24 2024 Water potential was affected by drought with a significant interaction among treatment, genotype, and date

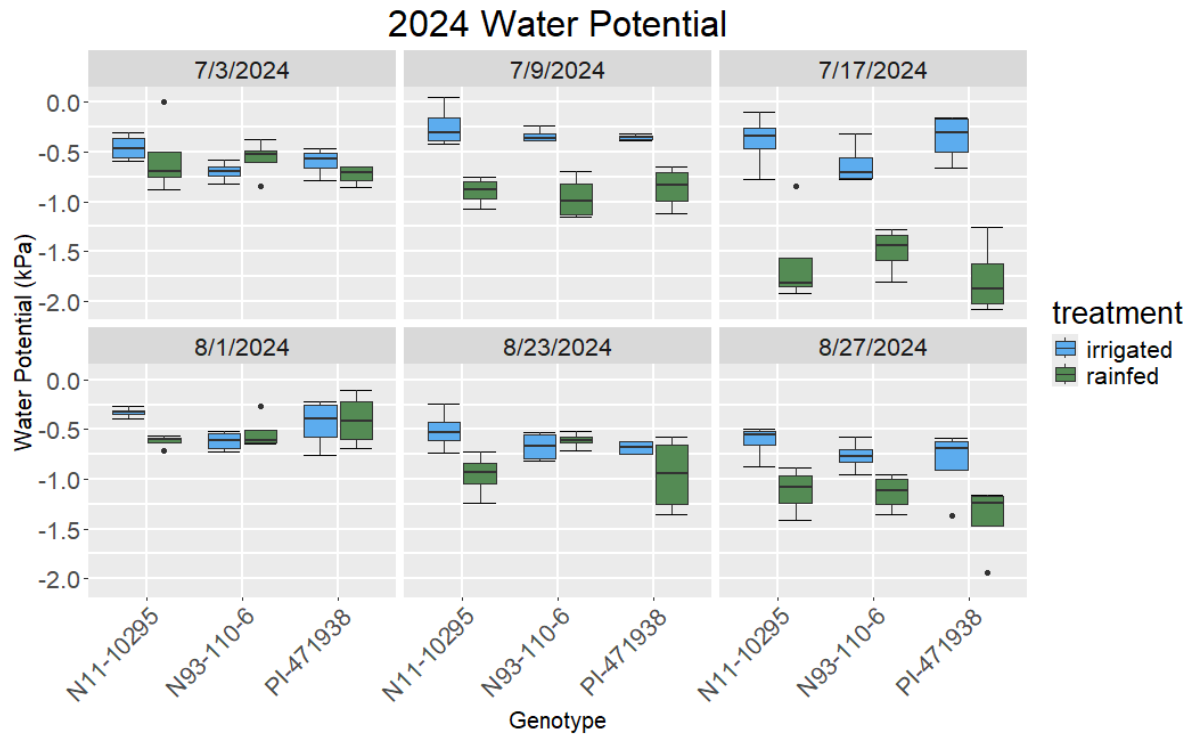


Figure 2.25 2023 Osmotic potential was affected by drought with a significant interaction among treatment, genotype, and date. On 7/20/2023 sampling day N93-110-6 was removed from graph due to psychrometers malfunctioning

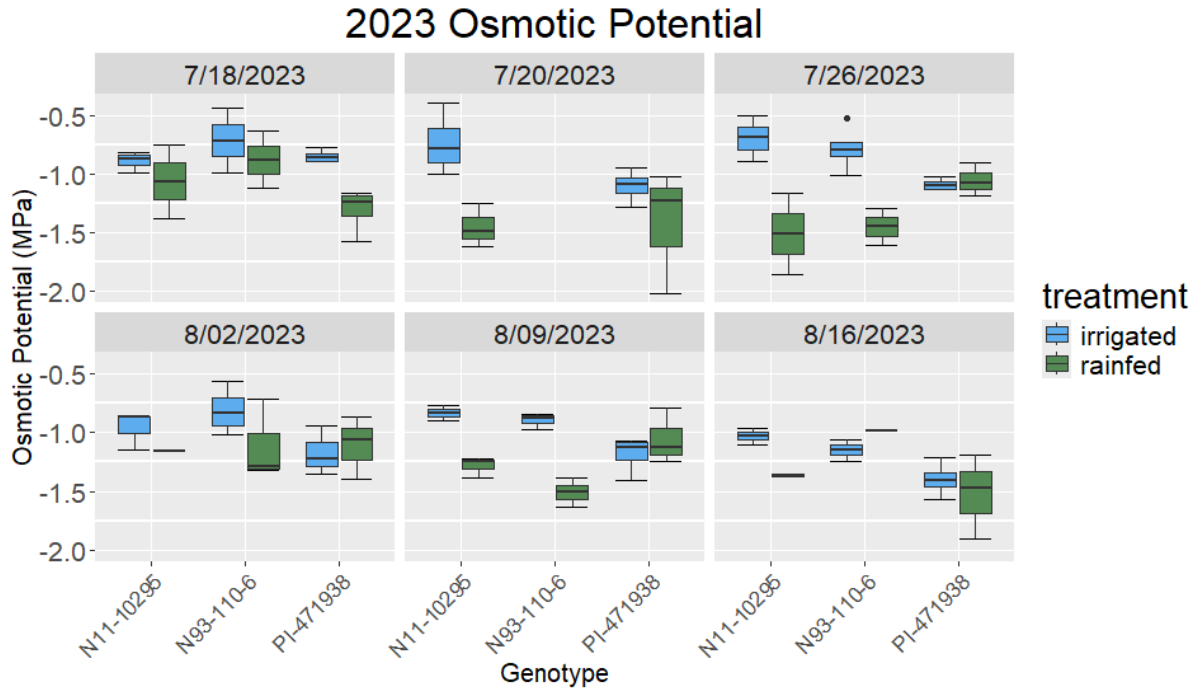


Figure 2.26 2024 Osmotic potential was affected by drought with a significant interaction among treatment, genotype, and date

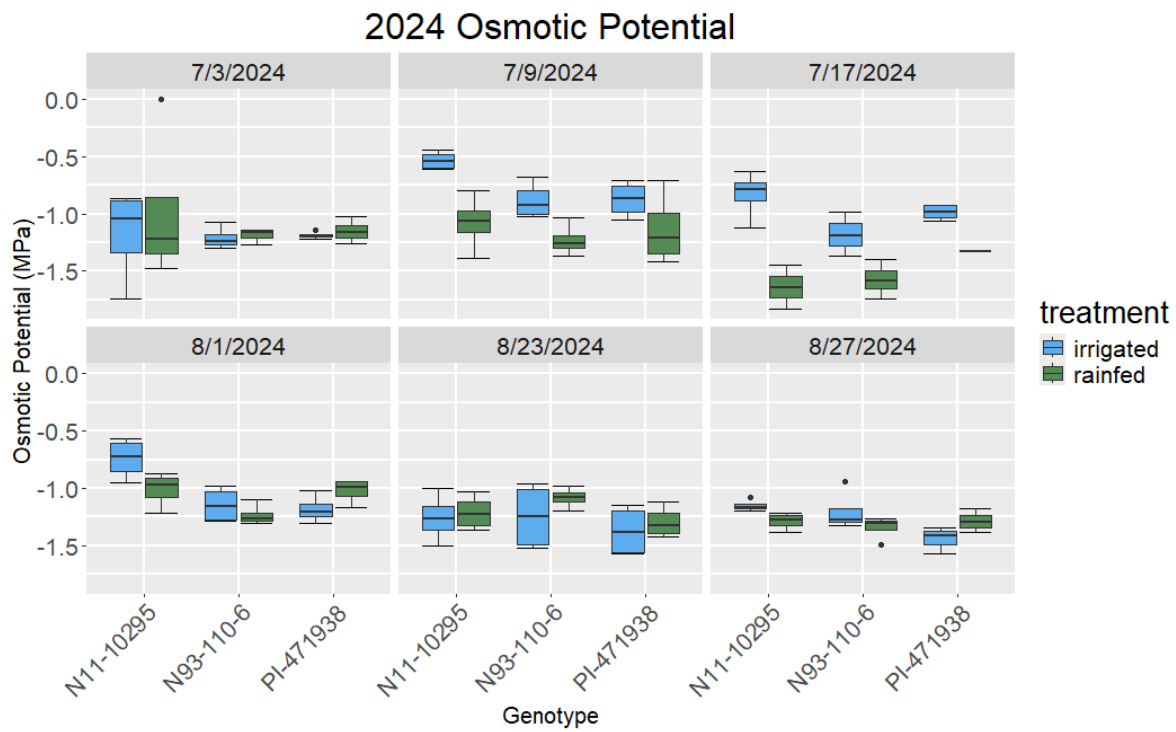


Figure 2.27 2023 Turgor pressure was not significant

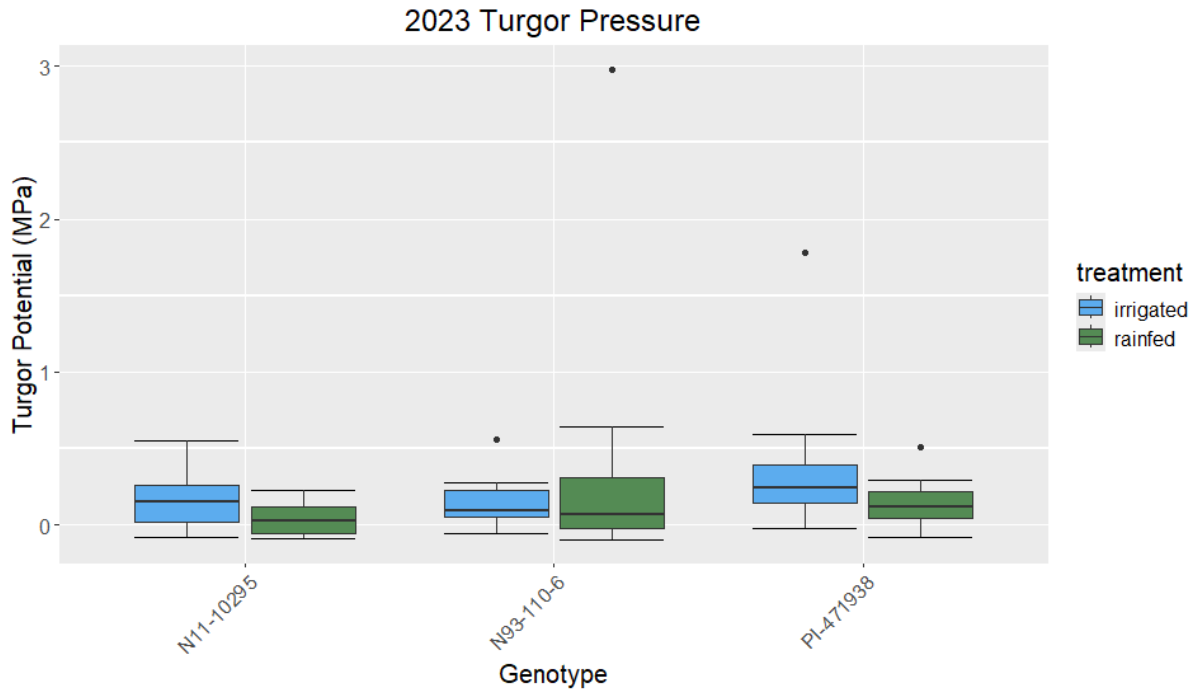


Figure 2.28 2024 Turgor pressure was affected by drought with a significant interaction among treatment, genotype, and date

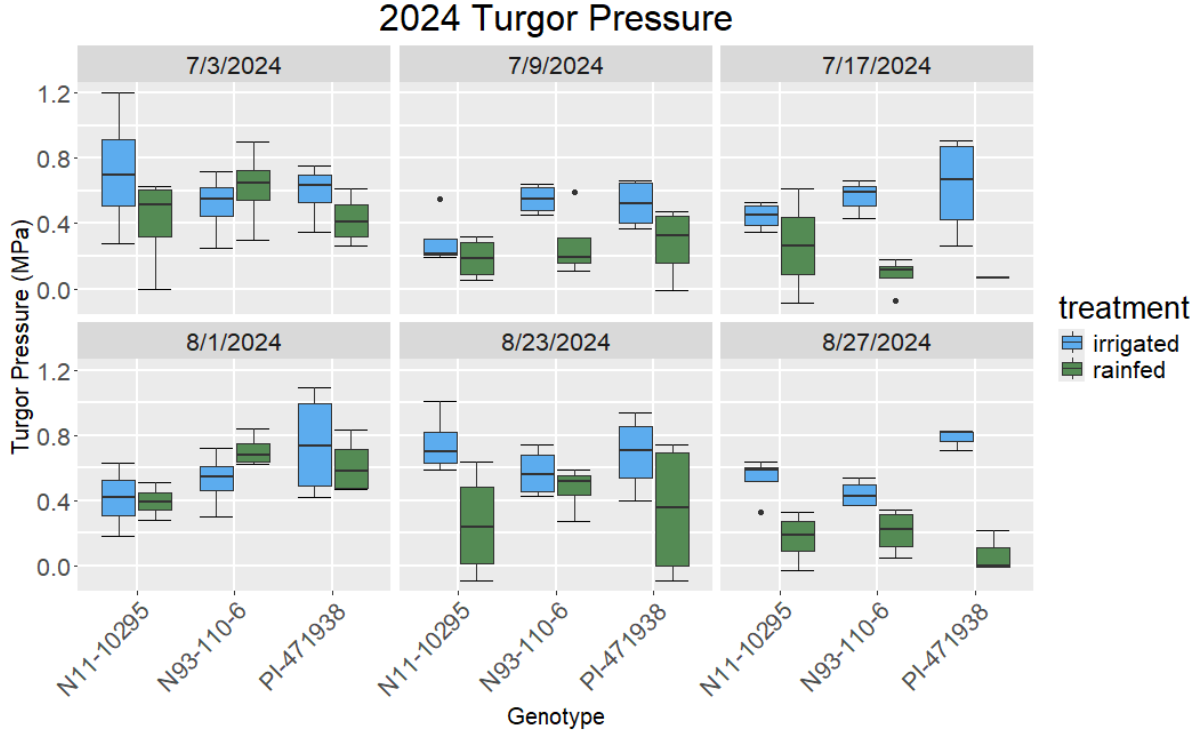


Figure 2.29 2023 Stomatal conductance (g_{sw}) was affected by drought and had a significant Treatment by VPD interaction

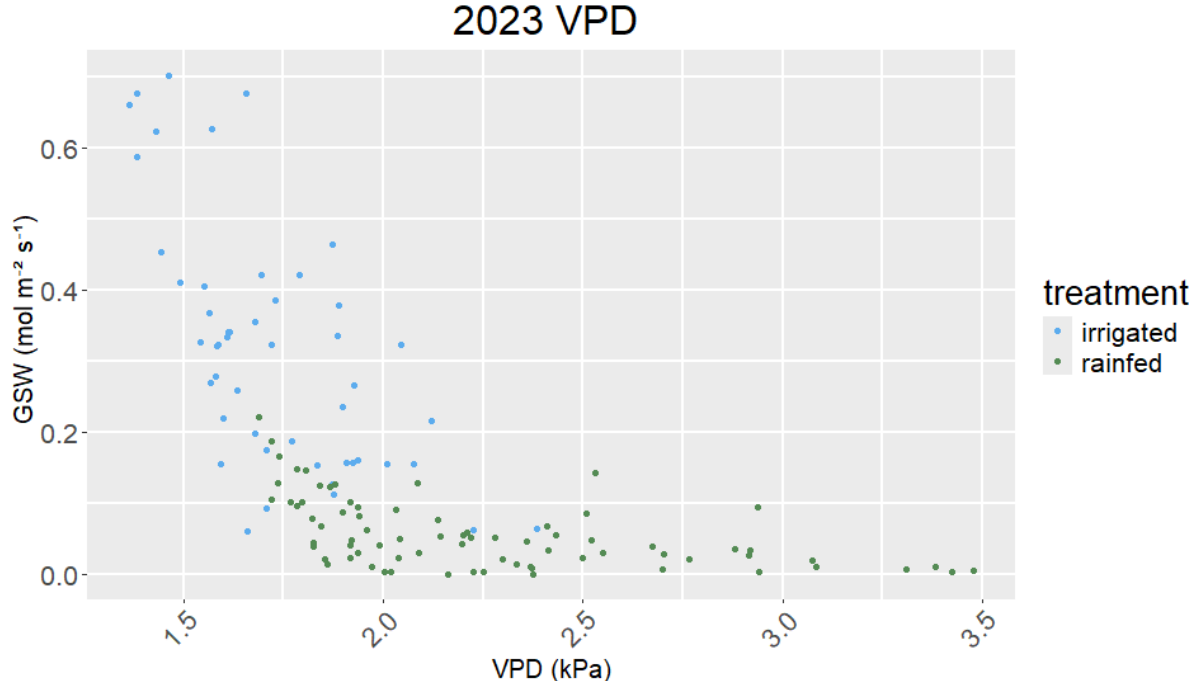


Figure 2.30 2024 Stomatal conductance (g_{sw}) was affected by drought and had a significant Treatment by VPD interaction

