Journal of Current Science and Technology, January-April 2024 Vol. 14 No. 1, Article 15

ISSN 2630-0656 (Online)

Cite this article: Pongtip, A., Thobunluepop, P., Nakasathien, S., Kongsil, P., Sarobol, E., Chitbanchong, W., & Pawelzik, E. (2024). Growth and physiological responses of maize (*Zea mays* L.) under drought stress at different development stages. *Journal of Current Science and Technology*, *14*(1), 1-10. Article 15. https://doi.org/10.59796/jcst.V14N1.2024.15

Journal of Current Science and Technology

Journal homepage: https://jcst.rsu.ac.th

Growth and Physiological Responses of Maize (*Zea mays* L.) under Drought Stress at Different Development Stages

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Received 10 August, 2023; Revised 20 September, 2023; Accepted 26 September, 2023

Abstract

Drought stress was a main problem of maize production in Thailand. This study aimed to evaluate the effect of drought stress at different development stages and maize varieties (*Zea mays* L.) on growth, physiological responses, and grain yield to maintain maize production. The experiment was arranged in split-plot in a Randomized Completely Block Design (RCBD) with four replications. The main plot was control (well-watered) and drought stress at different development stages (the vegetative phase (V5), before the reproductive phase (V12), and the grain filling phase (R3)). The sub-plot consisted of four maize varieties: TS1004, NS3, SW4452, and NK6248. Drought stress during the vegetative phase (V5) and before the reproductive phase (V12) was found to be a susceptible stage for maize because grain yield (GY) was decreased by the loss of crop growth rate (CGR) and total soluble sugar content (TSC) and it accumulated proline content. The NK6248 variety was found to be the most suitable for maize production because it had the highest grain yield (GY) and crop growth rate (CGR). In addition, it had low proline content (PC) under drought stress. In summary, under drought stress, it is advisable to select the NK6248 variety for crop production and avoid drought stress in the vegetative phase (V5) and before the reproductive phase (V12) because a mechanism by which maize could maintain its production of this study was the accumulation of total soluble sugar content to decrease proline content under drought stress condition.

Keywords: *Development stages; drought stress; grain yield; maize; physiological responses*

1. Introduction

Thailand's demand for maize in 2022 increased to 8.11 million tons, while the domestic supply of maize was insufficient. Thailand had a total yield of maize of about 4.85 million tons and a maize import of about 1.48 million tons. Maize yield was limited by cropping seasons and areas (Office of Agricultural Economics, 2019). The research on new sources of maize has received much attention currently. A new season of maize production was quite important. But the problem was that those new seasons were drought stress. Drought stress was one of the important problems in decreasing maize productivity. Maize production under drought stress resulted in decreased biomass yield, grain yield, leaf area, and root dry matter (Goswami et al., 2019; Laskari et al., 2022; Molla et al., 2014). Maize genotypes exhibited varying degrees of drought tolerance (Goswami et al., 2019). For instance, NK 40 varieties had higher dry matter content compared to Suwan 4452 varieties across all growth stages (Alam et al., 2014). However, several researches have shown that the timing of drought stress is critical for maize growth and yield (Song et al., 2019). Drought stress during the vegetative phase inhibited leaf area index and growth of maize production (Huang et al., 2023). Thus, drought stress at the vegetative phase (V10-V13) had a lower biomass yield than other phases (Aslam et al., 2015). The different growth stages of maize required varying amounts of water, with the mid-season growth stage having the highest crop coefficient (FAO, 2012). Furthermore, drought stress during the flowering and grain-filling periods resulted in reduced grain yield (Souza et al., 2016). The anthesis-silking interval had a high date from a different day of tassel and silking (Molla et al., 2014). These stages posed challenges in terms of maize's physiological responses and yield (Huang et al., 2023). Research on critical drought stress during the development stage of maize was important for maize production in Thailand to avoid factors of water stress in the crop season. Understanding the impact of these factors is crucial for predicting maize yields, which is the primary focus of this study.

