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# **RESEARCH ARTICLE**

# **Assessment of drought tolerance in six maize hybrids (***Zea mays* **L.) using biochemical indices**

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# **Edited by: ABSTRACT**

Maize (*Zea mays* L.) variety has diverse genetic characters; hence this study was led to assess drought tolerance of maize variety subjected to well water and water stress environments. Drought is one of major factor that results thoughtful influence plant development as well as productivity. The outcome becomes severe where rain fed agriculture is practiced. Evaluation of variety that can better tolerate such condition can help for selection and are important in reducing associated crop loss. The study was performed in greenhouse using CRD with three replications. Six maize varieties MH-140, Melkassa-2 (MH-2), Melkassa-3 (MH-3), Melkassa-4 (MH-4), Melkassa-6Q and MHQ138 developed by Melkassa Agricultural Research Center (MARC) were used and grown in normal and water scarce condition. The collected data were leaf proline, chlorophyll content, soluble sugar content, relative water content, leaf nitrogen content, protein content specific leaf area, specific leaf weight. Data were analyzed by ANOVA using SAS 9.4 software. Mean comparisons were implemented using LSD test at P < 0.01. The study outcomes revealed that high SLA and SLW value was recorded for, Melkassa-4 (MH-4) whereas, MHQ138 is recorded lower value. Leaf proline, chlorophyll content, soluble sugar content, relative water content, leaf nitrogen (N) content and protein content, value had revealed Melkassa-4 the most drought tolerant variety whereas MHQ138 is the least drought tolerant variety among the maize varieties used in this research study.

*Keywords:* biochemical parameters; drought tolerance; maize varieties; growth parameters

#### **INTRODUCTION**

Maize (*Zea mays* L.) is the most significant food crop in the world, having originated in America. It is also one of the most extensively dispersed crops on the planet (Leszek and Vincent, 2012). Maize is used in a variety of industries as a human meal, animal feed, biofuel, and feedstock material. It is a versatile crop that thrives in a variety of conditions. "It grows from 58°N to 40°S, from sea level to 3000 m above sea level, and in places with 250 mm to more than 5000 mm of annual rainfall" (Prasad, 2014). After wheat and rice, maize is the most important crop in the world economy, as it can be utilised as a feed, food, commerce, and industrial grain crop (Ongeti, 2012).

Maize is Ethiopia's most important and strategic cereal crops that have vital role in the country's food security. It is grown-up in 13 agro-ecological zones on 2 million ha (16.08%). At present, 39% of the total maize area in Ethiopia is now sowed with betterquality varieties (Eyob Bezabeh, 2015). Millions of people in Ethiopia depend on maize for their daily food especially in the area where maize is the major crop. Environmental abiotic strains severely impair plant progression and production worldwide. Water insufficiency is one of abiotic strains that strictly distress and lessen the yield and production of crops up to 70% worldwide but, plants responses to drought strain involves changes in their metabolism, morphology and structure (Lum et al., 2014). Drought is the problem of dry land regions in African countries like Ethiopia and leads to food insecurity, malnutrition and poverty.

One of the most important requirements for plants' survival, growth, and development is water. Due to a lack of water in the soil reserve, plant cells lose their turgidity and their stomata close.  $CO<sub>2</sub>$ absorption and nutrition uptake are hampered, and chemical energy production is reduced. Various biotic and abiotic stressors are reducing food productivity (Ahmadizadeh et al., 2011). Drought is one of the main reasons for Ethiopia's reduced maize production and food insecurity (Shiferaw et al., 2011). As a result, avoiding these losses is a critical concern for ensuring food security in a changing climate. The goal of this study was to determine the reasons for the low adoption of those varieties by evaluating their drought tolerance under controlled watering management and identifying key drought tolerance indices that could be used in future variety development programmes targeting Ethiopia's drought-prone areas.

#### **MATERIALS AND METHODS**

#### *Description of growth conditions*

From March to August 2018, the research was carried out in a greenhouse at the National Agricultural Biotechnology Research Center in Holeta. Holeta is in the Oromia special zone, which encompasses Addis Ababa. It is located at 9°3′N 38°3′E and has an elevation of 2391m above sea level. All of the types were cultivated in a controlled environment in a greenhouse. A soil was made by combining dirt (Vertisol) with manure in a 3:1 ratio and then filling a pot with it to grow the maize variety.

## *Experimental design and treatments*

The study was conducted utilising a three-replication Completely Randomized Design (CRD). MH-140, Melkassa-2, Melkassa-3, Melkassa-4, MHQ138, and Melkassa-6Q were the maize types employed in the study (hybrid varieties from Melkassa agricultural research center). Until drought stress and treatments were applied, each type was grown with the same water levels. On March 28, 2018, two seeds were put in each pot, and only one healthy seedling per pot was allowed to develop to eliminate competition. Two litters of water were used to irrigate the soil until it was completely saturated.

# *Water level determination*

In each pot 7 kg of soil was filled. Three liters of water were irrigated gently and consistently on upper of the soil in each of pot until the soil was entirely flooded. The water dripping was collected on tray after 48 hours. The collected water was measured with cylinder after 48 hours. The average of three repetitive times was taken as water volume for optimal plant growth. Through this method it was established that two litters of water were given to each plant/pot to keep the soil moisture. Plants were irrigated with this amount of water weekly (when maize needs water) from planting to physiological maturity unless otherwise watering was discontinued to execute drought stress (Eyerusalem Arusi, 2015). Well-watered (WW) = $100\%$  = FC (field capacity). = 2L Water stressed (WS) = watering was stopped for three weeks after flowering.

