THE EFFECTS OF DROUGHT STRESSON SILK EMERGENCE AND YIELD IN MAIZE IN THE TEXAS HIGHPLAINS

A Thesis

by

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Submitted to the Graduate and Professional School of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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August 2021

Major Subject: Plant Breeding

ABSTRACT

Water is a limiting factor when growing maize especially in arid and semi-arid environments such as the Texas High Plains. Maize (*Zea mays*, L.) kernel set remains vulnerable to drought stress occurring at flowering, and silk emergence is one of the key processes which limits kernel set under stress. Our objective of this research was to determine how much silk emergence differed in drought tolerant (TOL) and drought susceptible (SUS) maize hybrids and whether this was a significant genetic advantage driving yield under drought stress This study, conducted in the Texas Panhandle, compared five TOL and five SUS hybrids in a full irrigated (FI) and a drought stress (DS) environment over two years (2018, 2019) . Managed Stress Environment (MSE) techniques using sub-surface drip irrigation in this arid environment were used to impose drought stress bracketing the flowering window. Silk emergence, grain yield and yield components were evaluated for the TOL and SUS hybrids under FI and DS. The DS MSE resulted in significant reductions in silk emergence, yield, and yield components in both 2018 and 2019. Compared to the FI, the DS treatment reduced yield 33.9% over both years and all hybrids. Silk emergence was reduced 9.7% by the DS treatment. When evaluating the different genetic classes in DS, the TOL hybrid class out yielded the SUS class under DS, 9.21 Mg ha^{-1} vs. 8.06 Mg ha^{-1}, respectively ($p<0.0001$). This represents a 30.9% and a 37.0% reduction in yield of the TOL and SUS classes, compared to yields under FI. Similarly, the TOL class exhibited higher silk ear^{-1} than the SUS class, 461.8 vs. 426.6, respectively ($p<0.0004$). This represents a 6.2% and a 13,1% reduction in silk emergence for the TOL and SUS classes, compared to their silk emergence in FI. Linear regression between silk ear^{-1} and yield resulted in an $r^2 = 0.20$ ($p<0.0001$), suggesting that the more prolific silk emergence of the TOL hybrids was responsible for a significant portion of the observed yield variation under drought. These data suggest that ongoing efforts to select hybrids for improved silk emergence and yield under drought stress at flowering will improve hybrid maize performance when water limits grain yield potential in the Texas High Plains.

ACKNOWLEDGEMENTS

I would like to thank Corteva Agriscience for the resources and support of this research. I acknowledge Anderson, S.R., Farrington, R.L., Goldman, D.M., Hanselman, T.A., Hausmann, N.J., Schussler, J.R., Borras, L., and Edmeades, G. for their initial research of silk counting. I want to thank my committee, Steven Hague, Jeff Schussler, and Russell Jessup for their leadership and guiding input, as well as Scott Adair for his aid and inputs. I want to thank Qiao Hu for his algorithm knowledge and expertise. I could not have collected the critical silk number data without his help. I want to acknowledge the hard work of Caitlin Lakey and Jacob Ford, who aided with the collection of samples.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of Dr. Steven Hague of the Department of Soil and Crop Science and Dr. Jeffrey Schussler of Corteva Agriscience.

Initial research was done by Anderson, S.R., Farrington, R.L., Goldman, D.M., Hanselman, T.A., Hausmann, N.J., Schussler, J.R., Borras, L., and Edmeades, G. in 2010 for Corteva Agriscience.

All data collected was done by the student, but was aided by Scott Adair, Caitlin Lakey and Jacob Ford. All analyses and other work were done by the student.

Funding Sources

Graduate study was made possible by resources from Corteva Agriscience.

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1. INTRODUCTION

Maize is the world’s leading cereal grain. World production in 2019/20 was 1,116 million metric tons (Statista, 2021). Water is the main limiting factor for cultivated crops (Boyer, 1982). Of the major cereal crops, maize yield is particularly sensitive to the negative impacts of drought. Most of the world’s maize production is not irrigated, therefore development of drought tolerant maize hybrids is critical to improving overall genetic gain to meet the demands of a rising world population. By 2050 the world’s population will be roughly 9.7 billion (United Nations, 2019). To maintain food security for that projected growth, production of the major staple crops must increase. Enhanced grain yield stability under intermittent drought stress will contribute to that goal. Breeding for drought tolerance has become more prevalent in recent years. Commercial maize breeding programs (Campos et al, 2006, Cooper et al. 2014 and Gaffney et al., 2015) have been successful in selecting for drought tolerance in maize, contributing to overall improvements in genetic gain of that crop.

Maize drought research breeding has come a long way through the years. Most improvements in yield under drought have come indirectly from wide-area testing across the US Corn-belt. Stan Jensen, plant breeder for Pioneer Hi-Bred Int’l, was among the first to start running side-by-side drought trials in a Managed Stress Environment (MSE) in York, NE in the 1950s (Cooper, 2014). In 2006, Campos et al. conducted a study using 18 Pioneer hybrids from the 1950s to the early 2000s. In this study, there were five drought treatment intervals, each treatment interval subjected the crop to water deficit stress at various reproductive stages. These treatment intervals were flowering, early fill, mid fill, late fill, and terminal. Terminal is a prolonged stress, which begins at flowering and is continued until maturity is reached. The study showed that yield improved under drought conditions throughout the years due to progress of breeding programs. It has been well-documented that drought stress during flowering impacts maize yield primarily due to the steep increase of the anthesis-silking interval (ASI) (Bolanos and Edmeades, 1996). This disproportionate delay of the female ear (silking) vs the male tassel (pollen shed) has remained a physiological weakness that represents a target for drought tolerance selection. Campos et. al. (2006) found that the severe reductions in yield when stresses occurred at flowering and through mid-grain fill. DuPont Pioneer developed AQUAmax^{TM} hybrids via high precision phenotyping of silk emergence during severe flowering drought stress treatments in MSEs. During this work, QTLs associated with more rapid silk emergence, better kernel set and higher yield under drought were identified and incorporated with numerous other traits in a whole genome prediction (WGP) approach. DuPont Pioneer advanced AQUAmax hybrids that have improved performance under drought while maintaining parity performance under favorable growing conditions. (Cooper, 2014, Gaffney et al., 2015). Across thousands of small research plot trials and farm strip trials in North America and South America, Gaffney et al. (2015) reported a 5% to 9% yield advantage for AQUAmax hybrids exhibited in various drought conditions and a 2% to 3% advantage in favorable environments, compared to other commercial hybrids being planted at the time.

As mentioned earlier, ASI plays a large role in the effects of drought on grain yield. There is a direct correlation between the length of ASI and grain yield. Shorter ASI usually stabilizes yield under drought conditions (Bolaños and Edmeades, 1996). This makes sense when you consider the longer it takes a plant to exert silks the less pollen there is available, which is why Bolaños and Edmeades (1996) saw increased bareness in ears as ASI was extended. If the pollination window is missed, ovaries remain unpollinated and kernels ear^{-1} (KPE) are reduced. KPE can be reduced to zero (barren plant) if the stress is extreme (Schussler and Westgate, 1991). With each silk being a direct link to a kernel, the number of silks exerted during anthesis is a major breeding objective for the improvement of drought tolerance.

Drought not only reduces grain yield, but drought can also increase disease incidence and severity, which leads to decreased grain quality. Aflatoxins derived from *Aspergillus flavus* lessens the economic value of maize for growers and limits its marketability (Pekar et. al., 2019). Pekar observed aflatoxin levels were higher in Texas when drought conditions existed. Research done in Mississippi demonstrated that hotter and drier conditions increase aflatoxin levels (Abbas et. al., 2002). In the Texas High Plains, hot and dry conditions are a common occurrence, which can lead to higher than normal levels of aflatoxins. In 2017, the Texas High Plains experienced higher than normal levels of aflatoxin, including Potter County which saw levels reach 214 ppm (Herrman et. al. 2018). The drawback of aflatoxins in maize is their detrimental effect on grain quality. At high aflatoxins levels, grain can become unconsumable for food or feed, and if fed to livestock can cause sickness or death (Vardon, 2003). Breeding maize for improved resistance to aflatoxin development under drought stress is one way to limit the impact of aflatoxins in maize. In his research, Pekar identified several inbred lines that exhibited some natural level of resistance to aflatoxin accumulation while maintaining high grain yield potential. Continued genetic improvements will be needed to improve this issue in maize production in the Texas High Plains.

Justification for this project is the need for water use efficiency enhancement in maize grown in the semi-arid environment of the High Plains. In Plainview, Texas where this study is located, the average rainfall annually is 430 mm (West Texas Mesonet, 2021). While AQUAmax™ hybrids have been tested across North America, the target of this research is to further characterize AQUAmax hybrids in the Texas High Plains. Researchers with Texas A&M AgriLife Research compared the performance of AQUAmax hybrids to a leading non-drought tolerant hybrid using three ET treatments (100% ET, 75%ET, and 50% ET) (Hao et. al., 2015). This study suggested that AQUAmax hybrids significantly improved yield under limited ET environments in Texas, compared to standard hybrids (Hao et. al., 2015). The authors also showed that water use efficiency (WUE, kg grain mm^{-1} water applied) also was improved in the AQUAmax hybrids.