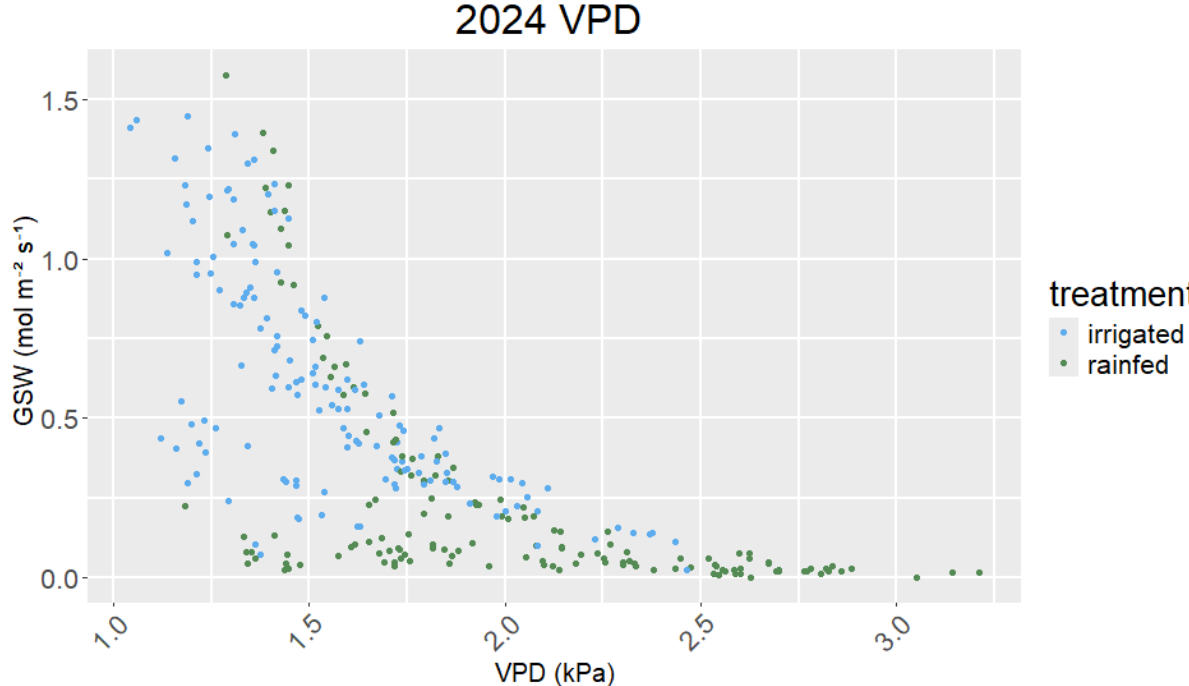


Figure 2.31 2023 Root depth only had a significant date effect

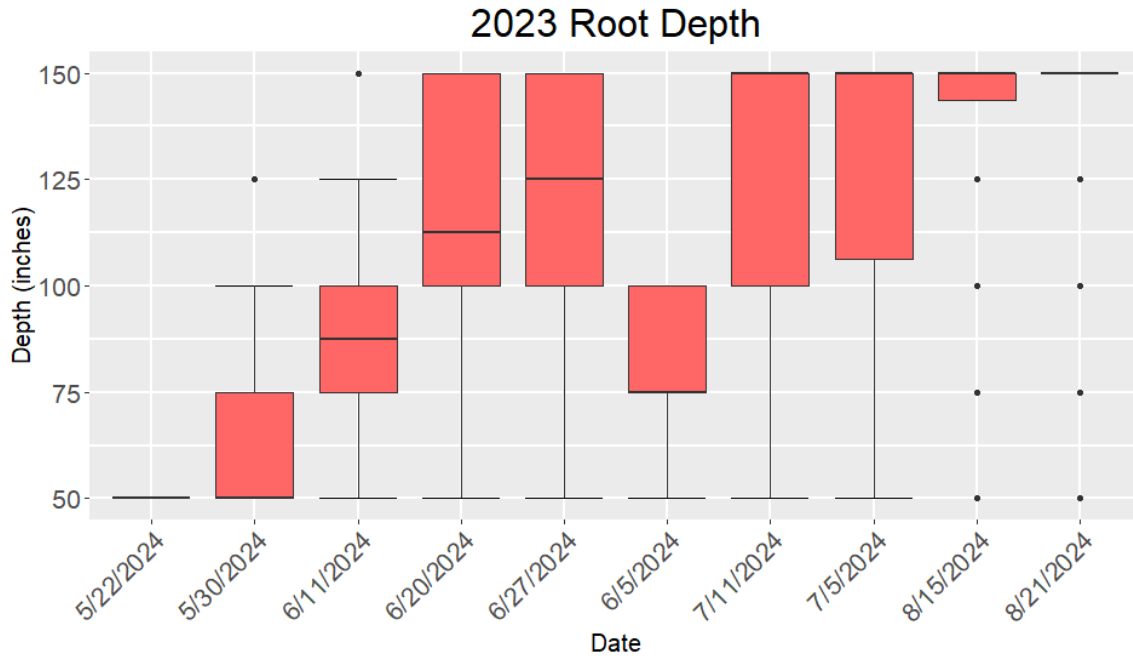


Figure 2.32 2024 Root depth was affected by drought with a treatment by date interaction

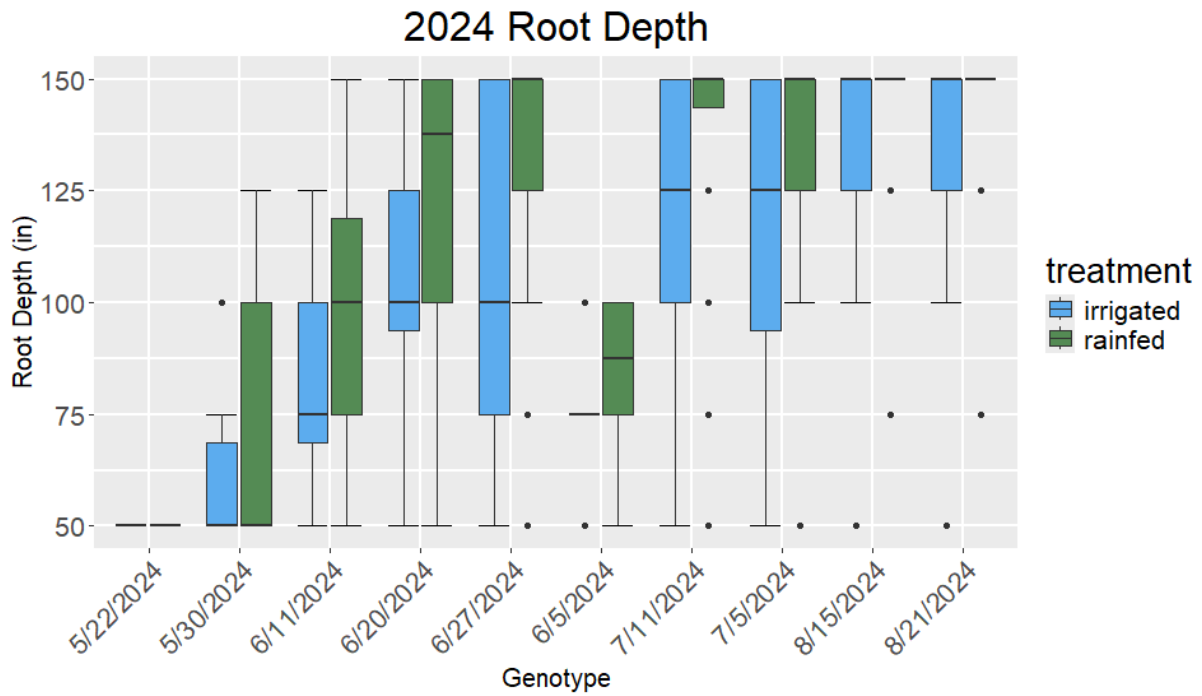


Figure 2.33 2023 Leaf area index (LAI) was affected by drought with a significant treatment by date interaction

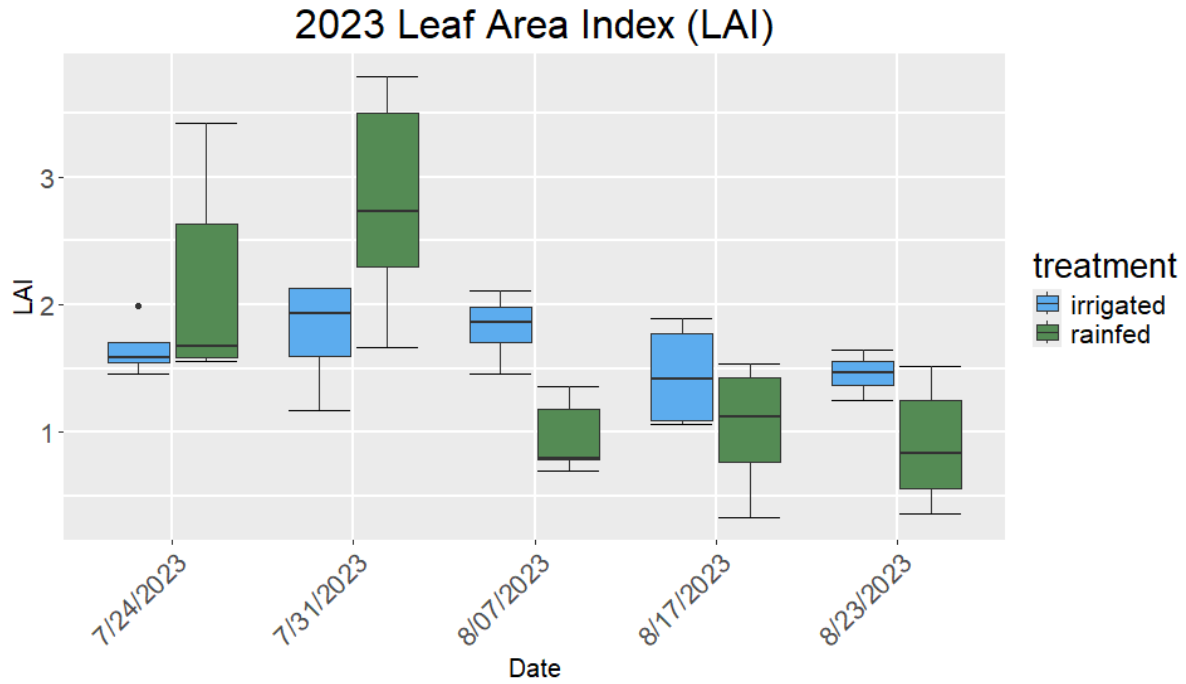


Figure 2.34 2024 Leaf area index (LAI) was affected by drought with a significant treatment, genotype, and date interaction

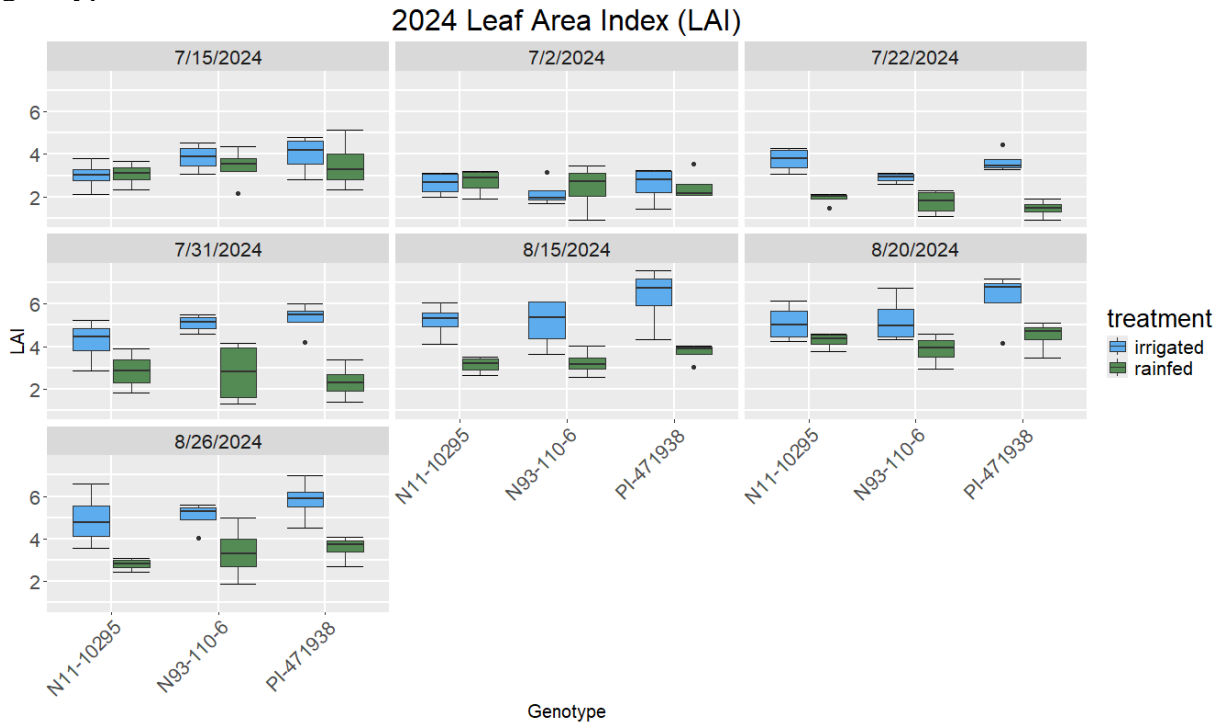


Figure 2.35 2023 % Nitrogen was affected by drought with a significant interaction between treatment and date

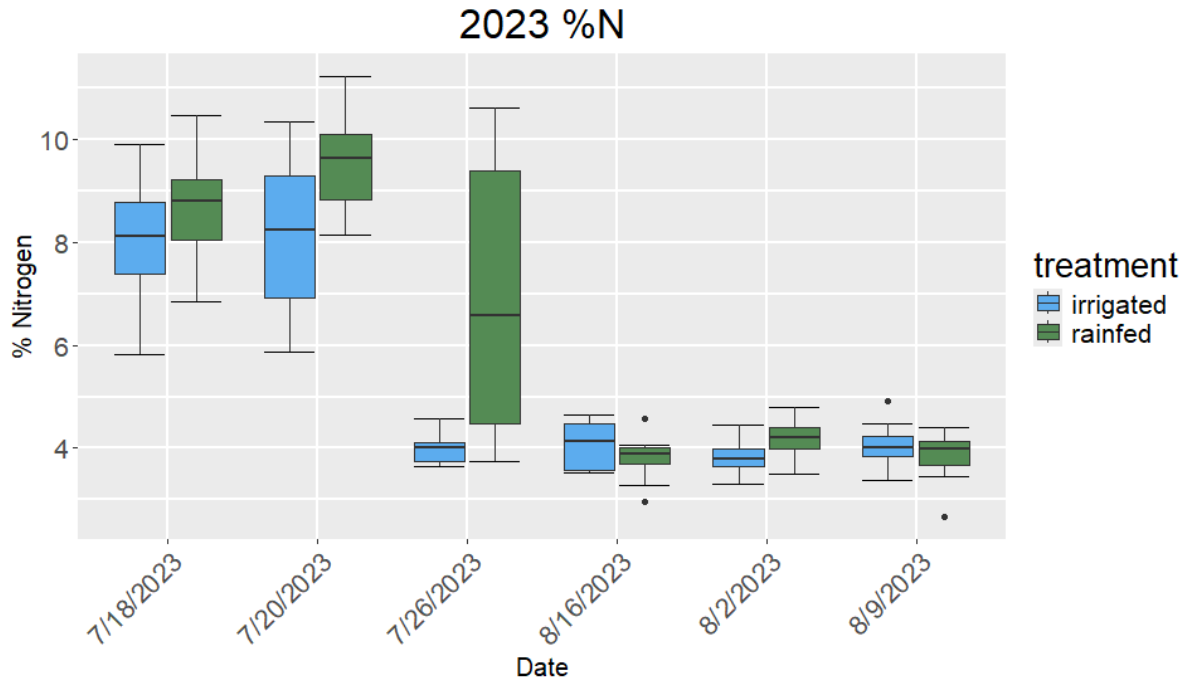


Figure 2.36 2024 % Nitrogen was affected by drought with a significant interaction between treatment, genotype, and date

