Anatomical Study on Leaves of Three Selected Varieties of *Zea mays* L. in Field Trial under Induced Water Stress

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Abstract

The anatomical character of three maize varieties: Sweet corn (V1), Sweet violet (V2) an Nga-cheik (V3) were investigated under water stress condition. In this study, two times of water stress (at 50% tasselling time and 50% earing time) and duration of water stress (for 10 days) were laid out in those three varieties using two treatments (water stress and control). The experiment was laid out in randomized complete block design with four replications. The following parameters were analyzed leaf area, thickness of lamina cuticle, and stomata index. Sweet corn (V1) showed that highest number of stomata index and thickness of lamina cuticle under water stress. It was followed by Sweet violet (V2) and Nga-cheik (V3). The maximum value of stoma pore size lower (length) at 50% tasselling time and at 50% earing time were found in Sweet violet (V2) under water stress. The higher value of stoma pore size upper and lower (length and width) was found in Nga-cheik (V3) at 50% earing time under water stress. Therefore, anatomical studied showed that Sweet corn (V1) was highly tolerant to water stress than Sweet violet (2) and Nga-cheik (V3).

Keywords: anatomical, varieties of *Zea mays* L., water stress

Introduction

Maize is one of the most important cultivated grain crops around the world and is widely used to provide food, forage, and industrial raw materials. Plant responses to drought depend on the physiological and anatomical properties of tissue components that regulate the transmission of stress effects to the tissues (Mostajeran and Rahimi-Eichi, 2008). In maize, the upper and lower surface of leave have stomata (paracytic type). Some cells of upper epidermis are larger in size. These are called bulliform cells or motor cells. Mesophyll is not differentiated in palisade and spongy parenchyma. These cells are spherical with only a few or no intercellular spaces.

Water stress is considered to be a moderate loss of water, which leads to stomatal closure and limitation of gas exchange. Plant tissues responses to water stress depend on the anatomical characteristics that regulate the transmission of the water stress effect to the cells (Matsuda and Rayan, 1990; Olmos *et al*., 2007).

Therefore, the effects of water stress on the anatomical characters of three selected varieties of *Zea mays* L. were investigated under water stress condition. In the study, two times of water stress inducing (at tasselling time and earing time) were laid out in those three maize varieties using two treatments (water stress and control). The objective of the present study was to measure the differences in morpho-anatomy of leaves, stems and roots of three maize varieties under drought and to evaluate drought tolerance of maize under water-stressed condition.

Materials and Methods

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An experiment was conducted on the three maize varieties in field at Vegetable and Research Development Centre (VFRDC), Yemon, Hlegu Township in Yangon Region. All fresh plant specimens were randomly collected from the field which treated with water stress and well-watered (control) plants for this study. For the water stress treatments, three maize varieties were induced water stress for 10 days at 50% of taselling time and 50% of earing time for about 10 days. The anatomical studies of the fresh specimens under drought condition were examined immediately after collection. In each treatment, the lamina at second leaf (upper) of main stem was used for the anatomical characters. In this study, the thickness of lamina, cuticle, upper epidermis, lower epidermis was recorded through this study. The stomata index, during water stress sere examined from both upper (adaxial) and lower (abaxial) surfaces of the lamina.

One microscopic field 0.0181 sq.mm (0.0181 cm^2) at 100X and 400X was used for the determination of stomatal index. The stomata were counted in three randomly chosen microscopic fields and thus a total of 9 microscopic fields were examined for determining the stomatal index in each treatment. The other parameters of lamina anatomy were also measured in the same method. Three glass sides were measured from three random fields of a slide and thus the total of 9 microscopic fields are studied.

The morphological characters were determined according to the following literatures: Esau (1965), Metcalfe and Chalk (1972) and Duke (1981). For the anatomical studies, transverse sections of leaves were rinsed in chloral-hydrate solution on the glass slides and observe the structure under the microscope. Chloral-hydrate solution was used for bleaching (Kokate, 2000).