In this study, a field site located in central Thailand was selected for this study. We controlled soil water through level irrigation and soil moisture content at a greenhouse. We evaluated the effects of drought stress at different development stages and maize varieties (*Zea mays* L.) on growth, physiological responses, and grain yield to maintain maize production under drought stress problems. In addition, it could improve water irrigation for maize in the dry season by reducing water use to save water for susceptible stages of maize.

2. Objectives

The objective of this study was to evaluate the effects of drought stress at different development stages and maize varieties (*Zea mays* L.) on growth, physiological responses, and grain yield to maintain maize production under drought stress problems.

3. Materials and methods

The field experiment was conducted at the greenhouse of Kasetsart University, Chatuchak, Bangkok, Thailand (13°51’006”N, 100°34’15.7”E, 2.5 m above sea level). Climatic conditions of the site were recorded at the Department of Agronomy, Faculty of Agriculture, Kasetsart University in April – July 2018. The high and low temperatures were collected every day by the thermometer in the greenhouse to calculate crop evapotranspiration for irrigation management during greenhouse experiments. (Figure 1) (Table 1) Crop evapotranspiration is a physical process in which water is lost from the soil and the plant to the atmosphere. Crop evapotranspiration (ETc) was calculated by the Blaney-Criddle method (FAO, 2012). (Table 1)

$$\mathrm{ETc} = \mathrm{Kc} \times \mathrm{ETo} \qquad (1)$$

where ETc is crop evapotranspiration, Kc is cropping coefficient, ETo is reference evapotranspiration.

$$\mathrm{ETo} = p\,(0.46\,\mathrm{Tmean} + 8) \qquad (2)$$

where p is the mean daily of annual daytime hours (%), and Tmean is the average temperature of daily day.

The soil type of the experiments was clay with a pH of 4.3; it contained the following: organic matter 2.25%, Total N 0.16%, P (Olsen) 18.98 mg kg^{-1}, and exchangeable K 184 mg kg^{-1}. The experimental design was a split-plot in Randomized Completely Block Design (RCBD) with four replications. The main plot was four drought stress different development stages (Control (well-watered), V5: Drought stress in the vegetative phase, V12: Drought stress before the reproductive phase, and R3: Drought stress in the grain filling phase) and sub-plot was four varieties (TS1004, NS3, SW4452, and NK6248). It began from April 2018 to July 2018.

Two seeds from each maize variety (which were later thinned down to one vigorous seedling seven days after planting) were sown in each of the pots filled with 25 kg of topsoil. The plot experiment was 2.0 X 1.2 m spacing, giving 12 plants per plot. The fertilizer of 15-15-15 (N-P-K, %) at the rate of 375 kg ha^{-1} was applied 5 days after sowing (DAS) 250 kg ha^{-1}, and 40 DAS 125 $\mathrm{kg.ha}^{-1}$. Nitrogen as urea fertilizer (46-0-0) at the rate of 281.25 kg ha^{-1} was applied 20 DAS 187.5 $\mathrm{kg.ha}^{-1}$ and 40 DAS 93.75 kg ha^{-1}. In the experiment, water management for the early establishment of the crop was continued up to drought stress treatments. Drought stress under the different development stages was imposed on plants by the designated 30 % soil moisture content: SMC at 7 days (V5: Drought stress in the vegetative phase (33-39 DAS), V12: Drought stress before the reproductive phase (58-64 DAS), and R3: Drought stress in the grain filling phase (83-89 DAS). After drought conditions followed by rewatering up to the physiological maturity stage (Table 1). Drought stress treatments (V5, V12, and R3) were three different growth stages that represented water stress (30%SMC at 7 days). Maize received reduced irrigation compared to normal irrigation (control) in different growth stages (Table 1). The data collection was conducted at 110 DAS, which collected growth analysis, physiological response, and yield.

3.1 Yield

For the sampling, four plants were selected by random from block then separated each plant part and dried. Four ears were sampled in paper bags and then taken to a hot air oven for drying at 65-70 °C 48 h. The drying was performed until the seeds reached approximately 14% moisture content. Data collection consisted of grain yield (g m^{-1}), and 100-seed weight (g).