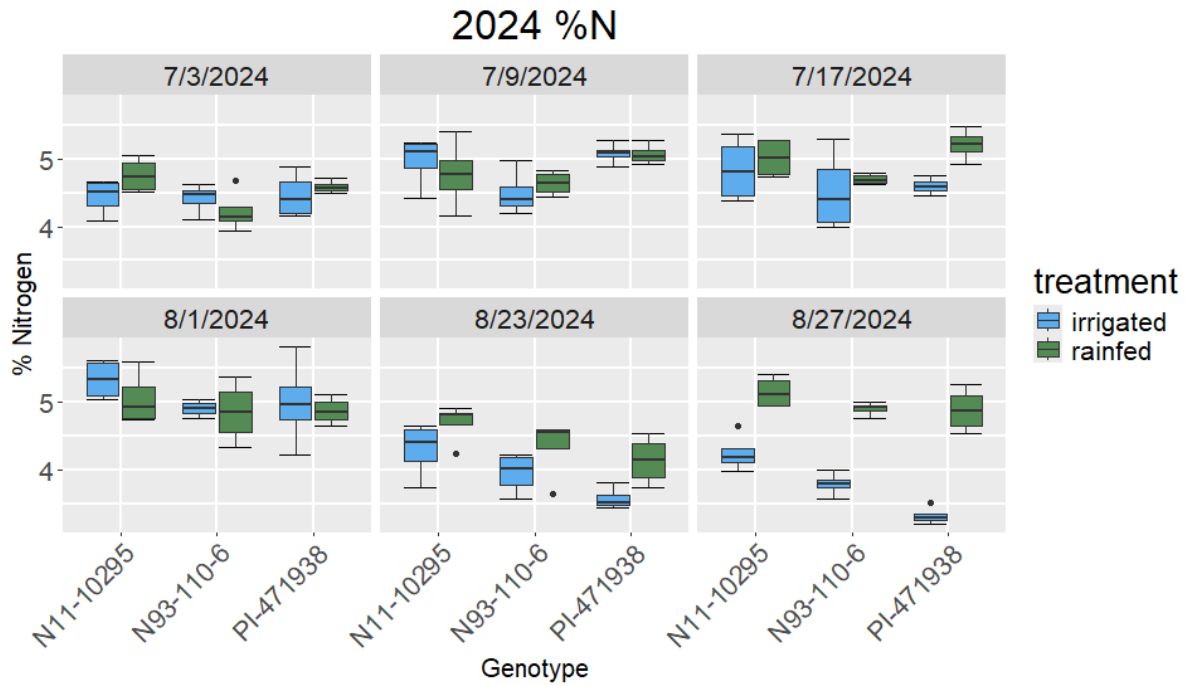


Figure 2.37 2023 Total Canopy Nitrogen was affected by drought with a significant interaction among treatment and genotype. There were not significant pairwise comparisons

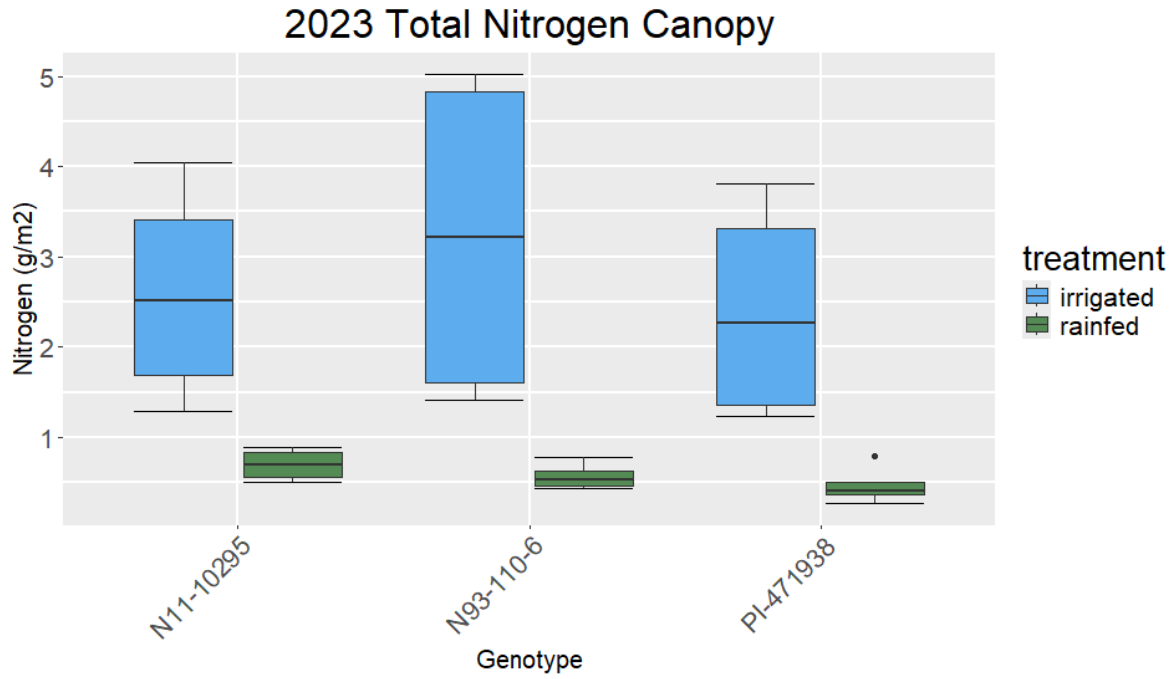


Figure 2.38 2024 Total Canopy Nitrogen as affected by drought with a significant interaction among treatments and genotypes. There were not significant pairwise comparisons

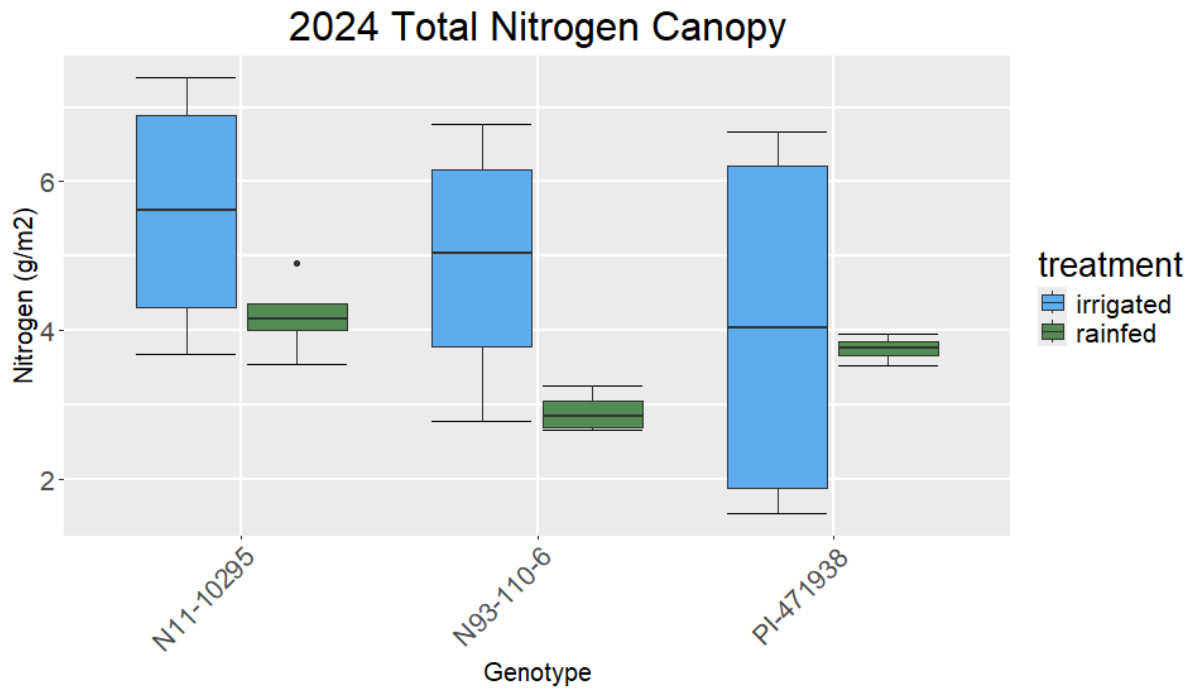


Figure 2.39 2023 Biomass was affected by drought with a significant treatment affect

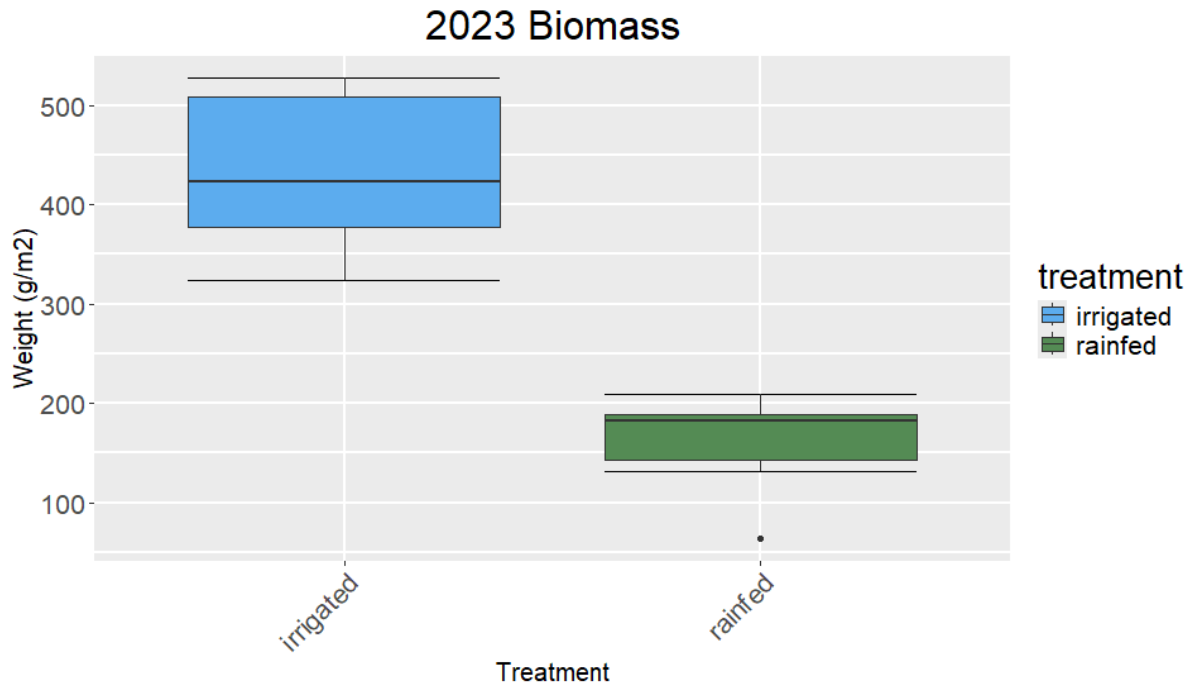


Figure 2.40 2024 Biomass was affected by drought with a significant interaction among treatments and genotypes. No pairwise comparisons significant

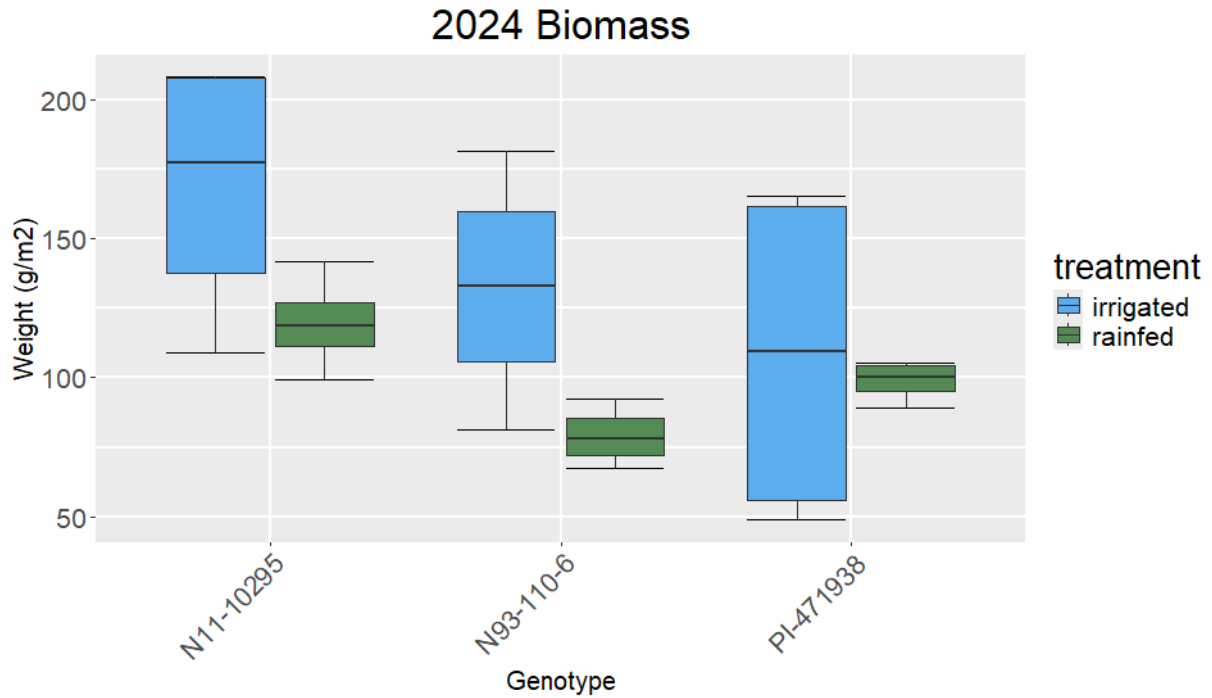


Figure 2.41 2023 Yield was affected by drought with a significant treatment affect

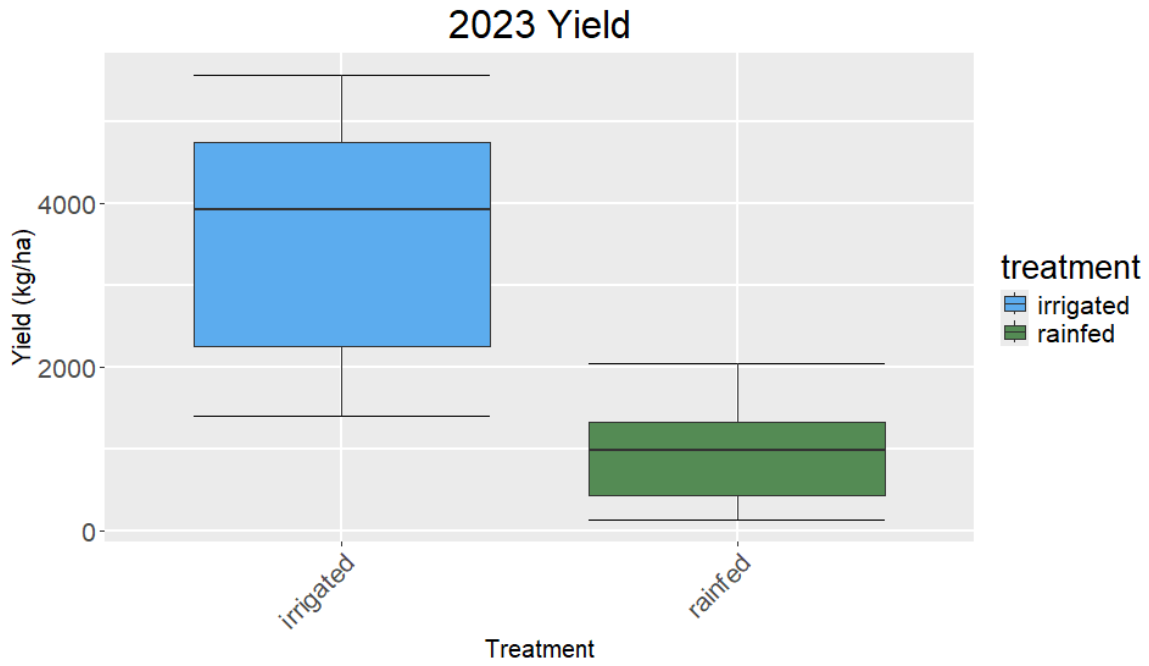


Figure 2.42 2024 Yield was not affected by drought but had a significant genotype affect

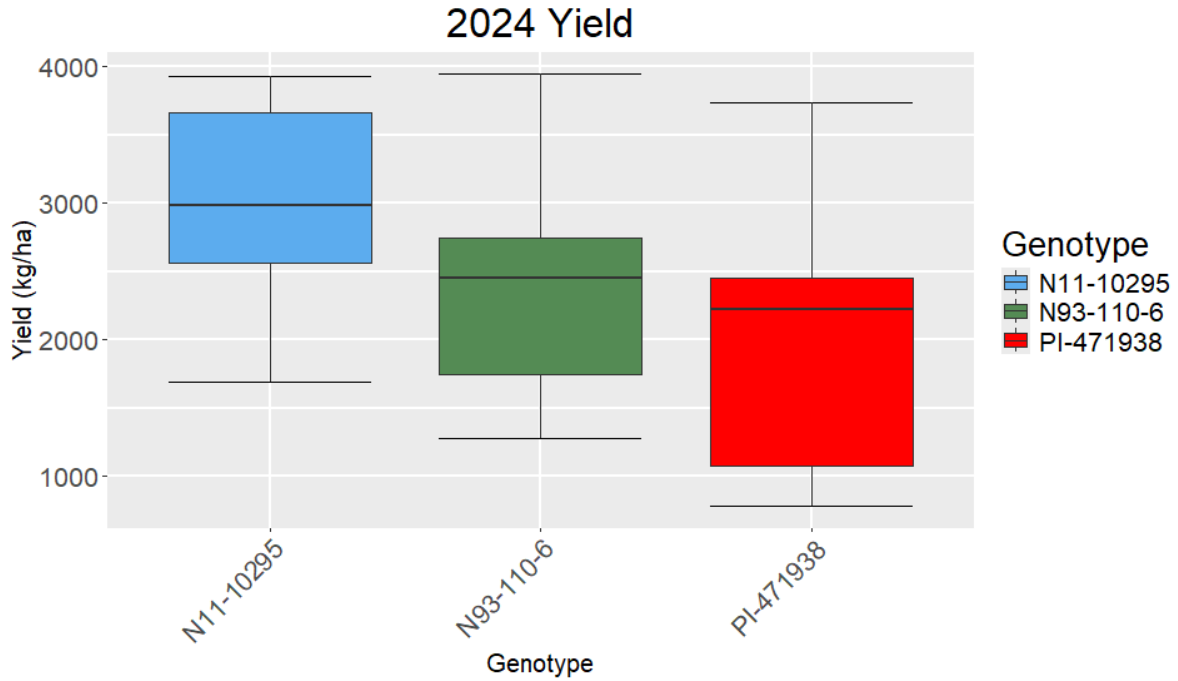


Figure 2.43 2023 Seed weight was affected by drought with a significant treatment by genotype interaction