Methods

Calculation of the field area

The microscopic field area was calculated as follows:

Eye piece	x	object		
10	x	40	=	4.8 big division
Diameter of the field			=	4.8 x 10
		=	48 small divisions (1 big div=10 small divs)	
45 small divisions	----------------?			
			=	0.48 mm in diameter
		r	=	d/2, (d = diameter)
		r	=	0.24
Field area		πr^2	=	$3.14 (0.24)^2$
		x	=	0.181 sq.mm, 0.0181 sq.cm (10 mm = 1cm)

According to (Santra *et al*, 1999) the following formula had been used.

i. Area of the field of division = x sq.cm

(Calculate it by the formula πr^2, where "r" is the radius of the field)

ii. Leaf area (LA) cm^2 = K x length (cm) x width (cm)
(K value for maize is (0.75) (Montgomery, 1911)

iii. Total leaf area = y sq.cm

iv. Stomata index (SI) = $\frac{No.of\ stomata\ in\ a\ given\ area\ (S)}{Total\ no.of\ epidermis\ cell\ (E) + S}$ x 100

= $\frac{S}{E+S}$ x 100

v. Stomatal density = $\frac{a}{x}$

Results

Effect of Water Stress on Leaves of Three Selected Varieties of *Zea mays* L.

Upper and lower surface of maize varieties

The anatomical characters of upper and lower surface of leaves were studied on three selected maize varieties (Sweet corn, Sweet violet and Nga-Cheik) during induced water stress for 10 days in 50% tasselling time and in 50% earing time.

(a) Total leaf area

In V1 (Sweet corn), the total leaf area of leaf of control were greater than that of water stress during tasselling time and earing time. In V2 (Sweet violet), the total leaf area of leaf of control were greater than that of water stress during taselling time and earing time. In V3 (Nga-Cheik), the total leaf area of leaf of control were greater than that of water stress during taselling time and earing time. When compared the three selected maize varieties, the maximum number of total leaf area were found in V1 (Sweet corn) at 10 days of 50% tasselling and 50% earing time. However, the minimum number of total leaf area was found in V3 (Nga-Cheik) at 10 days of 50% taselling and 50% earing time.

(b)Stomata density

In three varieties, number of stomata per microscopic field in water stress greater than control plants at 50% taselling and 50% earing time. In V1 (Sweet corn), the stomata density on upper and lower surface of leaf of water stress were greater than that of control during taselling time and earing time. In V2 (Sweet violet), the stomata density on upper surface of leaf of water stress were greater than that of control during taselling time and earing time. In V3 (Nga-Cheik), the total stomata density on upper surface of leaf of water stress were greater than that of control during taselling time and earing time. When compared the three selected maize varieties in upper surface, the highest stomata density of water stress were found in V1 (Sweet corn) at 10 days of 50% taselling and 50% earing time. However, the lowest stomata density of water stress was found in V3 (Nga-Cheik) at 10 days of 50% taselling and 50% earing time.

When compared the three selected maize varieties in lower surface, the highest stomata density were found in V1 (Sweet corn) at 10 days of 50% tasselling and 50% earing time. However, the lowest stomata density was found in V2 (Sweet-violet) at 10 days of 50% taselling and 50% earing time.

(c) Stomata Index

In three varieties, number of stomata index in water stress greater than control plants at 50% tasselling and 50% earing time. In upper surface leaf of water stress and control, the maximum number of stomata index was found in V1 (Sweet corn) at 50% tasselling and 50% earing time. The minimum number of stomata index was found in V3 (Nga cheik) at 50% tasselling and at 50% earing time.

In lower surface leaf of water stress and control, the maximum number of stomata index was found in V1 (Sweet corn) at 50% tasselling and 50% earing time. The minimum number of stomata index was found in V3 (Nga cheik) at 50% tasselling and at 50% earing time in water stress. In control, the minimum number of stomata index was found in V2 (Sweet corn) at 50% tasselling and at 50% earing time.

(d) Stoma pore size (µm) (Length and width)

In three varieties, stoma pore length and width size in control greater than water stress plants at 50% tasselling and 50% earing time. In upper surface leaf of water stress and control, the maximum number of stoma pore length was found in V1 (Sweet corn) at 50% tasselling and V3 (Nga-cheik) at 50% earing time. The minimum number of stoma pore length was found in V2 (Sweet corn) at 50% tasselling and V1 (Sweet corn) at 50% earing time.

In lower surface leaf of water stress and control, the maximum number of stoma pore length was found in V2 (Sweet violet) at 50% tasselling and V3 (Nga-cheik) at 50% earing time. The minimum number of stoma pore length in water stress was found in V1 (Sweet corn) at 50% tasselling and V2 (Sweet violet) at 50% eari time in control.

