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# Research Article
# Physiological Responses of Interspecific and Intergeneric Hybrids of Sugarcane under Early Drought Stress Conditions

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## Abstract
**Background and Objective:** Drought is one of the important problems that drastically affect sugarcane production in several countries. The objective of this experiment was to investigate certain physiological characteristics of drought-stressed sugarcane plants under plastic house conditions. **Materials and Methods:** The experiment was arranged in a completely randomized design with four replications. Five sugarcane genotypes were grown. These included three commercial cultivars [Khon Kaen 3 (drought tolerant), KpK98-40 (drought susceptible) and KK07-037] and two clones derived from *Saccharum spontaneum* and *Erianthus* sp. (KK08-214 and E08-4-019, respectively). The five sugarcane genotypes were subjected to water stress when they were 110 days old. The plants were divided into two groups: the controlled group regularly receiving water at the field capacity and the experimental group (under water stress) receiving 1/3 available water. After they were irrigated for 73 days, chlorophyll fluorescence (Fv/Fm), leaf greenness (SPAD value), water potential, electrolyte leakage and shoot fresh and dry weight were determined. **Results:** The results revealed that drought stress caused Fv/Fm and leaf water potential to decrease significantly ($p<0.05$) but electrolyte leakage in the leaves of all sugarcane genotypes to increase significantly ($p<0.05$). The leaf greenness of E08-4-019 was significantly ($p<0.05$) higher than that of the other genotypes with low electrolyte leakage during drought stress. **Conclusion:** E08-4-019 tolerated drought stress. Physiological traits can be used in selecting cultivars for increased efficiency in sugarcane breeding for drought tolerance.

Key words: Sugarcane, chlorophyll fluorescence, drought stress, electrolyte leakage

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Water-deficit stress or drought stress is one of the most important environmental stresses. Morphological and physiological responses of sugarcane plants vary, depending on the genotype, the growth stage, the intensity of stress and also the type of tissue affected[1-4]. The most common water stress responses in sugarcane are leaf rolling, stomatal closure, inhibition of stalk and leaf growth, leaf senescence and reduced leaf area[5-7]. Severe drought stress also inhibits the photosynthesis of plants by causing changes in chlorophyll content and by damaging the photosynthetic apparatus[1,8]. The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active reactive oxygen species[9-10]. Plants adapt to water stress by maintaining sufficient cell turgor. Generally, osmotic adjustment is a major mechanism to maintain cell turgor in many species as the water potential decreases[11]. According to Endres *et al.*[6], the tolerant sugarcane genotype was able to maintain photosynthetic activity better than the susceptible genotypes under low leaf water potential conditions.

Measurement of fluorescence from photosystem II has become a useful method for the determination of mechanisms of photosynthesis and for studying the effects of various environmental conditions on photosynthetic reactions. The integrated approaches are necessary for a better understanding of plant strategies for survival under environmental change. SPAD was suggested for estimating leaf chlorophyll concentration[12-14]. Zhao *et al.*[15] reported the differential decline of the SPAD index in sugarcane associated with soil physical properties. SPAD data were significantly correlated with chlorophyll content in sugarcane[16]. Several studies aimed to understand plant responses to water deficit[17-18]. Many drought response mechanisms have been investigated but their physiological responses to drought stress remain unknown. The utilization of physiological traits is one of the useful strategies to rapidly and economically screen for water use efficient and drought-tolerant cultivars. Thus, this study aimed to investigate the physiological traits associated with drought tolerance in five sugarcane genotypes subjected to drought under plastic house conditions. The physiological measurements are prone to providing effective selection criteria for drought-tolerant sugarcane cultivars.

## MATERIALS AND METHODS

**Plant material:** The experiment was arranged in a 2×5 factorial in a completely randomized design with four replications. Five sugarcane genotypes were provided by Khon Kaen Field Crops Research Center and the Department of Agronomy, Faculty of Agriculture, Khon Kaen University. They include Thailand's drought-tolerant sugarcane genotype: Khon Kaen 3, Thailand's drought-sensitive sugarcane genotype: KpK98-40, a Thailand's sugarcane genotype: KK07-037, an *S. spontaneum* clone: KK08-214 and an *Erianthus* clone: E08-4-019.