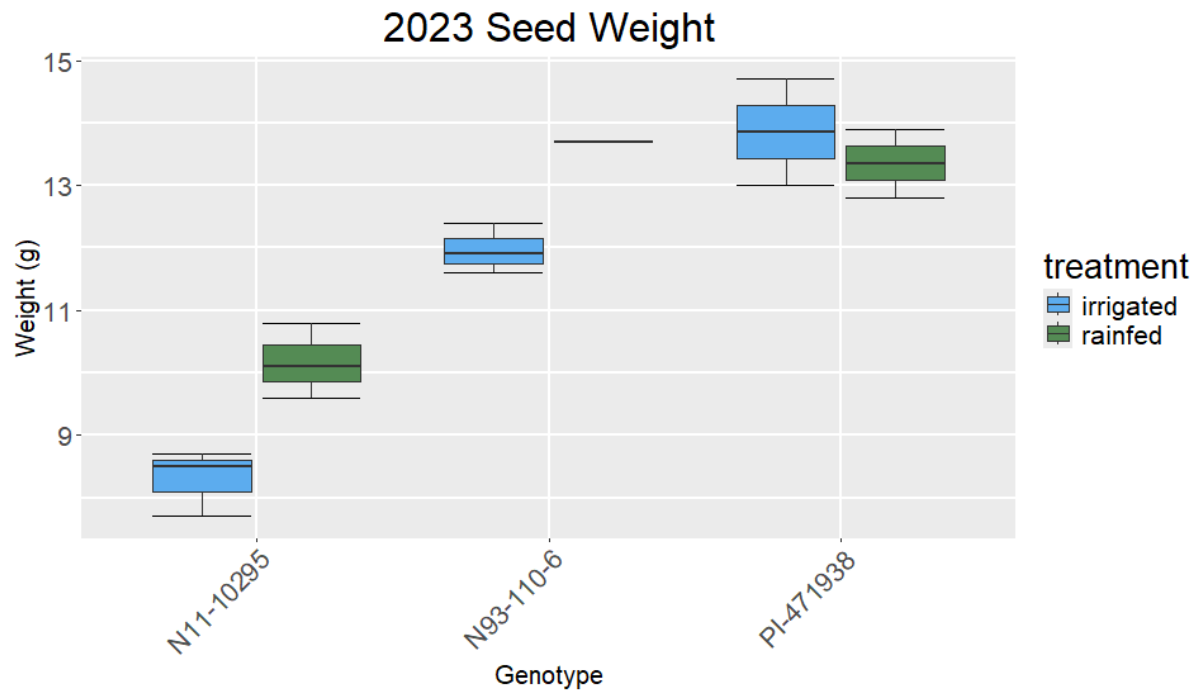


Figure 2.44 2024 Seed weight had a genotype and treatment affect
2024 Seed Weight

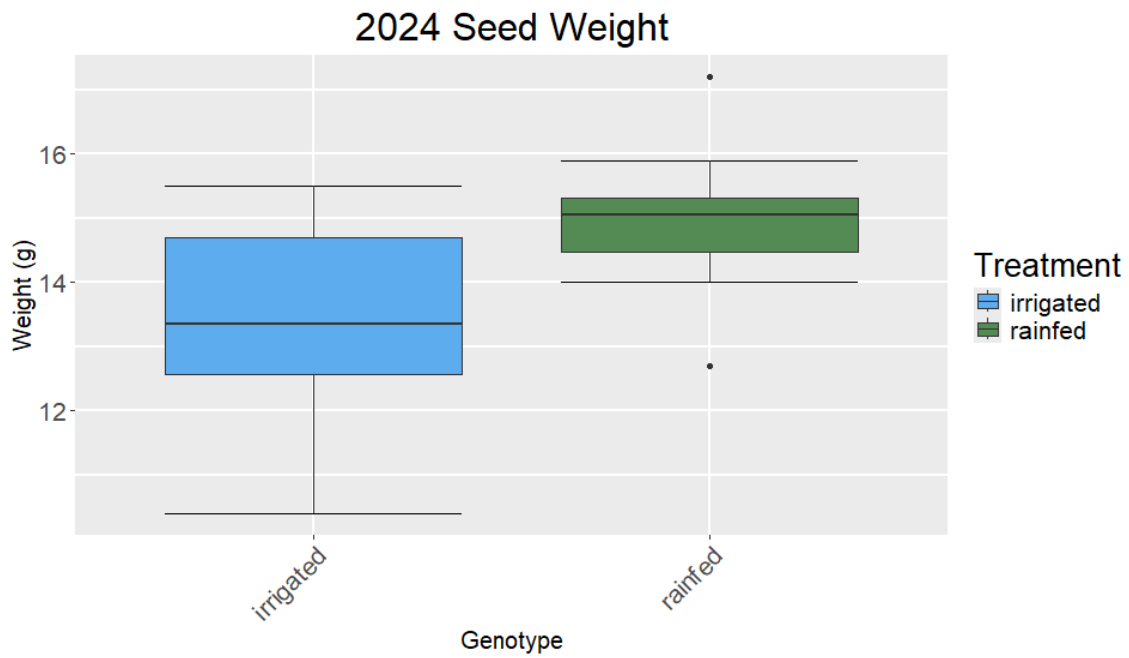
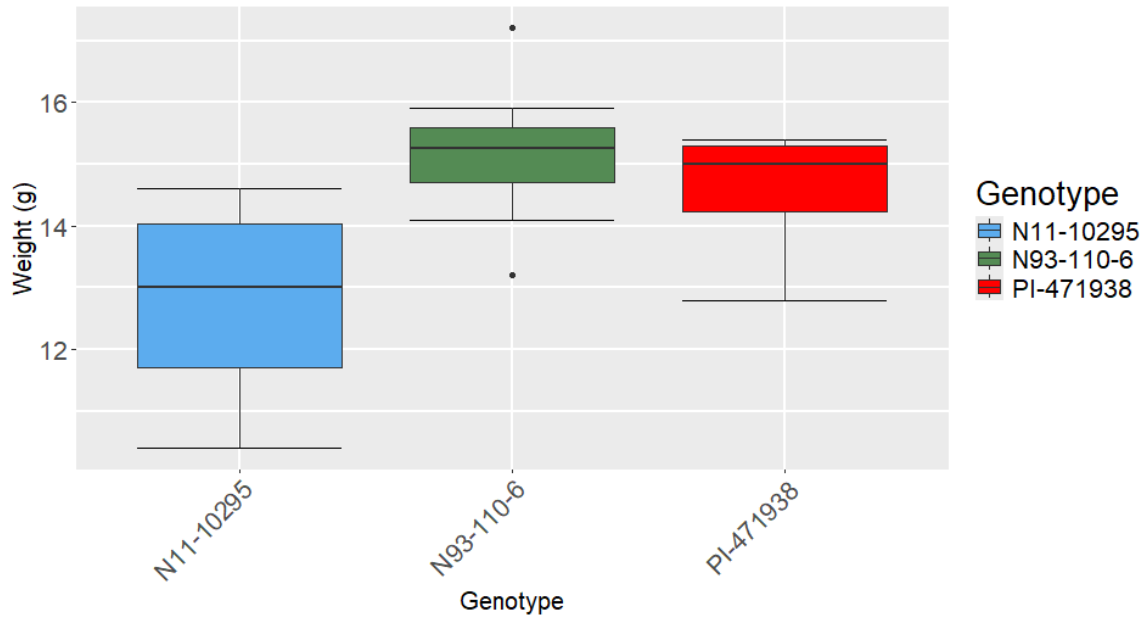


Figure 2.45 2023 Seed oil and protein was not significant in ANOVA

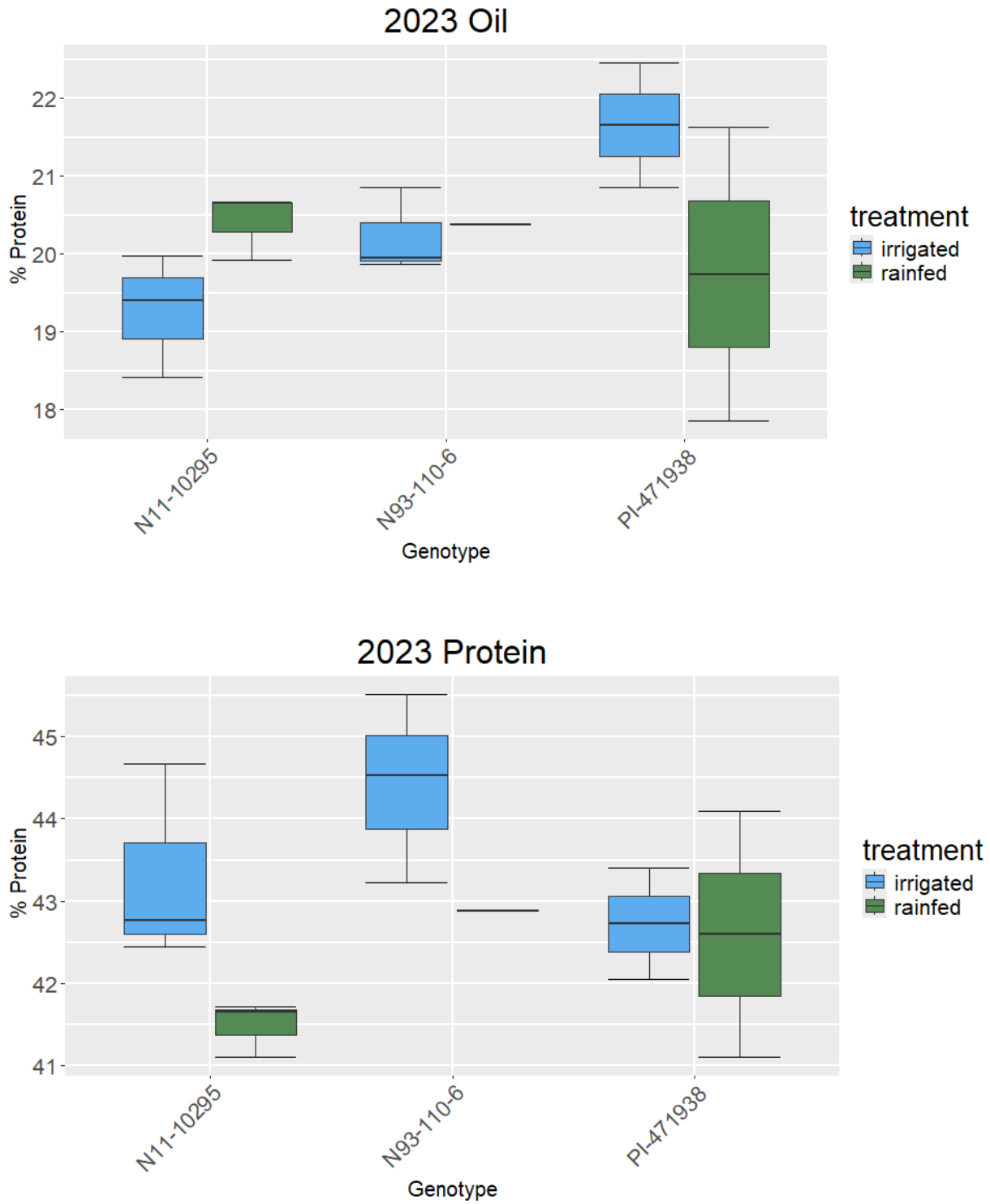


Figure 2.46 2024 Oil and protein had a genotype affect

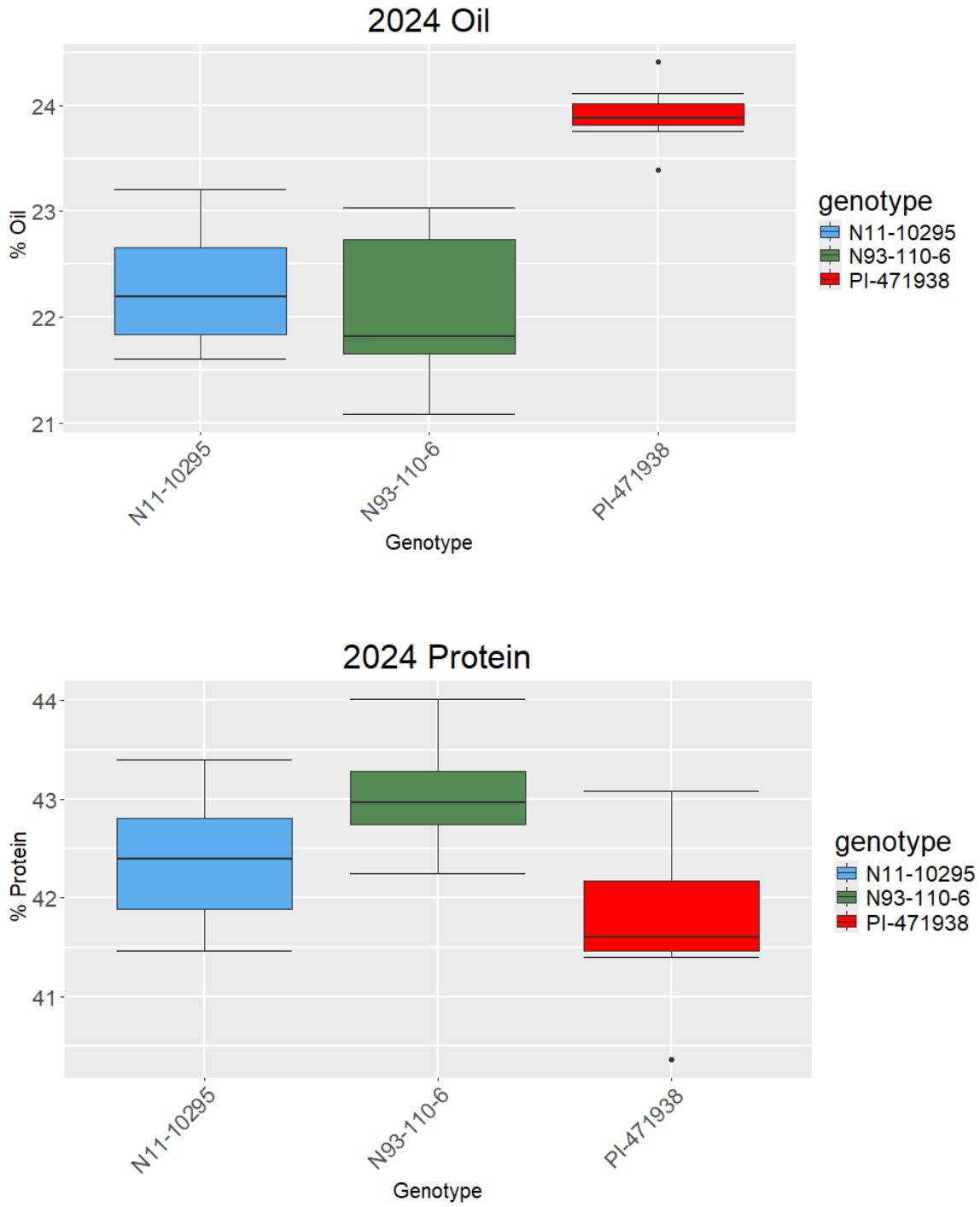


Figure 2.47 2024 Leaf nitrogen (g/cm^2) was affected by drought with a significant treatment, genotype, date interaction

