# Effects of Drought Stress on the Growth, Yield and Physiological Traits of Thai Super Sweet Corn

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#### ABSTRACT

Growth and yield performances of Thai Super Sweet (TSS) corn cultivar treated with various drought stress levels (25%, 50% and 100% Field Capacity, FC) at various growth stages (vegetative, pre-flowering and flowering) were investigated in an insect-proof net-house of Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan Campus. Drought treatments were applied at vegetative (14 to 34 days after sowing (DAS)), pre-flowering (35 to 55 DAS) and flowering (48 to 68 DAS) stages. Parameters measured and statistically analysed were growth, chlorophyll content, proline content and yield. The experiment was conducted as 3 x 3 factorial arrangements of treatments with a completely randomised design with four replications for each treatment. Length of first cob and fresh weight of the first cob showed interaction effects among the drought and growth stage treatments while chlorophyll content, number of cobs, cob girth, number of grains, 100 grains weight and proline content showed significant effects among drought levels treatments and cob girth showed significant effect among growth stages. Shorter flowering time (tasseling at 42 DAS) was found on drought treatment of 25% FC at the pre-flowering stage. It can be concluded that vegetative and flowering stages were sensitive to drought compared to the pre-flowering stage, where the yield components of vegetative and flowering stages were lower compared to the preflowering stage. During the pre-flowering drought period, TSS expressed tolerance response through the higher levels of chlorophyll and proline contents and hence was able to perform the best overall yield components.

Keywords: Chlorophyll, drought stress, growth stages, proline, sweet corn

### **INTRODUCTION**

Drought is a period without significant rainfall or a condition where water is limited (Shukla et al., 2012). It has been identified as the most common abiotic stress that limits the crop performance worldwide in terms of growth and productivity notably (Ali et al., 2017). Water scarcity is one of the serious environmental constraints to plant productivity and the most critical threat to global food security as water is an essential need for all plants throughout its growth (Lobell and Gourdji, 2012). It causes a variety of physiological, biochemical and metabolic changes such as changes in photosynthesis, respiration, nutrient uptake and growth promoters that trigger oxidative stress which further disrupts the plant metabolism, growth performance as well as the yield (Ehab, 2016). The exposure of the maize crops being planted annually in Malaysia, to a large range of biotic and abiotic stresses is matter of great concern due to the inability of maize to tolerate stress throughout their growth, especially water stress. In Malaysia, very little work has been done on these aspects as the maize stress physiology study receives little interest.

Corn (*Zea mays* L.) is one of the grain crops highly consumed as food and animal feed globally and being processed into biofuel (Malhotra, 2017). Apart from having a large record of corn consumption (4 million metric tonnes/year) in 2018, Malaysia is the largest import market of corn in Southeast Asia due to

insignificant corn production within the country (Wahab, 2019). In 2016, the exposure on the high import bill of corn reaching up to RM 13.1 billion per year had brought to the realisation that there is so little interest in the potential of local corn production. Water is essential for normal growth and development of corn crops in which studies reported that corn is sensitive to drought stress especially during growth and reproductive stage (Aslam et al., 2015; Valerie and Moses, 2016). Drought had been found to affect corn germination, early establishment, growth performance, reproductive stage and overall yield potential (Aslam et al., 2013; Partheeban et al., 2017).

In Malaysia, some corn production areas in the Peninsular Malaysia and Borneo are exposed to the threat of low growth performance and yield reduction during drought seasons. Observation on the response of corn plants to drought stress and the mechanism in resisting drought at different growth stages are needed in order to find out the extent of drought tolerance and water management in achieving higher corn crop productivity. Therefore, the effects of drought stress and different growth stages on the growth and yield of Thai Super Sweet corn were investigated in this study.

