STUDIES OF THE IMPACT OF DROUGHT STRESS ON POLLEN SHEDDING AND SILK DEVELOPMENT IN MAIZE (ZEA MAYS L.) TO ASSIST SELECTION OF STRESS TOLERANCE HYBRIDS BASED ON PHENOTYPIC TRAITS

A Capstone Project Thesis
Presented to the Faculty of the Graduate School
of Cornell University
in Partial Fulfillment of the Requirements for the Degree of
Master of Professional Studies in Agricultural and Life Sciences
Field of Plant Breeding & Genetics

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ABSTRACT

Maize (Zea mays L.) is the most valuable commodity crop in the United States and is second to rice in the world. With the upcoming challenges of extreme climates due to global warming, further study of abiotic stresses, including drought stress, are necessary to develop improved approaches for the selection of maize hybrids for stress tolerance. Understanding the performance under drought stress of the important growth stages, pollinating and silking, is critical for improving reproductive success of pollination and yield potential and in breeding hybrids with stress tolerance. This study investigated the impact of water stress on pollen shedding patterns, pollen quantity, pollen viability, and silk+ear growth across eight different hybrids. Assessment of pollen viability with tetrazolium compounds indicated unstable reactions and was inconsistent. However, the analysis of pollen quantity and silk growth provided direction and insights for breeding selection. By analyzing with two-way ANOVA and Emmeans functions through R studio on the pollen weight from the pollen shedding period, results showed that there was a significant difference of pollen weight among hybrids ($F_{(7df)}$=2.605, p=0.0534) and treatments ($F_{(1df)}$=6.165, p=0.0245). Drought significantly increased pollen weight in hybrids 11, 2, and 20, and hybrid 18 had high pollen weight in both control and stress conditions. In addition, drought reduced ear and silk growth and various silk and ear growth rates across hybrids. Among hybrids, 11, 13, 15, and 16 had greater silk+ear growth rate under drought stress. These observations provide insight regarding phenotypic traits in maize for drought stress tolerance breeding selection.

BIOGRAPHICAL SKETCH

Jialiang (Joe) Chen grew up in Guangzhou, the southern part of China. Joe received his Bachelor of Science degree in Agricultural & Consumer Economics with concentration of Agribusiness, Market, & Management and Crop Science and minor in Horticulture from University of Illinois at Champaign-Urbana. Joe expressed his passion in agronomy working in winter wheat breeding and maize breeding and field management during his internships and worked as research assistant under the supervision of Dr. Desiree in Prof. Sacks' lab and aimed to develop local hybrids for flooding tolerance on Miscanthus as biofuel crop by performing DNA extraction, PCR, and data collections in field trials. Currently, Joe is working toward a Master of Professional Studies degree in Integrative Plant Science from Cornell University, specializing in Plant Breeding & Genetics. Joe brings his experience of maize crossing and valuable knowledge of maize pollination to the maize selection project. During his capstone project, he worked closely under Professor Tim Setter and evaluated the impact of drought stress on pollen shedding and ear formation on maize for initial selection of maize hybrids with stress tolerance traits.

DEDICATION

This Capstone project is dedicated to everyone who has an interest and has influence in the field of plant breeding and genetics on maize. This project is also dedicated to my family members and Prof. Tim Setter.

ACKNOWLEDGEMENTS

First, I am deeply grateful to my research supervisor, Tim Setter, for his guidance and expertise, and encouragement throughout the capstone project, and Prof. Tim's feedback and insightful suggestions improved my effectiveness on analyzing process and taught me valuable knowledge of maize and provided information and materials for the capstone project during this past year.

Next, I would like to extend my sincere appreciation for the incredible support from faculties, people in MPS program, and friends at Cornell University, and I could learn valuable knowledge, perspectives, and more insights from people with different backgrounds for my future success of career and capstone project under such peaceful environment.

Also, I want to thank people who are outside of Cornell been mentally supporting me during my stress and challenging period.

Finally, I would like to express my gratitude heartfelt gratitude to my family for always supporting me physically and mentally on my academic path and career decision.

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INTRODUCTION

Maize (Zea mays L.) is a primary cereal crop which plays a pivotal role in food security and economic livelihoods worldwide. One of the most distinctive aspects of maize is its pollination mechanism. It predominantly undergoes cross-pollination in nature, meaning that pollen from the male flowers, known as tassels, travels to the female flowers via wind and gravity, located on the ear or cob, for successful fertilization. This unique reproductive process, combined with the plant's diverse genetics, allows development of a wide array of maize varieties with varying traits. Its reproductive system also allows corn to be easily self and cross-pollinated at various stages during selection and breeding. In the United States, a significant majority of maize varieties are F1 hybrids, underscoring the importance of inbreeding methods in breeding efforts. Corn is easily self- and cross-pollinated for reproduction methods, and it can be efficiently controlled by manipulating its male and female structures, known as the tassel and silk. This manipulation can be conducted in open fields or controlled environments like greenhouses. By carefully bagging the tassels and shoots overnight, a substantial quantity of fresh pollen is collected from the tassels, which can then be meticulously applied to the same or different plants to facilitate cross-pollination.

Climate change poses unprecedented challenges to global agricultural systems, with drought conditions emerging as a primary concern impacting crop production. The United States holds a substantial stake in global corn production, contributing 40% of the total annual production, with 15.3 billion bushels harvested in 2023 (Kim & Lee 2023). Maize is a valuable crop with multifaceted purposes in human consumption, animal feed, and industrial applications (Chen Jialiang, 2024). It faces significant challenges under climate change-induced drought conditions, which reduce yield and total production globally. Understanding the factors influencing the performance of maize is essential for plant breeders to develop drought-tolerance hybrids. One of the factors affecting the total kernel yield of maize under drought conditions is the number of pollen grains during the pollen shedding period to fertilize female reproductive structures to produce starch and protein in the endosperm and embryo, and fertile seeds (Chen Jialiang, 2024). According to the previous research by Hall, A. J. (1982), applying stress to maize at the beginning of the silking stage could lead to a low number of pollen grains and a reduction in kernel production. Other research found that the 4-day pollen shedding period is required to maximize kernel yield production (Tollenaar and Daynard 1978). Nevertheless, other studies have found that maize tends to produce an excess of pollen grains under non-stress conditions and pollen production does not limit yield (Uribelarrea et al., 2002; Westgate et al. 2003). However, pollen production might be reduced by drought stress and other environmental factors, so enough pollen produced by plants is needed to ensure pollination for yield production.

The relationship between maize (Zea mays L.) pollen production and kernel set is a crucial aspect of understanding the reproductive success and yield potential of maize crops. While it's generally known that maize is wind-pollinated, and pollen is essential for fertilizing the ovules to form kernels, the specifics of pollen production in modern hybrids and the impact of breeding for reduced tassel size are important areas of research. Traditionally, maize breeding has focused on improving yield, disease resistance, and other agronomic traits. The reduction in tassel size has been one of the strategies employed to enhance the crop's performance.

Tassels are the male reproductive structures of maize, and they produce and release pollen. Research has shown that, in some cases, maize pollen production may not be the limiting factor for kernel set. This means that the plant might produce enough pollen, but other factors such as environmental conditions, pollination efficiency, or availability of resources during the critical period of pollination and fertilization may influence the final kernel set. However, the information on pollen production in modern hybrids and the impact of breeding for reduced tassel size on this trait is indeed limited. It is important to note that different maize hybrids may exhibit varying characteristics, and the effects of breeding practices may vary. The objective of this study is to examine the relationship between pollen availability and silk performance in maize (Zea mays L.) under stress conditions. Through the application of rigorous measurements, including assessments of pollen availability, pollen shedding per plant, and silk growth rate, this research aims to quantify pollen production in the reproductive process. By utilizing these measurements as phenotypic traits, the study seeks to contribute toward a comprehensive understanding of how these traits contribute to the prediction of potential yields in maize hybrids, particularly under stress-inducing environmental conditions. The overarching goal is to provide valuable insights for breeders and researchers, facilitating the development of hybrids with enhanced stress tolerance and optimal reproductive performance, ultimately contributing to improved maize yields in challenging agricultural environments.

METHODS & MATERIALS

Greenhouse and plant materials:

My Master of Professional Studies (MPS) project was conducted in conjunction with an ongoing experiment of drought and shade by Professor Tim L. Setter. His experiment utilized ten hybrids that which were developed by CIMMYT in Mexico, as described by Cupertino-Rodrigues et al. (2020). The hybrids included were referenced according to the table below as hybrid 1, hybrid 2, hybrid 10, hybrid 11, hybrid 13, hybrid 15, hybrid 16, hybrid 17, hybrid 18, and hybrid 20. The levels of drought stress tolerance among those hybrids are currently being tested by CIMMYT. My contributions to the experiments were separated into three parts including pollen viability, pollen shedding, and silk growth. The studies were conducted at Cornell University in Ithaca, New York in the Guterman Greenhouse room 181e. Two treatment groups were established: a water stress treatment simulating drought conditions and a control treatment. Professor Setter grew the plants and subjected them to stress as described below, and I used these plants for my measurements of pollen production and ear+silk growth. Professor Setter collected additional data on the plants for his studies of transpiration, leaf carbohydrates and ABA, ear-tip gene expression, and ear mature-kernel yield. The greenhouse environment was thermostatically regulated to maintain temperatures ranging from 23°C (night) to 27°C (day) throughout the experiment. Combination of soil mixture of peat, vermiculite, and petite ratio of 1:1:1 with 6 g of pulverized limestone, 35 g of CaSO4, 1 g of fritted trace elements, 3 g of wetting agent, and 42 g of powered FeSO4+ is used as soil base for the experiments (Setter et al. 2001).

Hybrids No.	Genealogy
1	CSL1653&PHG29
2	CSL1653&PHG35
10	CML603&PHG29
11	CML550&LH198
13	CML576&PHW52
15	CML576&PHP85
16	CML576&PHEG9
17	CML576&PHG86
18	CSWL1789&H8431
20	CSL1661&2FACC

Table 1. Ten Hybrids from CIMMYT

Plant treatments:

Plants assigned randomly to the water stress or control treatment group. Controls were well-watered throughout, whereas the drought stressed plants were subjected to water deficit beginning on the day of silk exertion and continuing until 7 days after silking. The water deficit was maintained such that leaves rolled and transpiration was kept at about 10-30% of controls with small daily additions of 0.1 to 0.2 kg of water.

Pollen Viability Methods:

In previous studies, the viability of pollen in maize (Zea mays L.) was found to remain unaffected by stress at any developmental stage (Hall. A. J. 1982). However, despite pollen viability being maintained, alterations in the dynamics of flowering resulted in a reduction in the number of pollen grains available for pollination of the last ears to silk within stressed populations (Hall. A. J. 1982). The observation suggests that while the stress did not directly impact pollen viability, it did influence the timing or quantity of pollen shedding under water stress conditions. Due to the impact of pollen quantity reduction, it is necessary to observe the pollen availability between the control samples and plants under water stress conditions.

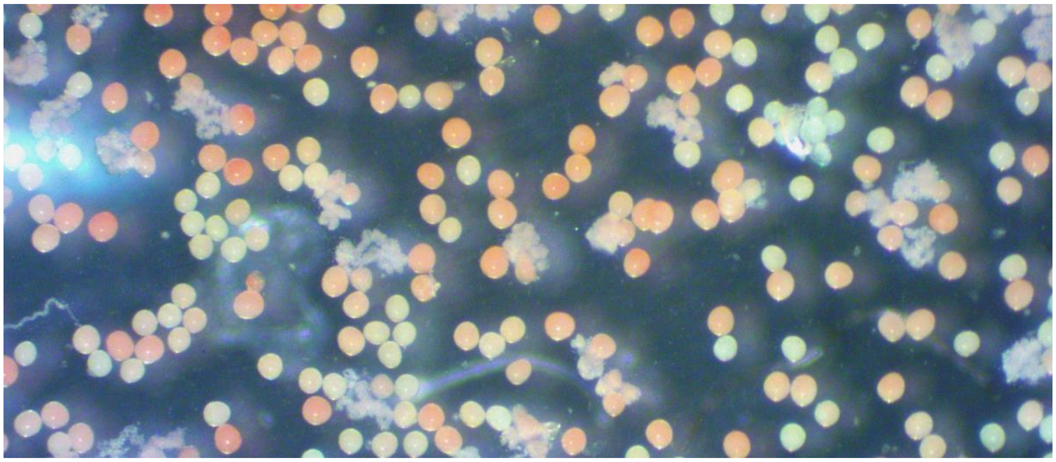
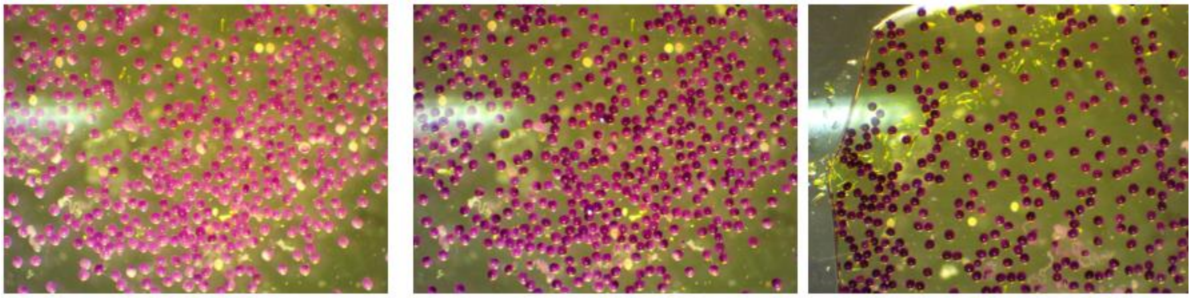


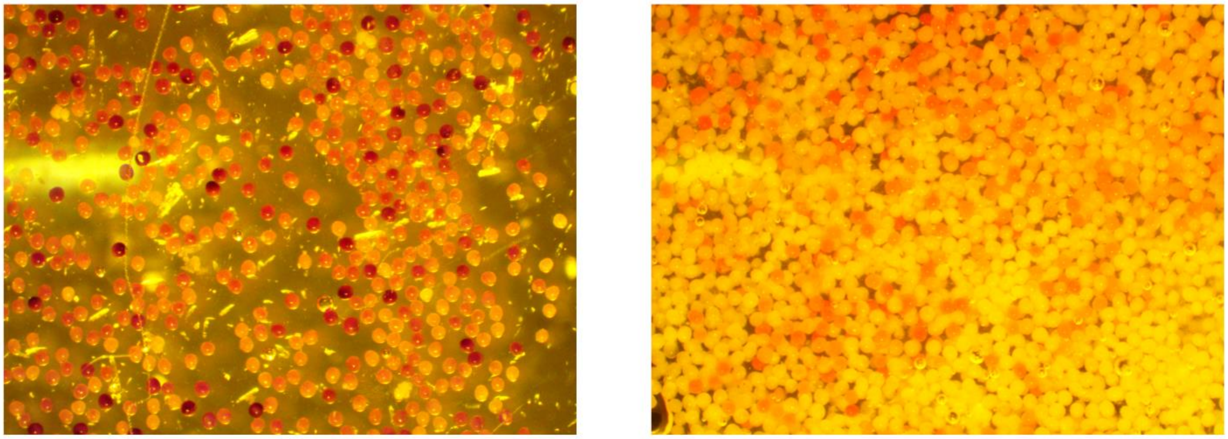
Illustration 1. Pollen with TTC treatment under room temperature

MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, is another indicator utilized first for estimating pollen viability in rice (Khatun S. and Flowers T.J 1994). After using MTT and including 1% sucrose(isotonic), it stains live pollen dark purple/black or orange, respectively. However, pollens showed distinct and unified purple to dark purple throughout the observation. Moreover, the cell explosion was reduced by 70% compared to TTC, and only a portion of pollens was found to crust after 10 minutes with the MTT.