3.2 Growth Parameters

Leaf area index (LAI) was determined using the ratio of the total leaf area (LA) to the ground area (GA) of a plant at a data collection time (Williams, 1946). Crop growth rate (CGR) was dry weight accumulation per ground area per unit time (g m^{-2} day^{-1}) (Watson, 1958).

$$LAI = LA/G \qquad (2)$$

$$CGR = 1/GA * dw/dt \qquad (3)$$

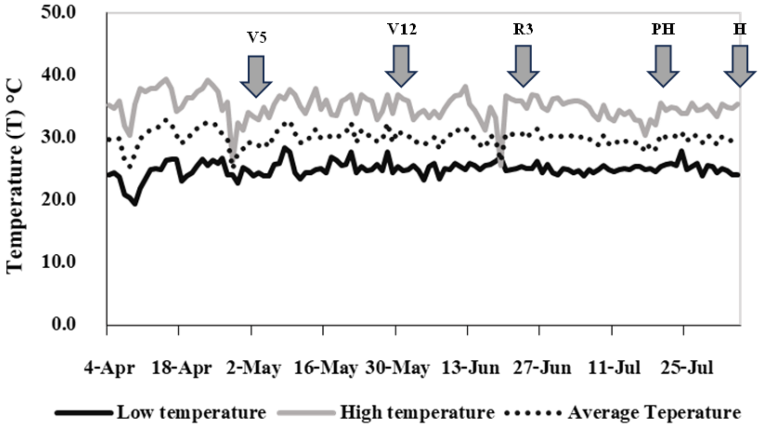

Figure 1 The temperature factors of the greenhouse experiment from April to July 2018. The temperature data were recorded with a weather station on site.

Notes: V5: Drought stress in the vegetative phase, V12: Drought stress before the reproductive phase, R3: Drought stress in the grain filling phase, PH: Data collection was conducted at 110 DAS, and H: Harvest was conducted at 125 DAS.

Table 1 The irrigation management during greenhouse experiments conducted from April to July 2018 during the growth season of maize.

Treatment	Growth stages				Total irrigation water (mm)
	Initial stage (20 days)	Crop development. Stage (35 days)	Mid-season stage (40 days)	Late season (30 days)	
ETc	49	177	291	123	640
Control	50	177	294	126	647
V5	50	137	294	126	607
V12	50	177	238	126	591
R3	50	177	234	126	587

Notes: ETc is crop evapotranspiration, Control is full irrigation (well-watered), and V5 is Drought stress in the vegetative phase (33-39 DAS). V12 is Drought stress before the reproductive phase (58-64 DAS), and R3 is Drought stress in the grain filling phase (83-89 DAS).

3.3 Physiological Parameters

Total soluble sugar content (TSC) was adapted from the Anthone method (Irigoyen et al., 1992) and proline content (PC) was adapted from the Bates method (Bates et al., 1973; Irigoyen et al., 1992). In the first step, 0.5 g of fresh leaves were homogenized in a mortar and 95% ethanol. The solution was filtrated and washed with 5 ml of 70% ethanol two times into a test tube. The alcoholic extract was kept in a refrigerator at 4 C° (Paquin, & Lechasseur, 1979).

Total soluble sugar content (TSC), 1 ml of alcoholic extract was mixed with 3 ml of 0.2% anthrone (150 mg anthrone, 100 ml of 72% sulphuric acid, W/W). The mixture was heated in a water bath at 100 °C for 10 minutes. After that, the reaction was broken into an ice solution for 5 minutes. Each sample was estimated at 620 nm using a spectrophotometer. The total soluble sugar was calculated by using the glucose standard and expressed in mg g^{-1} FW of leaves (Irigoyen et al., 1992).