**Irrigation treatments:** Five sugarcane genotypes were horizontally grown in 8×16 cm black plastic bags (each containing 500 g of a growing medium consisting of filter cake and loamy sand soil at the ratio of 1:1 by volume) for a month. Then, the sugarcane plants were transferred to pots (26.7 cm in diameter, each containing 2500 g of loamy sand soil) and maintained in a plastic house. The 110-day-old sugarcane plants were exposed to drought by withholding water until soil moisture dropped to 1/3 Available Water (AW). The sugarcane plants were divided into two groups: the controlled group regularly receiving water at the field capacity and the experimental group (under water stress) receiving 1/3 AW. Soil moisture was measured every 2 days using the gravimetric method. After withholding water for 73 days, growth and physiological parameters, shoot fresh and dry weight, chlorophyll fluorescence (Fv/Fm), leaf greenness (SPAD index), water potential and electrolyte leakage were determined.

**Soil moisture measurement:** Soil moisture content was sampled at 15 cm below the soil surface for irrigation treatments. The data on rainfall, temperature, solar radiation, relative humidity and wind were collected from the weather station located at the experimental field belonging to the Department of Agronomy, Faculty of Agriculture, Khon Kaen University.

**Plant growth and physiological measurements:** Stalk height was measured from the ground to the top visible dewlap leaf. Chlorophyll fluorescence, SPAD Chlorophyll Meter Reading (SCMR), leaf water potential and electrolyte leakage were determined in the Topmost Visible Dewlap (TVD) leaves. SPAD chlorophyll meter reading was measured using a SPAD-502 chlorophyll meter (Spectrum Technologies, USA). Chlorophyll fluorescence was measured with a chlorophyll fluorometer (Hansatech Instruments, England) after 30 min dark adaptation. Fv/Fm (maximum quantum efficiency of photosystem II) was determined from 9.00-10.30 am. Electrolyte leakage was determined according to the protocol outlined by Filex *et al.*[19].

**Statistical analysis:** The data were subjected to analysis of variance (ANOVA) and treatment means were compared by Least Significant Difference (LSD) test using Statistic 10 software.

## RESULTS

**Soil moisture content:** The soil moisture contents determined from soil physical properties were 14.54% for the Field Capacity (FC) and 4.75% for the Permanent Wilting Point (PWP). During 73 days of water stress treatment, the soil moisture content at 15 cm below the soil surface decreased to 1/3AW (8.01%). The average soil moisture content under FC conditions was 13.08%, while the value of this parameter gradually dropped to 5.94% under drought stress conditions in Fig. 1.

**Physiological characteristics of five sugarcane genotypes:** Mean squares for stalk height, shoot fresh weight, shoot dry weight and leaf water potential are shown in Table 1. Drought stress caused significant decreases in stalk height, shoot fresh weight, shoot dry weight and leaf water potential. However, the drought-stressed plants had higher electrolyte leakage than the well-watered plants. The interaction between water regime and genotype was observed in stalk height (Table 1). The significant differences in stalk height, shoot fresh weight, shoot dry weight and leaf water potential were found among sugarcane genotypes (Table 1). Mean squares for Fv/Fm, SPAD value and EL are shown in Table 2. The interaction between water regime and genotype (WXG) was not significant for Fv/Fm and SPAD value, however, the interaction between water regime and genotype was observed in EL (Table 2). Fv/Fm, SPAD value and EL were significantly different among water regimes. Moreover, there were significant differences in Fv/Fm, SPAD value and EL among sugarcane genotypes (Table 2). Increased EL in the stressed plants is one of the pieces of evidence indicating that water deficit affects membrane stability. Under FC conditions, the average EL was between 20.28 and 25.38%, while under 1/3 AW conditions, the value was between 19.74 and 40.94% in Table 3. A significant increase in EL was observed in KK3 and KpK98-40 under drought stress.