5 mins 10 mins 15 mins

Illustration 2. Pollen with MTT treatment under room temperature



MTT dead pollens 15 mins TTC dead pollens 15 mins

Illustration 3. Comparison of dead pollen under MTT and TTC treatments after 15 mins

Figure 1 shows the collected pollen from hybrid 18 stained using 0.5% 2,3,5-triphenyl tetrazolium chloride (TTC) with buffer solution from 0-15 minutes under the 40x microscope for pollen availability measurements (Luria G. et al. 2019). It was hard to distinguish between viable and nonviable pollen after their explosion. Since it concerns cytolysis and various colors on maize pollens, I concluded that TTC is not a stable technique for pollen viability observation in future experiments.

Furthermore, in the evaluation of pollen viability and performance, dead pollen stored at room temperature for 24 hours serves as a reliable test, comparing the outcomes between MTT and TTC. Figure 3 clearly demonstrated that MTT outperforms TTC in measurements, leading to reduced errors in results. The pollen materials used for this observational experiment were exclusively collected from hybrid 18 under a single condition-room temperature. To enhance the robustness of future observations and measurements, I included a high temperature killing control in later work to test the stability of indicators on maize pollen viability.

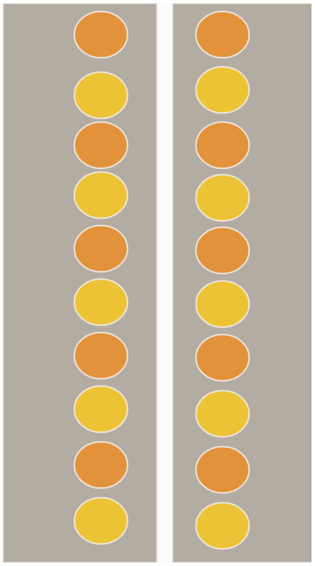


Illustration 4. Planting layout mixed with control and water stress treatments

Pollen Viability assessment

Pollen viability

During batch 1 of the experiment, small-scale pollen grains were collected from hybrids 2 and 11 under both water stress and control conditions. The viability test was performed following the measurement of pollen shedding using tubes at 9:00 am on March 23rd, 2024, at the 3rd replicate of the research. The test tubes were treated separately with three levels of temperature: 22.5 (room temperature), 45, and 65 to kill portion of pollens to observe the effectiveness of the indicator on pollen viability and the comparison between MTT and TTC in the incubator for 15 mins. After the three temperature treatments, a small amount of pollen was transported from the test tube to the eye slides, and 10 μL of MTT with 1% sucrose was added to the pollen for 10 minutes. Photos were taken after 10 minutes by using a light microscope with 40x magnification for evaluation.

Pollen shedding

After tassel emergence, pollen grains were collected daily from both treatment groups during the pollen-shedding period until the end using a funnel collector. Collection occurred between 8:00 am and 10:00 am to maintain consistency in sampling time during the pollination. The collection was performed using V-shaped cardboard about 50 cm X 40 cm, and tassels were tipped horizontal and shaken for about 30 sec so pollens fell and collected on the cardboard. Pollen grains collected from each treatment group were weighed using a precision scale to quantify pollen weight to 0. 01 g. Means and sums of pollen weight were calculated for each treatment group to assess pollination performance under normal water and water stress conditions. Statistical analysis was performed to determine if there were significant differences in pollen weight between the control and water stress treatments and hybrids.

Silk and ear growth

The silk of hybrids was bagged to prevent pollination during the early stage; silks were manually pollinated 7 days after silk emergence to estimate kernel production. During the batch 1 to 3, silk and ear growth were measured every day following the pollen shedding collection time around 10:00 am and 11:00 am. The length of the shank from the node to the base of the ear and the length of the shank+ear+silk from the node to the tip of the silk was measured initially. After that, the exposed silk was recorded and removed by pruning shear. After the data collection of silk and ear growth, the length of the ear+silk was calculated from the base of the ear to the silk and rates were calculated by dividing the ear+silk lengths by the time over which growth occurred.

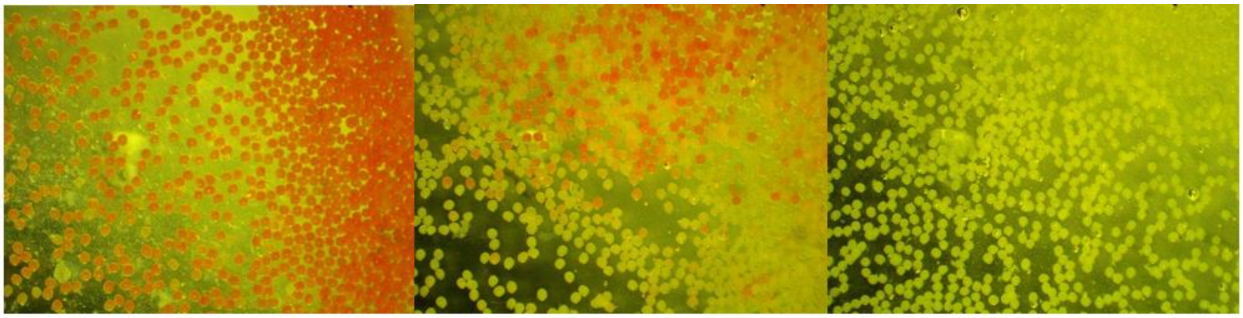
Statistical Methods

Multiple analyses were used during the evaluation including two-way ANOVA and Emmean function. The two-way ANOVA test was used to compare sums of pollen grain weight across different hybrids and treatments, with degrees of freedom (df) calculated based on the sample size. The ANOVA model included these sources of variation: hybrid, watering treatment and replicate batch. The significance level (p-value) was set at 5%. After the analysis, Emmeans function was used to further investigate the individual hybrid had significant increase pollen grain weight for future selection. In batch 3, the evaluation system focused on selecting maize hybrids with potential drought tolerance traits based on their silk and ear growth rates. Specifically, hybrids exhibiting a silk and ear growth rate in water stress greater than 10% of controls were identified as candidates for further investigation and potential selection. This threshold served as a criterion to differentiate hybrids with robust growth under water stress conditions, indicative of traits associated with drought tolerance. The utilization of a 10% growth rate threshold provided a clear and objective metric to assess the performance of hybrids under stress conditions. Hybrids surpassing this threshold demonstrated an ability to maintain reproductive vigor even under water-limited environments, suggesting adaptive traits that could contribute to improved yield stability in drought-prone regions.

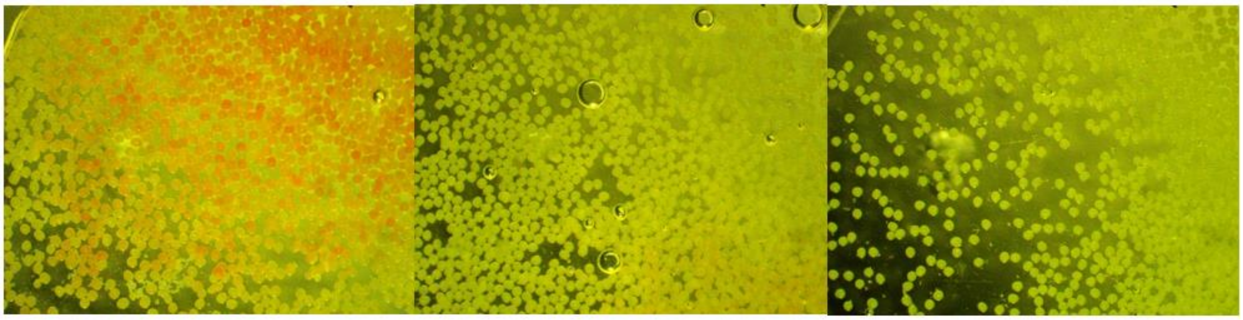
RESULTS

Pollen viability in batch 1

In the first batch, pollens were treated with TTC solution for 10 minutes for coloration and photos were taken under a microscope with 40x magnification, for evaluation. The photos revealed distinct levels of red coloration, indicative of pollen metabolism and viability, with over 50% of alive pollens in hybrids 2C, hybrid 2WS, and hybrid 11WS under room temperature as controls but not in hybrid 11 C. During the treatment with 45 ℃, most hybrids showed a small portion of pinkish color, which means there was low pollen viability, including hybrid 2C, hybrid 11C, and hybrid 11WS. At the last heat treatment with 65 ℃, none of the hybrids presented in pink and reddish color. However, the color of the live pollen was weak, and it was still hard to estimate the pollen viability.

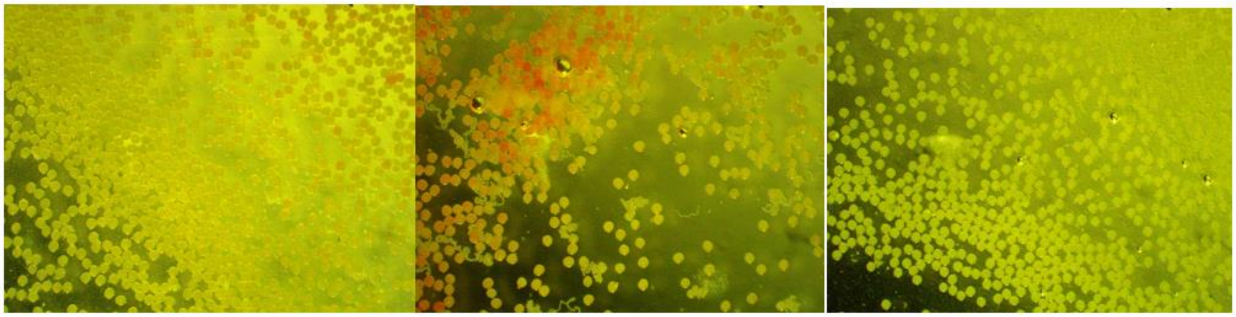


Hybrid 2C with 22.5°C Hybrid 2C with 45°C Hybrid 2C with 65°C

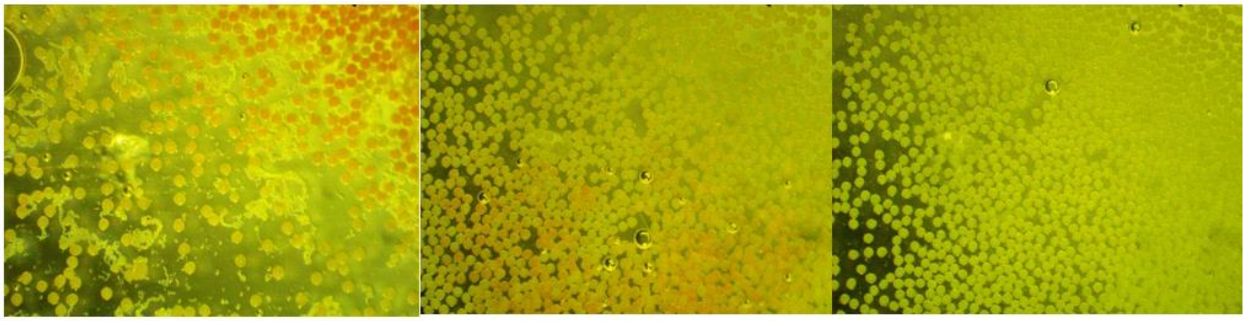


Hybrid 2WS with 22.5°C Hybrid 2WS with 45°C Hybrid 2WS with 65°C

Illustration 4. Hybrid 2 pollen viability with TTC in different temperatures

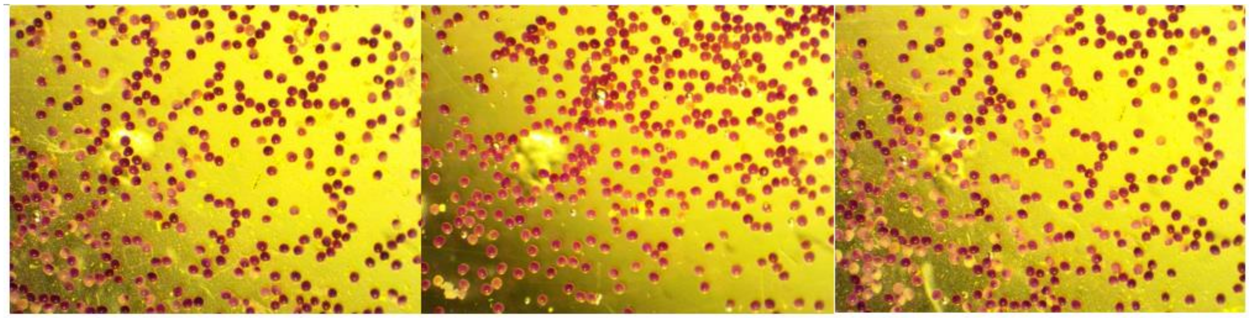


Hybrid 11C with 22.5°C Hybrid 11C with 45°C Hybrid 11C with 65°C



Hybrid 11WS with 22.5°C Hybrid 11WS with 45°C Hybrid 11WS with 65°C

Illustration 5. Hybrid 11 pollen viability with TTC in different temperatures



Hybrid 2C with 22.5°C   Hybrid 2C with 45°C   Hybrid 2C with 65°C

Hybrid 11C with 22.5°C   Hybrid 11C with 45°C   Hybrid 11C with 65°C

Illustration 6. Hybrid 2 & 11 pollen viability with MTT in different temperatures

In comparison with pollen in the TTC solution, pollen in all hybrids and temperatures treated with MTT solution were entirely dark purple and reddish in color in hybrid 2WS and hybrid 11WS, and more cytolysis occurred after the solution was added to the pollens for 9 minutes. MTT thus did not appear to provide a meaningful assessment of viability.