Proline content (PC), 1 ml of alcoholic extract was mixed with 10 ml of distilled water and 5 ml of ninhydrin (0.125 g ninhydrin, 2 ml of 6 mM NH_3PO_4, 3 ml of glacial acetic acid), and 5 ml of glacial acetic acid. The mixture was boiled in a boiling water bath for 45 min at 100ºC. The reaction was stopped in cold water. Finally, the samples were mixed with 10 ml benzene. Each sample was estimated at 520 nm using a spectrophotometer. The proline concentration was determined using a standard curve. Free proline content was expressed as µmole g^{-1} FW of leaves (Bates et al., 1973; Irigoyen et al., 1992).

Statistical Analysis: Analysis of variance was carried out using the statistics software package version 8 and the mean comparisons were done by using a least significant difference (LSD) test at the 5% level.

4. Results

The analysis of variance found that drought stress at different development stages was significantly different in leaf area index (LAI), crop growth rate (CGR), 100-grain weight, gain yield (GY), total soluble sugar content (TSC), and proline content (PC), while varieties were significantly different in LAI, CGR, 100-grain weight, gain yield, and total soluble sugar content (TSC). Interactions were significantly different in LAI, CGR, 100-grain weight, gain yield, and proline content (PC) (Table 2).

4.1 Growth Parameters

The effect of drought stress at different development stages showed that LAI and CGR decreased significantly under drought stress in the vegetative phase (V5) (1.38 and CGR 1.42 g m^{-2} day^{-1}, respectively). However, drought stress in the grain-filling phase (R3) increased significantly LAI (1.84) and CGR (2.43 g m^{-2} day^{-1}) because drought stress in the grain-filling phase was watered after drought stress at 83-89 DAS and had a regrowth plant (Figure 2). Maize water requirement in important stage is reduced by leaf area for maize survival under drought stress. In addition, leaf size and the number of leaves were reduced by drought stress, which reduced photosynthesis, transpiration, light interception, and biomass yield (Aslam et al., 2015). Drought stress in the vegetative phase (V10-V13) was a big problem in leaf area and biomass yield (Alam et al., 2014). Water stress in the vegetative phase decreased LAI of maize (Huang et al., 2023; Song et al., 2019).

Table 2 Analysis of variance (ANOVA) of growth, physiological responses, and yield of maize.

Source	**df**	**LAI**	**CGR**	**100-GW**	**GY**	**TSC**	**PC**
			g m^{-2} day^{-1}	**g**	**g m^{-2}**	**mg g^{-1} FW**	**µmole g^{-1} FW**
A	3	72.22^*	12.67^*	5.90^*	19.72^*	29.77^*	7.43^*
Error(A)	9						
B	3	14.97^*	10.15^*	8.50^*	14.58^*	3.08^*	2.38
A*B	9	2.21^*	2.23^*	6.72^*	6.58^*	2.01	5.27^*
Error(B)	36						
Total	64						

Notes: A: Drought stress at different development stages, B: Varieties, A*B: Stages*Varieties, LAI: Leaf area index, CGR: Crop growth rate, 100-GW: 100-grain weight, GY: Grain yield, TSC: total soluble sugar content, and PC: Proline content.
*: significantly different at $p<0.05$

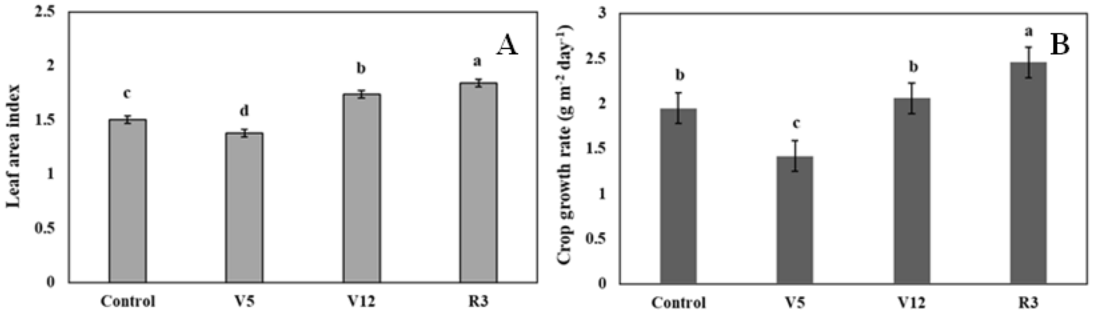

Figure 2 Effect of different drought stress regimes on LAI (A) and CGR (B) (error bars = ± SD and mean values with different superscript letters within each column denote significant ($p < 0.05$) differences between groups (LSD)).