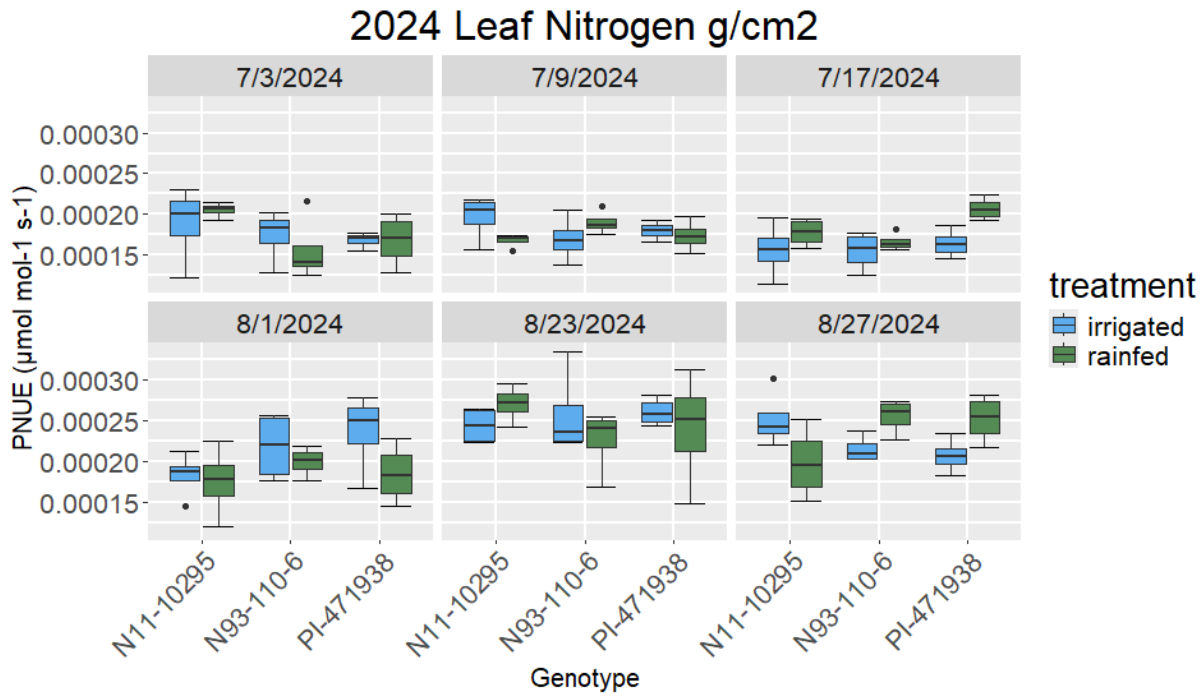


Figure 2.48 2024 Photosynthetic nitrogen use efficiency was affected by drought with a significant treatment, genotype, date interaction

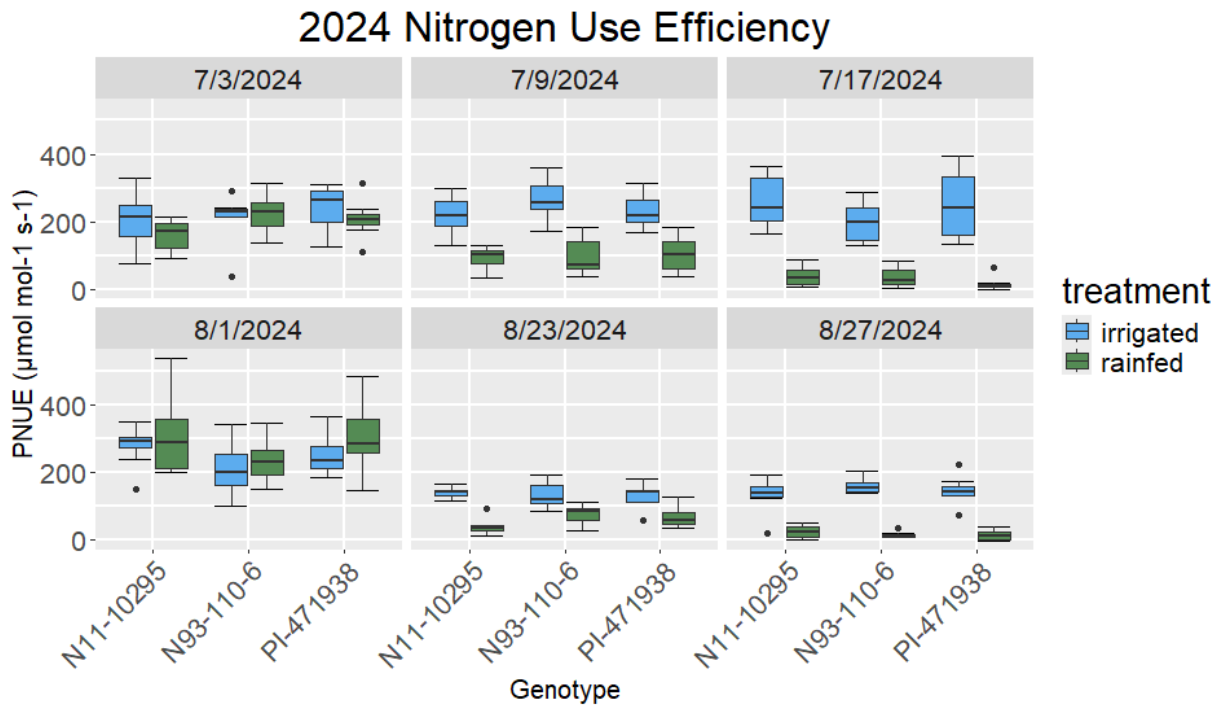


Table 2.1 Soybean Pedigree for genotypes N11-10295, N93-1106, and PI-471398

Genotype	Parents	Antecedent	Maturity Group
N11-10295	N01-11298 × N04-9646	12.5% PI 416937	VII
N93-110-6	Young × PI 416937	50% PI 416937	VI
PI 471938	Introduction		V

Table 2.2 2023 Daily total rain, maximum air temperature, and average air temperature data collected from NC ECONet Jackson station

Date	Total Rain (cm)	Max Air Temperature (C)	Average Air Temperature (C)
5-5-2023	0	24	16.6
5-6-2023	0	26.1	19.2
5-7-2023	0	25.9	20.1
5-8-2023	0	27.5	22
5-9-2023	0	30.2	23.1
5-10-2023	0	25.5	19.6
5-11-2023	0	27.8	20.5
5-12-2023	0	28.7	21.7
5-13-2023	0	27.6	22.2
5-14-2023	1.1	24.2	20.3
5-15-2023	0	26.2	20.8
5-16-2023	0.8	29.1	22.9
5-17-2023	0	26.7	21.7
5-18-2023	0	22.9	18.8
5-19-2023	0.6	19.4	16.2
5-20-2023	0	22.5	19.4
5-21-2023	0	22.6	19.4
5-22-2023	0	26.5	20.1
5-23-2023	0	24.8	19.2
5-24-2023	0	25.1	18.3
5-25-2023	0	24.5	18.1
5-26-2023	0	20.3	16.5
5-27-2023	1.3	14.8	13.7
5-28-2023	2	18.3	15.6
5-29-2023	0.2	21.3	17.7
5-30-2023	1.5	23.7	19
5-31-2023	0	25.4	20.3
6-1-2023	0	25.6	20.8
6-2-2023	0	27.7	22.3
6-3-2023	0	30.9	24.1
6-4-2023	0	20.9	17.9
6-5-2023	0	25	18.7
6-6-2023	0	28.4	21.6

Table 2.2 Continued

6-7-2023	0.4	26.1	19.8
6-8-2023	0	25.7	19.5
6-9-2023	0	26.6	20.2
6-10-2023	0	29.5	22.5
6-11-2023	0.3	30.6	21.8
6-12-2023	0.2	29.5	22.6
6-13-2023	0	27.9	22
6-14-2023	0	29.2	22.3
6-15-2023	0	30.2	24.1
6-16-2023	0	32.8	25.5
6-17-2023	0	29.9	23.5
6-18-2023	0	32.6	24.9
6-19-2023	0.5	30.8	23.4
6-20-2023	1.3	24.4	21.6
6-21-2023	2.7	23	20.3
6-22-2023	2.2	25.3	20.8
6-23-2023	0	27.5	23.6
6-24-2023	0	28.3	23.3
6-25-2023	0	31.6	26.1
6-26-2023	1.8	32.1	25.7
6-27-2023	0	31.2	24.9
6-28-2023	0	30.3	25.2
6-29-2023	0	31.4	25.1
6-30-2023	0	31.1	24.9
7-1-2023	0.1	32.6	26
7-2-2023	0	34.1	27.5
7-3-2023	0	32.5	28.3
7-4-2023	0	34.3	28.6
7-5-2023	0.1	32.6	26.3
7-6-2023	1.6	32.8	25.9
7-7-2023	0	33.3	26
7-8-2023	0.1	30.7	24.9
7-9-2023	2.1	31.7	24.6
7-10-2023	0	27.9	23.7
7-11-2023	0	31.7	26
7-12-2023	0	33.4	27.7
7-13-2023	0	34.3	27.7
7-14-2023	0	33.2	26.3
7-15-2023	0	31.2	25.5
7-16-2023	0.1	33.3	27.4
7-17-2023	0	32.1	26
7-18-2023	0	34.6	27.5
7-19-2023	0	34.8	28.2
7-20-2023	0	33.6	27.3
7-21-2023	0	35.1	28

Table 2.2 Continued

7-22-2023	0	30.3	25.4
7-23-2023	0	31.9	24.9
7-24-2023	0	31.8	25.4
7-25-2023	0	35.3	28.1
7-26-2023	0	35	28.3
7-27-2023	0	35.3	28.6
7-28-2023	0	35	28.1
7-29-2023	0	32.3	27.6
7-30-2023	0	34	26.9
7-31-2023	0	31.9	24.7
8-1-2023	0	31.6	24.8
8-2-2023	0	30.1	23.9
8-3-2023	0	25.7	22.3
8-4-2023	0.2	27.2	23
8-5-2023	0	33.9	26.4
8-6-2023	0.3	34.5	26.4
8-7-2023	0.1	34.8	25.1
8-8-2023	0	32.1	25.7
8-9-2023	0	32.4	26.4
8-10-2023	0.8	31	25.5
8-11-2023	0	31.6	26.3
8-12-2023	0	34.4	27.5
8-13-2023	0	36.3	28.9
8-14-2023	0.2	35.6	28.1
8-15-2023	0.2	37.2	28.3
8-16-2023	0	33.3	26.7
8-17-2023	0	32.5	26.5
8-18-2023	0	33	26.2
8-19-2023	0	33	25.8
8-20-2023	0	35.4	27.3
8-21-2023	0	35.9	28.9
8-22-2023	0	35.6	28.9
8-23-2023	0	31.5	25.9
8-24-2023	0	36.3	26.4
8-25-2023	0	35.3	27.5
8-26-2023	0	36.7	29.5
8-27-2023	2.2	34.8	26.5
8-28-2023	0.1	31.6	25.4
8-29-2023	5	30.9	24.2
8-30-2023	2.5	27.1	23.8
8-31-2023	0.3	28.3	22.8

Table 2.3 2024 Daily total rain, maximum air temperature, and average air temperature data collected from NC ECONet Jack station

Date	Total Rain (cm)	Max Air Temperature (C)	Average Air Temperature (C)
5-5-2024	1.1	26.5	21.5
5-6-2024	0.1	24.5	21.4
5-7-2024	0.2	29	23
5-8-2024	1.4	29.8	23
5-9-2024	1	25.2	20.5
5-10-2024	0	26.7	20.3
5-11-2024	0	22.4	17
5-12-2024	0	25.5	19.1
5-13-2024	0	24.7	19
5-14-2024	3.7	22.2	18.5
5-15-2024	3.3	26.5	18.9
5-16-2024	0	26.1	20.6
5-17-2024	1.3	26.6	22.4
5-18-2024	0.5	25.9	21.6
5-19-2024	0	21.5	18.1
5-20-2024	0	25.6	19.9
5-21-2024	0	29.7	21.8
5-22-2024	0	29.8	23.1
5-23-2024	0.3	31.1	24.3
5-24-2024	0.2	29.2	23.5
5-25-2024	0	29.9	24.7
5-26-2024	1.1	31.9	25.4
5-27-2024	0.6	29.3	24.2
5-28-2024	0	30.1	24.4
5-29-2024	0	29	23.6
5-30-2024	0	26.1	20.5
5-31-2024	0	24.5	19.1
6-1-2024	0	27.4	20.2
6-2-2024	0	24.2	20.7
6-3-2024	2.1	27.6	22.6
6-4-2024	0.8	30.7	23.8
6-5-2024 ⁵	0.3	29	22.9
6-6-2024	0.2	30.8	24.4
6-7-2024	0	30.3	25.1
6-8-2024	0	29.8	23.8
6-9-2024	0.8	30.4	24.4
6-10-2024	0	28	22.6
6-11-2024	0	28.3	22.7
6-12-2024	0	29.7	23.9
6-13-2024	0	30.9	24.9
6-14-2024	0	32.8	25.6

Table 2.3 Continued

6-15-2024	0	32.5	27.2
6-16-2024	0	31.9	26.6
6-17-2024	0	32.2	25.8
6-18-2024	0	32.2	25.4
6-19-2024	0	31.4	25.1
6-20-2024	0	31.9	25.2
6-21-2024	0	33.9	26.3
6-22-2024	0	34.6	28.2
6-23-2024	0	35.2	28.5
6-24-2024	0.1	34.8	29
6-25-2024	0	36.2	28.5
6-26-2024	0	36.8	29
6-27-2024	0	29.4	26.3
6-28-2024	0	33.8	26.7
6-29-2024	0	36.1	28.5
6-30-2024	2.6	34.5	27.7
7-1-2024	0	28.6	24.6
7-2-2024	0	30.1	23.2
7-3-2024	0	33.4	26.1
7-4-2024	0	35.7	28.8
7-5-2024	0	37.5	30.2
7-6-2024	0.7	36	27.7
7-7-2024	0	33.2	27
7-8-2024	0.2	32.7	26.9
7-9-2024	0	36.1	28.4
7-10-2024	0	35.4	29.5
7-11-2024	0	31.9	27.2
7-12-2024	0.3	28.8	24.6
7-13-2024	0	34.5	27.7
7-14-2024	0	36.9	29.4
7-15-2024	0	37.6	29.3
7-16-2024	0	36.9	29.1
7-17-2024	0	36.1	28.7
7-18-2024	0.3	35.1	26.6
7-19-2024	0.6	28.7	23.9
7-20-2024	0.4	30.6	24.5
7-21-2024	2	32	25.3
7-22-2024	1.6	30.2	24.9
7-23-2024	0.1	32.5	26.2
7-24-2024	0.3	32.6	26.2
7-25-2024	1.8	27.9	24.1
7-26-2024	0.4	26.4	23.9
7-27-2024	0	29.1	24.6
7-28-2024	0	31.3	24.9
7-29-2024	2.6	23.8	22

Table 2.3 Continued

7-30-2024	0	30.1	25.6
7-31-2024	0	31.6	26.2
8-1-2024	0	34.3	28.7
8-2-2024	0	33.7	27
8-3-2024	4.3	32.6	24.7
8-4-2024	2	30.9	24.8
8-5-2024	0	30.3	25.9
8-6-2024	0.5	24.7	23.2
8-7-2024	2.5	27	24.2
8-8-2024	7.7	25.8	23.6
8-9-2024	0.1	32.4	26
8-10-2024	0.1	32.8	27.2
8-11-2024	0	32.1	26.4
8-12-2024	2	28.8	24.9
8-13-2024	0.1	25.6	22.5
8-14-2024	0	29.8	23.8
8-15-2024	0	30.5	24.2
8-16-2024	0	31	25
8-17-2024	0	30.9	25.9
8-18-2024	0.4	31.1	24.8
8-19-2024	0	30.5	24.1
8-20-2024	0	27.2	22.4
8-21-2024	0	24.8	19.5
8-22-2024	0	26	19.4
8-23-2024	0	26.7	20.1
8-24-2024	0	28.3	21.2
8-25-2024	0	29.6	22.2
8-26-2024	0	31.7	24.3
8-27-2024	0	33.9	26.1
8-28-2024	0	34.9	26.9
8-29-2024	2.4	35.8	27.2
8-30-2024	1.2	33.9	26.1

Table 2.4 Type III analysis of variance results table with Satterthwaite's method for 2023 LI-COR 6800 data collection

		Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
<i>A</i>	Genotype	259.3	129.7	2	94	3.8743	0.02417*
	Treatment	5818.8	5818.8	1	94	173.8585	< 2e-16*
	Date	455.3	91.1	5	94	2.7208	0.02429*
	Genotype × Treatment	313.1	156.5	2	94	4.6769	0.01158*
	Genotype × Date	292.5	29.2	10	94	0.8739	0.56026
	Treatment × Date	398.9	79.8	5	94	2.3836	0.04405*
	Genotype × Treatment × Date	240.6	24.1	10	94	0.7190	0.70470
<i>g_{sw}</i>	Genotype	0.09118	0.04559	2	89	3.7768	0.02665*
	Treatment	1.39317	1.39317	1	89	115.4157	< 2e-16*
	Date	0.13417	0.02683	5	89	2.2230	0.05883*
	Genotype × Treatment	0.08962	0.04481	2	89	3.7121	0.02829*
	Genotype × Date	0.08687	0.00869	10	89	0.7197	0.70394
	Treatment × Date	0.18459	0.03692	5	89	3.0585	0.01356*
	Genotype × Treatment × Date	0.05974	0.00597	10	89	0.4949	0.88922
Φ PSII	Genotype	0.003279	0.001639	2	85	0.7937	0.455476
	Treatment	0.256750	0.256750	1	85	124.3160	< 2.2e16*
	Date	0.069023	0.01385	5	85	6.6841	2.697e05*
	Genotype × Treatment	0.010850	0.005425	2	85	2.6267	0.078184*
	Genotype × Date	0.023470	0.002347	10	85	1.1364	0.345412
	Treatment × Date	0.042895	0.008579	5	85	4.1539	0.002005*
	Genotype × Treatment × Date	0.035179	0.003518	10	85	1.7033	0.093066*
<i>i</i> WUE	Genotype	2915	1458	2	112	0.5803	0.5614
	Treatment	46510	46510	1	112	18.5172	3.619e-05*
	Genotype × Treatment	8967	4484	2	112	1.7851	0.1725