Pollen shedding in batch 2

In the 1st replicate, signs of calcium nutrition deficiency were observed in hybrids 1, 2, and 10, and the nutritional deficiency may have impacted those hybrids at the early stages of growth development, and especially the shoot apical meristem where the tassel is formed on maize. Most hybrids showed expected curves during the pollen shedding period, and by summing the pollen collected during this period and dividing by the days over which pollen was shed in each hybrid gave the mean per day of pollen shed. The mean of pollen shedding for control and water stress conditions suggested that there was no significant difference in the quantity of pollen grains in water stress compared to water stress conditions.

The duration of the pollen shedding period was monitored during water stress treatment, and double-peak patterns of pollen shed were observed in some hybrids, including hybrid 1 WS, hybrid 10 WS, and hybrid 20 WS. Hybrids 15 and 16 showed linear graphs of pollen shedding, with the maximum on the first day and declining after that, possibly due to Ca nutrition deficiency in these hybrids, or the collection of pollen might have started too late to catch the initial pollen shed. So data from the 1st replicate was excluded later as outlined in the two-way ANOVA test.

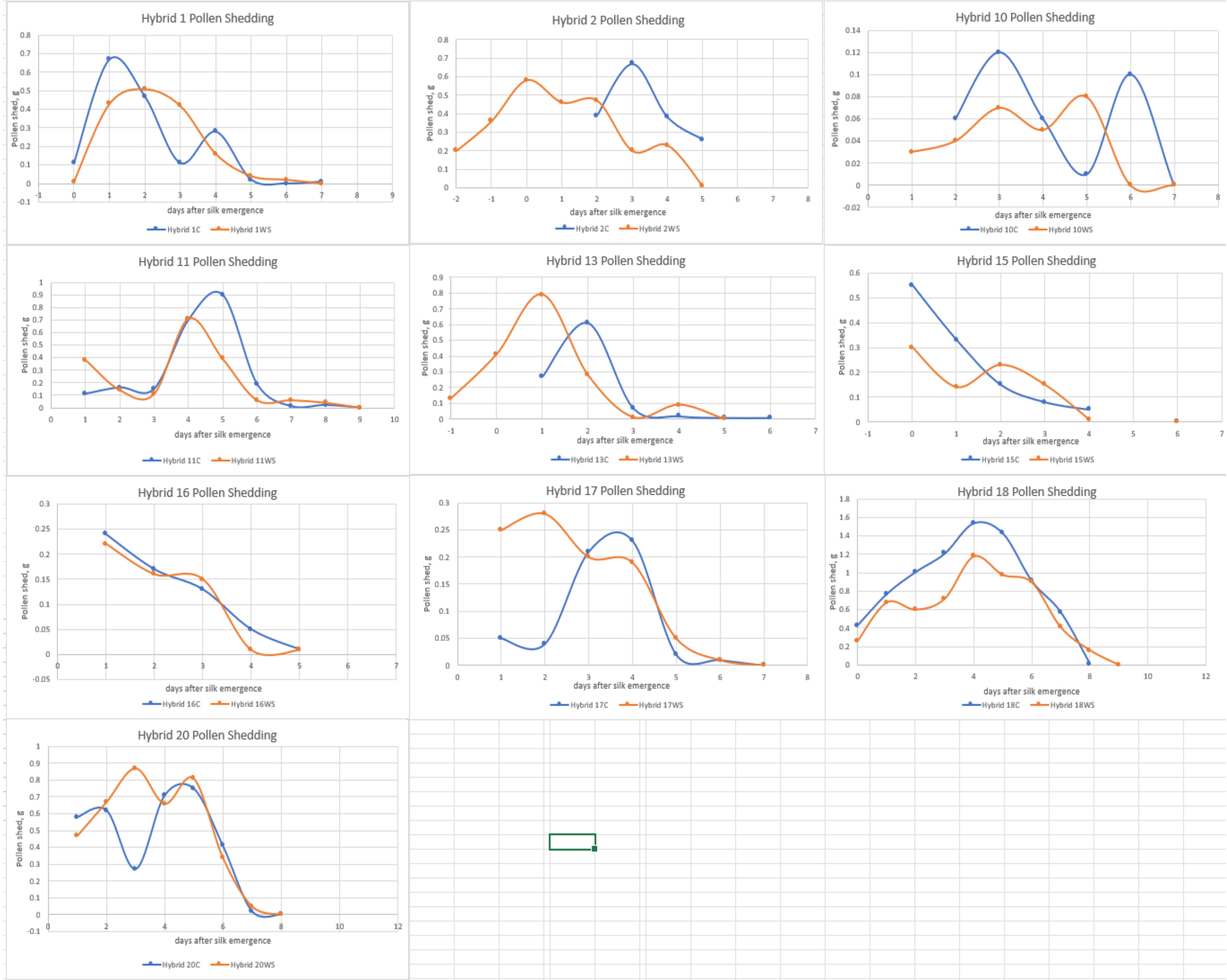


Figure 1. Pollen shedding in control and water stress treatment for 1^{st} replicate. Pollen collected daily from control (blue) and water stress (orange) treatments of 10 hybrids are shown.

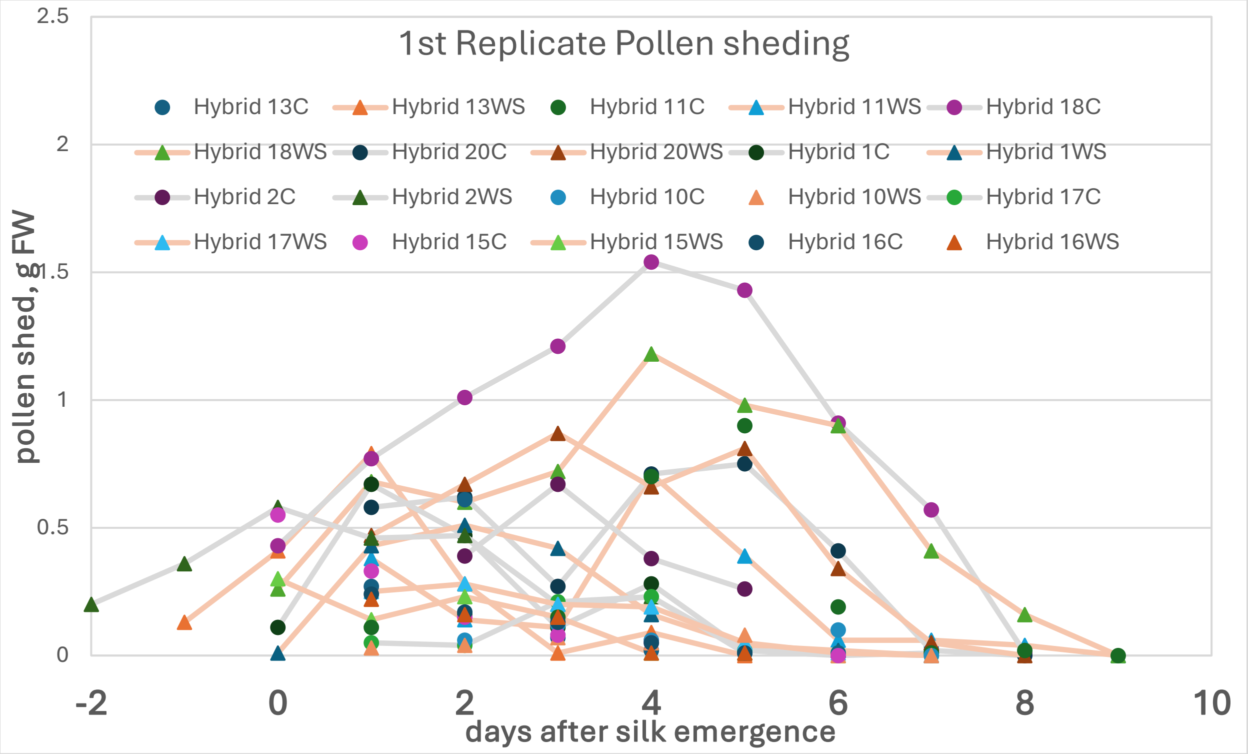


Figure 2. The hybrids pollen shedding

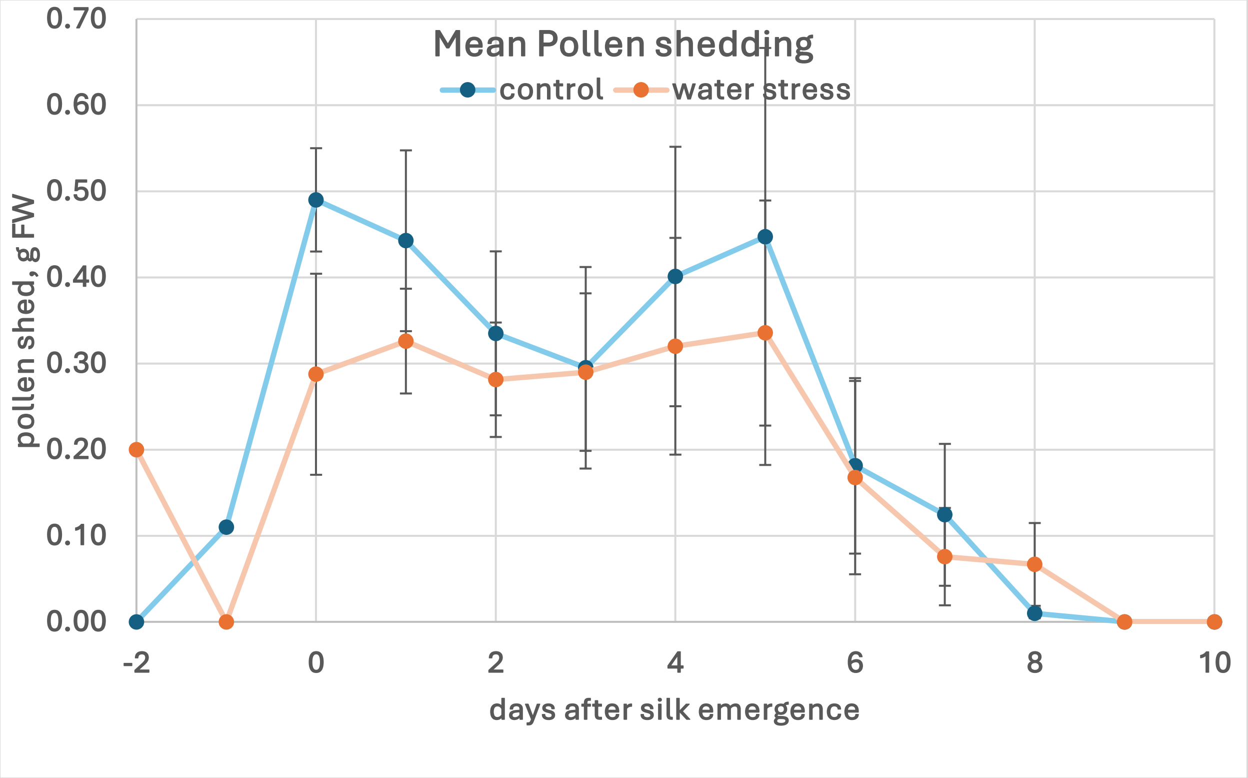
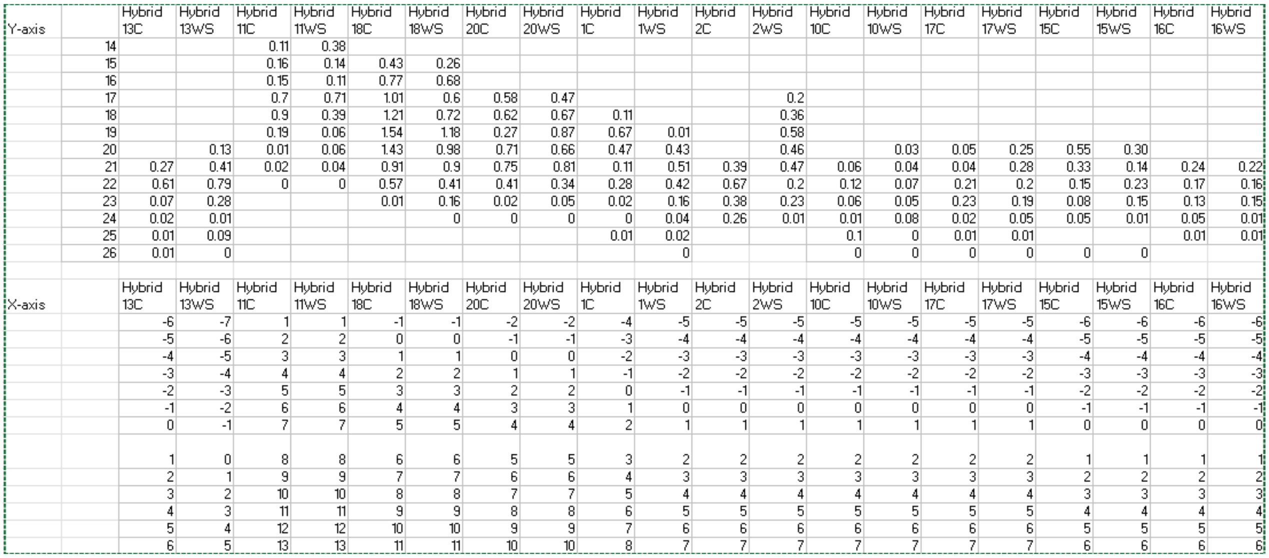