The major difference between Hao's research and the current study is the use of MSEs as opposed to a season long limited ET based irrigation. MSEs tend to show greater genetic separation than limited ET environments, due to the more intense selection pressure that can be applied with a more rapid onset acute water deficit stress. These MSEs more clearly demonstrate genetic variation for traits such as leaf wilting, silk emergence, ASI and early post pollination tip kernel abortion (TKA) than plants with ET based irrigation, which provides the opportunity for more rapid genetic advancement of hybrids with positive genetic variation for those traits (Campos et al. 2004). In 1989, Fischer et. al. conducted some of the first managed stress environment work in a wheat breeding program. He showed a significant separation in yield for his drought tolerant lines against both drought susceptible lines and the study's population. However, in 2004 Campos et al. defined the value of MSEs by highlighting maize's susceptibility to drought during flowering. Their research contributed to the overall DuPont Pioneer philosophy of utilizing MSEs to identify specific traits associated with improved reproductive resilience (Messina et al., 2020) under drought stress.

The objective of the current research was to confirm that in a MSE in the Texas High Plains, that drought tolerant (TOL) hybrids will have higher grain yield, exert more silks and produce more kernels per ear than drought susceptible (SUS) hybrids. There are four main questions we wanted to address in this research:

a. Confirm the genetic discrimination of the drought stress (DS) environments vs the full irrigation (FI) environments, by comparing maize hybrid performance across the two environments.
b. Confirm that drought tolerant (TOL) maize hybrids show less reduction in silk emergence, KPE and yield under the DS environment, compared to the drought susceptible (SUS) hybrids.
c. Compare the key trait of interest, silk number ear^{-1} in multiple matched CRM pairs of maize hybrids, with a TOL and SUS hybrid in each pair.

d. Evaluate differences between TOL and SUS maize hybrids under drought for yield components including KPE, grain weight plant^{-1}, and 100 KWT.

Answering these questions will improve confidence in recommending these TOL AQUAmax hybrids to growers in the Texas High Plains. Grain yield stability is critical for long-term economic sustainability and whole farm budget projections.

Experimental objectives of this research required the collection and processing/counting of roughly 1,200 silk bundle samples, which would be impracticable if counted manually. Manual counting is time consuming and prone to human error. Corteva Agrisciences developed a system to streamline the silk collection and counting process (Anderson et al., 2010). This tool cuts a 2 mm cross section of exposed silks from each ear (Figures 4 and 5). The sample is then stored in a vial of ethanol until processing. During processing, these vials of silk samples are poured into a standard petri dish and a digital image is ascertained (Figure 6). Those images are processed through an image analysis algorithm that counts silk pieces in each bundle (Figure 7). This high throughput method allows silks to be counted quickly and accurately, a necessity when incorporating such a phenotyping activity into a breeding program. All 1,200 samples for this project were processed through the image analysis algorithm in less than 24 hours. To ensure the algorithm's accuracy, a subset of samples was manually counted, and results were used to calibrate the algorithm. Image analysis software settings were adjusted until an r^2 of 0.97 was achieved. Use of this algorithm eliminated the time and accuracy issues associated with manual counts. It allowed many silk bundles to be processed, adding power to the data.

2. METHODS AND MATERIALS

2.1. Plant Materials

The ten hybrid entries were comprised of five nests with two hybrid entries per nest. Each nest was made up of a drought tolerant (TOL) and a non-drought tolerant hybrid (SUS). Hybrids within each nest were of similar corn relative maturity (CRM). The range of CRM nests included a 105-, 2 of 108-, a 109- and a 111-day CRM nest. Apart from one of the 108-day CRM nests, all hybrids were commercial hybrids. In one of the 108-day CRM nests, a wild type hybrid was compared to the same hybrid containing an experimental trait, designed to improve maize grain yield and grain yield stability. The study was planted on May 1, 2018 and May 1, 2019 in a clay loam soil (Olton Series) at Plainview, TX (34.233828, - 101.698769). Plots were planted with a PowerPlant^{TM} (Almaco, Nevada, IA) research vacuum planter to achieve a plant population density of 79,072 ha^{-1}. Plots received 168 kg ha^{-1} of N, prior to planting. P and K were added based on yearly soil test recommendations. Plots received an additional 112 kg ha^{-1} N split between two side-dress applications, one at V6-V8 growth stage, and one at the V15 stage, delivered through a subsurface drip irrigation (SDI) system. Total rainfall during the growing season was 430 mm for 2018 and 2019. However, it is worth noting that in 2018, only 215 mm of rain fell between January and August and 340 mm fell in the same timeframe in 2019. This location in the Texas High Plains does not support viable dryland/rainfed maize production, and so SDI was used to support growth during planting, emergence, early growth, and post anthesis growth.

2.2. Experimental Design

Entries were randomized within nests, and nests were randomized within each of 6 replicates in the field. Two environments were established in each year, a fully irrigated (FI) location and a drought stress (DS) location. Each environment contained 6 replicates in each of the two years. Each experimental unit consisted of 4 rows at 76 cm row spacing and 4.4 m in length.

2.3. Managed Drought Stress Treatment

To discriminate genetic variation for silk emergence, kernel set and yield under water limited conditions, water was withheld from the DS environment for approximately 22 days in 2018 and 23 days in 2019. Total water available from both natural rainfall and irrigation for the FI environment was 926 mm in 2018 and 843 mm in 2019 (January 1 through harvest). In comparison, reducing, or eliminating irrigations for approximately 5^{-1}0 days prior to silking and continuing to about 5^{-1}0 days post silking, resulted in a reduction of water available to 725 mm in 2018 and 729 mm in 2019 in the DS environments (Jan 1 through harvest). This treatment typically reduces rates of leaf and internode elongation, prior to flowering, resulting in reduced biomass production. Obvious drought stress symptoms including leaf wilting in the afternoons and eventually some premature leaf senescence was observed as plants moved into the flowering stage of development. This DS treatment is designed to slow the emergence of silks, compared to the FI treatments, resulting in reduced silk numbers available for pollination, reduced kernel number, and reduced yields. After silking and pollination, irrigation was resumed on this treatment, so that kernels established during the intermittent drought treatment could fill and mature. This stress management protocol provides the opportunity to identify genetic variation among maize hybrids for tolerance to stress during flowering.

2.4. Data Collection

Data collected included plant and ear height, stand count, silk counts, and grain yield. Grain yield was harvested from the center two rows of the 4-row plots, using a Wintersteiger Delta^{TM} (Wintersteiger AG, Innkreis, Austria) plot combine. Yield data was adjusted to 15.5% grain moisture. In addition, in 2019 kernel count per ear, kernel weight, and kernel weight per 100 kernels (100 KWT) were also collected from five sequential plants, which were the same plants used for silk counting at flowering. These ears were hand harvested and shelled. The kernels from each ear were counted and weighed. The 100 KWT calculated value is derived from the kernel counts and total kernel mass of each ear.

2.5. Silk Counting

To measure genetic variation for silk emergence under drought stress, a silk counting procedure was conducted on a sample of plants in each experimental unit. The silks were collected on five sequential plants in rows one or four of the 4-row plot. The five sequential plants for silk counts were identified before anthesis. The plants selected were equally spaced without gaps within the five plants or on either end of the five plants, toward the middle of the plot with at least five plants from either end. The silk samples were collected three days after 50% silk emergence of the plot, which was determined through daily observations.

An automated silk cutting device (Figure 4), developed by Corteva Agriscience (US 20100046792A1, 2010) was used to non-destructively collect silk samples from the ears. Its design allows the sampler to cut the silks while the ear is still attached to the plant. It utilizes a twin razor blade design that collects an entire cross section of the silk brush that is exposed from the ear shoot. This tool collects a 2 mm sample of each silk from the ear. Cut silk pieces are deposited in a 10 ml scintillation vial. Seventy percent isopropyl alcohol is used to rinse the cut silk pieces into the vial. Once samples have been collected, they can be immediately processed or can be stored in a 2 degrees Celsius refrigerated environment for up to a year. It is known that this process doesn't affect pollination or ear development. The hand harvested ears that were collected for total kernel ear^{-1} (TKE) data confirm this claim.

Processing of samples collected by the silk cutting device is easier and less time consuming than hand counting silks. Alcohol and silks from each vial are poured into a standard petri dish (Figure 6). That dish is placed in a specialized light box for taking digital pictures. Contents of the sample are spread over the dish so that no large clumps of silk cuttings remain. Digital images of the suspended silks are then captured. The images are loaded into a custom designed image analysis system that counts the samples and loads the results in a spreadsheet, as well as a processed image with the raw count listed on the image (Figure 7). To calibrate and verify the accuracy of the image analysis system, fifteen images were hand counted and compared to the system's output. Settings were adjusted and once the system's counts were within an acceptable range (greater than r^2= 0.95), all samples collected were processed. This final calibration curve (manual counts versus digital counts) had an r^2 = 0.97.

2.6. Data Analysis

Statistical analysis of the data was conducted in JMP Pro 15 (SAS, 2020). In JMP, a fit y by x was used to evaluate the data. This model generated a data output that included a student's T test and an LSD comparison. The P value generated by the student's T test was utilized for the comparisons generated in the data tables. Linear regressions were generated to show comparisons among silks and grain yield (Fig. 1), silks and kernels (Fig. 2), and kernels and grain yield (Fig.3). One-way ANOVA tables were created using combined years' data in each of tables 1-8 (Appendix).