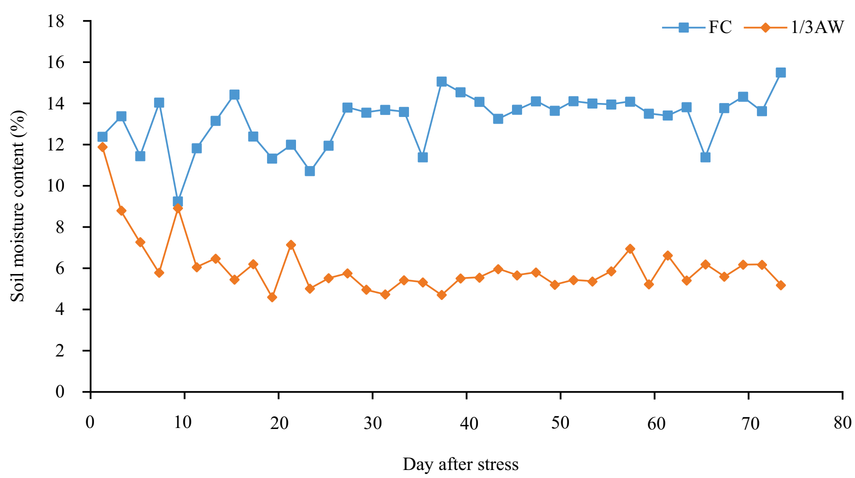


Fig. 1: Soil moisture content at 15 cm below the soil surface

Table 1: Mean squares for stalk height, shoot fresh weight, shoot dry weight and leaf water potential of five sugarcane genotypes under drought conditions

| Source of variance | df | Stalk height | Shoot fresh weight | Shoot dry weight | Leaf water potential |
|---|---|---|---|---|---|
| Water regime (W) | 1 | 6307.5** | 836286** | 26367** | 1.240** |
| Genotype (G) | 4 | 56130.9** | 624146** | 65867** | 0.861** |
| W×G | 10 | 3388.3** | 80964$^{ns}$ | 17752$^{ns}$ | 0.154$^{ns}$ |
| Pooled error | 18 | 2721.6 | 418324 | 47009 | 0.760 |

**and ns indicate significance at $p\leq0.01$ and non-significance, respectively

Table 2: Mean squares for Fv/Fm, SPAD value and EL of five sugarcane genotypes under drought conditions

| Source of variance | df | Fv/Fm | SPAD value | EL |
|---|---|---|---|---|
| Water regime (W) | 1 | 0.000396** | 168.51** | 217.18** |
| Genotype (G) | 4 | 0.000461** | 237.9** | 614.84** |
| W×G | 10 | 0.000094$^{ns}$ | 37.64$^{ns}$ | 552.42** |
| Pooled error | 18 | 0.0008410 | 152.66 | 89.61 |

**and ns indicate significance at $p\leq0.01$ and non-significance, respectively

Table 3: Stalk height and EL of five sugarcane genotypes under drought conditions

| | Stalk height (cm) | | EL (%) | |
|---|---|---|---|---|
| Genotype | FC | 1/3AW | FC | 1/3AW |
| KK08-214 | 177.33±6.81$^{Ab}$ | 111.00±18.25$^{Bb}$ | 25.38±0.98$^{Aa}$ | 19.74±2.03$^{Bc}$ |
| E08-4-019 | 208.33±13.05$^{Aa}$ | 194.67±8.08$^{Aa}$ | 21.52±1.40$^{Aab}$ | 21.29±1.96$^{Ac}$ |
| KK3 | 130.33±8.02$^{Ac}$ | 91.33±16.07$^{Bb}$ | 22.64±1.54$^{Bab}$ | 40.94±3.76$^{Aa}$ |
| KK07-037 | 122.33±18.00$^{Ac}$ | 112.33±12.01$^{Ab}$ | 20.28±3.57$^{Ab}$ | 22.96±0.76$^{Ac}$ |
| KpK98-40 | 78.67±6.66$^{Ad}$ | 62.67±12.70$^{Ac}$ | 24.34±2.45$^{Ba}$ | 36.13±1.46$^{Ab}$ |