Figure 3. Mean of pollen shedding for hybrids in 1st replicate



Y-axis		Hybrid 13C	Hybrid 13WS	Hybrid 11C	Hybrid 11WS	Hybrid 18C	Hybrid 18WS	Hybrid 20C	Hybrid 20WS	Hybrid 1C	Hybrid 1WS	Hybrid 2C	Hybrid 2WS	Hybrid 10C	Hybrid 10WS	Hybrid 17C	Hybrid 17WS	Hybrid 15C	Hybrid 15WS	Hybrid 16C	Hybrid 16WS
	14			0.11	0.38																
	15			0.16	0.14	0.43	0.26														
	16			0.15	0.11	0.77	0.68														
	17			0.7	0.71	1.01	0.6	0.58	0.47				0.2								
	18			0.9	0.39	1.21	0.72	0.62	0.67	0.11			0.36								
	19			0.19	0.06	1.54	1.18	0.27	0.87	0.67	0.01		0.58								
	20		0.13	0.01	0.06	1.43	0.98	0.71	0.66	0.47	0.43		0.46		0.03	0.05	0.25	0.55	0.30		
	21	0.27	0.41	0.02	0.04	0.91	0.9	0.75	0.81	0.11	0.51	0.39	0.47	0.06	0.04	0.04	0.28	0.33	0.14	0.24	0.22
	22	0.61	0.79	0	0	0.57	0.41	0.41	0.34	0.28	0.42	0.67	0.2	0.12	0.07	0.21	0.2	0.15	0.23	0.17	0.16
	23	0.07	0.28			0.01	0.16	0.02	0.05	0.02	0.16	0.38	0.23	0.06	0.05	0.23	0.19	0.08	0.15	0.13	0.15
	24	0.02	0.01				0	0	0	0	0.04	0.26	0.01	0.01	0.08	0.02	0.05	0.05	0.01	0.05	0.01
	25	0.01	0.09							0.01	0.02			0.1	0	0.01	0.01			0.01	0.01
	26	0.01	0								0			0	0	0	0	0	0		
X-axis		Hybrid 13C	Hybrid 13WS	Hybrid 11C	Hybrid 11WS	Hybrid 18C	Hybrid 18WS	Hybrid 20C	Hybrid 20WS	Hybrid 1C	Hybrid 1WS	Hybrid 2C	Hybrid 2WS	Hybrid 10C	Hybrid 10WS	Hybrid 17C	Hybrid 17WS	Hybrid 15C	Hybrid 15WS	Hybrid 16C	Hybrid 16WS
		-6	-7	1	1	-1	-1	-2	-2	-4	-5	-5	-5	-5	-5	-5	-5	-6	-6	-6	-6
		-5	-6	2	2	0	0	-1	-1	-3	-4	-4	-4	-4	-4	-4	-4	-5	-5	-5	-5
		-4	-5	3	3	1	1	0	0	-2	-3	-3	-3	-3	-3	-3	-3	-4	-4	-4	-4
		-3	-4	4	4	2	2	1	1	-1	-2	-2	-2	-2	-2	-2	-2	-3	-3	-3	-3
		-2	-3	5	5	3	3	2	2	0	-1	-1	-1	-1	-1	-1	-1	-2	-2	-2	-2
		-1	-2	6	6	4	4	3	3	1	0	0	0	0	0	0	0	-1	-1	-1	-1
		0	-1	7	7	5	5	4	4	2	1	1	1	1	1	1	1	0	0	0	0
		1	0	8	8	6	6	5	5	3	2	2	2	2	2	2	2	1	1	1	1
		2	1	9	9	7	7	6	6	4	3	3	3	3	3	3	3	2	2	2	2
		3	2	10	10	8	8	7	7	5	4	4	4	4	4	4	4	3	3	3	3
		4	3	11	11	9	9	8	8	6	5	5	5	5	5	5	5	4	4	4	4
		5	4	12	12	10	10	9	9	7	6	6	6	6	6	6	6	5	5	5	5
		6	5	13	13	11	11	10	10	8	7	7	7	7	7	7	7	6	6	6	6

Table 2. 1st replicate pollen shedding data

The 2nd replicate of plants followed curve patterns during the pollen shedding period. Hybrids1, 2, 13, 15 and 16 showed double-peak curve patterns under water stress, while other hybrids were not affected by water stress in this way. During the second replicate, an interesting phenomenon was recorded. Some hybrids under the water stress environment produced more pollen than those under the control environment, which might represent a drought stress tolerance trait in those hybrids.

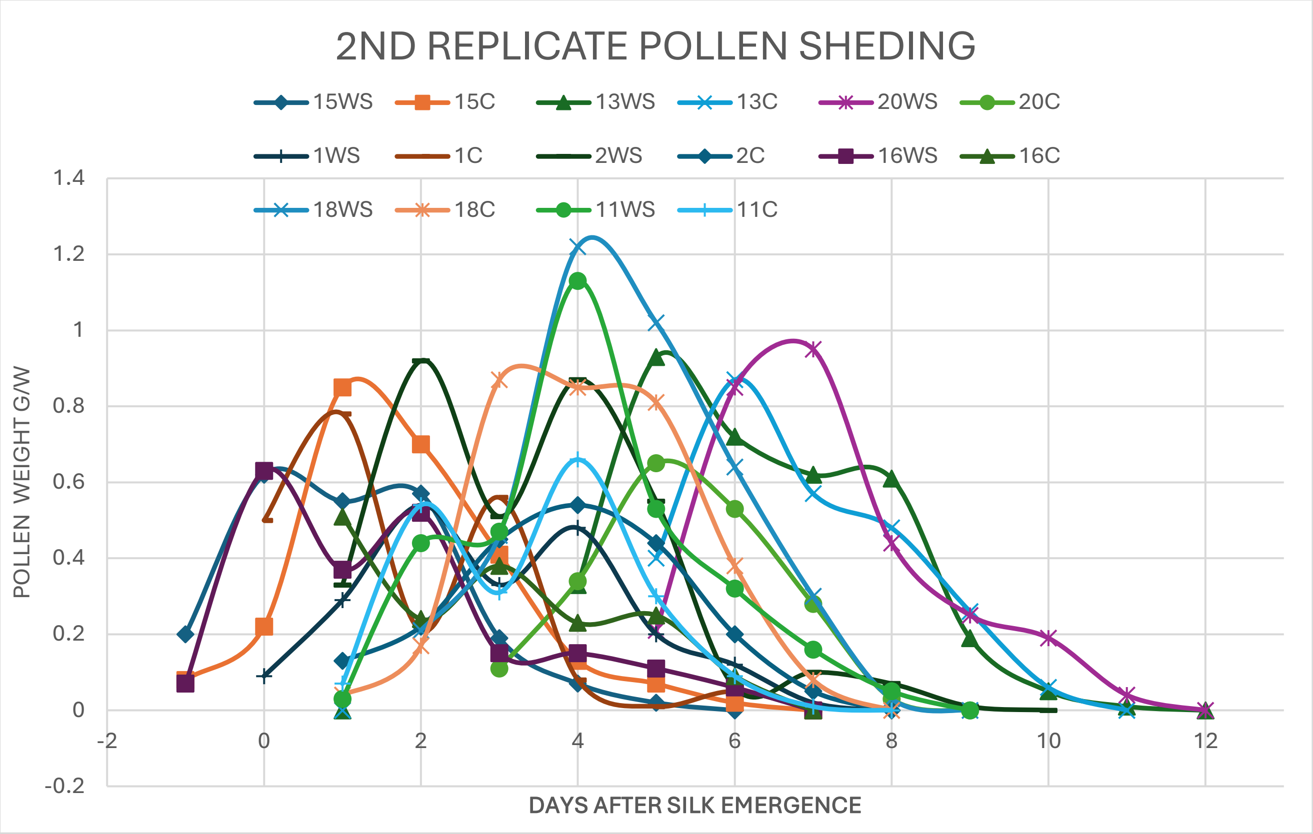
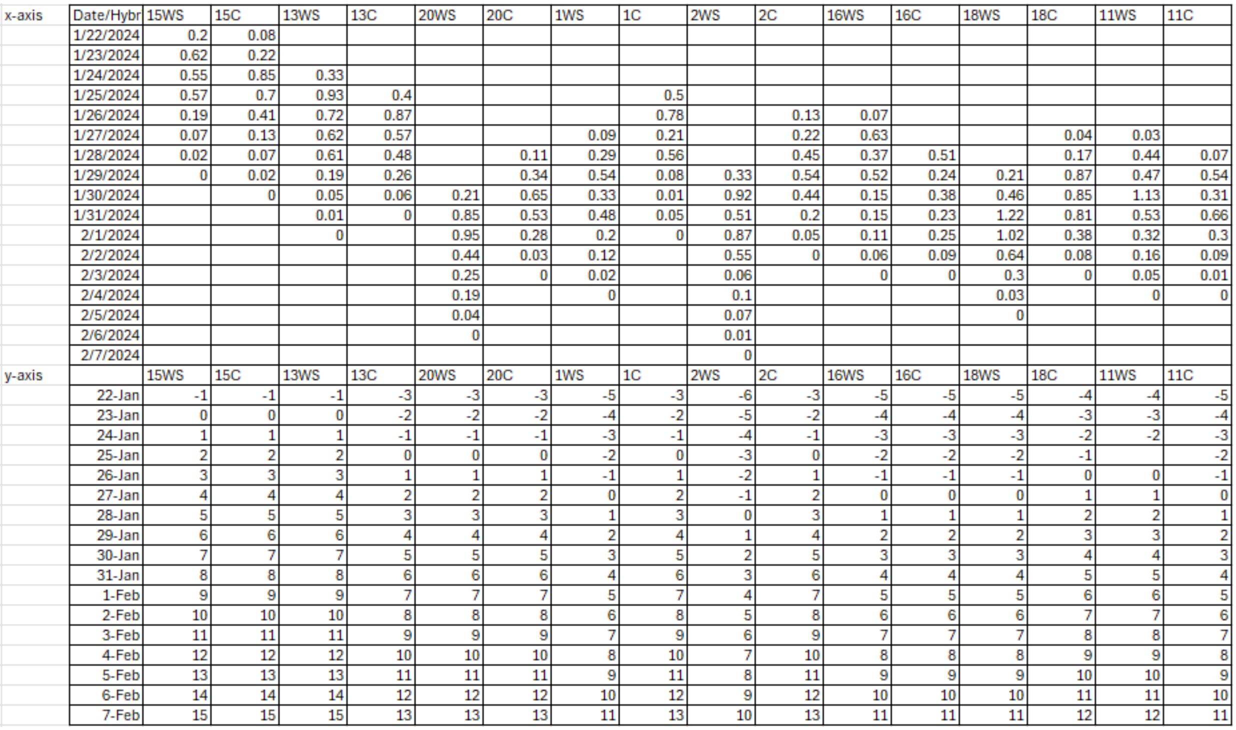


Figure 4. 2nd replicate pollen shedding for hybrids. Control and water stress for eight hybrids are shown.



x-axis	Date/Hybr	15WS	15C	13WS	13C	20WS	20C	1WS	1C	2WS	2C	16WS	16C	18WS	18C	11WS	11C
	1/22/2024	0.2	0.08														
	1/23/2024	0.62	0.22														
	1/24/2024	0.55	0.85	0.33													
	1/25/2024	0.57	0.7	0.93	0.4				0.5								
	1/26/2024	0.19	0.41	0.72	0.87				0.78		0.13	0.07					
	1/27/2024	0.07	0.13	0.62	0.57			0.09	0.21		0.22	0.63			0.04	0.03	
	1/28/2024	0.02	0.07	0.61	0.48		0.11	0.29	0.56		0.45	0.37	0.51		0.17	0.44	0.07
	1/29/2024	0	0.02	0.19	0.26		0.34	0.54	0.08	0.33	0.54	0.52	0.24	0.21	0.87	0.47	0.54
	1/30/2024		0	0.05	0.06	0.21	0.65	0.33	0.01	0.92	0.44	0.15	0.38	0.46	0.85	1.13	0.31
	1/31/2024			0.01	0	0.85	0.53	0.48	0.05	0.51	0.2	0.15	0.23	1.22	0.81	0.53	0.66
	2/1/2024			0		0.95	0.28	0.2	0	0.87	0.05	0.11	0.25	1.02	0.38	0.32	0.3
	2/2/2024					0.44	0.03	0.12		0.55	0	0.06	0.09	0.64	0.08	0.16	0.09
	2/3/2024					0.25	0	0.02		0.06		0	0	0.3	0	0.05	0.01
	2/4/2024					0.19		0		0.1				0.03		0	0
	2/5/2024					0.04				0.07				0			
	2/6/2024					0				0.01							
	2/7/2024									0							
y-axis		15WS	15C	13WS	13C	20WS	20C	1WS	1C	2WS	2C	16WS	16C	18WS	18C	11WS	11C
	22-Jan	-1	-1	-1	-3	-3	-3	-5	-3	-6	-3	-5	-5	-5	-4	-4	-5
	23-Jan	0	0	0	-2	-2	-2	-4	-2	-5	-2	-4	-4	-4	-3	-3	-4
	24-Jan	1	1	1	-1	-1	-1	-3	-1	-4	-1	-3	-3	-3	-2	-2	-3
	25-Jan	2	2	2	0	0	0	-2	0	-3	0	-2	-2	-2	-1		-2
	26-Jan	3	3	3	1	1	1	-1	1	-2	1	-1	-1	-1	0	0	-1
	27-Jan	4	4	4	2	2	2	0	2	-1	2	0	0	0	1	1	0
	28-Jan	5	5	5	3	3	3	1	3	0	3	1	1	1	2	2	1
	29-Jan	6	6	6	4	4	4	2	4	1	4	2	2	2	3	3	2
	30-Jan	7	7	7	5	5	5	3	5	2	5	3	3	3	4	4	3
	31-Jan	8	8	8	6	6	6	4	6	3	6	4	4	4	5	5	4
	1-Feb	9	9	9	7	7	7	5	7	4	7	5	5	5	6	6	5
	2-Feb	10	10	10	8	8	8	6	8	5	8	6	6	6	7	7	6
	3-Feb	11	11	11	9	9	9	7	9	6	9	7	7	7	8	8	7
	4-Feb	12	12	12	10	10	10	8	10	7	10	8	8	8	9	9	8
	5-Feb	13	13	13	11	11	11	9	11	8	11	9	9	9	10	10	9
	6-Feb	14	14	14	12	12	12	10	12	9	12	10	10	10	11	11	10
	7-Feb	15	15	15	13	13	13	11	13	10	13	11	11	11	12	12	11