3. RESULTS

In both 2018 and 2019, yields and emerged silks were both reduced in the DS environment, compared to the FI environment (Table 1). These environments were at the same physical location; therefore rainfall, temperature, and other environmental interactions were the same. Agronomic inputs such as fertilizer, tillage and pesticide treatments were similar. The only difference between the two locations was irrigation management bracketing the flowering period. The percent reduction from FI to DS is a good indicator of the severity of stress. This shows that in both years, the imposed drought stress was a significant factor in limiting grain yield. Over years, the DS treatment reduced grain yield 33.9%, compared to the FI treatment (Table 1), while emerged silk number was reduced 9.7%.

Genetic differences in response to the DS treatment were also observed (Table 2). When comparing hybrids classified as TOL, to those classified as SUS, several contrasts were observed.

We expected the yields of the TOL and SUS classes to be similar under full irrigation, but over years, the TOL class hybrid grain yield was slightly higher (0.42 Mg ha^{-1}, $P< 0.05$) than the SUS class. However, the TOL class hybrids displayed a much greater grain yield advantage compared to the SUS hybrids (1.15 Mg ha^{-1}, $P<0.001$) in the DS environments in both 2018 and 2019. This confirms there was genetic separation in grain yield stability between the TOL and SUS classes. Across years, DS reduced grain yield 30.9% and 37% in the TOL and SUS classes, respectively (Table 2). The DS treatment was slightly more severe in 2018, compared to 2019, where DS grain yields of the SUS class were 7.75 Mg ha^{-1} and 8.37 Mg ha^{-1}, respectively.

Emerged silk numbers (silks ear^{-1}) were also reduced by the DS treatment for both TOL and SUS classes. However, the inhibition of silk emergence was less severe for the TOL class (6.2%) vs. the SUS class (13.1%) when averaged across both seasons (Table 2). It is obvious that some of the grain yield reduction associated with the DS treatment was associated with limited silk emergence at flowering, resulting in a limitation on the number of kernels per ear, and that the SUS class demonstrated this vulnerability to a larger degree.

In the 2019 study, grain yield component data was collected to further explain the impact of the DS treatment on grain development. DS environment significantly reduced grain yield as well as emerged silks ear^{-1}, total kernels ear^{-1}, grain weight plant^{-1} and 100 KWT (Table 3, top). When comparing genetic backgrounds, the TOL hybrid class was less severely impacted by the DS treatment, compared to the SUS hybrid class (Table 3, bottom). The TOL class hybrids had significantly higher grain yield, emerged silks, kernels ear^{-1}, grain weight plant^{-1} and 100KWT, compared to the SUS hybrid class.

There are a few key observations that can be made about the grain yield component data. First, in the FI location, kernels ear^{-1} is higher than emerged silks ear^{-1}. This is true for both the overall environmental comparison (Table 3, top) as well as in the genetic hybrid class comparison (Table 3, bottom). As we know from maize physiology; each silk represents the potential for one kernel. A logical conclusion is that in the FI environment, silks continued to emerge after silk samples were collected at 3 days after 50% shed of the plot. However, looking at the DS environment, the relationship between silk number and kernels per ear is the opposite of the FI environment. Under the DS treatment, emerged silks tended to be higher than final kernels ear^{-1} (Table 3). After silk emergence in DS, some of those ovaries may have failed to pollinate, and others could have experienced kernel abortion after pollination, resulting in reduced sink size.

Tables 4, 5, 6, 7, and 8 display comparisons of the individual nested pairs. Both hybrids within each pair have the same CRM. Power to separate hybrids within each pair was limited due to the reduced sample size (6 reps in each environment), compared to the pooled data sets included in Tables 1 – 3.

Assessing the data from the 105 CRM pair (Table 4), there are several points to note. Across years, the TOL hybrid yielded more grain and had more emerged silks, compared to the SUS hybrid. In 2019, in the DS environment, emerged silks, grain yield, kernels ear^{-1}, grain wt. plant^{-1} and 100KWT all trended or were significantly higher in the TOL hybrid, compared to the SUS hybrid. Across years, the DS treatment only reduced silk emergence of the TOL hybrid 1.9% but reduced it 15.2% in the SUS hybrid. Thus, this SUS hybrid exhibited especially weak silking characteristics under DS, and so should be avoided because of instability when grown with limited irrigation on the Texas High Plains. The DS treatment reduced grain yield of the TOL hybrid 27.2%, but reduced it 32.4% in the SUS hybrid. These data confirm the drought stress classifications of these two hybrids.

The 108 CRM pair (Table 5) is an interesting grouping. As expected, the TOL hybrid yielded more grain than the SUS hybrid in the DS environment across years. The silk data, however, reflected characteristics that were unique. In this case, the SUS hybrid tended to have more silks emerged in most comparisons to the TOL hybrid. The SUS hybrid did lose a larger percentage of silks under the DS treatment over years, but it still had a higher overall silk number in DS over years. Grain yield component data, however, showed that the SUS hybrid had lower KPE, grain weight plant^{-1} and 100KWT compared to the TOL hybrid. Thus, even though this SUS hybrid emerged silks in DS relatively efficiently, it was unable to fill all the kernels, resulting in fewer kernels, and smaller kernels, leading to less grain production. This suggests that post-pollination processes such as tip kernel abortion and more rapid leaf senescence like also contributed to the grain yield reduction of this SUS hybrid under DS.

The TOL entry for the 109 CRM pair was changed between years due to seed availability (Table 6). Across years, the DS treatment reduced silk emergence 5.7% and 12.2% for the TOL and SUS hybrids, respectively, and the TOL hybrid emerged a significantly higher number of silks in DS, compared to the SUS hybrid. For grain yield, DS reduced yield of the TOL hybrid 33.1% compared to 38.7% for the SUS hybrid, and yield of the SUS was significantly lower than the TOL under DS. In the 2019 yield component DS data, it is apparent that even though the TOL hybrid emerged many more silks compared to the SUS, KPE of the two hybrids were not that different. This suggests that factors other than rate of silk emerge contributed to the genetic differences in drought tolerance between these two hybrids.

The 111 CRM pair, like other pairs, had mixed results. In terms of grain productivity, there is evidence of the susceptible hybrid being the strongest commercial hybrid of the other SUS entries. It outperformed all other susceptible hybrids in both the full irrigation and drought stressed locations. Across years, there was no significant difference between the TOL and SUS in the DS environment (Table 7). The DS treatment had relatively less impact on silk emergence in this CRM group, compared to other CRM groups. Grain yield was still reduced in the treatment at least 30%, but this must have been driven by additional yield component factors. The KPE in the FI location was higher in the TOL entry, but the grain weight and 100 KWT were higher for the SUS entry. This data can be interpreted to mean that in the FI, the SUS produced a large kernel.

The experimental pair in Table 8 is an isoline comparison of a hybrid with a novel grain yield stability gene and its wild type (maize hybrid without the target gene). At both locations in both years the TOL and SUS hybrids were at yield parity and there was no significant difference in the emerged silk number between the two entries (Table 8). The KPE trended higher for the TOL, but the difference was non-significant.

Three linear regressions were made to show various relationships in both environments. The comparisons that were made were: emerged silks versus yield (Figure 1), emerged silks versus kernel number (Figure 2) and kernel number versus yield (Figure 3). The relationship of silks ear^{-1} vs. yield (Figure 1) explained about 20% of the variation in in yield across environments (r^2 =0.197). However, there is an environment distinction that can be noted; most points above the fit line are from the FI environment and those below are from the DS environment. The strongest association was noted for kernel number vs. yield where $r^2 = 0.79$ (Figure 3a), confirming the known relationship between KPE and yield in maize.

4. DISCUSSION

The objective of this work was to measure the effects of drought on TOL and SUS commercial maize hybrids in the Texas High Plains by documenting genetic differences in silk emergence, KPE, 100 KWT and final yield.

One goal was to test the hypothesis that genetic differences in maize yield under stress can be partially explained by efficiency of silk emergence under stress at flowering. Performance of the TOL and SUS hybrids was not different under full irrigation conditions, but TOL hybrids were clearly superior to the SUS hybrids in terms of grain yield in the drought stress environment in both years (Table 2). It is known that the silking process in maize is still sensitive to drought stress and yield reductions due to stress can be particularly severe when they occur at this stage (Campos et. al. 2004, Shaw, 1977). The delay in silk emergence, compared to pollen shed is what defines anthesis to silking interval (ASI) (Bolaños and Edmeades, 1996). The pollen shed window is 5-7 days in good conditions but can be reduced to 3-5 days under drought stress. So, if ASI under stress increases to 2-5 days, there is a significant risk that many silks will not be exposed to viable pollen for pollination (Bassetti and Westgate 1993). ASI is variable across genotypes (Hall, 1982), and we also observed this in our individual TOL vs SUS CRM pairs. (Tables 4-8). In a worst-case scenario, extended ASI can result in a higher proportion of completely barren ears, and KPE of remaining ears will be severely reduced (Hall et. al., 1971, Herrero and Johnson, 1981, Du Plessis and Dijkhuis, 1967). The evidence of this can be seen in Table 1. Our data showed that a 9.7% reduction in silk emergence during the functional pollen shed window was associated with a 33.9% reduction in yield over all hybrids. Thus, the drought stress environment had a negative impact on silk emergence and the establishment of yield potential.