#### MATERIALS AND METHODS

In this study, Thai Super Sweet (TSS) corn variety (WH 21) from Wah Heng Hang Seeds & Co. was used. The experiment was carried out in insect-proof net-house of Faculty of Sustainable Agriculture, UMS. The experiment was laid out in Completely Randomized Design (CRD) with four replications. Different drought stress levels were adjusted using field capacity (FC) where 25% FC (severe) referred to as treatment 1 (T1), 50% FC (moderate) referred to as treatment 2 (T2), and 100% FC as control and also referred to as treatment 3 (T3) which were equivalent to soil moisture content (SMC) of 7.25%, 14.50% and 29.00%, respectively, were applied at different growth stages namely vegetative (V), pre-flowering (P) and flowering (F). The soil moisture was determined using a soil moisture sensor according to the particular Field Capacity (FC) (Cassel and Nielson, 1986). Field capacity was defined as the amount of water held in the soil after excess water had been drained away (Cassel and Nielson, 1986). To determine the optimum level of water reserve of the soil at 100% FC, field capacity test was conducted prior to the study. The soil (ultisols) was dried and sieved to a uniform size of 2 mm to be tested. The soil was then slowly wet to approximately half of their depth from the top by slowly adding water to saturation. The soil was ensured to be fully wet to saturation. However, care was taken not to overwater the soil, which could occur if the rate of water application was too much. The pots were then covered to prevent evaporation with a dark plastic cover. The containers were left for 24 hours for the water to fully drain. After 24 hours, the soil was at 100% FC and quickly weighed (wet weight). FieldScout Soil Sensor Reader (Spectrum Technologies, Inc.) was then used to record the percentage of water content (% WC) in the soil. The field capacity water content in kilogramme was then calculated by using the formula below and converted into a litre of water:

Field capacity water content (L) = weight of wet soil - weight of dry soil \*1 kg = 1 L

Drought treatment was applied to the corn plants at the respective growth stages for 20 days. There was no drought stress applied to the corn plants before and after the treatment period, which indicated that the plants were re-watered after the 20 days of drought treatment. The data collection was carried out during planting until harvesting at 63 days after transplanting (DAT). Number of leaves, plant height, stem girth, and flowering time were recorded throughout the planting, while the number of cobs, the height of the first cob, length of the first cob, fresh weight of the first cob, cob girth, number of grains and 100 grains weight were measured once at harvest. Relative chlorophyll content was measured from the second uppermost collared leaves using SPAD-502 Plus chlorophyll meter at the final week of planting. The proline accumulation in fresh ear-leaves was extracted and quantified using the protocol of Bates et al. (1973). The middle section of the ear-leaves was used to test the proline content from all corn plants. The collected data

were subjected to two-way analysis of variance (ANOVA) with Statistical Analysis System Software (SAS 9.4). Means were separated by using the Least Significant Difference (LSD) test at 0.05 level of probability.

#### **RESULTS AND DISCUSSION**

The results showed that there were interaction effects among the drought and growth stages treatments for the length of the first cob (Figure 1A) and fresh weight of first cob (Figure 1B), while number of cobs (Figure 2A), cob girth (Figure 2B), number of grains (Figure 2C), 100 grains weight (Figure 2D), relative chlorophyll content (Figure 3A) and proline content (Figure 3B) showed significant effects among drought levels treatments and cob girth showed significant effect among growth stages (Figure 4).

The length of the first cob showed an interaction effect among the treatments (p < 0.01) and the longest mean length of the first cob was PT1 (25% FC at pre-flowering) (18.45 cm) (Figure 1A). In contrast, plants at 50% FC (T2) at vegetative and flowering stages were recorded shorter mean cob length of 13.23 cm and 13.08 cm, respectively. Compared to other growth stages, plants at the flowering stage maintained low readings of the length of the first cob throughout all levels of drought stress. It deviates from other study findings that record the reduction of yield components of corn plant being imposed on drought stress at the same growth stage (Khayatnezhad and Gholamin, 2012; Aslam et al., 2015). However, the findings of lower mean cob's length of VT1 and PT2 showed the correlation of drought stress levels and different growth stages in causing the reduction of the length of cob (Otegui et al., 1995; Farooq et al., 2009). Longer cobs bring to the improvement of other important yield components like the total number of grains per cob and higher fresh weight (Claassen and Shaw, 1970; Çakir, 2004). Minimum cob length as obtained by T1 due to drought stress imposed at vegetative was in accordance with the similar findings of cob length reduction when the maize plant was exposed to drought at a five-leaf stage (Zamir et al., 2015). However, 50% FC (T2) condition during all growth stages remarkably records the shorter length of cobs among all plants.

The fresh weight of the first cob showed an interaction effect among the treatments (p < 0.01) and the highest mean fresh weight of first cobs (g) was at PT1 (25% FC at pre-flowering) of 118.75 g (Figure 1B). In contrast, plants under 25% FC (T1) and 50% FC (T2) at vegetative stage produced cobs with a smaller mean fresh weight of only 47.5 g and 40.0 g, respectively. Weight of cobs was attributed to other yield components such as cob girth, cob length, number of grains and grain weight. The highest fresh weight recorded by plants under 25% FC at pre-flowering (PT1) was also in correlation with its increase in cob girth, length of cob, the number of grains and 100 grains weight. PT1 was recorded having higher cob fresh weight despite being induced under severe drought (25% FC) during pre-flowering and deviated from previous findings that had recorded the reduction of yield components of the corn plant under drought stress at the same growth stage (Khayatnezhad and Gholamin, 2012; Aslam et al., 2015). However, lower fresh weight of VT1 (25% FC at vegetative) and PT2 (50% FC at pre-flowering) showed the correlation of drought and growth stages in causing the reduction of its fresh weight of first cob (Otegui et al., 1995; Farooq et al., 2009).