Transverse section of Lamina

(a) Thickness of cuticle

In three varieties, thickness of cuticle in water stress greater than control plants at 50% tasselling and 50% earing time. In water stress and control, the maximum number of thickness of cuticle was found in V1 (Sweet corn) at 50% tasselling and 50% earing time. The minimum number of thickness of cuticle was found in V3 (Nga cheik) at 50% tasselling and at 50% earing time.

Transverse section of Midrib

(a) Vascular bundle size and vascular bundle sheath size

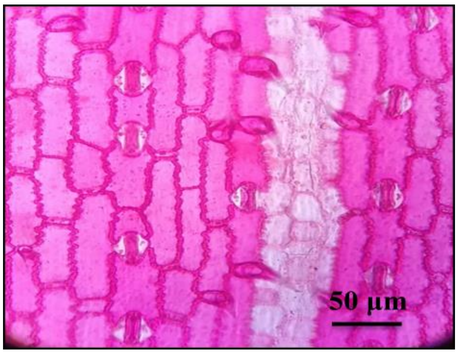
In three varieties, thickness of mesophyll layer in water stress greater than control plants at 50% taselling and 50% earing time. In water stress and control, the maximum number of vascular bundle size was found in V1 (Sweet corn) at 50% tasselling time and earing time. The minimum number of vascular bundle size were found in V2 (Sweet violet) at 50% tasselling time and at 50% earing time.

In water stress, the maximum number of vascular bundle size was found in V2 (Sweet violet) and the minimum number of vascular bundle size was found in V3 (Nga cheik) at 50% tasselling time and the maximum number of vascular bundle size was found in V1 (Sweet corn) and the minimum number of vascular bundle size was found in V3 (Nga cheik) at 50% taselling time in control. The maximum number of vascular bundle size was found in V1 (Sweet corn) and the minimum number of vascular bundle size was found in V3 (Nga cheik) at 50% earing time in water stress and control plants.

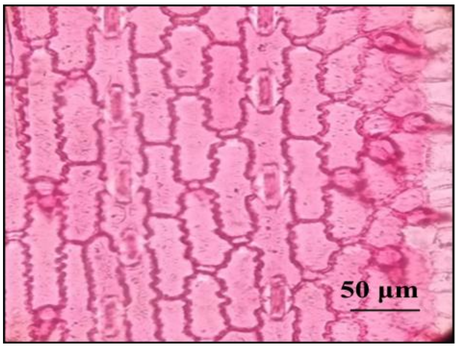
(b) Thickness metaxylem and protoxylem

In three varieties, thickness of metaxylem and protoxylem in water stress greater than control plants at 50% taselling and 50% earing time. In water stress and control, the maximum number of thickness metaxylem was found in V1 (Sweet corn) at 50% taselling time and earing time. The minimum number of thickness metaxylem were found in V3 (Nga-cheik) at 50% taselling time and at 50% earing time.

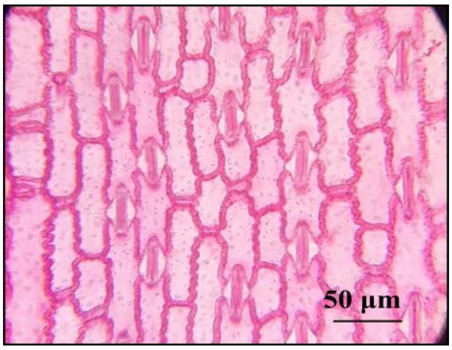
In water stress and control, the maximum value of thickness of protoxylem was found in V1 (Sweet corn) at 50% taselling time. The minimum value of thickness of protoxylem were found in V2 (Sweet violet) at 50% taselling time in water stress and V3 (Nga-cheik) in control. The maximum value of thickness of protoxylem was found in V1 (Sweet corn) and the minimum value was found in V2 (Sweet violet) at 50% earing time.