Table 3. 2^{nd} replicate pollen shedding data

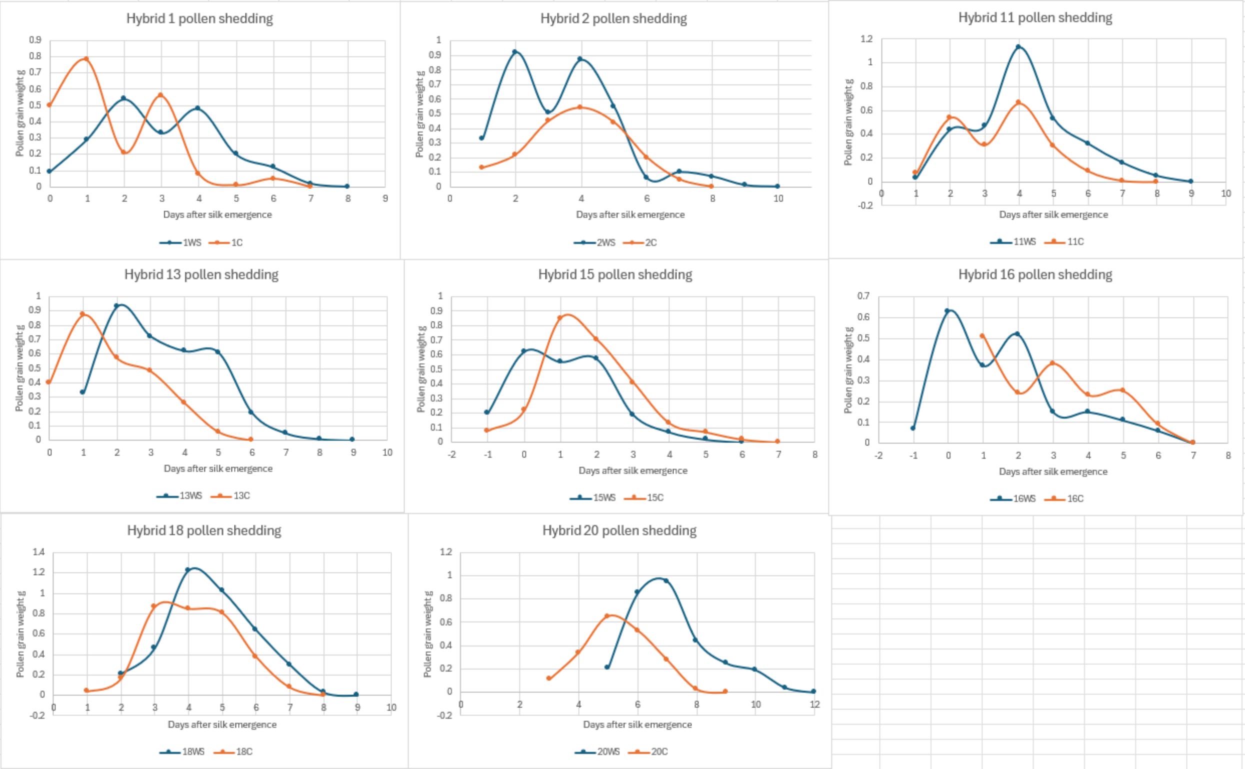


Figure 5. Pollen shedding for individual hybrid in the second replicate. Pollen collected daily from control (orange) and water stress (blue) treatments of 8 hybrids are shown.

Pollen shedding in batch 3

In the 3rd replicate, based on the graphs, hybrid 2 WS, 11 WS, 13WS, 16 WS, 18WS, and 20WS was the hybrids which appeared to respond a lot to water stress, while the others had similar curved patterns in control and water stress (Figure 5). Stress responses of pollen shedding varied across different hybrids. In the 2nd replicate and 3rd replicate, more pollen was produced in some hybrids under water stress. The sum of pollen shedding amount was analyzed with 2-way ANOVA through R studio. The results showed that across treatments, hybrids differed significantly ($F_{(7df)}$=2.605, p=0.0534), and across hybrids, water stress significantly increased pollen production ($F_{(1df)}$=6.165, p=0.0245), but there was no interaction between hybrids and treatments ($F_{(7df)}$=1.954, p=0.1265). Using Emmeans statistical procedure, it was revealed that only certain hybrids showed a significant increase in pollen grain weight under water stress treatments, including hybrid 2 (df=16, p=0.0139), hybrid 11 (df=16, p=0.0345), and hybrid 20 (df=16, p=0.0269) (Figure 8). Most of the other hybrids had no significant difference of pollen grain weight between treatments, including hybrid 1 (df=16, p=0.7888), hybrid 13 (df=16, p=0.6184), hybrid 15 (df=16, p=4636), hybrid 16 (df=16, p=0.8905), and hybrid 18 (df=16, p=0.8677). However, among those hybrids, hybrid 18 showed high amounts of pollen grain weight under both control and stress and thus, in addition to hybrids 2, 11 and 20, was identified for future investigation as candidates with germplasm contributing a potential drought tolerance trait according to Figure 8.

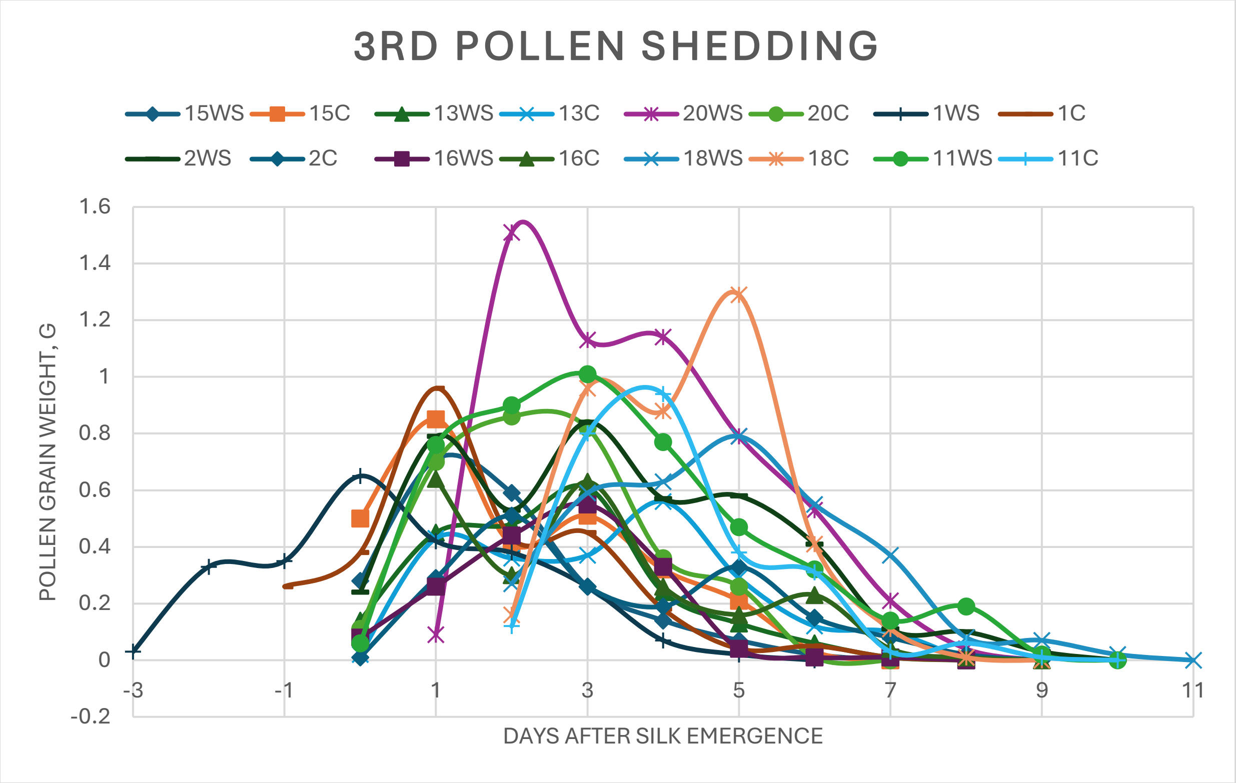
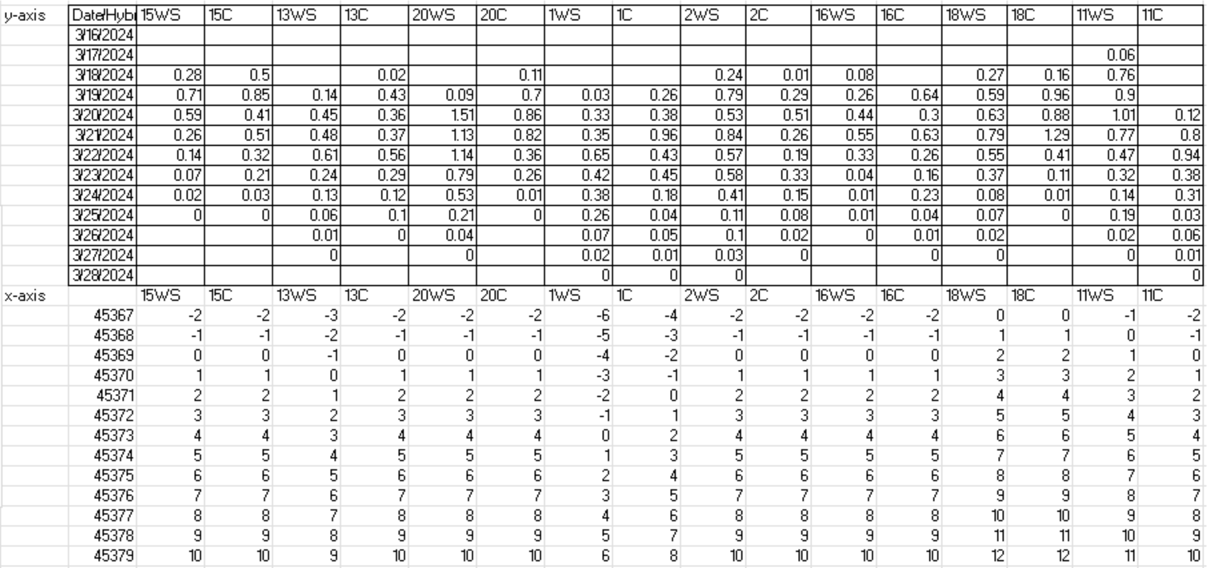


Figure 6. Third replicate pollen shedding for hybrids. Control and water stress for eight hybrids are shown.



y-axis	Date/Hyb	15WS	15C	13WS	13C	20WS	20C	1WS	1C	2WS	2C	16WS	16C	18WS	18C	11WS	11C
	3/16/2024																
	3/17/2024															0.06	
	3/18/2024	0.28	0.5		0.02		0.11			0.24	0.01	0.08		0.27	0.16	0.76	
	3/19/2024	0.71	0.85	0.14	0.43	0.09	0.7	0.03	0.26	0.79	0.29	0.26	0.64	0.59	0.96	0.9	
	3/20/2024	0.59	0.41	0.45	0.36	1.51	0.86	0.33	0.38	0.53	0.51	0.44	0.3	0.63	0.88	1.01	0.12
	3/21/2024	0.26	0.51	0.48	0.37	1.13	0.82	0.35	0.96	0.84	0.26	0.55	0.63	0.79	1.29	0.77	0.8
	3/22/2024	0.14	0.32	0.61	0.56	1.14	0.36	0.65	0.43	0.57	0.19	0.33	0.26	0.55	0.41	0.47	0.94
	3/23/2024	0.07	0.21	0.24	0.29	0.79	0.26	0.42	0.45	0.58	0.33	0.04	0.16	0.37	0.11	0.32	0.38
	3/24/2024	0.02	0.03	0.13	0.12	0.53	0.01	0.38	0.18	0.41	0.15	0.01	0.23	0.08	0.01	0.14	0.31
	3/25/2024	0	0	0.06	0.1	0.21	0	0.26	0.04	0.11	0.08	0.01	0.04	0.07	0	0.19	0.03
	3/26/2024			0.01	0	0.04		0.07	0.05	0.1	0.02	0	0.01	0.02		0.02	0.06
	3/27/2024			0		0		0.02	0.01	0.03	0		0	0		0	0.01
	3/28/2024							0	0	0							0
x-axis		15WS	15C	13WS	13C	20WS	20C	1WS	1C	2WS	2C	16WS	16C	18WS	18C	11WS	11C
	45367	-2	-2	-3	-2	-2	-2	-6	-4	-2	-2	-2	-2	0	0	-1	-2
	45368	-1	-1	-2	-1	-1	-1	-5	-3	-1	-1	-1	-1	1	1	0	-1
	45369	0	0	-1	0	0	0	-4	-2	0	0	0	0	2	2	1	0
	45370	1	1	0	1	1	1	-3	-1	1	1	1	1	3	3	2	1
	45371	2	2	1	2	2	2	-2	0	2	2	2	2	4	4	3	2
	45372	3	3	2	3	3	3	-1	1	3	3	3	3	5	5	4	3
	45373	4	4	3	4	4	4	0	2	4	4	4	4	6	6	5	4
	45374	5	5	4	5	5	5	1	3	5	5	5	5	7	7	6	5
	45375	6	6	5	6	6	6	2	4	6	6	6	6	8	8	7	6
	45376	7	7	6	7	7	7	3	5	7	7	7	7	9	9	8	7
	45377	8	8	7	8	8	8	4	6	8	8	8	8	10	10	9	8
	45378	9	9	8	9	9	9	5	7	9	9	9	9	11	11	10	9
	45379	10	10	9	10	10	10	6	8	10	10	10	10	12	12	11	10