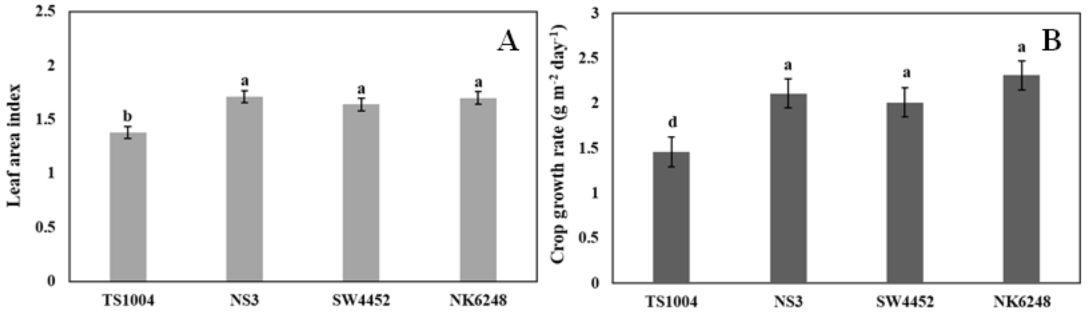

Figure 3 Effect of varieties on LAI (A) and CGR (B) under drought stress (error bars = ± SD and mean values with different superscript letters within each column denote significant ($p < 0.05$) differences between groups (LSD)).

Table 3 The interaction of drought stress at different development stages and varieties on LAI and CGR

Drought stages	**LAI**				**CGR**			
	Varieties							
	TS1004	**NS3**	**SW4452**	**NK6248**	**TS1004**	**NS3**	**SW4452**	**NK6248**
Control	1.29^{i}	$1.74^{\text{a-e}}$	$1.60^{\text{e-h}}$	1.40^{hi}	1.44^{hi}	$2.20^{\text{b-f}}$	$2.02^{\text{c-h}}$	$2.15^{\text{c-g}}$
V5	1.05^{j}	$1.40^{\text{g-i}}$	$1.43^{\text{g-i}}$	$1.68^{\text{c-f}}$	1.00^{i}	$1.54^{\text{f-i}}$	1.46^{hi}	$1.69^{\text{e-h}}$
V12	$1.50^{\text{f-i}}$	$1.88^{\text{a-c}}$	$1.71^{\text{b-f}}$	$1.85^{\text{a-c}}$	$1.83^{\text{d-h}}$	$2.33^{\text{b-e}}$	$1.51^{\text{g-i}}$	$2.55^{\text{a-c}}$
R3	$1.68^{\text{c-f}}$	$1.81^{\text{a-d}}$	1.93^{a}	1.92^{ab}	$1.57^{\text{f-i}}$	$2.37^{\text{b-c}}$	3.07^{a}	2.84^{ab}
Mean	1.61				1.97			
LSD 0.05	0.22^{*}				0.685^{*}			
%CV	10.03				23.16			

Notes: a, b, c, d, e and f compared with LSD ($P<0.05$)

The effect of varieties showed that LAI and CGR decreased significantly under TS1004 varieties (1.38 and 1.46 g m^{-2} day^{-1}, respectively) (Figure 3). Because the TS1004 variety maize had an early harvest day (95 days) (Nakhon Sawan Field Crops Research Center, 2017). LAI is an important parameter for maize selection under drought stress (Hajibabaee et al., 2012).