When comparing TOL and SUS classes of hybrids under drought stress (Table 2), significant differences in yield and emerged silks were observed. This indicates there was genetic separation between the two hybrid classes. While both classes exhibited reductions in silk emergence, KPE, 100 KWT and grain yield due to the drought stress treatment (Table 2 and bottom of Table 3) the SUS hybrids suffered proportionately greater losses in silk emergence and yield. For example, SUS hybrids suffered a 37% reduction in yield in the DS environment, while the TOL hybrids lost only 30.9% yield under drought stress (Table 2.). The difference is roughly .62 Mg ha^{-1}, which is roughly 10 bu acre^{-1}. At a corn price of t \$7.48 bu per acre (Business Insider, 2021) that 10 bu acre^{-1} improvement in TOL hybrids would provide a substantial financial advantage to a dryland/water-limited grower. Selecting the correct drought tolerant hybrid could mean the difference between a grower making a profit or having a negative return-on-investment from a maize crop. This drought stability did not come at a price of reduced yield potential under full irrigation conditions (Table 1). This is consistent with the previous reports of yield parity of AQUAmax^{TM} maize hybrids under optimum conditions, with improved yield stability in water-limited environments (Gaffney et al., 2015).

Even though maize is susceptible to acute drought stress at anthesis (Campos et.al. 2004, Shaw, 1977), it is possible that the SUS hybrids might have suffered some slight tip kernel abortion due to transient diurnal drought stress occurring in the FI block on hot, windy days in Plainview because apical kernels are the first to abort even in lighter stress situations (Si Shen et. al., 2018). These trends in the data presented in Table 2 are in line with expected outcomes of the research. These data indicate that the TOL hybrid group yielded better and produced more silks at anthesis than the SUS hybrid group. The DS environment was an effective discriminating environment, which allowed us to separate the TOL and SUS hybrid groups.

When interpreting yield component data (Table 3), we see similar trends to those shown in Tables 1 and 2. In the environment comparison (top of Table 3) the three yield components all show a significant reduction when comparing the DS to the FI. The reduction in kernel number ear^{-1}, grain weight plant^{-1}., and 100 KWT shows that when maize is lacking water during anthesis, kernel number, kernel weight and kernel depth are all reduced. KPE could have been reduced due to a combination of reduced silk emergence and post-anthesis tip kernel abortion. One study showed under drought conditions ASI was increased, due to a delay in silk emergence (Bolaños and Edmeades,1996). Silk growth is known to arrest more rapidly under stress, compared to optimum conditions (Oury et al., 2016). All these factors contributed to increased kernel abortion (Oury, 2016). Reductions in photosynthesis or reduced carbohydrate availability could be a reason for 100 KWT reductions in the DS environment. Schussler and Westgate (1991) showed that in severe water-deficit conditions photosynthesis is completely inhibited and that developing kernel growth was limited by reduced in assimilate availability, which was responsible for kernel loss. In Schussler and Westgate's (1991) moderate water deficit environment, kernel set was reduced by 48%. Some of this impact was due to direct impact of reduced tissue water potential, however, since some kernel abortion still occurred even when measured kernel sugars were adequate (Schussler and Westgate, 1995). Si Shen et. al. in 2018 did similar work and came to similar conclusions that low levels of assimilates along with delayed pollination are main causes of kernel abortion under water-deficit conditions. Delayed silk exertion, reduced growth rate, and growth arrest are other factors that could have contributed to kernel abortion (Oury, 2016). Several factors: photosynthesis inhibition, low levels of assimilates, increased ASI, silk growth rate, silk growth arrest, and decrease in days of available pollen in the DS environment all likely contributed to the reduction in these yield components.

It is interesting to compare kernel number and emerged silks. Since maize produces one silk for every kernel, the emerged silks and kernel numbers should be similar no matter what the environment. In FI there are more kernels that silks. This is likely explained by additional silk emergence occurring after the day of silk cutting. Our silk cutting protocol called for the harvesting of silk samples at three days post 50% silk of all plants in the plot. This protocol was written for another location where plants do not exert silks after three days post silking date. Our environment has been known to exert silks up to 4 days after 50% silk. Therefore, taking the silk samples one day later at the Plainview location would likely have provided a closer relationship between emerged silks and final KPE. In the DS environment, both TOL and SUS kernel numbers were less than emerged silks. In this case, some emerged silks may not have received pollen, or kernels could have been aborted soon after pollination. As discussed earlier, we know that there is an increase in ASI and a decrease in days of pollen availability under drought (Bassetti and Westgate

1993). This “gap” can reduce silk receptivity in severe stress, resulting in barren ears (Schussler and Westgate, 1991, Bassetti and Westgate 1993). As part of the managed stress environment, the crop was completely rehydrated after the imposed stress period. However, if the rehydration wasn’t soon enough, kernel abortion may have already begun. Once a plant begins aborting a kernel, there is no reversal.

Tables 4 through 8 show the data of individual pairs and this data is inconsistent. One reason for discrepancies is when making the comparison within a CRM pair there were only six replications per environment. Power to separate these two entries was much lower, compared to pooled data over hybrids, and this resulted in fewer distinct differences in TOL and SUS individual comparisons.

Four of the five hybrid sets did separate as expected for yield, with only the experimental pair (Table 8) not producing expected results in the DS environment. One potential explanation of this is when comparing the data (Table 2), for combined yield levels for the TOL class in the DS environment, we see that the average yield is 9.21 Mg ha^{-1} compared to the SUS class which was 8.06 Mg ha^{-1}. However, the yields for the wild type (SUS, WT) and the traited (TOL) hybrids were 9.12 and 9.04 Mg ha^{-1} respectively (Table 8). Thus, it appears that the WT SUS hybrid yielded more similarly to a TOL hybrid than a SUS hybrid. This may explain why we did not detect any effect of the experimental trait in this isogenic pair. Additional research in the future might include the evaluation of the trait in hybrids that are truly characterized as susceptible.

When looking at silk numbers, four of the five hybrid sets separate out as expected. However, those differences were statistically significant in only two of the 5 sets. Except for the CRM pair in Table 8, these pairs were made up of completely different genotypes, which likely express multiple trait differences. Hall et al., (1982) confirmed drought stress had different effects on various genetic backgrounds. The original criteria for selecting TOL and SUS pairs was selecting hybrids from the same CRM, one that had a high drought rating and one that had a low drought rating (Pioneer corn sales brochures). In the future, additional care must be exercised in selecting hybrids with clear differences in TOL and SUS historical performance. It is getting more difficult to find SUS hybrids for these kinds of studies, since most of them are discarded during the plant breeding development process.

characterize the many mechanisms responsible more fully for better performance of the TOL class, compared to the SUS class of maize hybrids under drought stress.

5. CONCLUSIONS

This project was successful in confirming the main hypothesis. First, DS environments reduced both silks ear^{-1} and yield ha^{-1}, compared to the FI environment. Second, when comparing TOL and SUS maize hybrids, there was significant separation between the two groups in the DS environment, confirming the improved reproductive resilience of the TOL hybrids. Thus, recommendations to preferentially use the TOL commercial hybrids in the Texas High Plains are justified. Improved silk emergence of TOL hybrids contributed to their improved yield, but other factors including reduced kernel abortion and better canopy photosynthesis also likely contributed to the superior performance of the TOL class. There are multiple mechanisms that influence performance of maize under drought stress and relying on only one of those factors for selection may not always lead to success (Messina, 2019). Thus, additional studies are required to characterize the many mechanisms responsible more fully for better performance of the TOL class, compared to the SUS class of maize hybrids under drought stress.