Table 4. 3rd replicate pollen shedding data

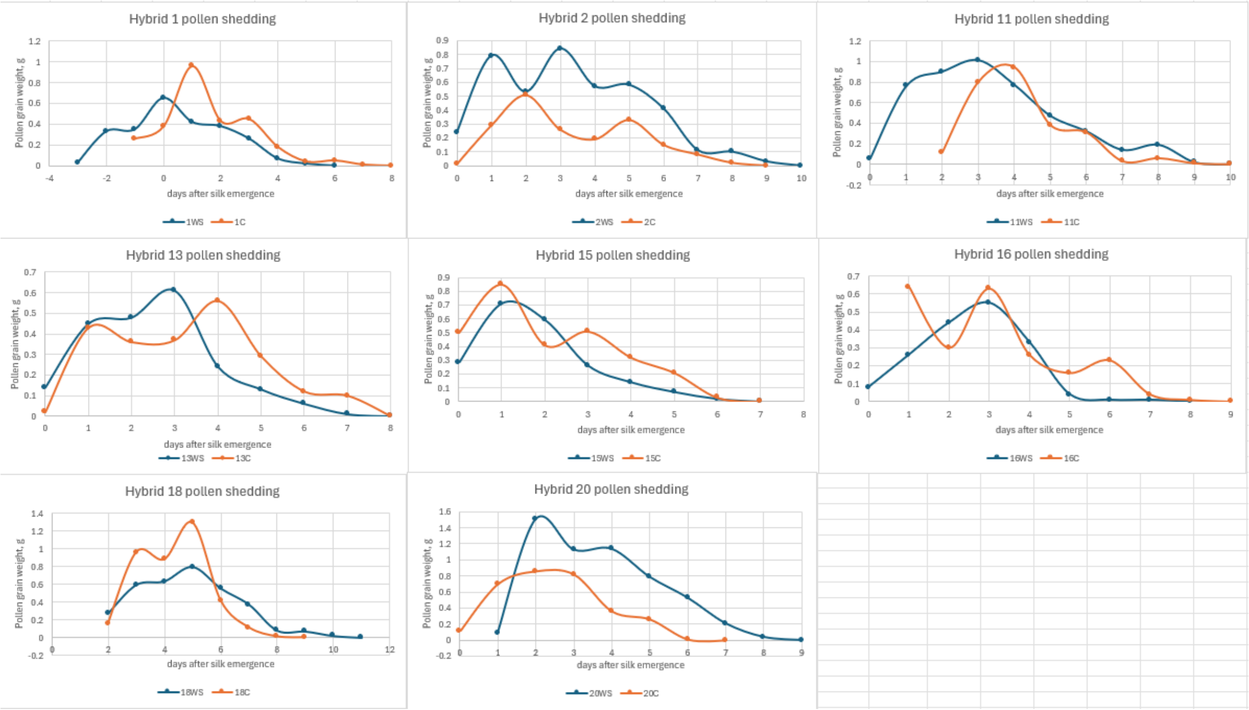
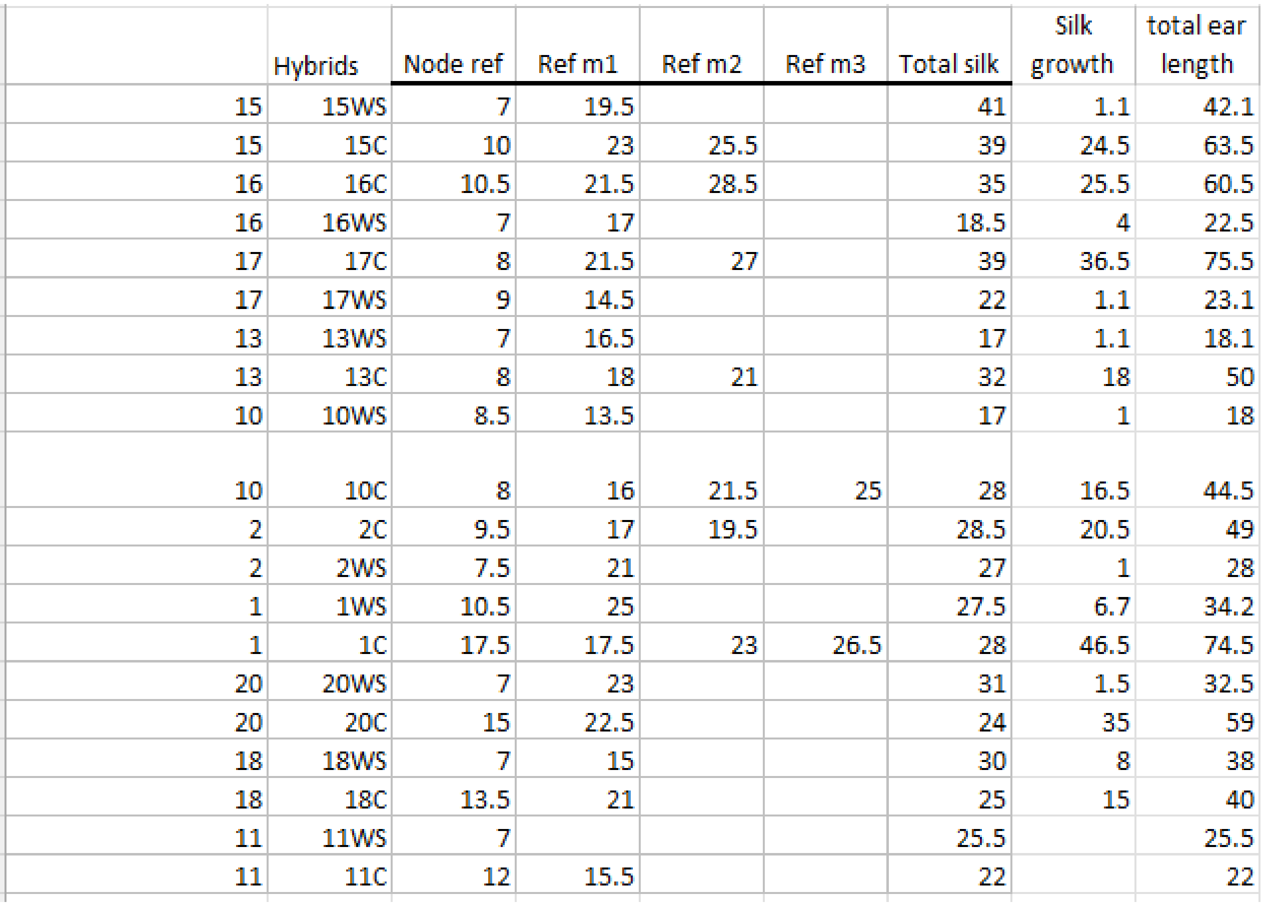


Figure 7. Pollen shedding for individual hybrid in the third replicate. Pollen collected daily from control (orange) and water stress (blue) treatments of 8 hybrids are shown.



	Hybrids	Node ref	Ref m1	Ref m2	Ref m3	Total silk	Silk growth	total ear length
15	15WS	7	19.5			41	1.1	42.1
15	15C	10	23	25.5		39	24.5	63.5
16	16C	10.5	21.5	28.5		35	25.5	60.5
16	16WS	7	17			18.5	4	22.5
17	17C	8	21.5	27		39	36.5	75.5
17	17WS	9	14.5			22	1.1	23.1
13	13WS	7	16.5			17	1.1	18.1
13	13C	8	18	21		32	18	50
10	10WS	8.5	13.5			17	1	18
10	10C	8	16	21.5	25	28	16.5	44.5
2	2C	9.5	17	19.5		28.5	20.5	49
2	2WS	7.5	21			27	1	28
1	1WS	10.5	25			27.5	6.7	34.2
1	1C	17.5	17.5	23	26.5	28	46.5	74.5
20	20WS	7	23			31	1.5	32.5
20	20C	15	22.5			24	35	59
18	18WS	7	15			30	8	38
18	18C	13.5	21			25	15	40
11	11WS	7				25.5		25.5
11	11C	12	15.5			22		22

Table 5. 1st replicate silk growth data

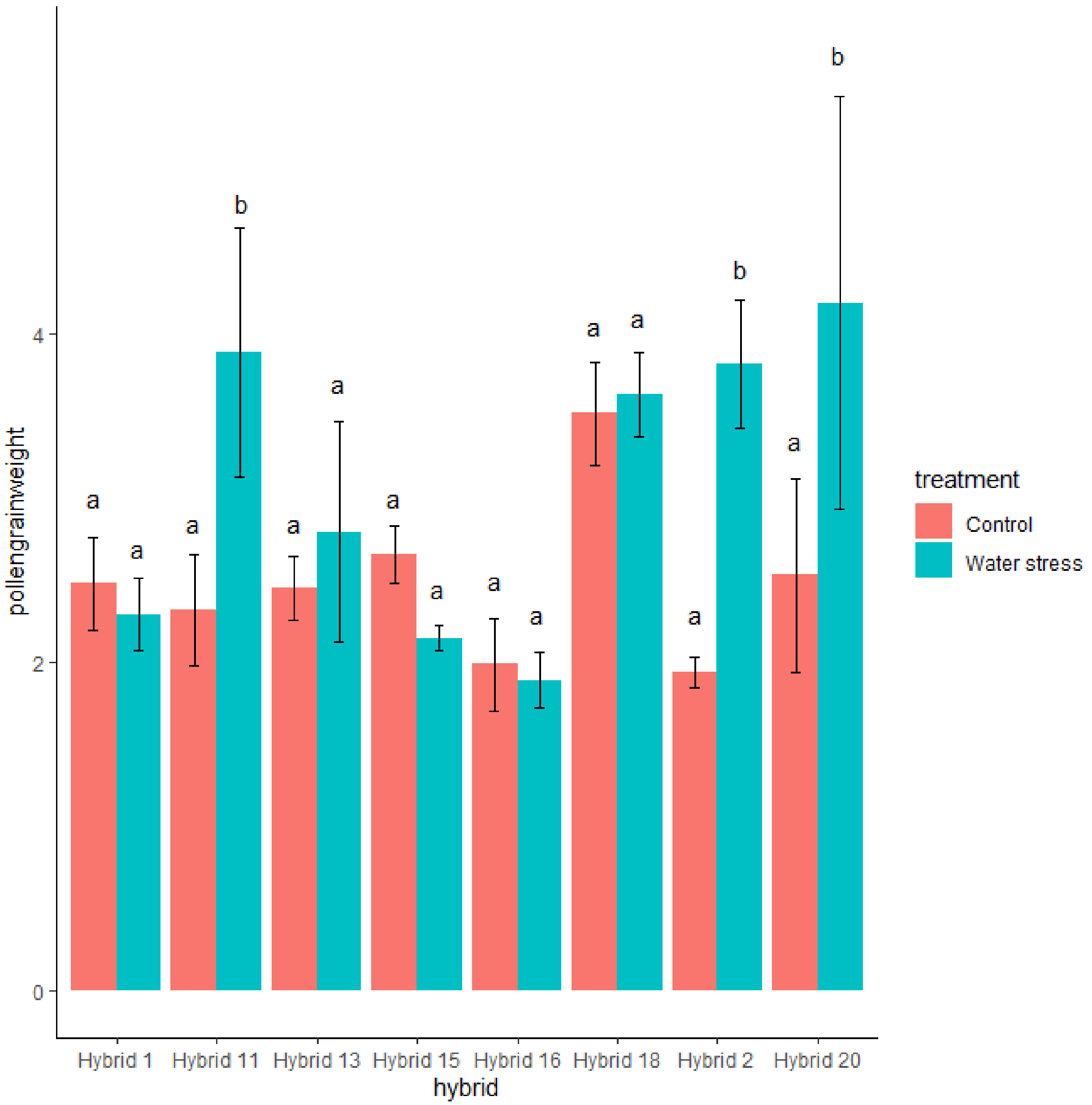


Figure 8. Pollen production in eight hybrids evaluated under water stress and control treatments in the 2nd and 3rd replicates. In pairwise comparisons of water stress (blue) versus control (red), bars with different letters were significantly different (P<0.05).

Silk+ ear growth in batches 2 and 3

Results from the measurement of silk+ ear growth indicated that there was no interaction between hybrids and treatments ($F_{(7df)}$=0.852, p=0.554) and no significant difference in silk+ ear growth between hybrids ($F_{(7df)}$=0.772, p=0.615). However, there was a considerable difference in silk+ ear growth in water stress treatment compared to the well-watered control treatment according to 2-way ANOVA through R studio ($F_{(1df)}$=83.402, p=1.99e^-10) (Figure 9, 10 and 11). The analysis from the Emmean function indicated a significant difference in silk+ear growth rates between treatments within each hybrid (Figure 9). Furthermore, selection was made by evaluating the strip chart of silk+ ear growth rates, expressed as a fraction of control in each batch, between treatments within each hybrid, and fast silk growth rates were identified by the graph (Figure 12). Some of the hybrids which maintained silk growth rates in water stress of at least 10% or greater relative to the corresponding control in two or more replicates were identified as candidates for selection, and other hybrids with 0% silk growth rate or less than 10% silk growth rate in two replicates were considered as hybrids lacking the silk+ear drought tolerance trait. By evaluating the silk+ear growth rate and this criteria, hybrid 11, hybrid 13, hybrid 15, and hybrid 16 were identified as hybrids that may have this drought tolerance trait.