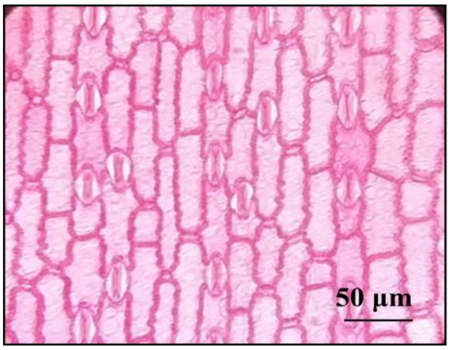
Upper surface of leaf of water stress V1 (10 days) TT



Upper surface of leaf of water stress V1 (10 days) ET

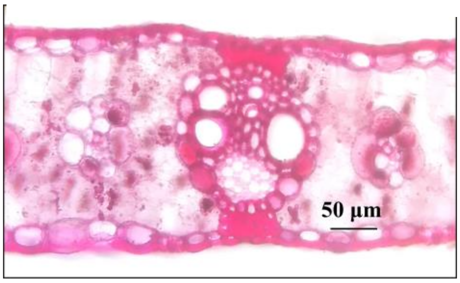


Lower surface of leaf of water stress V1 (10 days) TT

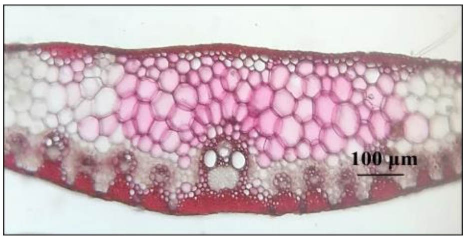


Lower surface of leaf of water stress V1 (10 days) ET

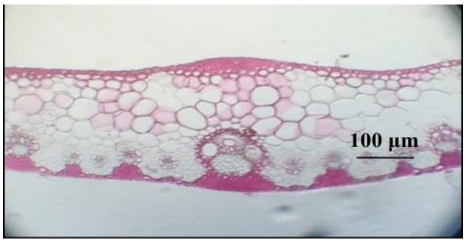
T.S of Lamina of water stress V1 (10 days) TT



T.S of Lamina of water stress V1 (10 days) ET

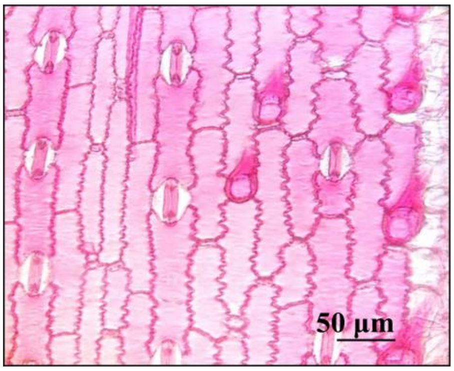


T.S of midrib of water stress V1 (10 days) TT

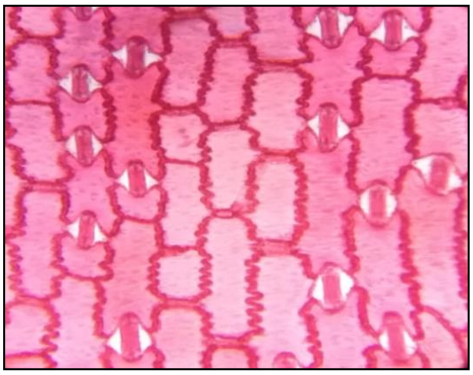


T.S of midrib of water stress V1 (10 days) ET

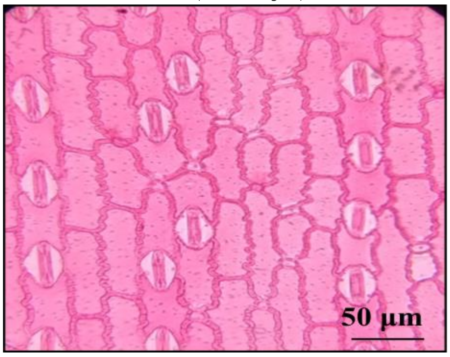
Figure 1 Water stress induced plant (50% tasselling time) and (50% earing time) of var. Sweet corn (V1)



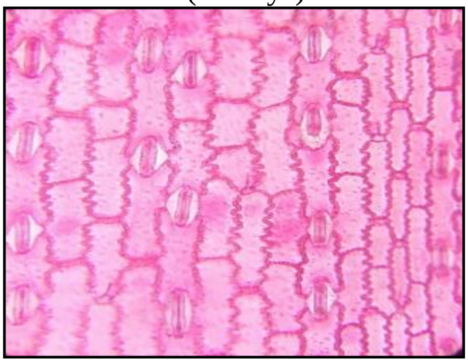
Upper surface of leaf of water stress V2 (10 days) TT