Interactions between drought stress at different development stages and varieties showed that LAI decreased significantly under interactions between drought stress in the vegetative phase (V5) and TS1004 and NS3 varieties. In addition, CGR decreased significantly under interactions between drought stress in the vegetative phase (V5) and TS1004 varieties. However, LAI and CGR had non-significant interactions between drought stress before the reproductive phase (V12) and the grain filling phase (R3) and varieties (Table 3).

4.2 Physiological Parameters

Drought stress in the vegetative phase (V5) and before the reproductive phase (V12) had lower total soluble sugar content (TSC) (39.340 and 32.937 mg g^{-1} FW, respectively) than the control (Figure 4A). On the other hand, drought stress at V5 had the highest proline content (PC) (18.148 µmole g^{-1} FW) (Figure 4B). Also, Drought stress at V16-VT accumulated total soluble sugar content and proline content of leaves (Huang et al., 2023). Moreover, Suwan 4452 variety had the highest TSC (51.212 mg g^{-1} FW) and had higher PC (16.644 µmole g^{-1} FW) than the NK6248 variety (Figure 5). Total soluble sugar was accumulated to help maintain normal cell osmotic pressure or osmotic adjustment (Huang et al., 2023; Li et al., 2017; Liu et al., 2015). At the same time, proline was accumulated to protect maize plant tissues from oxidative damage (Anjum et al., 2016). The high proline was recorded under 25% field capacity at pre-flowering. To maintain cell turgor, Maize accumulated organic and inorganic solutes in the cytosol (proline, sucrose, and soluble carbohydrates) under drought stress to control osmotic potential (Rou et al., 2020).

Interactions between drought stress at different development stages and varieties showed that PC increased significantly under interactions between drought stress in the vegetative phase (V5) and SW4452 varieties (Table 4). Proline was accumulated by maize under drought stress, for it stabilized membranes and maintained the conformation of proteins (Chandrasekar et al., 2004).

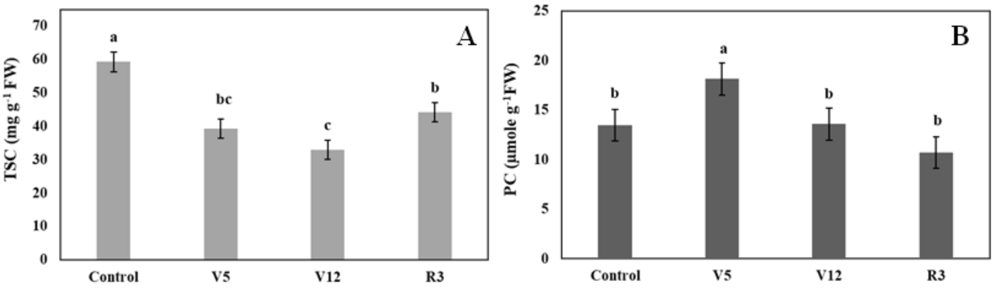

Figure 4 Effect of different drought stress regimes on total soluble sugar content (A) and Proline content (B) (error bars = ± SD and mean values with different superscript letters within each column denote significant ($p < 0.05$) differences between groups (LSD)).

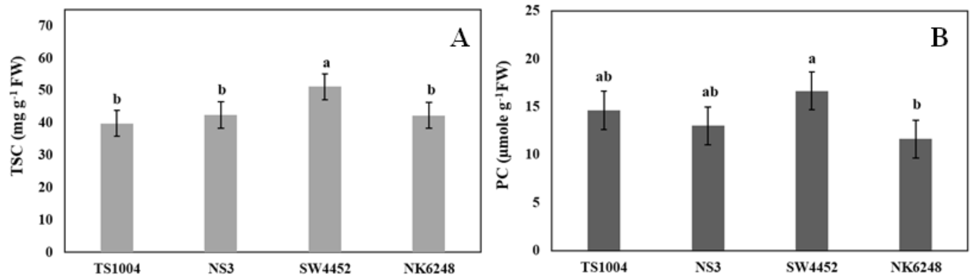

Figure 5 Effect of varieties on total soluble sugar content (A) and proline content (B) under drought stress (error bars = ± SD and mean values with different superscript letters within each column denote significant ($p < 0.05$) differences between groups (LSD)).