Table 1: Silk Number and Yield Data in 2018 and 2019 in Full Irrigation and Drought Stress Environments

	Environment Comparison				
			Statistical Analyses		
Collected Data	**Full Irrigation**	**Drought Stress**	**P Value**	**LSD**	**% Reduced (FI to DS)**
2018					
Silk Number (Silk Ear^{-1})	472	427.4***	<0.0001	27.1	9.50%
Yield (Mg ha^{-1})	12.43	8.54***	<0.0001	3.4	31.30%
2019					
Silk Number (Silk Ear^{-1})	510.7	457.1***	<0.0001	35.4	10.50%
Yield (Mg ha^{-1})	13.68	8.72***	<0.0001	4.6	36.20%
Combined					
Silk Number (Silk Ear^{-1})	491.2	443.5***	<0.0001	34.9	9.70%
Yield (Mg ha^{-1})	13.05	8.63***	<0.0001	4.1	33.90%

Table 2: Silk Number and Yield for TOL and SUS classes in 2018 and 2019 in Full Irrigation and Drought Stress Environments

	TOL versus SUS Comparison					
			Statistical Analyses			
Collected Data	TOL	SUS	P Value	LSD	% Reduced (FI to DS) TOL Class	% Reduced (FI to DS) SUS Class
2018						
Full Irrigation						
Silk Number (Silk Ear^{-1})	464.7	479.4	0.1499	ns		
Yield (Mg ha^{-1})	12.67	12.20*	0.0358	0.03		
Drought Stress						
Silk Number (Silk Ear^{-1})	459.2	413.2***	0.0008	18.8	1.20%	13.80%
Yield (Mg ha^{-1})	9.33	7.75***	<0.0001	0.94	26.30%	36.50%
2019						
Full Irrigation						
Silk Number (Silk Ear^{-1})	517.7	503.4	0.2925	ns		
Yield (Mg ha^{-1})	13.97	13.38*	0.0259	0.003		
Drought Stress						
Silk Number (Silk Ear^{-1})	475.2	439.2**	0.005	11	8.20%	12.80%
Yield (Mg ha^{-1})	9.08	8.37***	0.0006	0.32	35.00%	37.50%
Combined						
Full Irrigation						
Silk Number (Silk Ear^{-1})	491.3	491	0.9776	ns		
Yield (Mg ha^{-1})	13.32	12.79*	0.02	0.09		
Drought Stress						
Silk Number (Silk Ear^{-1})	460.8	426.6***	0.0004	15.2	6.20%	13.10%
Yield (Mg ha^{-1})	9.21	8.06***	<0.0001	0.77	30.90%	37.00%

Table 3: Silk number, Yield, Kernel per Ear, Kernel weight, and 100 Kernel weight for TOL and SUS classes in 2018 and 2019 in Full Irrigation and Drought Stress Environments. FI: Full Irrigation DS: Drought Stress TOL: Tolerant hybrids SUS: Susceptible hybrids

	2019 Yield Component Data					
			Statistical Analyses			
Collected Data	**TOL**	**SUS**	**P Value**	**LSD**	**% Reduced (FI to DS)**	
Environment Comparison						
Silk Number (Silk Ear^{-1})	510.7	457.1***	<0.0001	35.4	10.50%	
Yield (Mg ha^{-1})	13.68	8.72***	<0.0001	4.6	36.20%	
Kernel Number (Kernels Ear^{-1})	568.4	379.2***	<0.0001	169.5	33.30%	
Grain Wt. (g plant^{-1})	175.2	107.6***	<0.0001	61.4	38.60%	
100 KWT (g)	30.9	28.5***	<0.0001	1.6	7.80%	
TOL versus SUS Comparison						
Full Irrigation						
Silk Number (Silk Ear^{-1})	517.7	503.4	0.2925	ns		
Yield (Mg ha^{-1})	13.97	13.38*	0.0259	0.002		
Kernel Number (Kernels Ear^{-1})	576.5	560.3	0.3327	ns		
Grain Wt. (g plant^{-1})	181.2	169.2*	0.0238	1.692		
100 KWT (g)	31.5	30.3*	0.0273	-0.02		
					% Reduced (FI to DS) TOL Class	**% Reduced (FI to DS) SUS Class**
Drought Stress						
Silk Number (Silk Ear^{-1})	475.2	439.2**	0.005	11	8.20%	12.80%
Yield (Mg ha^{-1})	9.08	8.37***	0.0006	0.32	35.00%	37.50%
Kernel Number (Kernels Ear^{-1})	391.6	366.7*	0.0221	3.7	32.10%	34.60%
Grain Wt. (g plant^{-1})	113.8	101.3***	0.0001	6.5	37.20%	40.30%
100 KWT (g)	29.2	27.8*	0.0382	0.08	7.30%	8.30%

Table 4: 105 CRM Pair. Silk number, Yield, Kernel per Ear, Kernel weight, and 100 Kernel weight for TOL and SUS classes in 2018 and 2019 in Full Irrigation and Drought Stress Environments. FI: Full Irrigation DS: Drought Stress TOL: Tolerant hybrids SUS: Susceptible hybrids

	TOL versus SUS Comparison					
			Statistical Analyses			
Collected Data	**TOL**	**SUS**	**P Value**	**LSD**	**% Reduced (FI to DS) TOL Class**	**% Reduced (FI to DS) SUS Class**
2018						
Full Irrigation						
Silk Number (Silk Ear^{-1})	445.2	463.6	0.3985	ns		
Yield (Mg ha^{-1})	12.16	10.89*	0.0026	0.61		
Drought Stress						
Silk Number (Silk Ear^{-1})	451.9	374.6*	0.0158	15	$^{-1}$.50%	19.20%
Yield (Mg ha^{-1})	9.58	6.73***	<0.0001	2.05	21.20%	38.20%
2019						
Full Irrigation						
Silk Number (Silk Ear^{-1})	457.5	495	0.1564	ns		
Yield (Mg ha^{-1})	13.53	11.62***	<0.0001	1.36		
Kernel Number (Kernels Ear^{-1})	562.1	540.1	0.3479	ns		
Grain Wt. (g plant^{-1})	168.8	147.5*	0.0497	1.1		
100 KWT (g)	30	27.4**	0.0096	0.8		
Drought Stress						
Silk Number (Silk Ear^{-1})	467.9	438.5	0.1793	ns	-2.30%	11.40%
Yield (Mg ha^{-1})	9.14	8.48	0.0629	ns	32.00%	27.00%
Kernel Number (Kernels Ear^{-1})	397.5	388.8	0.6126	ns	29.30%	28.00%
Grain Wt. (g plant^{-1})	116.7	100.9*	0.0154	3.7	30.90%	31.60%
100 KWT (g)	29.4	25.9***	<0.0001	2.7	2.00%	5.50%
Combined						
Full Irrigation						
Silk Number (Silk Ear^{-1})	451.5	479.3	0.1034	ns		
Yield (Mg ha^{-1})	12.85	11.25***	<0.0001	0.97		
Drought Stress						
Silk Number (Silk Ear^{-1})	460.2	406.5**	0.0061	15.7	$^{-1}$.90%	15.20%
Yield (Mg ha^{-1})	9.36	7.61***	<0.0001	1.02	27.20%	32.40%

Table 5: 108 CRM Pair. Silk number, Yield, Kernel per Ear, Kernel weight, and 100 Kernel weight for TOL and SUS classes in 2018 and 2019 in Full Irrigation and Drought Stress Environments. FI: Full Irrigation DS: Drought Stress TOL: Tolerant hybrids SUS: Susceptible hybrids

	TOL versus SUS Comparison					
			Statistical Analyses			
Collected Data	**TOL**	**SUS**	**P Value**	**LSD**	**% Reduced (FI to DS) TOL Class**	**% Reduced (FI to DS) SUS Class**
2018						
Full Irrigation						
Silk Number (Silk Ear^{-1})	447.7	566.0***	<0.0001	84		
Yield (Mg ha^{-1})	12.79	12.38	0.3098	0.44		
Drought Stress						
Silk Number (Silk Ear^{-1})	468.4	438.7	0.317	ns	-4.60%	22.50%
Yield (Mg ha^{-1})	8.49	6.54**	0.0017	0.93	33.60%	47.20%
2019						
Full Irrigation						
Silk Number (Silk Ear^{-1})	519.5	630.9***	<0.0001	67.3		
Yield (Mg ha^{-1})	14.12	14.64	0.094	ns		
Kernel Number (Kernels Ear^{-1})	611.4	678.7	0.0824	ns		
Grain Wt. (g plant^{-1})	195.6	188.5	0.4943	ns		
100 KWT (g)	32.2	27.8**	0.0036	2.3		
Drought Stress						
Silk Number (Silk Ear^{-1})	445.7	503.8*	0.0269	7	14.20%	20.10%
Yield (Mg ha^{-1})	9.76	8.41***	<0.0001	0.91	30.90%	42.53%
Kernel Number (Kernels Ear^{-1})	429.9	385.4*	0.04	2.7	29.70%	43.20%
Grain Wt. (g plant^{-1})	121.5	101.0*	0.019	4.7	37.90%	46.40%
100 KWT (g)	28.2	26.2*	0.0313	0.3	12.40%	5.80%
Combined						
Full Irrigation						
Silk Number (Silk Ear^{-1})	483.6	598.5***	<0.0001	84.7		
Yield (Mg ha^{-1})	13.46	13.51	0.9159	ns		
Drought Stress						
Silk Number (Silk Ear^{-1})	455.6	472.4	0.3968	ns	5.80%	21.10%
Yield (Mg ha^{-1})	9.12	7.47***	0.0007	0.79	32.20%	44.70%

Table 6: 109 CRM Pair. Silk number, Yield, Kernel per Ear, Kernel weight, and 100 Kernel weight for TOL and SUS classes in 2018 and 2019 in Full Irrigation and Drought Stress Environments. FI: Full Irrigation DS: Drought Stress TOL: Tolerant hybrids SUS: Susceptible Hybrids