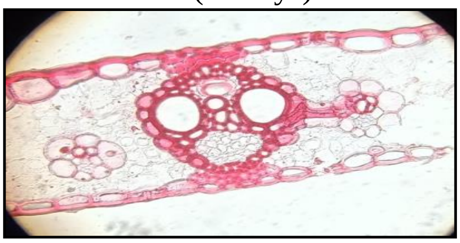
Upper surface of leaf of water stress V2 (10 days) ET



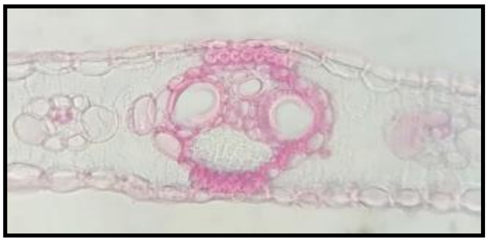
Lower surface of leaf of water stress V2 (10 days) TT



Lower surface of leaf of water stress V2 (10 days) ET

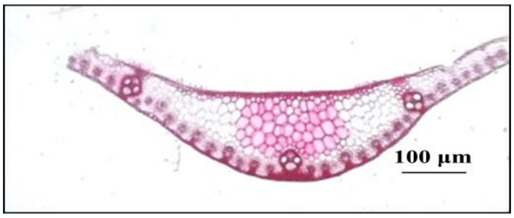


T.S of lamina of water stress V2 (10 days) TT



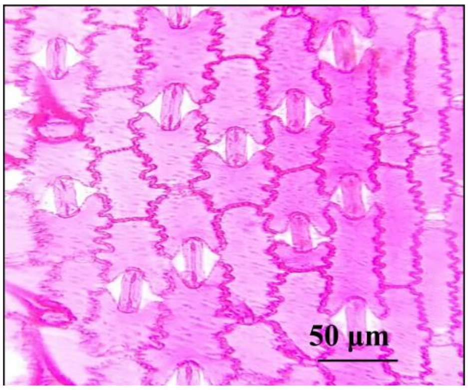
T.S of lamina of water stress V2 (10 days) ET

T.S of midrib of water stress V2 (10 days) ET

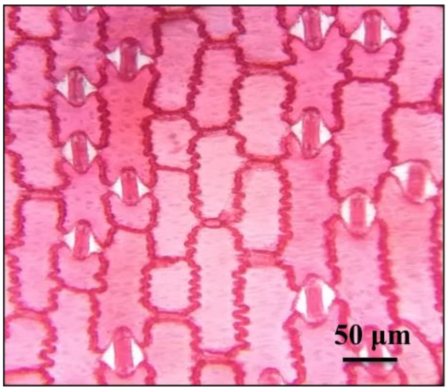


T.S of midrib of water stress V2 (10 days) ET

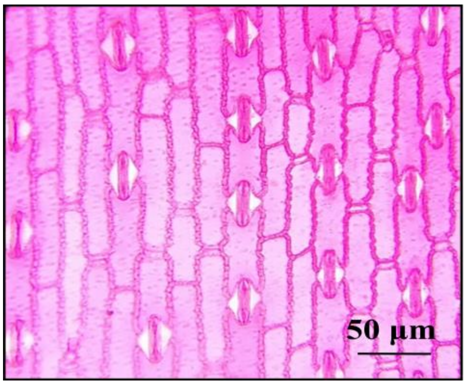
Figure 2 Water stress induced plant (50% tasselling time) (50% tasselling time) of var. Sweet violet (V2)



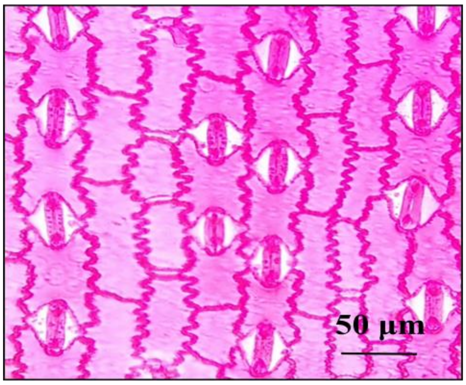
Upper surface of leaf of control V3 (10 days) TT