* Significant *p* value

2.5 Type III analysis of variance results table with Satterthwaite's method for 2024 LI-COR 6800 data collection

		Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Assimilation							
	Genotype	90.3	45.2	2	246	1.1812	0.3086
	Treatment	3799.3	3799.3	1	6	99.3608	5.8999e-05 *
	Date	17048.5	3409.7	5	246	89.1721	< 2.2e-16 *
	Genotype × Treatment	74.0	37.0	2	246	0.9671	0.3816
	Genotype × Date	534.3	53.4	10	246	1.3974	0.1818
	Treatment × Date	5194.3	1038.9	5	246	27.1690	< 2.2e-16 *
	Genotype × Treatment × Date	187.0	18.7	10	246	0.4891	0.8964
<i>g_{sw}</i>							
	Genotype	0.3542	0.1771	2	246	4.6889	0.010035 *
	Treatment	0.9549	0.9549	1	6	25.2837	0.002384 *
	Date	16.0277	3.2055	5	246	84.8795	< 2.2e-16 *
	Genotype × Treatment	0.1925	0.0963	2	246	2.5491	0.080215
	Genotype × Date	0.8474	0.0847	10	246	2.2437	0.016061 *
	Treatment × Date	5.6916	1.1383	5	246	30.1417	< 2.2e-16 *
	Genotype × Treatment × Date	0.2666	0.0267	10	246	0.7060	0.718572
ΦPSII							
	Genotype	0.1489	0.0745	2	246	1.6782	0.18884
	Treatment	1.4000	1.4000	1	6	31.5487	0.00136 *
	Date	24.0066	4.8013	5	246	108.1998	< 2e-16 *
	Genotype × Treatment	0.2527	0.1263	2	246	2.8473	0.05991 *
	Genotype × Date	0.6860	0.0686	10	246	1.5460	0.12373
	Treatment × Date	7.4186	1.4837	5	246	33.4363	< 2e-16 *
	Genotype × Treatment × Date	0.2182	0.0218	10	246	0.4918	0.89462

Table 2.5 Continued

	Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
iWUE						
Genotype	1314	657.1	2	244.035	1.0484	0.3520650
Treatment	25288	25288.2	1	6.019	40.3487	0.0007
Date	110915	22182.9	5	244.045	35.3941	< 2.2e-16
Genotype × Treatment	6031	3015.7	2	244.035	4.8117	0.0090 *
Genotype × Date	11818	1181.8	10	244.044	1.8856	0.0478 *
Treatment × Date	68209	13641.9	5	244.045	21.7664	< 2.2e-16 *
Genotype × Treatment × Date	4637	463.7	10	244.044	0.7398	0.6865482

* Significant p-value

Table 2.6 Type III analysis of variance results table with Satterthwaite's method for 2023 leaf water potential, turgor pressure, and osmotic potential

	Sum of Squares	Mean Squares	Num DF	Den DF	F-value	Pr(>F)
Ψ_{leaf}						
Genotype	0.7886	0.3943	2	102	2.0777	0.1304802
Treatment	6.9358	6.9358	1	6	36.5486	0.0009270*
Date	4.4246	0.8849	5	102	4.6631	0.0007109*
Genotype \times Treatment	0.2862	0.1431	2	102	0.7540	0.4730769
Genotype \times Date	3.3854	0.3385	10	102	1.7840	0.0728136
Treatment \times Date	1.0394	0.2079	5	102	1.0954	0.3675985
Genotype \times Treatment \times Date	2.3412	0.2341	10	102	1.2337	0.2787868
Ψ_{P}						
Genotype	0.30944	0.154720	2	64.369	1.2039	0.3067
Treatment	0.07226	0.072256	1	4.602	0.5622	0.4899
Date	0.46749	0.093497	5	62.843	0.7275	0.6054
Genotype \times Treatment	0.48460	0.242298	2	64.561	1.8853	0.1600
Genotype \times Date	1.22185	0.122185	10	60.579	0.9507	0.4948
Treatment \times Date	0.17352	0.034704	5	62.767	0.2700	0.9278
Genotype \times Treatment \times Date	1.31867	0.146519	9	60.469	1.1401	0.3496
Ψ_{II}						
Genotype	0.38027	0.19013	2	64.714	3.6223	0.03227*
Treatment	1.44874	1.44874	1	6.175	27.6022	0.00175*
Date	0.66574	0.13315	5	63.584	2.5366	0.03718*
Genotype \times Treatment	0.48109	0.24054	2	64.851	4.5826	0.01375*
Genotype \times Date	0.48214	0.04821	10	61.899	0.9185	0.52234
Treatment \times Date	0.53405	0.10681	5	63.528	2.0348	0.08561*
Genotype \times Treatment \times Date	0.92364	0.10263	9	61.823	1.9551	0.06029*

* Significant p value

Table 2.7 Type III analysis of variance results table with Satterthwaite's method for 2024 leaf water potential, turgor pressure, and osmotic potential

	Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Ψ_{leaf}						
Genotype	0.2199	0.1099	2	107.185	2.1063	0.12669
Treatment	4.7693	4.7693	1	8.786	91.3665	6.178e-06*
Date	5.8751	1.1750	5	102.792	22.5100	3.469e-15*
Genotype \times Treatment	0.4421	0.2210	2	107.198	4.2343	0.01699 *
Genotype \times Date	0.4233	0.0423	10	102.792	0.8109	0.61865
Treatment \times Date	5.4398	1.0880	5	102.792	20.8421	2.502e-14*
Genotype \times Treatment \times Date	0.3930	0.0393	10	102.792	0.7529	0.67328
Ψ_{P}						
Genotype	0.13552	0.06776	2	98.225	1.5103	0.2259334
Treatment	1.49521	1.49521	1	12.221	33.3261	8.231e-05*
Date	1.19884	0.23977	5	96.348	5.3441	0.0002229*
Genotype \times Treatment	0.24262	0.12131	2	98.387	2.7038	0.0719241*
Genotype \times Date	0.38300	0.03830	10	96.236	0.8537	0.5788502
Treatment \times Date	0.78468	0.15694	5	96.284	3.4979	0.0059980*
Genotype \times Treatment \times Date	0.48413	0.04841	10	96.286	1.0791	0.3858692
Ψ_{II}						
Genotype	0.47038	0.23519	2	95.623	5.5973	0.005026*
Treatment	0.58562	0.58562	1	6.440	13.9370	0.008525*
Date	1.87908	0.37582	5	94.025	8.9440	5.604e-07*
Genotype \times Treatment	0.24911	0.12455	2	94.990	2.9642	0.056394*
Genotype \times Date	0.54124	0.05412	10	93.806	1.2881	0.248481
Treatment \times Date	1.54800	0.30960	5	93.898	7.3682	7.263e-06*
Genotype \times Treatment \times Date	0.35748	0.03575	10	93.911	0.8508	0.581588

* Significant P-value

Table 2.8 Type III analysis of variance table with Satterthwaite's method for 2023 VPD data

	Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Genotype	0.01060	0.00530	2	112.58	0.7487	0.47530
VPD	0.33588	0.33588	1	110.23	47.4571	3.646e-10*
Treatment	0.20740	0.20740	1	111.93	29.3041	3.568e-07*
Genotype × VPD	0.00661	0.00330	2	111.44	0.4669	0.62817
Genotype × Treatment	0.03454	0.01727	2	112.58	2.4400	0.09176
VPD × Treatment	0.14243	0.14243	1	110.23	20.1237	1.789e-05*
Genotype × VPD × Treatment	0.03167	0.01583	2	111.44	2.2372	0.11153

Table 2.9 Type III analysis of variance table with Satterthwaite's method for 2024 VPD data

	Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Genotype	0.2590	0.1295	2	276	1.6942	0.1857
VPD	12.8902	12.8902	1	276	168.6060	< 2.2e-16*
Treatment	2.0946	2.0946	1	276	27.3984	3.284e-07*
Genotype × VPD	0.2202	0.1101	2	276	1.4399	0.2387
Genotype × Treatment	0.0198	0.0099	2	276	0.1297	0.8785
VPD × Treatment	1.5584	1.5584	1	276	20.3838	9.394e06*
Genotype × VPD × Treatment	0.0323	0.0161	2	276	0.2109	0.8099

Table 2.10 2023 Type II analysis of variance results with Satterthwaite's method for g_{sw} response to soil moisture at 30.5 cm depth with PI 471938 removed from analysis

	Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Genotype	0.0000152	0.0000152	1	15.133	0.0015	0.9695
30.5 cm	0.0114739	0.0114739	1	21.247	1.1440	0.2968
Genotype \times 30.5 cm	0.0007211	0.0007211	1	14.426	0.0719	0.7924

¥ No significant p values

Table 2.11 2023 Type II analysis of variance results with Satterthwaite's method for 2023 g_{sw} response to soil moisture at 61 cm depth with PI 471938 removed from analysis

	Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Genotype	0.013857	0.013857	1	22.306	1.4964	0.23399
61 cm	0.030182	0.030182	1	19.703	3.2595	0.08632
Genotype \times 61 cm	0.019312	0.019312	1	13.101	2.0856	0.17218

¥ No significant p values

Table 2.12 2024 Type II analysis of variance results with Satterthwaite's method for 2023 g_{sw} response to soil moisture at 30.5 cm depth

	Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Genotype	1.2054	0.60270	2	175.97	5.3451	0.005577*
30.5 cm	0.6011	0.60112	1	125.50	5.3311	0.022584*
Genotype \times 30.5 cm	3.6645	1.8322	2	117.751	16.2493	5.863e-07*

Table 2.13 2024 Type II analysis of variance results with Satterthwaite's method for 2023 g_{sw} response to soil moisture at 61 cm depth

	Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Genotype	0.64028	0.32014	2	186.66	2.8326	0.06139
30.5 cm	0.39076	0.39076	1	178.25	3.4574	0.06462
Genotype \times 61 cm	1.03622	0.51811	2	172.38	4.5843	0.01149 *

Table 2.14 Type III analysis of variance results table with Satterthwaite's method for 2023 root depth

	Sum Squares	Mean Squares	Num DF	Den DF	F-value	Pr(>F)
Genotype	188	93.9	2	36.514	0.2545	0.7766
Treatment	149	149.5	1	5.983	0.4054	0.5479
Date	43426	10856.6	4	138.152	29.4441	< 2e-16 *
Genotype × Treatment	224	111.8	2	36.514	0.3033	0.7402
Genotype × Date	937	117.1	8	138.254	0.3177	0.9583
Treatment × Date	1198	299.6	4	138.152	0.8125	0.5192
Genotype × Treatment × Date	2046	255.7	8	138.254	0.6936	0.6967

* Significant p-value

Table 2.15 Type III analysis of variance results table with Satterthwaite's method for 2024 root depth

	Sum Squares	Mean Squares	Num DF	Den DF	F-value	Pr(>F)
Genotype	660	329.9	2	36.737	1.1333	0.332980
Treatment	197	196.7	1	6.266	0.6755	0.441300
Date	247442	27493.5	5	291.656	94.4321	< 2.2 e-16 *
Genotype × Treatment	590	295.0	2	34.616	1.0132	0.373572
Genotype × Date	4510	250.6	18	291.625	0.8607	0.627091
Treatment × Date	6609	734.3	9	291.660	2.5222	0.008509 *
Genotype × Treatment × Date	1750	109.4	16	291.420	0.3758	0.987058

* Significant p-value

Table 2.16 Type III analysis of variance results with Satterthwaite's method for 2023 LAI

	Sum of Squares	Mean Squares	Num DF	Den DF	F-value	Pr(>F)
Genotype	0.9811	0.49053	2	25	1.7528	0.193915
Treatment	0.5094	0.50937	1	25	1.8201	0.189390
Date	6.8147	1.70368	4	25	6.0878	0.001457*
Genotype × Treatment	1.2881	0.64404	2	25	2.3013	0.120951
Genotype × Date	0.5895	0.07369	8	25	0.2633	0.972126
Treatment × Date	3.4646	0.86614	4	25	3.0950	0.033668*
Genotype × Treatment × Date	1.9412	0.24266	8	25	0.8671	0.556204

* Significant *p* value

Table 2.17 Type III analysis of variance results with Satterthwaite's method for 2024 LAI

	Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Genotype	6.029	3.0144	2	120	4.7061	0.0107740*
Treatment	23.356	23.3563	1	6	36.4642	0.0009326*
Date	23.356	20.1571	6	120	31.4696	< 2.2e-16*
Genotype × Treatment	3.089	1.5443	2	120	2.4111	0.0940553*
Genotype × Date	8.378	0.6982	12	120	1.0900	0.3746357
Treatment × Date	31.657	5.2762	6	120	8.2373	1.793e-07*
Genotype × Treatment × Date	2.874	0.2395	12	120	0.3739	0.9703883

* Significant *p*-value

Table 2.18 Type II analysis of variance results table with Satterthwaite's method for 2023 biomass

	Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Genotype	21660	8308	2	5.8114	2.8967	0.13405
Treatment	115891	115891	1	1.3539	40.4093	0.05699 *
Genotype × Treatment	6806	3403	2	5.8114	1.1865	0.36974

* Significant *p* value

Table 2.19 Type II analysis of variance results table with Satterthwaite's method for 2024 biomass

	Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Genotype	8204.6	4102.3	2	16	17.5980	9.101e-05 *
Treatment	169.8	169.8	1	2	0.7283	0.48332
Genotype × Treatment	2302.8	1151.4	2	16	4.9392	0.02135 *

* Significant *p*-value among genotype and treatment interaction

Table 2.20 Type II analysis of variance results table with Satterthwaite's method for 2023 biomass canopy nitrogen and carbon concentrations

		Sum Squares	Mean Squares	Num DF	Den DF	F-value	Pr(>F)
% N	Genotype	28.532	14.266	2	15.9999	1.0163	0.3842
	Treatment	15.363	15.363	1	2.0004	1.0944	0.4053
	Genotype × Treatment	42.791	21.396	2	15.9999	1.5242	0.2478
% C	Genotype	28.532	14.266	2	15.9999	1.0163	0.3842
	Treatment	15.363	15.363	1	2.0004	1.0944	0.4053
	Genotype × Treatment	42.791	21.396	2	15.9999	1.5242	0.2478

‡ No significant *p* values

Table 2.21 Type II analysis of variance results table with Satterthwaite's method for 2024 biomass canopy nitrogen and carbon concentration

		Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
% N	Genotype	0.40891	0.204453	2	12	7.0350	0.009511*
	Treatment	0.01722	0.017220	1	2	0.5925	0.521930
	Genotype × Treatment	0.04633	0.023167	2	12	0.7972	0.473086
% C	Genotype	1.2924	0.6462	2	16	0.4255	0.66063
	Treatment	18.3913	18.3913	1	2	12.1096	0.07358 *
	Genotype × Treatment	0.3202	0.1601	2	16	0.1054	0.90056

Significant *p* value

Table 2.22 Type III analysis of variance table with Satterthwaite's method for 2023 leaf carbon and nitrogen concentration

		Sum Squares	Mean Squares	Num DF	Den DF	F-value	Pr(>F)
% N	Genotype	0.07	0.033	2	102	0.0256	0.97474
	Treatment	16.77	16.766	1	6	12.9098	0.01146 *
	Date	636.49	127.297	5	102	98.0190	< 2.2e-16 *
	Genotype × Treatment	0.85	0.425	2	102	0.3273	0.72161
	Genotype × Date	4.06	0.406	10	102	0.3124	0.97642
	Treatment × Date	41.55	8.309	5	102	6.3980	3.293e-05 *
	Genotype × Treatment × Date	3.96	0.396	10	102	0.3046	0.97853
% C	Genotype	4	2.1	2	106	0.0268	0.9736
	Treatment	194	194.2	1	2	2.4744	0.2564
	Date	64126	12825.2	5	106	163.3731	< 2.2e-16 *
	Genotype × Treatment	3	1.6	2	106	0.0206	0.9796
	Genotype × Date	139	13.9	10	106	0.1769	0.9975
	Treatment × Date	2931	586.2	5	106	7.4679	4.889e-06 *
	Genotype × Treatment × Date	115	11.5	10	106	0.1463	0.9989

* Significant *p* value

Table 2.23 Type III analysis of variance table with Satterthwaite's method for 2024 leaf carbon and nitrogen concentration

		Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
% N	Genotype	2.6031	1.3016	2	105.960	12.4554	1.386e-05*
	Treatment	3.4639	3.4639	1	7.651	33.1474	0.0005014*
	Date	12.2046	2.4409	5	103.523	23.3585	1.208e-15*
	Genotype × Treatment	0.3793	0.1896	2	105.959	1.8148	0.1678943
	Genotype × Date	2.7925	0.2792	10	103.523	2.6723	0.0060196*
	Treatment × Date	6.9810	1.3962	5	103.523	13.3610	4.773e-10*
	Genotype × Treatment × Date	0.8030	0.0803	10	103.523	0.7685	0.6586198
% C	Genotype	2.484	1.2419	2	106.809	0.6472	0.5255
	Treatment	1.858	1.8580	1	5.853	0.9683	0.3640
	Date	103.379	20.6759	5	100.015	10.7748	2.643e-08*
	Genotype × Treatment	7.120	3.5598	2	106.833	1.8551	0.1614
	Genotype × Date	15.407	1.5407	10	100.015	0.8029	0.6262
	Treatment × Date	8.077	1.6154	5	100.015	0.8418	0.5232
	Genotype × Treatment × Date	30.651	3.0651	10	100.015	1.5973	0.1182