The number of cobs recorded not only including the first cob, but also the developing cob on the plants. There was no interaction between the drought stress levels and growth stages (p > 0.05) for the number of cobs, but there was a significant effect among the drought stress levels (p < 0.05) on the number of cobs formed in which the highest mean number of cobs was produced by the plants under T3 (100% FC) with two cobs produced (Figure 2A). Control plants (T3) recorded the highest mean of the potential cob number of two cobs per plant and this set the yield potential of TSS cultivar under normal practices. In this study, drought deviated the plants from their maximum potential yield especially at severe drought by limiting the cob production to only one cob per plant. However, different growth stages where drought occurred did not affect the number of cobs produced among the treatments.

There was no interaction effect (p > 0.05) among the treatments, but a significant main effect of drought stress levels (p < 0.01, Figure 2B) and growth stages (p < 0.01, Figure 4) which affected the mean cob girth. The highest cob girth was recorded by plants undergoing drought stress at the pre-flowering stage

(P) with cob girth of 14.12 cm. Corn plants underwent drought stress during pre-flowering were usually subjected to yield component reduction but this differed from this study since the cob girth remained unaffected by the water-limited growth at the reproductive stage. On the other hand, the cobs with the smallest girth were recorded from plants undergoing stress during the vegetative stage (V) with only 12.26 cm. Drought during vegetative stage had been reported to cause fewer kernel rows and reduced number of kernels per row that correlated with the development of cob girth (Claassen and Shaw, 1970). Furthermore, drought stress that happened at tasseling affected the ear initiation and delayed silking and might have induced the reduction of cob girth while being imposed to stress at the time of cob development. This explained the low record of cob girth (12.87 cm) by the plants imposed with drought during flowering (F).

The cob girth is one of the important components to be measured in determining corn yield potential. Drought stress was reported to affect yield components of corn including girth (Khodarahmpour and Hamidi, 2012). The control plants recorded the highest cob girth of 14.52 cm whereas the shortest mean cob girth of 12.43 cm was exhibited by plants undergoing severe stress of T1 (25% FC) (Figure 2B). The results showed that the cobs under T3 (100% FC) recorded the highest mean cob girth compared to other plants being treated within the same growth stage. Higher cob girth commonly correlated with the increase of other grain components such as increased number of kernel row numbers, cob diameter, number of grains and overall cob size (Çakir, 2004; Khodarahmpour and Hamidi, 2012). In this study, the increase of T3 cobs girth was correlated with the increase in other yield components such as number of grains, longer cob length and higher fresh weight after harvest.

The number of grains was significantly affected by different drought stress levels (p < 0.05) and the highest mean number of grains was recorded by T3 (100% FC) with 312 grains per cob followed by T1 (25% FC) with 260 grains per cob (Figure 2C). The cobs with the lowest mean number of grains were recorded from T2 (50% FC) with only 234 grains per cob (Figure 2C). The drought was reported to affect yield and its components other than the plant growth itself (Atteya, 2003; Žalud et al., 2017). Grain yield reductions at drought was reported to be within the range of 10 to 76% depending on the severity of water stress and the growth stage in which the stress had occurred (Bolaños et al., 1993; Khodarahmpour and Hamidi, 2012). Grain yield of corn under severe drought was reported to be significantly reduced compared to the normal and less severe drought conditions (Shi et al., 2017). This was in accordance with the low mean number of grains recorded from plants at 25% and 50% FC (T1 and T2), respectively. The number of grains per cob actually depended on other aspects such as row number per cob, length of cobs, cob diameter, grain width and grain depth (Khodarahmpour and Hamidi, 2012). As the T3 cobs (100% FC) recorded longer length and a larger circumference of cobs compared to other drought stress treatments, these might contribute to the cobs having the highest number of grains among the treatments.



Figure 1. Interaction effects of drought stress levels and different growth stages of Thai Super Sweet corn variety at 63 DAT. (A) Mean length of the first cob and (B) Mean fresh weight of the first cob, at 25% FC (T1), 50% FC (T2) and 100% FC (T3), at vegetative (V), pre-flowering (P) and flowering (F) stages.