Upper surface of leaf of water stress V3 (10 days) ET

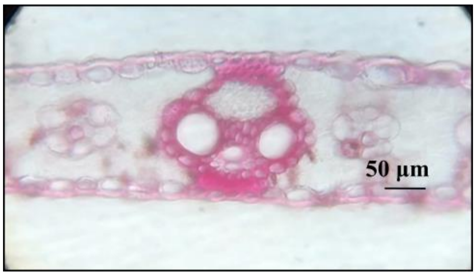


Lower surface of leaf of control V3 (10 days) TT

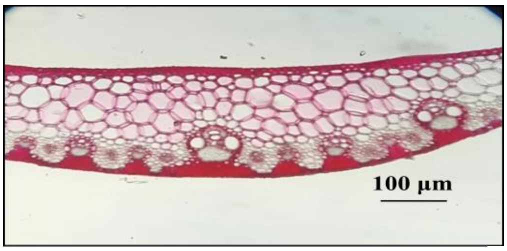


Lower surface of leaf of control V3 (10 days) ET

T.S of lamina of water stress V3 (10 days) TT



T.S of lamina of water stress V3 (10 days) ET



T.S of midrib of water stress V3 (10 days) ET

T.S of midrib of water stress V3 (10 days) TT

Figure 3 Water stress induced plant (50% tasselling time) and (50% tasselling time) of var. Sweet violet (V3)

Discussion and Conclusion

The study was carried out to investigate the anatomical characteristics and responses of drought-induced maize plants. Three maize varieties: Sweet corn (V1), Sweet violet (V2) and Nga-cheik (V3) were used to induce the drought stress (water stress) at their 50% tasselling and 50% earing time for short duration (10) days of drought inducing this study.

In terms of, the leaf area was less in water stress plants than control of Sweet corn (V1), Sweet violet (V2) and Nga-cheik (V3). Verelst *et al.* (2013), who reported the leaf size decreased under drought stress. Drought reduces leaf area by slowing leaf expansion and causes decreased cell development and diminished cell division which in long run influences the leaf size by lessening mature cell size.

In term of stomata density, the maximum distribution on both surfaces of leaf at 50% tasselling and 50% earing time of water stress plant noted than in control plants. The highest number of stomata density was found in Sweet corn (V1) and Sweet violet (V2) at 50% tasselling and 50% earing time under water stress plant. The lowest number of stomata density distribution of both surface of leaf was found in Nga-cheik (V3). This finding was agreement with Nayeem *et al*. (1989) who stated that drought resistance wheat had greater stomatal density than irrigated conditions.

In this study, the stomata index was more in water stress plants than control plants on both surface of leaf in Sweet corn (V1) and Sweet violet (V2). This finding agreed with Quarrie and Jones, (1977) has indicated that stomata index increase with water stress plants. However, stomata index was lesser in water stress plants of both surfaces of leaf in Nga-cheik (V3) at 50% tasselling and 50% earing time.

In the upper surface of stoma pore size in water stress plants was shorter and narrower than control plants of Sweet corn (V1). In the lower surface of stoma pore size in water stress plants was shorter and narrower than well-watered plants at 50% tasselling time was found in Sweet violet (V2) and 50% earing time of var. Nga-cheik (V3). Zhang *et al*. (2006) reported that water deficit leads to decrease in stoma pore size.

Cuticle of lamina was significantly thicker in the water stress plants than in the control at 50% tasselling and 50% earing time of three varieties. Schohers *et al*. (1979) reported that cuticles were much thicker on leaves developed in the sun and xerophytes. The thickest cuticle was found in water stress plants in Sweet corn (V1) followed by Sweet violet (V2) and Nga-cheik (V3).

It can be concluded that drought stress are quite similar in their response to some anatomical parameters. It is linked to changes in leaf anatomy and ultrastructure. Shrinkage in the size of leaves, decrease in the number of stomata; thickening of lead cell walls, cutinization of leaf surface and under development of the conductive system increase in the number of large vessels, submersion of stomata in succulent plants and in xerophytes and induction of early senescence are the other reported morphological changes. But it differ also in some other anatomical parameters aspects in three maize varieties. From the results, the varieties Sweet corn (V1) is more drought resistant than Sweet violet (V2) and Nga-cheik (V3). The results suggested that V1 (Sweet corn) was the most drought tolerant varieties followed by V2 (Sweet violet) and V3 (Nga-cheik). The V1 (Sweet corn) maize variety is recommended to use in developing water tolerance in maize breeding programmes based on their higher performances.

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