Table 4 The interaction of drought stress at different development stages and varieties on proline content

Drought stages	**Proline content (µmole g^{-1} FW)**			
	Varieties			
	TS1004	**NS3**	**SW4452**	**NK6248**
Control	14.283 ^{cd}	14.170 ^{cd}	14.392 $^{b\text{-}d}$	11.087 ^{cd}
V5	10.464 ^{cd}	12.520 ^{cd}	32.424 ^{a}	17.183 ^{bc}
V12	22.208 ^{b}	14.737 $^{b\text{-}d}$	9.114 ^{d}	8.284 ^{d}
R3	11.561 ^{cd}	10.658 ^{cd}	10.646 ^{cd}	9.114 ^{d}
Mean	13.980			
LSD 0.05	7.835*			
%CV	40.07			

Notes: a, b, c, d, e and f compared with LSD ($P<0.05$)

The control and drought stress in the grain filling phase (R3) significantly increased and had the highest 100-grain weight (22.37 and 22.33 g, respectively) and gain yield (222.03 and 170.04 g m^{-2}, respectively). And NK6248 variety significantly increased and had the highest 100-grain weight (23.14 g) and grain yield (187.10 g m^{-2}, respectively) (Table 5). Also, water stress in the vegetative phase and flowering phase had a high impact on the yield component of maize (Rou et al., 2020). And Huang et al. (2023) reported that the 100-grain weight of waxy corn was decreased by drought stress at V6-VT. and drought stress at VE-V8 was a dangerous drought phase for loss yield maize (Song et al., 2019). Interactions between drought stress at different development stages and varieties showed that 100-grain weight increased significantly under interactions between the control and drought stress in the grain filling phase (R3) and NK6248 varieties. In addition, grain yield increased significantly under interactions between drought stress in the grain filling phase (R3) and NK6248 varieties. However, the control and drought stress in the grain filling phase (R3) and all varieties had greater grain weight than interactions between drought stress in the vegetative phase (V5) and before the reproductive phase (V12) and all varieties (Table 6).

The correlation of crop growth rate (CGR), total soluble sugar content (TCS), and proline content (PC) significantly correlated to grain yield including with coefficients 0.345, 0.345, and -0.310, respectively. Moreover, correlation of Leaf area index (LAI) significantly correlated to CGR and PC with coefficients 0.743, and -0.276, respectively (Table 7).

Table 5 Effect of drought at development stages and varieties on 100-grain weight (100-GW), and grain yield (GY).

Treatment	100-GW (g)	GY ($g\ m^{-2}$)
Drought stress (A)		
Control	22.37 ^{a}	222.03^{a}
V5	20.69 ^{b}	78.18 ^{b}
V12	20.10 ^{b}	78.47 ^{b}
R3	22.33 ^{a}	170.04 ^{a}
Mean	21.375	142.18
LSD 0.05	1.52*	52.36*
%CV	8.91	41.08
Varieties (B)		
TS1004	21.10 ^{b}	129.06 ^{b}
NS3	20.13 ^{b}	114.73 ^{b}
SW4452	21.13 ^{b}	112.56 ^{b}
NK6248	23.14 ^{a}	187.10 ^{a}
Mean	21.375	142.18
LSD 0.05	1.24*	36.388*
%CV	8.10	35.69

Notes: Means followed by the same letter are not significantly different, at a significance level $p < 0.05$, according to the LSD criterion

Table 6 The interaction of drought stress at different development stages and varieties on 100-grain weight (100-GW), and grain yield (GY).