Figure 2. Effects of drought stress levels on the yield of Thai Super Sweet corn variety at 63 DAT. (A) Mean number of cobs, (B) mean cob girth, (C) mean number of grains, and (D) mean 100 grains weight at different field capacities. Values followed by the different letters are significantly different at p < 0.05 by the Least Significant Difference test.

There was no interaction effect among the treatments (p > 0.05) for the 100 grains weight but there was a significant effect of different drought stress levels (p < 0.01) for the 100 grains weight. The cobs from T3 (100% FC) recorded the highest 100 grains weight of 16.31 g whereas T1 (25% FC) showed the lowest 100 grains weight of 10.56 g (Figure 2D). In accordance with the results, grain weight had been reported as one of the important yield components affected by drought. Weight of grains from fresh cob was commonly measured in the evaluation of drought stress effect towards the yield characteristics of maize but the number of grains to be weighed was not specified across the study (Çakir, 2004; Khodarahmpour and Hamidi, 2012). Drought stress had been reported to affect the number of grains per cob and 100-grains weight thus affecting the overall yield (Khalili et al., 2013). Corn grain yield was determined by several factors such as the growth and development potential of a corn plant, photosynthetic performance at growth and efficiency of photosynthate to be partitioned into the grains (Milander, 2015). Grain number reductions were reported to be proportional to the reduction in grain weight (Eck, 1986). Our results showed that T1 (25% FC) differed in terms of 100 grains weight compared to the control treatment, T3 (100% FC), as they developed smaller grain sizes. Smaller grain size usually attributed by smaller grain width and shorter grain depth in the cob cavity that contribute to a lighter grain weight (Khayatnezhad and Gholamin, 2012). Reduction of assimilate partitioning, sucrose and starch synthesis activities following drought hampered the grain filling and dry matter partitioning as reported by Anjum et al. (2011) and thus, they may be the contributing factors to smaller and fewer grains of T1. The lower mean of 100 grains weight recorded from T1 cobs was supported by the findings on the reduction of grains weight during a severe drought (Fatemi et al., 2006; Khalili et al., 2010).

Chlorophyll content has been used as one of the physiological indicators in evaluating the limiting factors of plant growth in drought stress conditions (Maisura et al., 2014). The data of relative chlorophyll content at 63 DAT showed a significant effect of the drought stress level (p < 0.01) towards all plants. The plants under drought stress of T1 (25% FC) showed the highest mean relative chlorophyll content (41.05 SPAD unit) followed by plants under T2 (50% FC) with a mean relative chlorophyll content of 35.16 SPAD unit. In contrast, the plants under 100% FC (T3) gave the lowest mean relative chlorophyll content of 33.71 SPAD units (Figure 3A). The relative chlorophyll content of 50% FC (T2) was increased 4.30% compared to 100% FC (T3) control treatment, while there was an increase of 21.77% of relative chlorophyll content for 25% FC (T1) compared to 100% FC (T3). This indicated that severe drought increased the relative chlorophyll content and could cause the corn plants to become more stress-tolerant. Drought stress had been reported to influence the number of chlorophyll contents being expressed by the plants (Sanchez-diaz and Kramer, 2017). Duration and severity of drought were reported to affect the plant response towards drought by the reduction or unchanged chlorophyll level (Anjum et al., 2011). However, a different level of chlorophyll had been recorded in the study of resistant maize cultivars that showed a significant increase of chlorophyll contents during drought stress compared to the normal cultivars (Khayatnezhad and Gholamin, 2012). The study was similar to the current results in which the plants under severe drought of 25% FC (T1) exhibited the highest amount of chlorophyll compared to the control (T3) (Figure 3A). Higher chlorophyll content was reported having a significant direct effect and indirectly contributed to grain yield per plant via increase in cob length and cob weight in accordance with yield components findings (Ali et al., 2017). In accordance with the higher chlorophyll level in plants under water stress level of 25% FC (T1), the finding was also in accordance with another study conducted on maize plants of various tolerance levels towards water deficit. The study reported that two resistant maize cultivars expressed a higher level of chlorophyll and the response was then contributed to the higher tolerance ability of the cultivars compared to others (Khayatnezhad and Gholamin, 2012; Fanaei et al., 2015). Resistant genotypes of wheat and corn were also found with higher chlorophyll content under drought condition by other studies (Pastori and Trippi, 1992; Zaefyzadeh et al., 2009). Through this correlation, higher chlorophyll expressions may be recognised as one of the physiological responses of TSS maize towards water stress and also identified as the cultivar characteristics towards stress tolerance potential. Water-saturated soil was reported to reduce the chlorophyll contents in the plant, and this might explain the low levels of mean chlorophylls of plants under 100% FC (T3) (Khayatnezhad and Gholamin, 2012). Variation in SPAD readings of maize chlorophyll contents was reported to be highest during the early stage of plant growth compared to the later stages by Argenta et al. (2004).