* Significant *p* value

Table 2.24 Type II analysis of variance table with Satterthwaite's method for 2023 total canopy nitrogen g m⁻²

	Sum Squares	Mean Squares	NumDF	DenDF	F-value	Pr(>F)
Genotype	0.85695	0.42847	2	16	3.5706	0.05222 *
Treatment	0.34692	0.34692	1	2	2.8910	0.23118
Genotype × Treatment	0.73506	0.36753	2	16	3.0628	0.07478 *

* Significant *p* value

Table 2.25 Type II analysis of variance results table with Satterthwaite's method for 2024 total canopy nitrogen g m⁻²

	Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Genotype	5.1309	2.56544	2	16	8.4520	0.003126 *
Treatment	0.1503	0.15032	1	2	0.4952	0.554501
Genotype × Treatment	2.9220	1.46100	2	16	4.8133	0.023090 *

* Significant *p* value

Table 2.26 Type III analysis of variance table with Satterthwaite's method for 2024 leaf nitrogen g cm² content samples

	Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Genotype	3.2140e-06	1.6069e-06	2	107.107	3.5943	0.030843 *
Treatment	4.3500e-07	4.3520e-07	1	10.537	0.9734	0.336531
Date	1.2465e-04	2.4930e-05	5	98.517	55.7625	< 2.2e-16 *
Genotype × Treatment	7.5100e-07	3.7540e-07	2	107.104	0.8397	0.434645
Genotype × Date	9.8810e-06	9.8810e-07	10	98.517	2.2102	0.023067 *
Treatment × Date	8.6350e-06	1.7270e-06	5	98.517	3.8628	0.003075 *
Genotype × Treatment × Date	6.7170e-06	6.7170e-07	10	98.517	1.5023	0.149835

* Significant *p* value**Table 2.27** Type III analysis of variance results table with Satterthwaite's method for 2024 photosynthetic nitrogen-use efficiency

	Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Genotype	3759	1880	2	246	0.5902	0.55500
Treatment	307153	307153	1	6	96.4414	6.422e-05 *
Date	1156172	231234	5	246	72.6040	< 2.2e-16 *
Genotype × Treatment	2813	1406	2	246	0.4416	0.64352
Genotype × Date	72036	7204	10	246	2.2618	0.01518 *
Treatment × Date	454063	90813	5	246	28.5138	< 2.2e-16 *
Genotype × Treatment × Date	30298	3030	10	246	0.9513	0.48682

* Significant *p* value

Table 2.28 Type II analysis of variance results with Satterthwaite's method for 2023 yield (kg ha⁻¹)

	Sum of Squares	Mean Squares	Num DF	Den DF	F-value	Pr(>F)
Genotype	406657	203329	2	8	2.6517	0.130774
Treatment	1050357	1050357	1	8	13.6980	0.006033 *
Genotype × Treatment	27411	13705	2	8	0.1787	0.839576

* Significant *p* value**Table 2.29** Type II analysis of variance table with Satterthwaite's method for 2024 yield (kg ha⁻¹)

	Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Genotype	3764294	1882147	2	12	16.0705	0.0004 *
Treatment	44982	44982	1	6	0.3841	0.5582
Genotype × Treatment	648467	324234	2	12	2.7684	0.1027

* Significant *p*-value**Table 2.30** Type II analysis of variance table with Satterthwaite's method for 2023 100 seed weight (g)

	Sum of Squares	Mean Squares	Num DF	Den DF	F-value	Pr(>F)
Genotype	53.68.9	26.8446	2	8	58.6234	1.665e-05*
Treatment	3.203	3.2033	1	8	6.9955	0.02949*
Genotype × Treatment	3.742	118712	2	8	4.0862	0.05988*

* Significant *p* value**Table 2.31** Type II analysis of variance table with Satterthwaite's method 2024 100 seed weight (g)

	Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Genotype	25.1233	12.5617	2	12	32.8410	1.359e-05 *
Treatment	2.5765	2.5765	1	6	6.7359	0.04092 *
Genotype × Treatment	1.2133	0.6067	2	12	1.5861	0.24480

* Significant *p*-value

Table 2.32 Type II analysis of variance results table with Satterthwaite's method for 2023 oil and protein NIR analysis

		Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Protein ‡							
	Genotype	1.9442	0.97209	1	2.4281	2.4308	0.2632
	Treatment	1.4120	1.41202	2	5.4797	3.5309	0.1139
	Genotype × Treatment	3.8590	1.92949	2	2.4281	4.8249	0.1426
Oil ‡							
	Genotype	1.3472	0.6736	2	2.8427	0.6634	0.5802
	Treatment	0.0294	0.0294	1	2.2790	0.0290	0.8787
	Genotype × Treatment	6.3756	3.1878	2	2.6285	3.1392	0.2011

‡ No significant *p* values**Table 2.33** Type II analysis of variance results table with Satterthwaite's method for 2024 oil and protein

		Sum of Squares	Mean Square	Num DF	Den DF	F-value	Pr(>F)
Protein	Genotype	6.5408	3.2704	2	18	8.3390	0.002735 *
	Treatment	1.2240	1.2240	1	18	3.1211	0.094239
	Genotype × Treatment	1.3171	0.6586	2	2	1.6792	0.214452
Oil	Genotype	16.2569	8.1284	2	12	35.3868	9.284e-06*
	Treatment	0.9572	0.9572	1	6	4.1669	0.08729
	Genotype × Treatment	0.6984	0.3492	2	2	1.5203	0.25793

* Significant P-value

Table 2.34 2023 WUE, yield, biomass, water potential, VPD contrasts between irrigated and rainfed treatments with a significant p value < 0.05

	Estimate	SE	DF	T-ratio	P-value
Water Use Efficiency	-44.680	11.8417	2.3910	-3.7731	0.0478
Yield	572.2109	208.5872	2.7602	2.7432	0.0780
Biomass	266.0434	50.4653	1.9261	5.2718	0.0370
VPD	-0.3848	0.0860	110.23	-4.4724	1.8883e-05
Ψ_{leaf}	0.5206	0.0861	6	6.0455	0.0009

Table 2.35 2024 Seed weight contrasts between irrigated and rainfed treatments with a with a significant p value < 0.05

Estimate	SE	DF	T-ratio	P-value
-1.5417	0.59401	6	-2.5954	0.0409

Table 2.36 2024 VPD contrasts between irrigated and rainfed treatments with a significant p value < 0.05

Estimate	SE	DF	T-ratio	P-value
-0.4176	0.0962	165.0912	-4.3417	2.4592

No significant P -value

Table 2.37 2023 Root depth contrasts among dates with a significant p value < 0.05

Contrast	Estimate	SE	DF	T-ratio	P-value
7/7/23 - 7/12/23	-15.802	4.8784	138.1317	-3.2392	0.0019
7/7/23 - 7/21/23	-21.8957	4.8356	138.7036	-4.528	2.5411e-05
7/7/23 - 7/27/23	-32.254	4.8807	138.9522	-6.6084	2.5572e-09
7/7/23 - 8/10/23	-47.9425	4.8859	138.3673	-9.8125	1.4112e-16
7/12/23 - 7/27/23	-16.4519	4.1556	137.9064	-3.959	0.0002
7/12/23 - 8/10/23	-32.1405	4.2011	137.6795	-7.6505	1.5676e-11
7/21/23 - 7/27/23	-10.3583	3.995	137.0871	-2.5928	0.0117
7/21/23 - 8/10/23	-26.0468	4.0814	137.5902	-6.3818	6.2202e-09
7/27/23 - 8/10/23	-15.6885	4.1133	137.7282	-3.8141	0.0003

Table 2.38 2024 Minirhizotron root depth contrasts among dates with a significant *p* value <0.05

Contrast	Estimate	SE	DF	T-ratio	P-value
5/30/2024 - 6/5/2024	-11.9071	4.3586	293.4437	-2.7319	0.0078
5/30/2024 - 6/11/2024	-23.7802	4.2328	293.8535	-5.618	7.0145e-08
5/30/2024 - 6/20/2024	-48.7795	4.2365	294.0995	-11.5141	3.6698e-25
5/30/2024 - 6/27/2024	-57.6465	4.1591	294.2686	-13.8602	2.0633e-33
5/30/2024 - 7/5/2024	-64.0453	4.1591	294.2686	-15.3987	7.1488e-39
5/30/2024 - 7/11/2024	-66.8191	4.1348	294.6878	-16.1602	1.2034e-41
5/30/2024 - 8/15/2024	-78.7983	4.1348	294.6878	-19.0574	4.5661e-52
5/30/2024 - 8/21/2024	-79.3191	4.1348	294.6878	-19.1833	3.1080e-52
6/5/2024 - 6/11/2024	-11.8731	3.9539	293.4494	-3.0029	0.0035
6/5/2024 - 6/20/2024	-36.8725	3.9554	293.6928	-9.322	6.3926e-18
6/5/2024 - 6/27/2024	-45.7394	3.8749	293.9094	-11.8041	4.1958e-26
6/5/2024 - 7/5/2024	-52.1382	3.8749	293.9094	-13.4555	5.7249e-32
6/5/2024 - 7/11/2024	-54.9121	3.8486	294.3911	-14.2679	7.2505e-35
6/5/2024 - 8/15/2024	-66.8912	3.8486	294.3911	-17.3805	4.2438e-46
6/5/2024 - 8/21/2024	-67.4121	3.8486	294.3911	-17.5158	1.7657e-46
6/11/2024 - 6/20/2024	-24.9993	3.7682	293.2164	-6.6342	2.8288e-10
6/11/2024 - 6/27/2024	-33.8663	3.6867	293.5007	-9.186	1.6170e-17
6/11/2024 - 7/5/2024	-40.2651	3.6867	293.5007	-10.9216	3.7900e-23
6/11/2024 - 7/11/2024	-43.039	3.6592	294.0316	-11.7619	5.4110e-26
6/11/2024 - 8/15/2024	-55.0181	3.6592	294.0316	-15.0356	1.2150e-37
6/11/2024 - 8/21/2024	-55.539	3.6592	294.0316	-15.178	4.1166e-38
6/20/2024 - 6/27/2024	-8.867	3.6738	293.4239	-2.4136	0.0179
6/20/2024 - 7/5/2024	-15.2658	3.6738	293.4239	-4.1553	5.6872e-05
6/20/2024 - 7/11/2024	-18.0396	3.6461	293.9578	-4.9476	1.8997e-06
6/20/2024 - 8/15/2024	-30.0188	3.6461	293.9578	-8.2331	1.14110e-14
6/20/2024 - 8/21/2024	-30.5396	3.6461	293.9578	-8.376	4.5496e-15
6/27/2024 - 7/11/2024	-9.1727	3.5364	293.5803	-2.5938	0.0112
6/27/2024 - 8/15/2024	-21.1518	3.5364	293.5803	-5.9811	1.0522e-08
6/27/2024 - 8/21/2024	-21.6727	3.5364	293.5803	-6.1284	4.8882e-09
7/5/2024 - 8/15/2024	-14.753	3.5364	293.5803	-4.1717	5.5178e-05
7/5/2024 - 8/21/2024	-15.2738	3.5364	293.5803	-4.319	3.0910e-05
7/11/2024 - 8/15/2024	-11.9792	3.483	293.018	-3.4394	0.0008
7/11/2024 - 8/21/2024	-12.5	3.483	293.018	-3.5889	0.0005

Table 2.39 2024 % nitrogen biomass, seed protein, seed oil, 100 seed weight, biomass carbon g m⁻² and yield contrasts among genotypes with a significant *p* value < 0.05

	Genotype	Estimate	SE	DF	T-ratio	P-value
ΦPSII	N11-10295 - PI 471938	-0.0172	0.0072	246.5628	-2.3938	0.0453
	N93-110-6 - PI 471938	-0.0157	0.0072	246.0038	-2.1804	0.0453
% N	N11-10295 - N93-110-6	-0.2657	0.0852	12	-3.1175	0.0133
	N11-10295 - PI 471938	-0.2868	0.0852	12	-3.3652	0.0133
% Protein	N93-110-6 - PI 471938	1.2788	0.3131	12	4.0839	0.0046
% Oil	N11-10295 - PI 471938	-1.6188	0.2396	12	-6.7550	3.0439e-05
	N93-110-6 - PI 471938	-1.8500	0.2396	12	-7.7200	1.6213e-05
100 Seed Weight	N11-10295 - N93-110-6	-2.400	3.0923	12	-7.7611	1.5363e-05
	N11-10295 - PI 471938	-1.825	3.0923	12	-5.9017	0.0001
	N93-110-6 - PI 471938	0.5575	3.0923	12	1.8594	0.0876
Carbon g m ⁻²	N11-10295 - N93-110-6	4.7003	1.3134	12	3.5788	0.0057
	N11-10295 - PI 471938	5.1629	1.3134	12	3.931	0.0057
Yield kg ha ⁻¹	N11-10295 - N93-110-6	584.693	171.1128	12	3.417	0.0077
	N11-10295 - PI 471938	962.7238	171.1128	12	5.6263	0.0003
	N93-110-6 - PI 471938	378.0308	171.1128	12	2.2092	0.0473

Table 2.40 2023 Seed weight contrast between irrigated and rainfed treatments among genotypes with a significant p value < 0.05

Genotype	Estimate	SE	DF	T-value	P-value
N11-10295	-1.8667	0.5900	8	-3.1640	0.0133

Table 2.41 2023 Total canopy nitrogen contrasts between treatments and among genotypes. None of the contrasts were significant even though the ANOVA interaction is significant

Genotype	Estimate	SE	DF	T-ratio	P-value
N11-10295	1.8928	1.2852	2.1003	1.4728	0.2730
N93-110-6	2.6531	1.2852	2.1003	2.0644	0.1690
PI 471938	1.9298	1.2852	2.1003	1.5016	0.2663

Table 2.42 2024 Biomass (g m^{-2}) and total canopy nitrogen contrasts between irrigated and rainfed treatments among genotypes. None of the contrasts were significant even though the ANOVA interaction is significant

	Genotype	Estimate (g m^{-2})	SE	DF	T-ratio	P-value
Biomass	N11-10295	48.3685	44.2268	2.1683	1.0936	0.3806
	N93-110-6	53.1724	44.2268	2.1683	1.2023	0.3441
	PI 471938	9.421	44.2268	2.1683	0.213	0.8497
Total canopy nitrogen	N11-10295	1.39	1.7856	2.1329	0.7784	0.513
	N93-110-6	2.004	1.7856	2.1329	1.1223	0.3721
	PI 471938	0.3154	1.7856	2.1329	0.1766	0.8751

Table 2.43 2023 Water potential contrast with a significant interaction among genotypes and dates with a significant p value < 0.05

Date	Contrast	Estimate	SE	DF	T-ratio	P-value
8/2/2023	N11-10295 - N93-110-6	-0.7662	0.2178	102	-3.5179	0.0012
8/2/2023	N93-110-6 - PI 471938	0.5263	0.2178	102	2.4161	0.0262

Table 2.44 2024 G_{sw} and soil moisture contrast with a significant interaction among genotypes and soil moisture depths with a significant p value < 0.05

	Contrast	Estimate	SE	DF	T-value	P-value
30.5 cm	N11-10295 - N93-110-6	4.6142	1.0688	138.8228	4.3170	8.8262e-05
	N93-110-6 - PI 471938	-4.6641	1.1732	136.7397	-3.9755	3.3181e-04
61 cm	N11-10295 - PI 471938	-2.7534	0.9384	164.5429	-2.9345	0.0106
	N93-110-6 - PI 471938	-2.7629	1.0314	176.0840	-2.6788	0.0219

Table 2.45 2023 Leaf area index (LAI), Leaf % nitrogen, and % carbon content pairwise comparison between irrigated and rainfed among dates with a significant p value < 0.05

	Date	Estimate	SE	DF	T-ratio	P-value
LAI	8/7/2023	0.8808	0.3775	23.0831	2.3332	0.0287
% N	7/20/2023	-1.3348	0.4799	72.1703	-2.7812	0.0069
	7/26/2023	-2.8889	0.4799	72.1703	-6.0192	6.6504e-08
% C	7/26/2023	-25.37	5.102	5.8623	-4.9726	0.0027

Table 2.46 2024 Assimilation, Φ PSII, and root depth contrasts between irrigated and rainfed treatments among dates with a significant p value < 0.05

	Date	Estimate	SE	DF	T-ratio	P-value
Assimilation	7/9/2024	18.2536	2.0927	36.6	8.7226	1.8193e-10
	7/17/2024	22.3341	2.0927	36.6	10.6725	8.6472e-13
	8/23/2024	14.0071	2.0927	36.6	6.6934	7.6788e-08
	8/27/2024	20.4975	2.0927	36.6	9.7948	9.0370e-12
Φ PSII	7/9/2024	0.1042	0.0144	134.1818	7.2444	3.0649e-11
	7/17/2024	0.1653	0.0144	134.1818	11.4956	1.0269e-21
	8/23/2024	0.0426	0.0144	134.1818	2.9630	3.606e-03
	8/27/2024	0.1633	0.0144	134.1818	11.3540	2.3455e-21
Root depth	6/11/2024	-19.0301	10.5194	10.2384	-1.8091	0.0999
	6/20/2024	-20.8172	10.5012	10.1686	-1.9824	0.0751