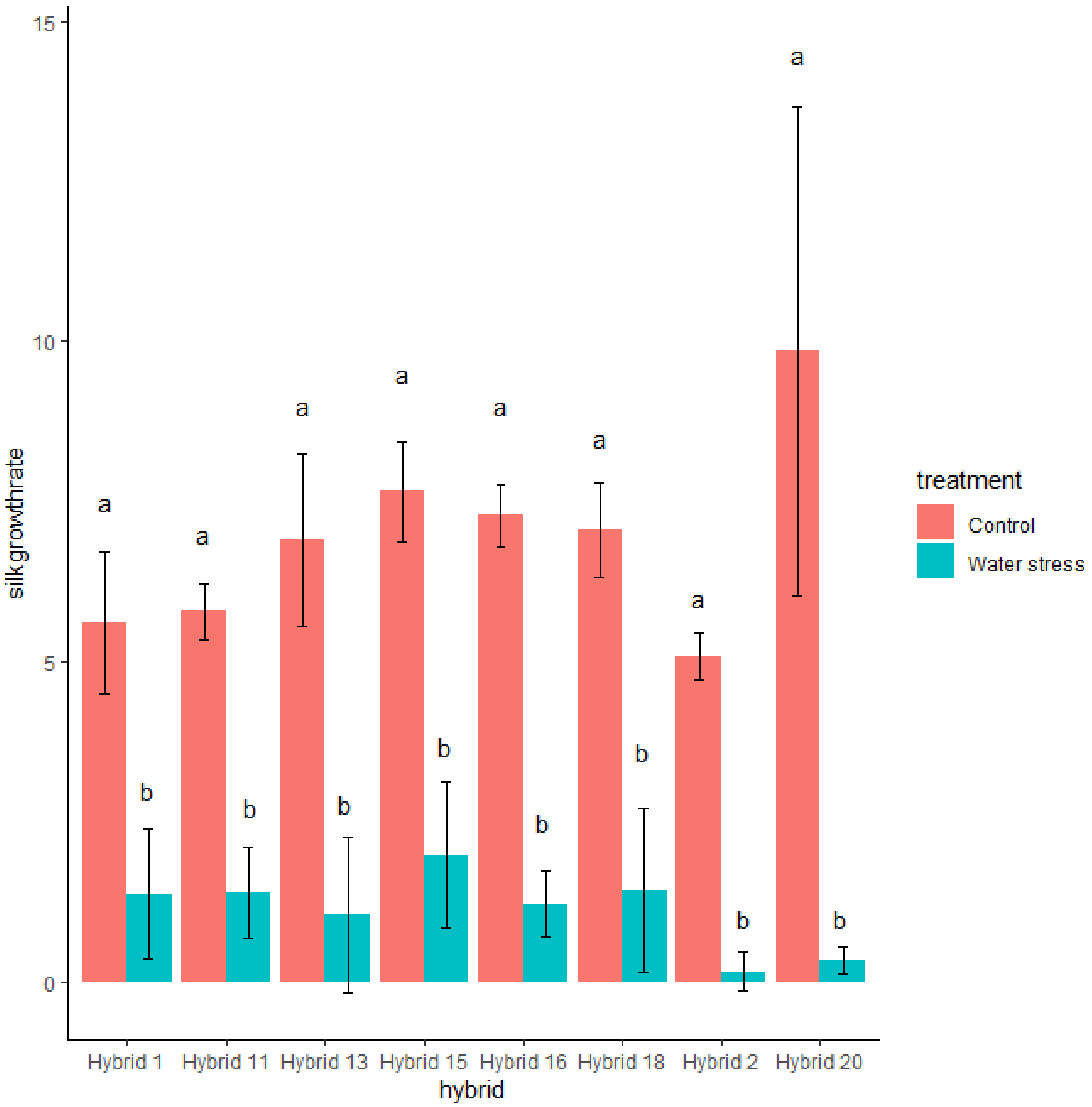


Figure 9. Silk+ear growth rate in eight hybrids evaluated under water stress and control treatments in the 1st, 2nd and 3rd replicates. Silk+ear represents the combined length of the ear+silk length, measured from the ear base to the longest silk tip. In pairwise comparisons of water stress (blue) versus control (red), bars with different letters were significantly different ($P<0.05$).

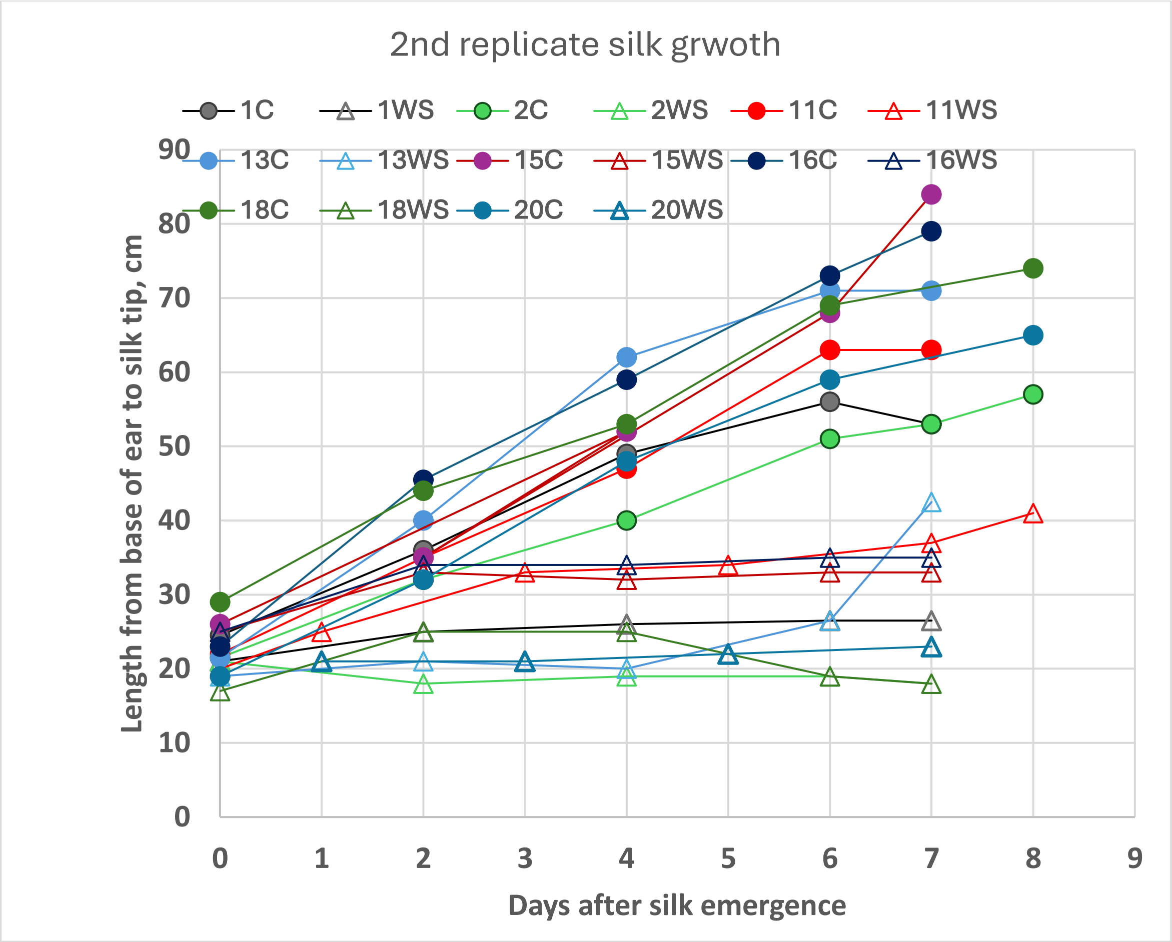


Figure 10. Silk+ear growth rate in eight hybrids evaluated under water stress (triangles) and control (circles) treatments in the 2nd replicate. Silk+ear represents the combined length of the ear+silk length, measured from the ear base to the longest silk tip.

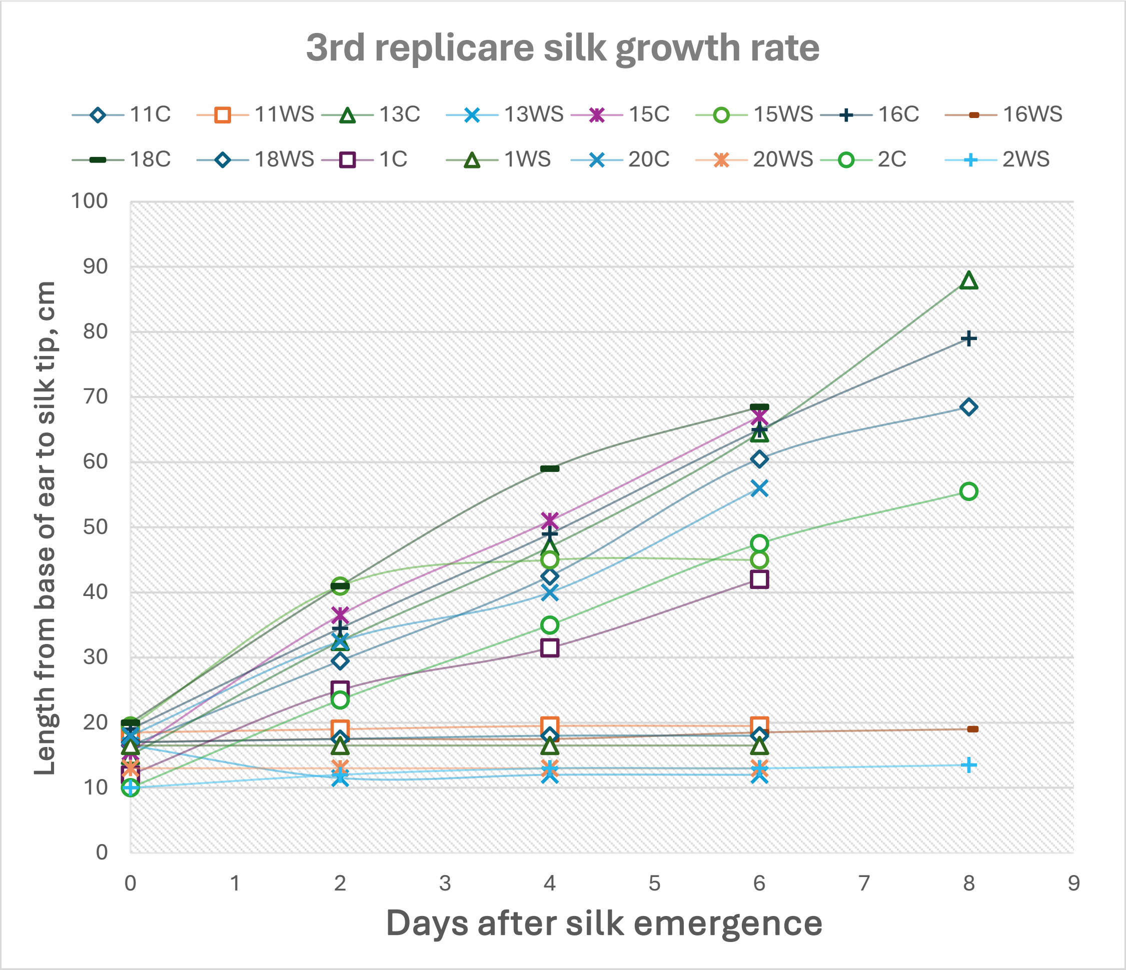


Figure 11. Silk+ear growth rate in eight hybrids evaluated under water stress (triangles) and control (circles) treatments in the 3rd replicate. Silk+ear represents the combined length of the ear+silk length, measured from the ear base to the longest silk tip.

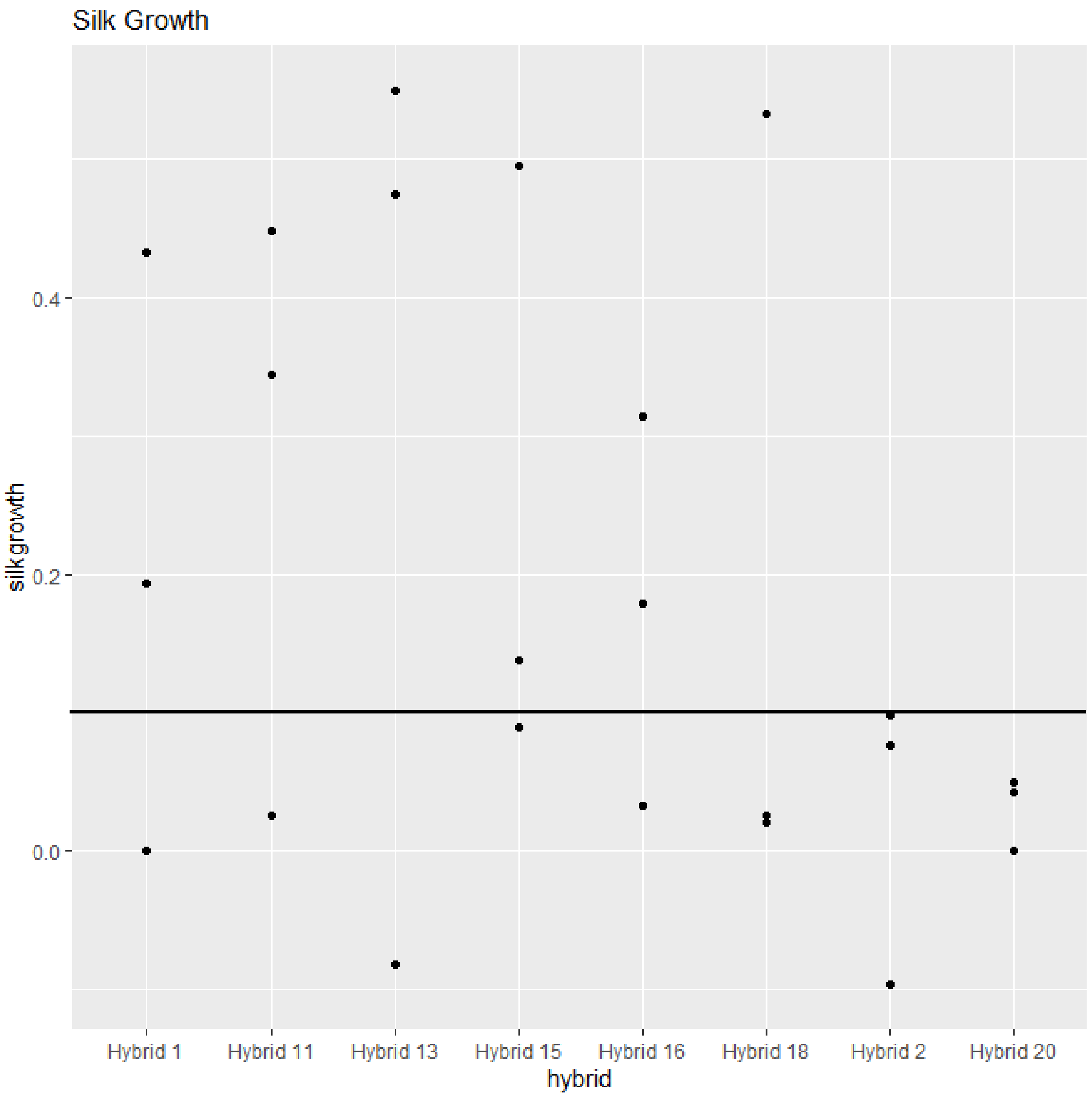


Figure 12. Silk+ear growth rate in eight hybrids evaluated under water stress and control treatments in the three replicates with 10% growth rate cut off. Shown are the growth rates of the water stress treatments, expressed as a fraction of the control growth rates. Silk+ear represents the combined length of the ear+silk length, measured from the ear base to the longest silk tip, over the period of observation, which was usually from 0 to 6 days after silk emergence.