Figure 3. Effects of drought stress levels on the content of Thai Super Sweet corn variety at 63 DAT. (A) Mean relative chlorophyll content and (B) mean proline content at different field capacities. Values followed by the different letters are significantly different at p < 0.05 by the Least Significant Difference





Figure 4. Effects of different growth stages on the mean cob girth of Thai Super Sweet corn variety at 63 DAT. Values followed by the different letters are significantly different at p < 0.05 by the Least Significant Difference test.

The results showed a significant effect among the drought stress levels (p < 0.01) on the proline content (Figure 3B). The highest mean proline content was recorded from ear leaves of T1 plants (25% FC) with 237.57 µmole g<sup>-1</sup> fresh weight (FW), while the lowest proline content of 110.85 µmole g<sup>-1</sup> FW was found in T3 plants (100% FC) (Figure 3B). Accumulation of proline under stress had been related to stress tolerance of many plant species with a report of higher concentration in stress-tolerant than in stress-susceptible plant species (Anjum *et al.*, 2011). To maintain cell turgor, plants accumulate different types of organic and inorganic solutes in the cytosol such as proline, sucrose, soluble carbohydrates and glycine betaine in the response towards lower osmotic potential during drought (Farhoudi et al., 2014; Pandey and Shukla, 2015). Plants exposed to drought induced higher accumulation of proline in order to reduce the injury to cells (Anjum et al., 2011). The results were in accordance with studies that identified an increase of the biosynthetic rate of proline following osmotic stress especially in maize (Boggess et al., 1976; Neisiani et al., 2009). Proline accumulation is a common metabolic response of plants exposed to a dry period in order to reduce the injury to cells (Fahad et al., 2017). This finding correlated with the

accumulation of high mean proline content in the ear leaves of all plants exposed to 25% FC (T1) compared to others (Figure 3B). The higher proline content recorded in the ear leaves of T1 (25% FC) might contribute to its recovery from stress at the vegetative stage. In accordance with higher proline accumulations, T1 plants were able to recover from the 20 days of stress and performed optimally during flowering and yield developments. Proline is the most common compound besides glycine betaine that accumulates in high concentration in plants under water and salt stress (Caseiro et al., 2004). Proline accumulation is denoted as one of the important adaptation strategies of plants over short periods to withstand water stress conditions (Jäger and Meyer, 1977).

The comparison of corn cobs between treatments after harvest was shown in Figure 5. The first anthesis and silking were recorded by PT1 (25% FC at pre-flowering) at 42 and 47 DAS, respectively. Most of the other corn plants started flowering with obvious tassel development at 48 to 51 DAS.



Figure 5. Comparison of corn cobs between treatments after harvest.

Apart from the above parameters, there were several parameters which showed no significant interaction and single effects, including the plant height, stem girth and height of the first cob of all the treatments (Table 1).

Interaction		Plant height (cm)	Stem girth (cm)	Height of the first cob (cm)
Growth stages	Field capacity (%)	_		
Vegetative	25	196.63	6.25	91.03
	50	183.73	6.38	99.73
	100	201.00	6.03	98.55
Pre-flowering	25	186.45	6.98	87.65
	50	194.80	5.68	93.73
	100	196.98	5.93	90.38
Flowering	25	200.23	6.10	89.05
	50	198.85	6.33	88.55
	100	199.50	5.83	94.40

Table 1. Effects of drought stress on plant height, stem girth and height of the first cob on different growth stages

\*Values are mean of four replications.

### CONCLUSIONS

Shorter flowering time (tasseling at 42 DAS) was found on drought treatment of 25% FC at the preflowering stage. Vegetative and flowering stages were the critical stages of drought especially under severe drought as the yield components of both stages were lower compared to the pre-flowering stage. Thus, farmers should be aware and avoid drought or limitation of water during the cultivation of corn at these critical growth stages with the goal of achieving optimum yield potential. During the pre-flowering drought period, TSS expressed tolerance response through the higher levels of chlorophyll and proline contents and hence was able to perform the best overall yield components. Further study is required to determine the physiological, biochemical responses and yield performance regarding the drought tolerance ability of corn at the pre-flowering stage.

## **AUTHOR CONTRIBUTION**

AZN conceived, designed and performed the analysis, wrote the paper, and checked and approved the submission.

## **CONFLICT OF INTEREST**

The authors declare there are no conflict of interests.

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