Drought stages	100-grain weight (g)				Grain yield ($g\ m^{-2}$)			
	Varieties							
	TS1004	NS3	SW4452	NK6248	TS1004	NS3	SW4452	NK6248
Control	21.985 ^{bc}	19.953 ^{cd}	21.122 $^{b-d}$	25.957 ^{a}	212.65 $^{b-d}$	154.30 $^{c-f}$	159.65 $^{c-e}$	225.52 ^{bc}
V5	20.780 $^{b-d}$	20.490 $^{b-d}$	22.678 ^{b}	18.810 ^{de}	97.97 $^{e-h}$	61.76 ^{h}	77.90 ^{gh}	75.11 ^{gh}
V12	19.785 $^{c-e}$	17.460 ^{e}	19.953 ^{cd}	21.152 $^{b-d}$	66.71 ^{gh}	74.33 ^{gh}	93.23 ^{h}	79.61 $^{f-h}$
R3	19.785 ^{cd}	22.157 ^{bc}	20.775 $^{b-d}$	26.620 ^{a}	138.89 $^{d-g}$	245.50 ^{b}	140.58 $^{d-g}$	371.15 ^{a}
Mean	21.375				142.18			
LSD 0.05	63..2*				78.346*			
%CV	8.10				35.69			

Notes: a, b, c, d, e and f compared with LSD ($P<0.05$)

Table 7 The correlation of growth, physiological response, and yield under drought stress

	100-GW	**GY**	**LAI**	**CGR**	**TSC**
GY	0.692*				
LAI	ns	ns			
CGR	ns	0.345*	0.743*		
TSC	ns	0.345*	ns	ns	
PC	ns	-0.310*	-0.276	ns	ns

Notes: LAI: Leaf area index, CGR: Crop growth rate, 100-GW: 100-grain weight, GY: Grain yield, TSC: total soluble sugar content, and PC: Proline content

*: Significant difference and ns: not significant difference in correlation of growth, physiological response, and yield, at significance level $p < 0.05$, according to the LSD criterion

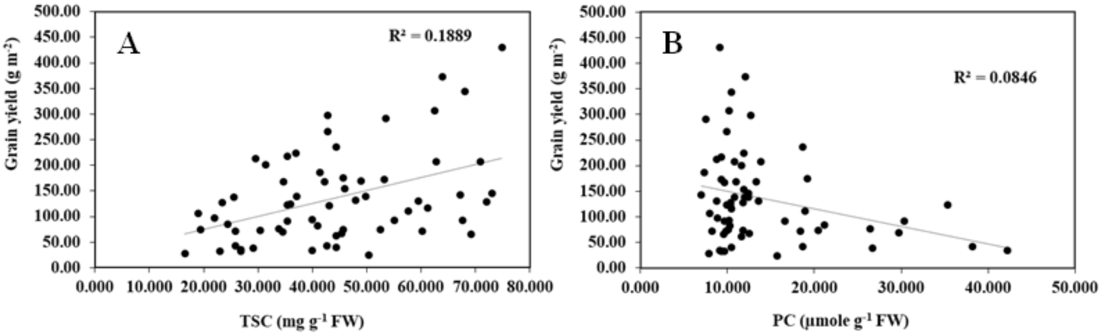

Figure 6 The correlation of total soluble sugar content (A) and Proline content (B) with grain yield (B)

Consequently, grain yield was increased by increased crop growth rate (CGR) and total soluble sugar content (TSC). However, grain yield (GY) was increased by increased proline content (PC) under drought stress (Figure 6).

5. Conclusion

Drought stress in the vegetative phase (V5) and before the reproductive phase (V12) was a susceptible stage of maize for drought stress because grain yield was decreased by the loss of Crop growth rate (CGR) and total soluble sugar content (TSC). And it had high proline content (PC). The variety of NK6248 was optimized for maize produce because it had the highest grain yield and CGR. In addition, it had low PC in drought stress. In summary, under drought stress, it is advisable to select the NK6248 variety for crop production and avoid drought stress in the vegetative phase (V5) and before the reproductive phase (V12). Because a mechanism by which maize could maintain yield production of this study was the accumulation of total soluble sugar content to decrease proline content under drought stress condition.

6. Acknowledgements

Faculty of Agriculture, Kasetsart University for experimental field and plant physiology and renewable energy laboratory Department of Agronomy and Faculty of Agriculture, Kasetsart University.

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