Different uppercase letters indicate significant differences among irrigation treatments in each genotype, different lowercase letters indicate significant differences among genotypes in each irrigation treatment by LSD at $p \leq 0.05$. EL: Electrolyte leakage and FC: Field capacity

Table 4: Shoot fresh weight, shoot dry weight, leaf water potential, Fv/Fm and SPAD value of five sugarcane genotypes under drought conditions

| Treatment | Shoot fresh weight (g) | Shoot dry weight (g) | Leaf water potential (MPa) | Fv/Fm | SPAD value |
|---|---|---|---|---|---|
| **Water regime (W)** | | | | | |
| FC | 960.92±245.78$^{A}$ | 255.48±68.53$^{A}$ | -0.93±0.24$^{A}$ | 0.794±0.005$^{A}$ | 40.53±2.66$^{A}$ |
| 1/3 FC | 626.99±142.97$^{B}$ | 196.19±68.79$^{B}$ | -1.33±0.27$^{B}$ | 0.786±0.009$^{B}$ | 35.79±9.12$^{B}$ |
| **Genotype (G)** | | | | | |
| KK08-214 | 813.36±142.74$^{b}$ | 224.43±64.56$^{b}$ | -1.37±0.31$^{c}$ | 0.791±0.010$^{ab}$ | 36.96±3.47$^{b}$ |
| E08-4-019 | 998.57±237.99$^{a}$ | 311.95±55.83$^{a}$ | -1.29±0.37$^{bc}$ | 0.793±0.004$^{a}$ | 43.38±4.13$^{a}$ |
| KK3 | 827.58±341.84$^{ab}$ | 209.81±83.99$^{b}$ | -1.05±0.23$^{ab}$ | 0.793±0.004$^{a}$ | 37.36±2.70$^{b}$ |
| KK07-037 | 782.31±215.30$^{b}$ | 213.24±47.28$^{b}$ | -1.02±0.31$^{a}$ | 0.783±0.009$^{b}$ | 34.96±4.77$^{b}$ |
| KpK98-40 | 547.96±169.06$^{c}$ | 169.75±43.83$^{b}$ | -0.93±0.24$^{a}$ | 0.791±0.007$^{ab}$ | 38.14±4.49$^{b}$ |
| **F-test** | | | | | |
| W | ** | ** | ** | ** | ** |
| G | ** | ** | ** | ** | ** |
| W×G | ns | ns | ns | ns | ns |

**and ns indicate significance at $p \leq 0.01$ and non-significance, respectively

Decreased or unchanged stalk height during drought stress was observed (Table 3). The stalk height of KK08-214 and KK3 significantly decreased after exposure to drought. KpK98-40 showed the lowest stalk height under 1/3 AW conditions. Drought stress decreased shoot fresh weight by 34.76%. The highest average shoot fresh weight was observed in E08-4-019 (988.57 g), while the lowest value was noted in KpK98-40 (547.96 g) in Table 4. Shoot dry weight varied from 169.75-311.95 g. The decrease in shoot dry weight was 23.21% due to drought stress. E08-4-019 had a greater shoot dry weight than the other genotypes (Table 4). Leaf water potential was lower in KK08-214 and E08-4-019 than in the other genotypes (Table 4). The Fv/Fm of all five sugarcane genotypes was affected by drought stress. The lowest Fv/Fm was noticed in KK07-037 (Table 4). Drought stress induced the change in the SPAD value of sugarcane leaves. The SPAD value was reduced with an average reduction of 21.41%. E08-4-019 had a higher SPAD value than the other genotypes (Table 4).

## DISCUSSION

Drought stress can interrupt cell division and cell elongation[20]. Stem and leaf elongation are the most seriously affected growth processes[21-22]. Under water stress conditions, leaf area and stalk growth decrease and plant height also reduces[2,5]. The drought-susceptible genotype KpK98-40 showed the lowest stalk height and shoot fresh weight.