Table 2.47 2023 A contrast between irrigated and rainfed treatments among genotype and date with a significant p value < 0.05

Date	Genotype	Estimate	SE	DF	T-ratio	P-value
7/18/2023	N11-10295	20.3753	3.5427	79.2006	5.7514	1.5935e-07
7/20/2023	N11-10295	21.8028	3.5427	79.2006	6.1543	2.9102e-08
7/20/2023	N93-110-6	14.7905	5.0506	79.2006	2.9284	0.0044
7/20/2023	PI 471938	14.0584	6.0313	79.2006	2.3309	0.0223
7/26/2023	N11-10295	23.8360	3.6314	80.1422	6.5636	4.7978e-09
7/26/2023	N93-110-6	17.1966	5.0506	79.2007	3.4048	0.0010
7/26/2023	PI 471938	24.2599	7.2877	89.6126	3.3289	0.0013
8/2/2023	N11-10295	17.8948	3.5427	79.2006	5.0512	2.7527e-06
8/9/2023	N11-10295	15.8461	3.5427	79.2006	4.4729	2.5482e-05
8/9/2023	N93-110-6	10.9617	5.0506	79.2006	2.1704	0.0330
8/9/2023	PI 471938	13.3960	6.0313	79.2006	2.2211	0.0292
8/16/2023	N11-10295	18.0344	3.6316	80.1422	4.9660	3.7858e-06
8/16/2023	N93-110-6	18.4481	5.0506	79.2006	3.6526	0.0005
8/16/2023	PI 471938	18.4234	5.4729	81.5862	3.3663	0.0012

Table 2.48 2023 g_{sw} contrast between irrigated and rainfed treatments among genotype and date with a significant p value < 0.05

Date	Genotype	Estimate	SE	DF	T-ratio	P-value
7/18/2023	N11-10295	0.3907	0.0689	74.4611	5.6677	2.5910e-07
7/18/2023	PI 471938	0.2319	0.1150	73.5024	2.0168	0.0474
7/20/2023	N11-10295	0.2998	0.0673	73.5024	4.4561	2.9340e-05
7/20/2023	N93-110-6	0.2402	0.0960	73.5024	2.5015	0.0146
7/20/2023	PI 471938	0.2439	0.1150	73.5024	2.1219	0.0372
7/26/2023	N11-10295	0.3808	0.0745	75.1352	5.1116	2.3585e-06
7/26/2023	N93-110-6	0.2348	0.0960	73.5024	2.4467	0.0168
8/2/2023	N11-10295	0.2340	0.0672	73.5024	3.4782	0.0009
8/9/2023	N11-10295	0.1781	0.0690	74.4611	2.5812	0.0118
8/16/2023	N11-10295	0.4000	0.0690	74.4611	5.7997	1.5106e-07
8/16/2023	N93-110-6	0.4085	0.0960	73.5024	4.2570	6.0379e-05
8/16/2023	PI 471938	0.2897	0.1042	75.9363	2.7818	0.0068

Table 2.49 2024 g_{sw} contrast between irrigated and rainfed treatments among genotypes and date with a significant p value < 0.05

Date	Genotype	Estimate	SE	DF	T-ratio	P-value
7/3/2024	N11-10295	0.2931	0.119	40.7785	2.4625	0.0181
7/9/2024	N11-10295	0.9696	0.119	40.7785	8.1446	4.3713e-10
7/9/2024	N93-110-6	0.9024	0.119	40.7785	7.5806	2.6152e-09
7/9/2024	PI 471938	0.778	0.119	40.7785	6.5354	7.6766e-08
7/17/2024	N11-10295	0.5788	0.119	40.7785	4.8623	1.7642e-05
7/17/2024	N93-110-6	0.3739	0.119	40.7785	3.1409	0.0031
7/17/2024	PI 471938	0.5653	0.119	40.7785	4.7483	2.5365e-05
8/23/2024	N11-10295	0.2853	0.119	40.7785	2.3963	0.0212
8/27/2024	N11-10295	0.4071	0.119	40.7785	3.4202	0.0014
8/27/2024	N93-110-6	0.3083	0.119	40.7785	2.59	0.0132
8/27/2024	PI 471938	0.3288	0.119	40.7785	2.7616	0.0086

Table 2.50 2023 Osmotic Potential contrast between irrigated and rainfed treatments among genotype and date with a significant p value < 0.05

Date	Genotype	Estimate	SE	DF	T-ratio	P-value
7/18/2023	PI 471938	0.4525	0.1641	65.5471	2.7581	0.0075
7/20/2023	N11-10295	0.7078	0.1777	65.6985	3.9845	0.0002
7/26/2023	N11-10295	0.7947	0.2135	65.9388	3.7225	0.0004
7/26/2023	N93-110-6	0.657	0.2022	65.8803	3.2491	0.0018
8/9/2023	N11-10295	0.437	0.2135	65.9579	2.0469	0.0447
8/9/2023	N93-110-6	0.621	0.2134	65.9379	2.9098	0.0049

Table 2.51 2023 Φ PSII contrast between irrigated and rainfed treatments among genotype and date with a significant p value < 0.05

Date	Genotype	Estimate	SE	DF	T-ratio	P-value
7/18/2023	N11-10295	0.1269	0.0294	73.1499	4.3154	4.9178e-05
7/20/2023	N11-10295	0.1321	0.0372	82.5487	3.5549	0.0006
7/20/2023	N93-110-6	0.1536	0.0511	82.2374	3.0078	0.0035
7/26/2023	N11-10295	0.1923	0.0285	71.7751	6.7396	3.3680e-09
7/26/2023	N93-110-6	0.135	0.0397	70.88	3.4009	0.0011
7/26/2023	PI 471938	0.2289	0.0574	80.8303	3.9854	0.0001
8/2/2023	N11-10295	0.1248	0.0278	70.88	4.484	2.7575e-05
8/2/2023	N93-110-6	0.0918	0.0397	70.88	2.3129	0.0236
8/9/2023	N11-10295	0.1155	0.0278	70.88	4.1506	9.0989e-05
8/9/2023	PI 471938	0.1137	0.0476	70.88	2.3871	0.0197
8/16/2023	N11-10295	0.1079	0.0285	71.7751	3.7826	0.0003
8/16/2023	N93-110-6	0.1091	0.0397	70.88	2.7468	0.0076
8/16/2023	PI 471938	0.1865	0.0432	73.1499	4.3179	4.8729e-05

Table 2.52 2024 Water potential, turgor potential, an osmotic potential contrast between irrigated and rainfed treatments among genotypes and dates with a significant p value < 0.05

	Date	Genotype	Estimate	SE	DF	t-ratio	p-value
Ψ_{leaf}	7/9/2024	N11-10295	0.6381	0.1661	105.053	3.8428	0.0002
	7/9/2024	N93-110-6	0.6184	0.1631	107.3559	3.7929	0.0002
	7/9/2024	PI 471938	0.4935	0.1655	107.2433	2.9819	0.0035
	7/17/2024	N11-10295	1.2031	0.1661	105.053	7.2454	7.5180e-11
	7/17/2024	N93-110-6	0.8634	0.1631	107.3559	5.2955	6.3571e-07
	7/17/2024	PI 471938	1.3985	0.1655	107.2433	8.4498	1.5698e-13
	8/23/2024	N11-10295	0.4356	0.1661	105.053	2.6233	0.0100
	8/27/2024	N11-10295	0.4881	0.1661	105.053	2.9395	0.0040
	8/27/2024	N93-110-6	0.3684	0.1631	107.3559	2.2597	0.0259
	8/27/2024	PI 471938	0.5485	0.1655	107.2433	3.3142	0.0012
Ψ_{P}	7/17/2024	N93-110-6	0.471	0.163	98.8491	2.8893	0.0047
	7/17/2024	PI 471938	0.5366	0.2408	99.0587	2.2289	0.0281
	8/23/2024	N11-10295	0.4856	0.1535	97.2871	3.1626	0.0021
	8/23/2024	PI 471938	0.3388	0.1532	99.2686	2.2112	0.0293
	8/27/2024	N11-10295	0.3581	0.1535	97.2871	2.3322	0.0218
	8/27/2024	PI 471938	0.6925	0.1789	98.2669	3.8707	0.0002
Ψ_{II}	7/9/2024	N11-10295	0.5435	0.1471	97.8053	3.6942	0.0004
	7/9/2024	N93-110-6	0.3469	0.1456	99.2	2.3821	0.0191
	7/17/2024	N11-10295	0.811	0.1793	99.9101	4.523	1.6813e-05
	7/17/2024	N93-110-6	0.3957	0.1573	98.839	2.515	0.0135

Table 2.53 2024 WUE pairwise comparison between irrigated and rainfed treatments among genotypes and dates for water use efficiency significant p value < 0.05

Date	Genotype	Estimate	SE	DF	T-ratio	P-value
7/3/2024	N11-10295	-41.2864	13.5019	115.8841	-3.0578	0.0028
7/3/2024	N93-110-6	-39.6028	13.5019	115.8841	-2.9331	0.0040
7/3/2024	PI 471938	-40.7097	13.5019	115.8841	-3.0151	0.0032
7/9/2024	N11-10295	-100.5459	13.5019	115.8841	-7.4468	1.8456e-11
7/9/2024	N93-110-6	-76.0527	13.5019	115.8841	-5.6327	1.2610e-07
7/9/2024	PI 471938	-93.8239	13.5019	115.8841	-6.9489	2.2967e-10
7/17/2024	N11-10295	-42.264	13.5019	115.8841	-3.1302	0.0022
8/1/2024	N93-110-6	28.971	13.5019	115.8841	2.1457	0.0340
8/23/2024	N11-10295	-72.2597	13.5019	115.8841	-5.3518	4.4535e-07
8/23/2024	N93-110-6	-43.7769	13.5019	115.8841	-3.2423	0.0015
8/23/2024	PI 471938	-53.958	13.5019	115.8841	-3.9963	0.0001
8/27/2024	N11-10295	-37.6999	13.5019	115.8841	-2.7922	0.0061

Table 2.54 2024 LAI pairwise comparison between irrigated and rainfed treatments among genotypes and dates with a significant p value < 0.05

Date	Genotype	Estimate	SE	DF	T-ratio	P-value
7/22/2024	N11-10295	1.825	0.5979	103.5423	3.0523	0.0029
7/22/2024	PI 471938	2.2125	0.5979	103.5423	3.7004	0.0003
7/31/2024	N11-10295	1.395	0.5979	103.5423	2.3331	0.0216
7/31/2024	N93-110-6	2.3225	0.5979	103.5423	3.8844	0.0002
7/31/2024	PI 471938	2.98	0.5979	103.5423	4.9841	2.5081e-06
8/15/2024	N11-10295	2.08	0.5979	103.5423	3.4788	0.0007
8/15/2024	N93-110-6	1.88	0.5979	103.5423	3.1443	0.0022
8/15/2024	PI 471938	2.635	0.5979	103.5423	4.407	2.5648e-05
8/20/2024	N93-110-6	1.41	0.5979	103.5423	2.3582	0.0202
8/20/2024	PI 471938	1.7225	0.5979	103.5423	2.8809	0.0048
8/26/2024	N11-10295	2.1125	0.5979	103.5423	3.5332	0.0006
8/26/2024	N93-110-6	1.67	0.5979	103.5423	2.7931	0.0062
8/26/2024	PI 471938	2.25	0.5979	103.5423	3.7631	0.0003

Table 2.55 2024 Leaf % nitrogen content pairwise comparison between irrigated and rainfed treatments among genotypes and dates with a significant p value < 0.05

Date	Genotype	Estimate	SE	DF	T-ratio	P-value
7/17/2024	PI 471938	-0.6142	0.2315	107.2745	-2.653	0.0092
8/23/2024	PI 471938	-0.565	0.2315	107.2745	-2.4405	0.0163
8/27/2024	N11-10295	-0.9013	0.2313	105.909	-3.8962	0.0002
8/27/2024	N93-110-6	-1.1105	0.2294	107.1827	-4.8404	4.3752e-06
8/27/2024	PI 471938	-1.5577	0.2315	107.2745	-6.7289	8.6720e-10

Table 2.56 2024 Leaf nitrogen $g\ cm^{-2}$ content pairwise comparison between irrigated and rainfed treatments among genotypes and dates with a significant p value < 0.05

Date	Genotype	Estimate	SE	DF	T-ratio	P-value
8/23/2024	N93-110-6	0.001	5e-04	107.0349	2.0119	0.0467
8/23/2024	PI 471938	0.0012	5e-04	107.9961	2.3855	0.0188
8/27/2024	N11-10295	0.0018	5e-04	106.7502	3.5389	0.0006

Table 2.57 2024 Photosynthetic nitrogen use efficiency (PNUE) $\mu mol\ CO_2\ mol^{-1}\ s^{-1}$ pairwise comparison between irrigated and rainfed treatments among genotypes and dates with a significant p value < 0.05

Date	Genotype	Estimate	SE	DF	T-ratio	P-value
7/9/2024	N11-10295	126.5304	28.8791	203.387	4.3814	1.8879e-05
7/9/2024	N93-110-6	167.3598	28.8791	203.387	5.7952	2.5698e-08
7/9/2024	PI 471938	129.1718	28.8791	203.387	4.4728	1.2827e-05
7/17/2024	N11-10295	220.6334	28.8791	203.387	7.6399	8.3805e-13
7/17/2024	N93-110-6	160.8223	28.8791	203.387	5.5688	8.0533e-08
7/17/2024	PI 471938	231.4988	28.8791	203.387	8.0161	8.4142e-14
8/23/2024	N11-10295	102.3533	28.8791	203.387	3.5442	0.0005
8/23/2024	PI 471938	62.8576	28.8791	203.387	2.1766	0.0307
8/27/2024	N11-10295	108.9586	28.8791	203.387	3.7729	0.0002
8/27/2024	N93-110-6	143.1916	28.8791	203.387	4.9583	1.4966e-06
8/27/2024	PI 471938	132.1682	28.8791	203.387	4.5766	8.2157e-06

Table 2.58 Estimated marginal means for 2023 Biomass, yield, 100 seed weight, protein and oil concentrations based on genotype and treatment excluding plots affected by nematode

Genotype	Treatment	Biomass g/m²	Seed Yield Kg ha⁻¹	100 seed weight kg	Protein %	Oil %
N11-10295	Irrigated	445	881.9	8.3	43.5	19.2
N11-10295	Rainfed	159	281.1	10.2	41.1	20.6
N93-110-6	Irrigated	486	1036.3	12.0	44.7	20.1
N93-110-6	Rainfed	171	358.7	13.7	42.1	20.7
PI 471938	Irrigated	334	499.2	13.8	42.6	21.7
PI 471938	Rainfed	137	60.8	13.3	42.8	19.7

Table 2.59 Estimated marginal means for 2024 biomass, yield, 100 seed weight, protein and oil concentrations based on genotype and treatment

Genotype	Treatment	Biomass g/m²	Seed Yield Kg ha⁻¹	100 seed weight kg	Protein %	Oil %
N11-10295	Irrigated	167.9	2536	11.7	42.4	21.8
N11-10295	Rainfed	119.6	3372	13.8	42.4	22.8
N93-110-6	Irrigated	132.2	2240	14.5	43.1	22.4
N93-110-6	Rainfed	79.0	2499	15.8	43.0	23.1
PI 471938	Irrigated	108.2	1960	14.0	42.3	24.3
PI 471938	Rainfed	98.8	2022	15.2	41.2	24.4

Table 2.60 2023 Developmental stages for rainfed plots during drought treatment

Genotype	7/12/2024	7/21/2024	7/27/2024	8/7/2024	8/17/2024	8/23/2024
N11-10295	V10	R2	R2	R2	R2/R3/R4	R2/R3/R4
N93-110-6	V10	R2	R2	R2	R2/R3/R4	R2/R3/R4
PI-471938	V10	R2	R2	R2	R2/R3/R4	R2/R3/R4

Table 2.61 2023 Developmental stages for irrigated plots during drought treatment

Genotype	7/12/2024	7/21/2024	7/27/2024	8/7/2024	8/17/2024	8/23/2024
N11-10295	V10	R2	R2	R3	R3/R4	R4
N93-110-6	V10	R2	R2	R3	R3/R4	R4
PI-471938	V10	R2	R2	R3	R3/R4	R4

Table 2.62 2024 Developmental stages for rainfed plots during drought treatment

Genotype	7/2/2024	7/22/2024	7/30/2024	8/12/2024	8/20/2024	8/26/2024
N11-10295	R1	R2	R2	R3	R4	R4/R5
N93-110-6	R1	R2	R2	R3	R4	R4/R5
PI-471938	R1	R2	R3	R4	R4	R5

Table 2.63 2024 Developmental stages for rainfed plots during drought treatment

Genotype	7/2/2024	7/22/2024	7/30/2024	8/12/2024	8/20/2024	8/26/2024
N11-10295	R1	R2	R2	R3	R4	R4/R5
N93-110-6	R1	R2	R2	R3	R4	R4/R5
PI-471938	R1	R2	R3	R4	R4	R5