	TOL versus SUS Comparison					
			Statistical Analyses			
Collected Data	**TOL**	**SUS**	**P Value**	**LSD**	**% Reduced (FI to DS) TOL Class**	**% Reduced (FI to DS) SUS Class**
2018						
Full Irrigation						
Silk Number (Silk Ear^{-1})	454.2	430.7	0.396	ns		
Yield (Mg ha^{-1})	12.62	10.88	<0.0001	1.3		
Drought Stress						
Silk Number (Silk Ear^{-1})	418	376	0.2548	ns	8.00%	12.20%
Yield (Mg ha^{-1})	9.57	6.67***	<0.0001	2	24.20%	38.70%
2019						
Full Irrigation						
Silk Number (Silk Ear^{-1})	556	415.6***	<0.0001	74.9		
Yield (Mg ha^{-1})	14.09	11.74***	<0.0001	1.59		
Kernel Number (Kernels Ear^{-1})	537.6	481.9*	0.0183	12.1		
Grain Wt. (g plant^{-1})	175.5	148.9***	0.0007	14.8		
100 KWT (g)	32.6	30.9**	0.0061	0.64		
Drought Stress						
Silk Number (Silk Ear^{-1})	529.6	367.5***	<0.0001	98.3	4.70%	11.60%
Yield (Mg ha^{-1})	8.3	7.19***	0.0001	0.74	41.10%	38.70%
Kernel Number (Kernels Ear^{-1})	397.3	362.1	0.0545	ns	26.10%	24.90%
Grain Wt. (g plant^{-1})	105.6	92.3*	0.018	2.717	39.80%	38.00%
100 KWT (g)	26.7	27	0.7542	ns	18.10%	12.60%
Combined						
Full Irrigation						
Silk Number (Silk Ear^{-1})	504.3	423.4***	0.0004	37		
Yield (Mg ha^{-1})	13.36	11.31***	<0.0001	1.39		
Drought Stress						
Silk Number (Silk Ear^{-1})	475.8	371.8***	<0.0001	54.2	5.70%	12.20%
Yield (Mg ha^{-1})	8.94	6.93***	<0.0001	1.4	33.10%	38.70%

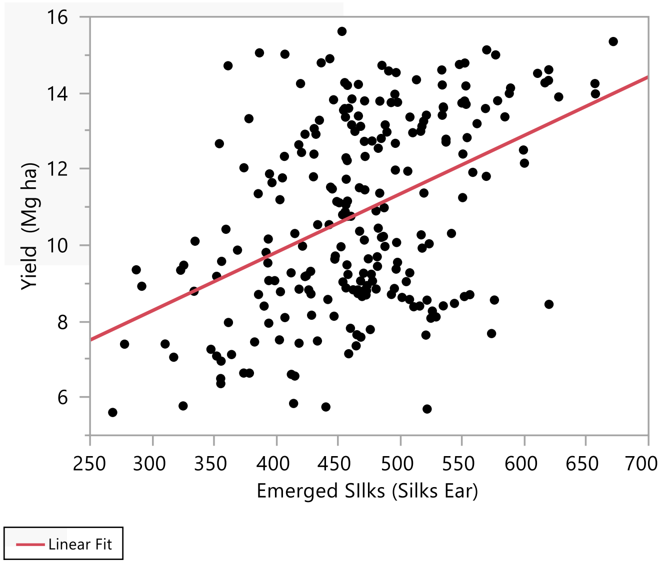
Table 7: 111CRM Pair. Silk number, Yield, Kernel per Ear, Kernel weight, and 100 Kernel weight for TOL and SUS classes in 2018 and 2019 in Full Irrigation and Drought Stress Environments. FI: Full Irrigation DS: Drought Stress TOL: Tolerant hybrids SUS: Susceptible hybrids

	TOL versus SUS Comparison					
			Statistical Analyses			
Collected Data	TOL	SUS	P Value	LSD	% Reduced (FI to DS) TOL Class	% Reduced (FI to DS) SUS Class
2018						
Full Irrigation						
Silk Number (Silk Ear^{-1})	455.6	442.1	0.5009	ns		
Yield (Mg ha^{-1})	12.6	13.73*	0.0123	0.38		
Drought Stress						
Silk Number (Silk Ear^{-1})	396.9	444.3	0.1655	ns	12.90%	-0.50%
Yield (Mg ha^{-1})	9.63	9.3	0.3676	0.45	23.60%	32.20%
2019						
Full Irrigation						
Silk Number (Silk Ear^{-1})	497.2	410.2***	<0.0001	49.4		
Yield (Mg ha^{-1})	14.39	15.02*	0.0187	0.13		
Kernel Number (Kernels Ear^{-1})	606.8	566.8*	0.0156	9.4		
Grain Wt. (g plant^{-1})	190.9	194.4	0.4482	ns		
100 KWT (g)	31.4	34.3***	0.0003	1.6		
Drought Stress						
Silk Number (Silk Ear^{-1})	467.8	401.4**	0.0102	16.5	4.90%	2.10%
Yield (Mg ha^{-1})	9.51	9	0.2018	ns	33.90%	40.00%
Kernel Number (Kernels Ear^{-1})	388.6	378.2	0.6019	ns	36.00%	33.30%
Grain Wt. (g plant^{-1})	113.9	104.5	0.1577	ns	40.30%	46.20%
100 KWT (g)	29.3	27.7	0.2311	ns	6.70%	19.20%
Combined						
Full Irrigation						
Silk Number (Silk Ear^{-1})	478	426.4***	0.0003	24		
Yield (Mg ha^{-1})	13.5	14.37*	0.0342	0.08		
Drought Stress						
Silk Number (Silk Ear^{-1})	432.3	421.3	0.6036	ns	9.60%	1.20%
Yield (Mg ha^{-1})	9.57	9.15	0.1053	ns	29.10%	36.30%

Table 8: Experimental Pair. Silk number, Yield, Kernel per Ear, Kernel weight, and 100 Kernel weight for TOL and SUS classes in 2018 and 2019 in Full Irrigation and Drought Stress Environments. FI: Full Irrigation DS: Drought Stress TOL: Tolerant hybrids SUS: Susceptible hybrids

	TOL versus SUS Comparison					
			Statistical Analyses			
Collected Data	**TOL**	**SUS**	**P Value**	**LSD**	**% Reduced (FI to DS) TOL Class**	**% Reduced (FI to DS) SUS Class**
2018						
Full Irrigation						
Silk Number (Silk Ear^{-1})	517.3	492.7	0.1042	ns		
Yield (Mg ha^{-1})	13.15	13.11	0.8838	ns		
Drought Stress						
Silk Number (Silk Ear^{-1})	492.5	439.3	0.0631	ns	4.80%	10.80%
Yield (Mg ha^{-1})	9.39	9.49	0.8691	ns	28.60%	27.60%
2019						
Full Irrigation						
Silk Number (Silk Ear^{-1})	559.5	562.6	0.8943	ns		
Yield (Mg ha^{-1})	13.73	13.9	0.4462	ns		
Kernel Number (Kernels Ear^{-1})	564.6	533.8	0.266	ns		
Grain Wt. (g plant^{-1})	175.2	166.7	0.2891	ns		
100 KWT (g)	31.1	31.4	0.2802	ns		
Drought Stress						
Silk Number (Silk Ear^{-1})	465.7	483.4	0.5278	ns	16.80%	14.10%
Yield (Mg ha^{-1})	8.69	8.75	0.8676	ns	36.70%	37.10%
Kernel Number (Kernels Ear^{-1})	344.7	319.2	0.3184	ns	39.00%	40.20%
Grain Wt. (g plant^{-1})	111.6	102.5	0.2875	ns	36.30%	38.50%
100 KWT (g)	32.3	32.1	0.658	ns	-3.90%	-2.20%
Combined						
Full Irrigation						
Silk Number (Silk Ear^{-1})	538.4	524.5	0.3378	ns		
Yield (Mg ha^{-1})	13.44	13.51	0.7431	ns		
Drought Stress						
Silk Number (Silk Ear^{-1})	479.1	462.5	0.399	ns	11.00%	11.80%
Yield (Mg ha^{-1})	9.04	9.12	0.8263	ns	32.70%	32.50%

Bivariate Fit of Yield (Mg ha) By Emerged Silks (Silks Ear)



Linear Fit

Yield (Mg ha) = 3.6608648 + 0.015373*Emerged Silks (Silks Ear)

Summary of Fit

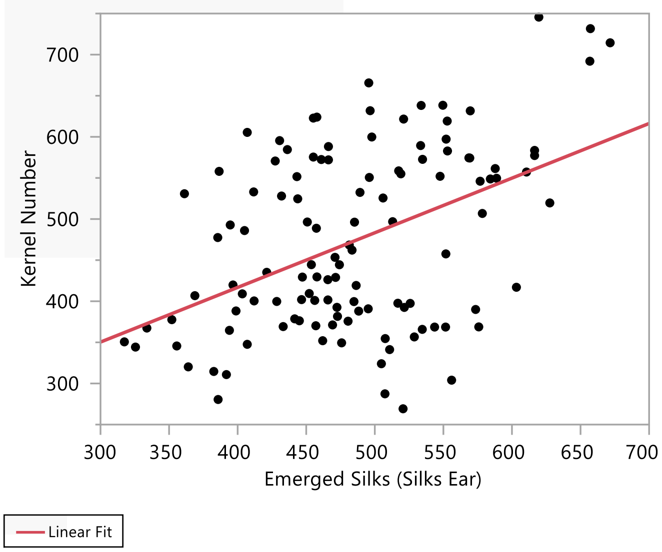
RSquare	0.196741
RSquare Adj	0.193337
Root Mean Square Error	2.27252
Mean of Response	10.83692
Observations (or Sum Wgts)	238