DISCUSSION

Pollen Viability

The observed differences in pollen viability and shedding across maize hybrids under different temperature and stress treatments highlight the sensitivity of maize reproductive processes to environmental conditions. The coloration of pollens post-treatment with TTC and MTT solutions in the first trial suggested there may be differences among hybrids, particularly under heat stress (45 °C and 65 °C). Notably, the decline in pollen viability and cytolysis observed in certain hybrids, like 11C and hybrids 2C, 11C, and 11WS under heat stress, suggests there may be vulnerability of these hybrids to temperature extremes. However, the inconsistency in the findings between the first and second trials using these dyes indicates more work will be needed to use them in future evaluations.

Pollen Shedding and Quantity of Pollen

The current results showed that hybrids differed in the quantity of pollen produced. This agrees with previous observations that hybrids differ in their tassel size (length and number of branches). Interestingly, drought significantly increased pollen amounts in several hybrids. It is not known what the caused this outcome. The water stress treatments were begun at the time of silk emergence when tassels and pollen grain development inside the anthers were already formed. One can speculate that greater amounts of pollen were shed during water stress due to an increased ability of pollen grains to be shaken out of anthers because pollen grains are less plump when partially dehydrated.

Silk and Ear Growth:

The analysis of silk and ear growth under water stress conditions versus controls underscores the significant influence of water availability on reproductive development in maize hybrids. The absence of interaction effects between hybrids and treatments suggests that water stress affects silk and ear growth consistently across the hybrids tested. Notably, the selection criteria based on silk growth rates highlight specific hybrids as potentially exhibiting drought tolerance characteristics.

Implications and Future Directions:

The findings from these studies have some implications for maize breeding and selection for drought tolerance. Hybrids showing resilience in production of pollen and their viability and under stress conditions merit further investigation as potential candidates for breeding programs aimed at developing drought-tolerant maize varieties. The observed variations in pollen shedding and silk and ear growth among hybrids underscore the complex genetic and physiological factors underlying maize responses to environmental stress. Future studies could focus on elucidating the genetic mechanisms responsible for the observed traits, potentially leveraging molecular approaches such as marker-assisted selection to expedite breeding efforts. Furthermore, additional investigations into better indicator solution for pollen viability and developing more trials in field or in greenhouse would provide deeper insights into optimizing maize productivity under varying environmental conditions.

ADDENDUM

Illustration 7. Transplants

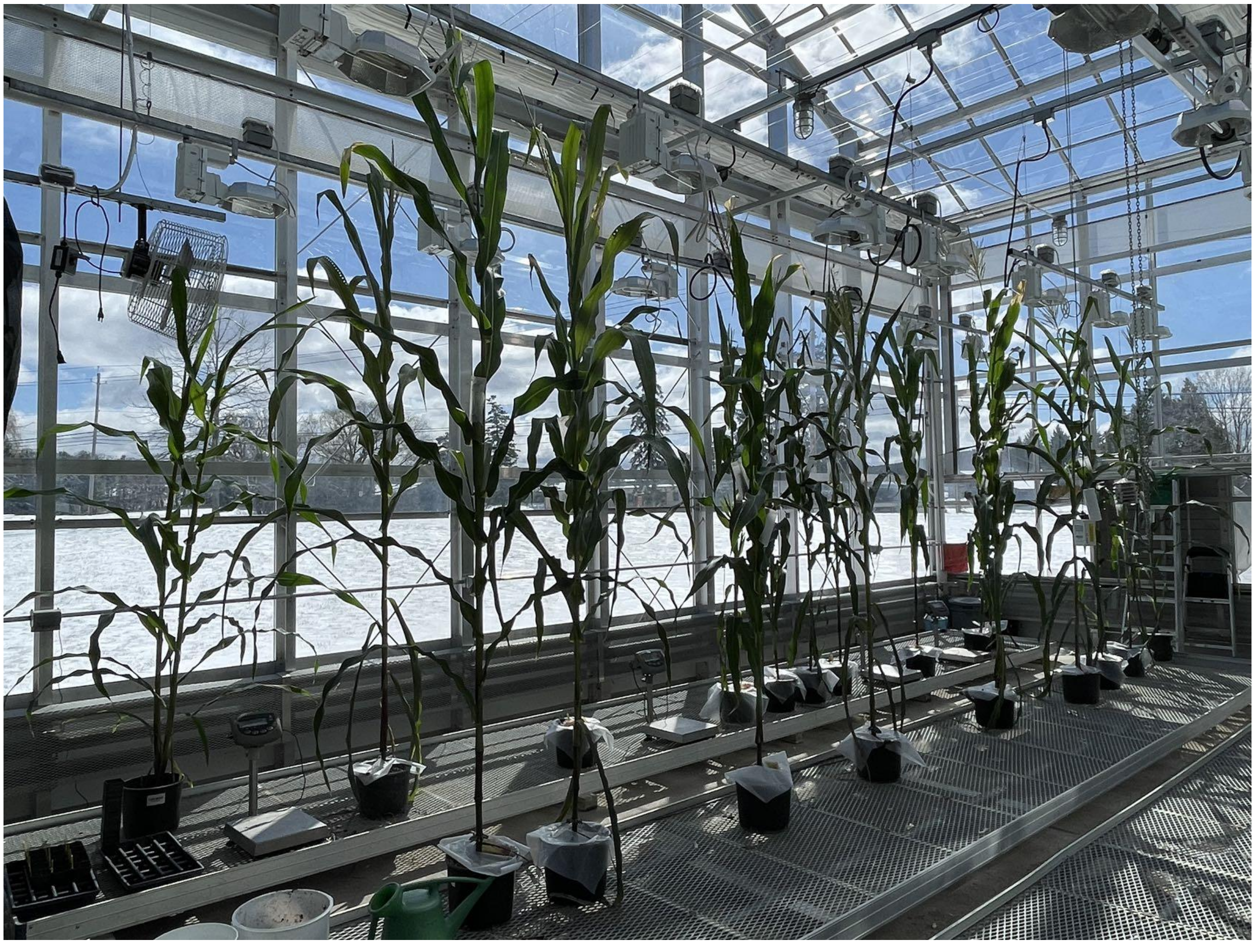


Illustration 8. 2nd replicate of 8 hybrids in Guterman Greenhouse

Illustration 9. Bagging silk hybrid 1 control treatment

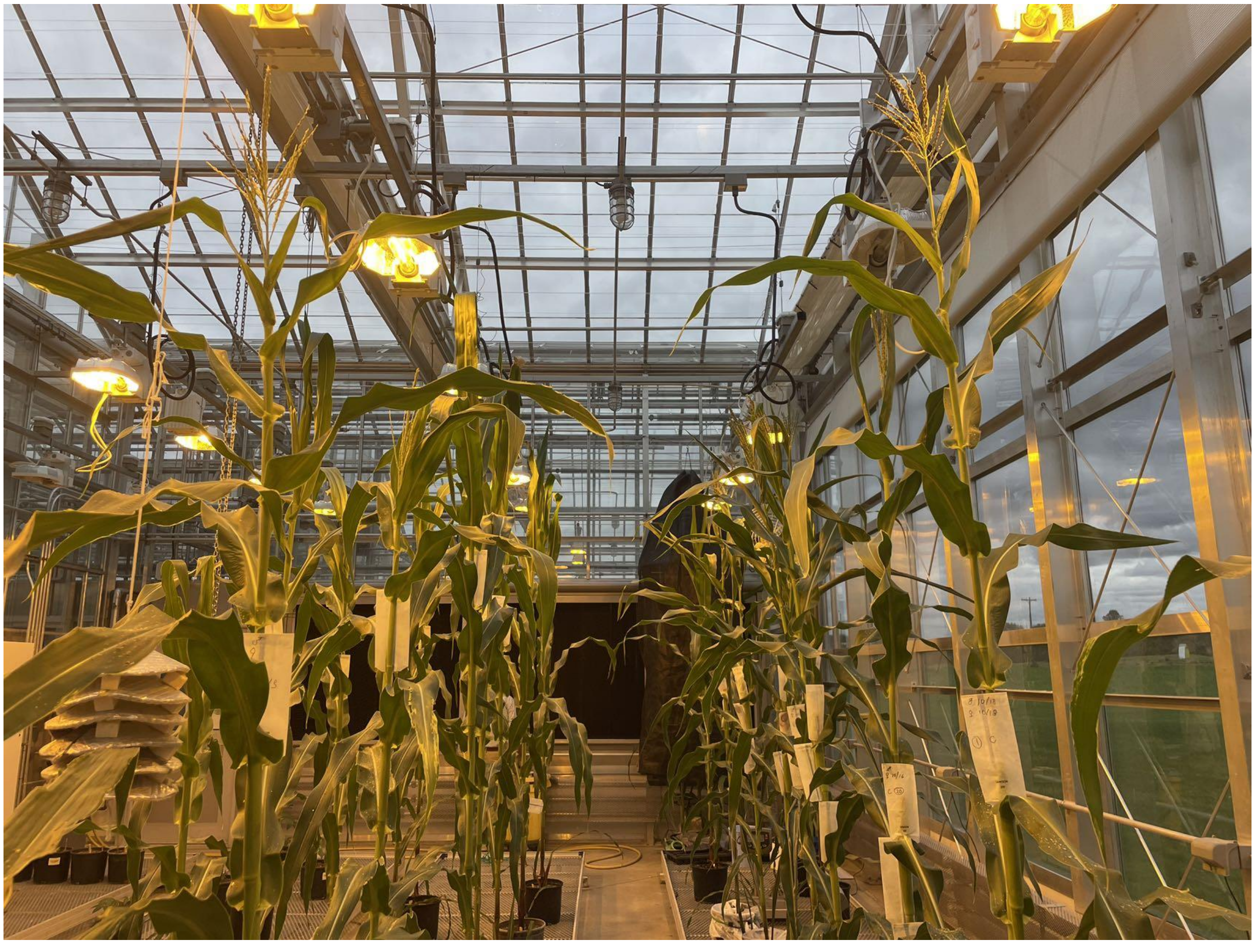


Illustration 10. Bagging silks for 8 hybrids

Illustration 11. Collecting pollen

Illustration 12. Removal silk potion under control treatment

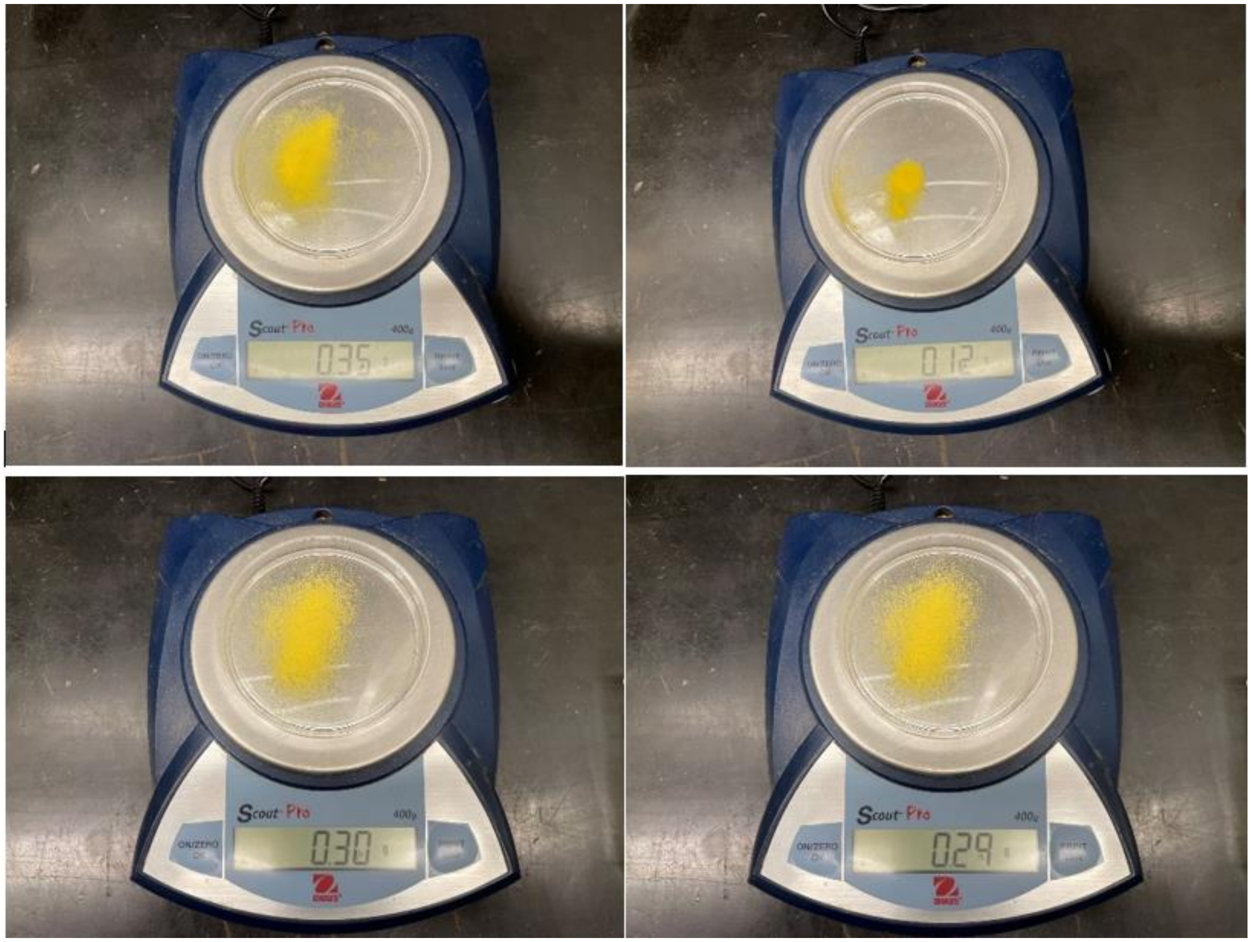


Illustration 13. Pollen measurement on scale

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