Electrolyte Leakage (EL) has been used as an indicator of membrane stability for the degree of cell membrane injury[19]. The increase in EL in the sensitive sugarcane during drought is consistent with the previous report by Sudhakar *et al.*[23] and Reyes *et al.*[4]. In this study, the increase in EL was observed in KK3 and KpK98-40 under drought stress.

The chlorophyll content of sugarcane decreases as a result of drought stress[8,24]. The SPAD value is significantly correlated with leaf chlorophyll content in sugarcane[16]. It was observed that drought induced a 21% decrease in SPAD value. Similarly, drought caused a decline in the SPAD value of sugarcane leaves after exposure to drought for 10-15 days, while the greater SPAD value declines under drought stress in the drought-sensitive genotype[25]. Silva *et al.*[24] reported that sugarcane productivity was associated with the ability to maintain physiological functions such as SPAD value and Fv/Fm under water deficit.

Drought stress caused stomatal closure, reduced leaf net $CO_2$ assimilation rate and decreased photochemical yield of open photosystem II (PSII) centers, which contributed to the decrease in photosynthesis[26-27]. The efficiency of photosynthesis can be measured by the efficiency of PSII photochemistry. Chlorophyll fluorescence is considered to be suitable for the measurement of the activity of photosynthesis[28]. The Fv/Fm parameter is wildly used as the drought stress indicator in various crops such as durum and bread wheat, barley[29] and *Arabidopsis*[30]. In contrast, no change in Fv/Fm was detected in American chestnut[31], strawberry[32] and soybean[33] grown under drought conditions. In snap bean, the Fv/Fm decreased significantly only in the most sensitive primary leaves and the effective quantum yield was also significantly reduced in the more sensitive genotypes[34]. The decrease in Fv/Fm under water deficit conditions has been reported in sugarcane[1,24,27]. Graça *et al.*[1] found that drought-tolerant cultivars showed better quantum efficiency photosystem II than sensitive cultivars. The quantum efficiency photosystem II of drought-tolerant cultivars was more stable in the last days of the experimental treatment, suggesting that the decline in relative water content stimulated an adjustment of photosynthetic capacity to tolerate the change in water availability. The measurement of chlorophyll fluorescence as a rapid non-destructive method can be easily used to investigate plant drought stress.

Drought stress alters leaf water relations by decreasing leaf water potential. According to Silva *et al.*[24], sugarcane leaf water potential showed a reduction by 46 and 26% in the drought-susceptible and drought-tolerant genotypes, respectively after 60 days of water deficit. The water potential reduced significantly during drought stress treatment and it was lower in the sensitive cultivar than in the tolerant cultivar[7,35,36]. In this study, the average leaf water potential of the controlled plants was lower than that of the stressed plants. The maintenance of leaf water potential under drought allows the plants to maintain physiological functions such as stomatal opening, $CO_2$ assimilation, cell expansion and development[37].

## CONCLUSION

Drought stress decreased shoot weight, chlorophyll fluorescence and SPAD chlorophyll meter reading. However, a significant increase in electrolyte leakage was found in the sugarcane plants under drought stress. The *Erianthus* clone: E08-4-019 had high biomass and showed several drought tolerance characteristics. SPAD chlorophyll meter reading and electrolyte leakage could be used as the key selection tools for drought tolerance. These physiological traits also have great potential as selection criteria for improving crop productivity in drought-prone environments.

## SIGNIFICANCE STATEMENTS

The present study revealed that drought stress decreased the shoot weight, chlorophyll fluorescence and SPAD chlorophyll meter reading of the five sugarcane genotypes grown under plastic house conditions. Using physiological characteristics will become increasingly strategic to improve drought-adaptive traits and sugarcane cultivars. SPAD chlorophyll meter reading and electrolyte leakage could be used as the key selection tools for drought tolerance. The interspecific and intergeneric hybrids of *Saccharum* and *Erianthus* contribute to the drought tolerance characteristics. The physiological measurements are prone to providing effective selection criteria for drought-tolerant sugarcane cultivars. The utilization of physiological traits is one of the useful strategies to rapidly and economically screen sugarcane cultivars for drought tolerance.

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