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	298.5154	298.515	57.8031
Error	236	1218.7855	5.164	**Prob > F**
C. Total	237	1517.3009		<.0001*

Figure 1: Linear regression of silks and yield for 2018, 2019 in all environments

Bivariate Fit of Kernel Number by Emerged Silks (Silks Ear)



Linear Fit

Kernel Number = 150.91273 + 0.664774*Emerged Silks (Silks Ear)

Summary of Fit

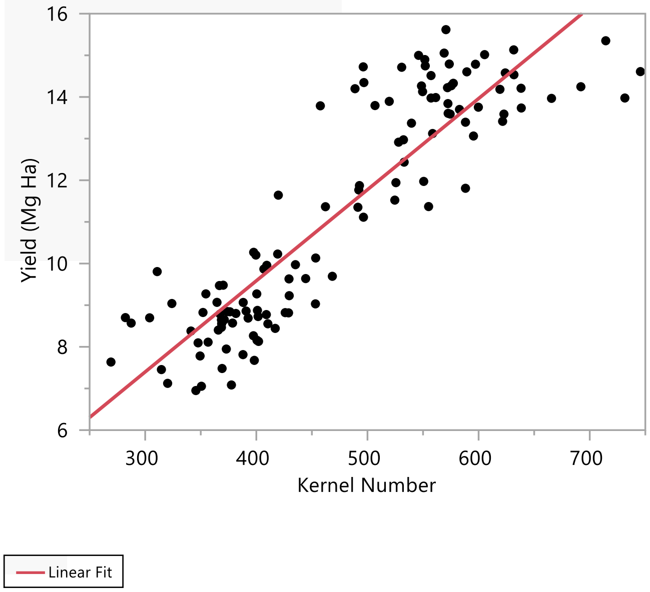
RSquare	0.206229
RSquare Adj	0.199445
Root Mean Square Error	98.58031
Mean of Response	472.6076
Observations (or Sum Wgts)	119

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	295407.8	295408	30.3978
Error	117	1137015.0	9718	**Prob > F**
C. Total	118	1432422.8		<.0001*

Figure 2: Linear regression of silks and kernel number for 2019 in all environments

Bivariate Fit of Yield (Mg Ha) By Kernel Number



Linear Fit

Yield (Mg Ha) = 0.8334153 + 0.0218811*Kernel Number

Summary of Fit

RSquare	0.794776
RSquare Adj	0.793037
Root Mean Square Error	1.221119
Mean of Response	11.20024
Observations (or Sum Wgts)	120

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	681.41974	681.420	456.9819
Error	118	175.95343	1.491	**Prob > F**
C. Total	119	857.37317		<.0001*

Figure 3:Linear regression of kernel number and yield for 2019 in all environments

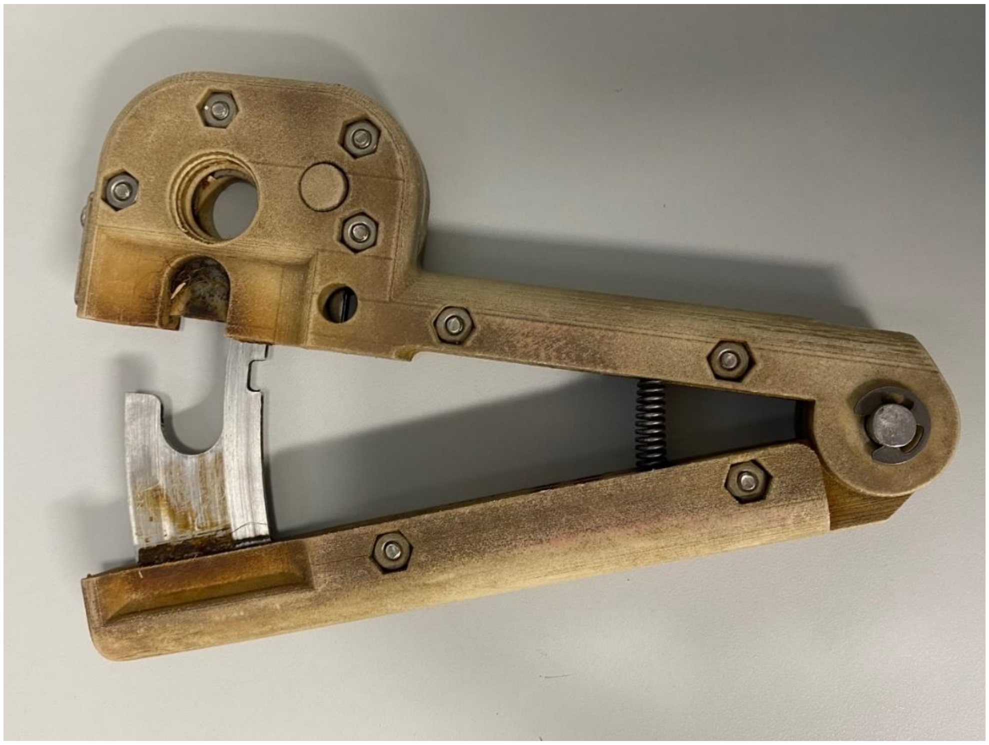


Figure 4: Silk cutting device developed by Corteva Agriscience

Figure 5: Utilizing the silk cutting device to harvest a silk sample

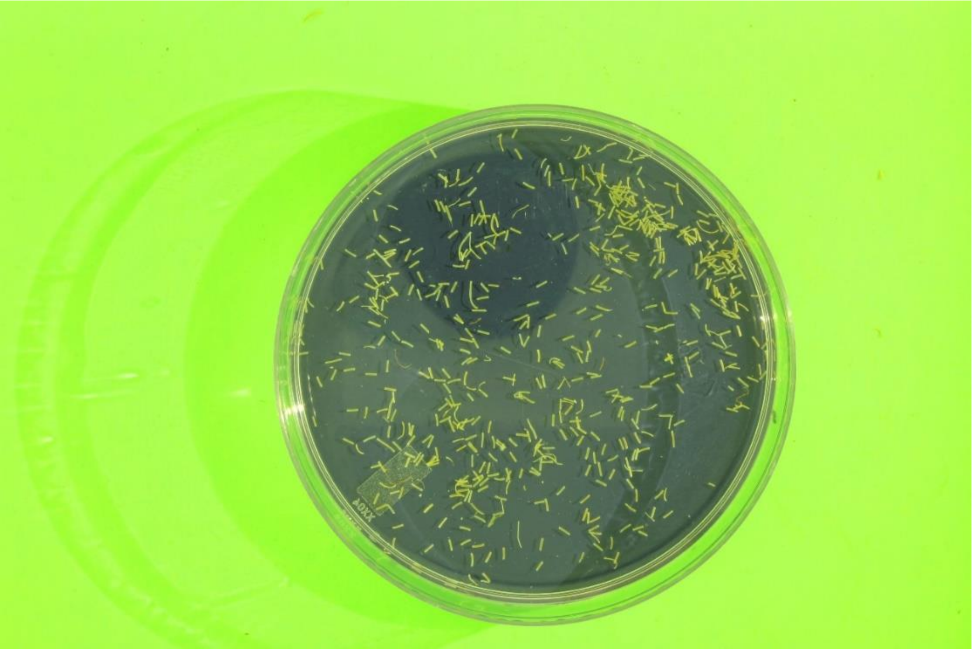


Figure 6: Digital image of unprocessed silk sample

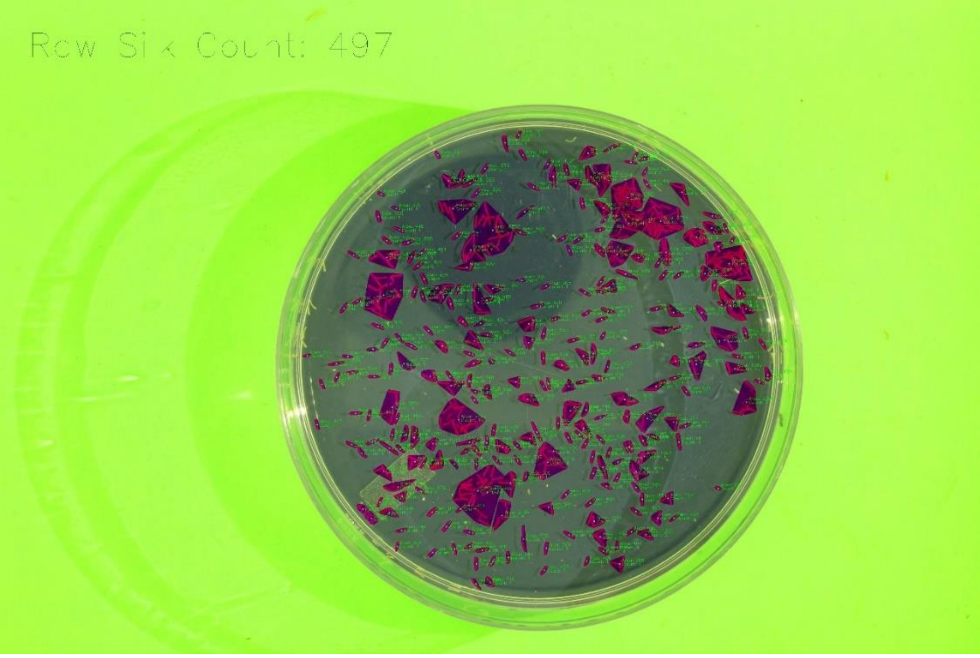


Figure 7: Digital Image of the same silk sample once it has been processed and a count is attached

Figure 8: Maize showing little to no effects of drought stress

Figure 9: Maize under drought stress showing heavy leaf rolling/folding

Figure 10: Maize under drought stress showing leaf rolling and lower canopy senescence

Figure 11: Maize under heavy drought stress showing upper and lower canopy senescence

Figure 12: Comparison of five drought tolerant ears (top) and five drought susceptible ears (below) in a heavily stressed environment

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APPENDIX A

ANOVA TABLES FOR DATA TABLES 1-8

ANOVA for emerged silks (silk ear^{-1}) by environment over years (Table 1)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Environment	1	651994	651994	53.9457	<.0001*
Error	1147	13862793	12086		
C. Total	1148	14514787			

ANOVA for yield (Mg ha) by environment over years (Table 1)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Location Name	1	1174.3667	1174.37	785.5940	<.0001*
Error	238	355.7808	1.49		
C. Total	239	1530.1475			

ANOVA for emerged silks (silk ear^{-1}) comparison of TOL vs SUS in DS environment over years (Table 2)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	164891.5	164891	12.4507	0.0005*
Error	562	7442899.5	13244		
C. Total	563	7607791.0			

ANOVA for emerged silks (silk ear^{-1}) comparison of TOL vs SUS in FI environment over years (Table 2)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	8.5	8.5	0.0008	0.9775
Error	583	6254993.0	10729.0		
C. Total	584	6255001.5			

ANOVA for yield (Mg ha) comparison of TOL vs SUS in DS environment over years (Table 2)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	39.61986	39.6199	36.0704	<.0001*
Error	118	129.61164	1.0984		
C. Total	119	169.23150			

ANOVA for yield (Mg ha) comparison of TOL vs SUS in FI environment over years (Table 2)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	8.45223	8.45223	5.6001	0.0196*
Error	118	178.09706	1.50930		
C. Total	119	186.54929			

ANOVA for Kernel Number by environment (Table 3)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Environment	1	1073937.0	1073937	362.8023	<.0001*
Error	118	349293.7	2960		
C. Total	119	1423230.7			

ANOVA for Kernel WT by environment (Table 3)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Environment	1	137232.65	137233	457.5683	<.0001*
Error	118	35390.24	300		
C. Total	119	172622.89			

ANOVA for 100 KWT by environment (Table 3)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Environment	1	176.92359	176.924	30.0328	<.0001*
Error	118	695.13921	5.891		
C. Total	119	872.06279			

ANOVA for Kernel Number comparison of TOL vs SUS in DS environment (Table 3)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	9269.05	9269.05	5.5352	0.0221*
Error	58	97125.38	1674.58		
C. Total	59	106394.43			

ANOVA for Kernel Number comparison of TOL vs SUS in FI environment (Table 3)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	3948.76	3948.76	0.9585	0.3316
Error	58	238950.50	4119.84		
C. Total	59	242899.26			

ANOVA for Kernel WT comparison of TOL vs SUS in DS environment (Table 3)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	2356.204	2356.20	17.5272	<.0001*
Error	58	7796.993	134.43		
C. Total	59	10153.197			

ANOVA for Kernel WT comparison of TOL vs SUS in FI environment (Table 3)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	2160.320	2160.32	5.4297	0.0233*
Error	58	23076.722	397.87		
C. Total	59	25237.042			

ANOVA for 100 KWT comparison of TOL vs SUS in DS environment (Table 3)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	28.50488	28.5049	4.5062	0.0381*
Error	58	366.88922	6.3257		
C. Total	59	395.39410			

ANOVA for 100 KWT comparison of TOL vs SUS in FI environment (Table 3)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	18.84061	18.8406	3.8901	0.0533
Error	58	280.90449	4.8432		
C. Total	59	299.74510			

ANOVA for 105 CRM pair emerged silks (silk ear^{-1}) comparison of TOL vs SUS in DS environment over years (Table 4)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	80678.9	80678.9	7.8456	0.0060*
Error	110	1131165.4	10283.3		
C. Total	111	1211844.3			

ANOVA for 105 CRM pair emerged silks (silk ear^{-1}) comparison of TOL vs SUS in FI environment over years (Table 4)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	22606.01	22606.0	2.6820	0.1042
Error	115	969294.30	8428.6		
C. Total	116	991900.31			

ANOVA for 105 CRM pair yield (Mg ha) comparison of TOL vs SUS in DS environment over years (Table 4)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	18.427037	18.4270	24.5968	<.0001*
Error	22	16.481639	0.7492		
C. Total	23	34.908676			

ANOVA for 105 CRM pair yield (Mg ha) comparison of TOL vs SUS in FI environment over years (Table 4)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	15.193816	15.1938	28.5649	<.0001*
Error	22	11.701902	0.5319		
C. Total	23	26.895718			

ANOVA for 108 CRM pair emerged silks (silk ear^{-1}) comparison of TOL vs SUS in DS environment over years (Table 5)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	7811.1	7811.1	0.7281	0.3954
Error	109	1169404.5	10728.5		
C. Total	110	1177215.6			

ANOVA for 108 CRM pair emerged silks (silk ear^{-1}) comparison of TOL vs SUS in FI environment over years (Table 5)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	395830.5	395831	57.0011	<.0001*
Error	118	819423.3	6944		
C. Total	119	1215253.9			

ANOVA for 108 CRM pair yield (Mg ha) comparison of TOL vs SUS in DS environment over years (Table 5)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	16.334276	16.3343	15.7269	0.0007*
Error	22	22.849715	1.0386		
C. Total	23	39.183992			

ANOVA for 108 CRM pair yield (Mg ha) comparison of TOL vs SUS in FI environment over years (Table 5)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	0.014232	0.01423	0.0115	0.9157
Error	22	27.328857	1.24222		
C. Total	23	27.343088			

ANOVA for 109 CRM pair emerged silks (silk ear^{-1}) comparison of TOL vs SUS in DS environment over years (Table 6)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	313721.5	313722	17.1078	<.0001*
Error	114	2090521.5	18338		
C. Total	115	2404243.0			

ANOVA for 109 CRM pair emerged silks (silk ear^{-1}) comparison of TOL vs SUS in FI environment over years (Table 6)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	191058.5	191058	13.3237	0.0004*
Error	115	1649071.4	14340		
C. Total	116	1840129.9			

ANOVA for 109 CRM pair yield (Mg ha) comparison of TOL vs SUS in DS environment over years (Table 6)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	24.187399	24.1874	46.6326	<.0001*
Error	22	11.410972	0.5187		
C. Total	23	35.598371			

ANOVA for 109 CRM pair yield (Mg ha) comparison of TOL vs SUS in FI environment over years (Table 6)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	25.304306	25.3043	41.2473	<.0001*
Error	22	13.496504	0.6135		
C. Total	23	38.800810			

ANOVA for 111 CRM pair emerged silks (silk ear^{-1}) comparison of TOL vs SUS in DS environment over years (Table 7)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	3353.3	3353.3	0.2713	0.6036
Error	108	1335106.2	12362.1		
C. Total	109	1338459.5			

ANOVA for 111 CRM pair emerged silks (silk ear^{-1}) comparison of TOL vs SUS in FI environment over years (Table 7)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	77075.85	77075.9	13.7446	0.0003*
Error	114	639278.04	5607.7		
C. Total	115	716353.89			

ANOVA for 111 CRM pair yield (Mg ha) comparison of TOL vs SUS in DS environment over years (Table 7)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	1.0455304	1.04553	2.8540	0.1053
Error	22	8.0595270	0.36634		
C. Total	23	9.1050574			

ANOVA for 111 CRM pair yield (Mg ha) comparison of TOL vs SUS in FI environment over years (Table 7)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	4.576657	4.57666	5.1984	0.0327*
Error	22	19.368668	0.88039		
C. Total	23	23.945325			

ANOVA for Experimental pair emerged silks (silk ear^{-1}) comparison of TOL vs SUS in DS environment over years
(Table 8)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	7933.7	7933.7	0.7240	0.3966
Error	113	1238232.8	10957.8		
C. Total	114	1246166.5			

ANOVA for Experimental pair emerged silks (silk ear^{-1}) comparison of TOL vs SUS in FI environment over years
(Table 8)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	5552.75	5552.75	0.9471	0.3325
Error	113	662513.89	5862.95		
C. Total	114	668066.64			

ANOVA for Experimental pair yield (Mg ha) comparison of TOL vs SUS in DS environment over years (Table 8)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	0.040018	0.040018	0.0493	0.8263
Error	22	17.850199	0.811373		
C. Total	23	17.890217			

ANOVA for Experimental pair yield (Mg ha) comparison of TOL vs SUS in FI environment over years (Table 8)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Drought Rating	1	0.0284916	0.028492	0.1104	0.7428
Error	22	5.6768265	0.258038		
C. Total	23	5.7053182			