# **Data collection** *Specific leaf area (SLA)*

Data for SLA was taken at post-flowering stage when full growth (after 120 days after planting) was attained. Two completely expanded leaves were

selected arbitrarily from the middle part of a single plot and a total of 6 leaves were taken from the 3 replications. LA (Leaf area) was determined by CI-202 Leaf area meter as cited in Zhao *et al*. (2016). Then, dry weight estimation was done by digital sensitive balance after leaves were dried for 24 hours at 70°C. Finally, SLA was estimated as leaf area (cm2)/Leaf dry weight (g).

# *Specific leaf weight (SLW)*

Data SLW was taken at post-flowering stage when maximum development (after 120 days after planting) was attained. Two totally expanded leaves were selected randomly from the middle part of a single plot and a total of 6 leaves were taken from the 3 replicas. LA (leaf area) was determined by CI-202 area meter. Then, dry weight determination was done by sensitive balance after leaf was dried for 24 hours at 700C. Finally, SLW was estimated as Leaf dry weight  $(g)/$ Leaf area (cm<sup>2</sup>) as cited in (Eyerusalem Arusi, 2015).

# *Biochemical analysis*

Chlorophyll content was estimated from leaf collected at extreme temperature and at low water. SPAD-502 meter was used to assessment of Chlorophyll content. The estimation was taken on the flag leaf. Three flag leaf quantities were taken per pot (five estimations per plant). "The results were then averaged to give in a single value to represent the plot as cited in" (Van den Berg and Parkins, 2004). RWC % was calculated according to the methods mentioned Bedada et al. (2016). Study of proline content in the leaves was measured following method of Bates *et al*. (1973). Leaf N% was estimated using Kjeldahl technique used by Pawar (2007). Protein percentage calculated by following formulae, Protein  $(\%) = \%$  Nitrogen x conversion factor (6.25). The soluble sugar was assessed by the method Dey (1990).

# *Data Analysis*

Data was analyzed using analysis of variance with SAS 9.4 software. Mean comparisons was accomplished using LSD test at P < 0.01 and correlation analysis was performed following the method of Pawar (2007).

# **RESULTS AND DISCUSSION**

# *Specific Leaf Area and Specific Leaf Weight*

The mean comparison result showed that the water stress had the lowest levels of SLA and well-watered had the highest amount of SLA (Table 1). SLA obtained from Melkassa-4 was significantly different under water stress condition. However, Melkassa-2, MHQ138 and Melkassa-3 showed non-significant difference in their SLA under water stress conditions. Under water stressed condition, MH140 and MHQ138 was recorded the highest SLA. Significantly Melkassa-4 was recorded the lower SLA when compared with other variety (Table 1). The mean result showed that the water stress had the lowest levels of SLW and well-watered treatment had the highest amount of SLW (Table 1). SLW obtained from Melkassa-4 was significant under water stress condition. Under water stressed condition, MHQ138 had recorded the lower SLW. Significantly Melkassa-4 had showed higher SLW when compared with other variety (Table 1).

Variety	Mean SLA		Mean SLW	
	WS	WW	WS	WW
MH140	45.1 <sub>ba</sub>	47.1aa	$0.022$ bbc	$0.048$ abc
Melkassa-2	38.2 <sub>bc</sub>	4 <sub>Rac</sub>	0.026 <sup>ba</sup>	$0.060$ aa
Melkassa-3	38.6ba	$45.1$ aa	0.021 <sub>bc</sub>	$0.035$ ac
Melkassa-4	33.6bba	$48.7$ aba	$0.03$ bbc	$0.045$ abc
Melkassa-6Q	44.8ba	49.9aa	$0.022$ bc	$0.040$ <sup>ac</sup>

**Table 1.** SLA and SLW values of maize variety under well-watered and water stressed.

Means assigned with the same later have no significant difference at  $P < 0.01$ . WW= well water, WS= water stress, CV= coefficient of variation, SLA = Specific leaf area and SLW= specific leaf weight, LSD= List Significance Difference.

MHQ138 48.9**bbc** 44.4**abc** 0.02**bba** 0.038**aba**  $CV\%$  22.38 6.39 15.9 19.3

LSD 7.91 0.006

SLA is an important growth parameter in response to favorable and unfavorable condition (Campos et al., 2004), associated with physiological activity (photo assimilation) (Pawar, 2007). SLA indirectly tells the leaf thickness. The lower SLA value shows a reduction of growth in comeback to water shortage is one of the tolerance mechanisms (Anjum et al., 2011). Plants with high SLA have advanced metabolic activity than those with lower SLA. Higher leaf area may cause more water losses as a result of more evapotranspiration from the leaf surface. Similar to our finding, Painawadee et al. (2009) revealed that plant with lower SLA value indicates the higher drought resistant genotype and the plant with higher SLA value indicates the less drought tolerant genotype.

SLW varies considerably in maize variety (Table 1). The decrease of SLW in water scarcity is linked with decreased assimilate formation in leaves and leaf growth rate (Mehmood-Ul-Hassan et al., 2013). A survey of literature of Misra (1995) revealed that SLW and leaf dry matter are the key parameters used in selection of drought tolerance crops. Maize variety Melkassa-4 was recorded the lower SLA and higher SLW than other variety used in the present study so this variety is more drought tolerant than other variety, while MH140 and MHQ138 maize variety was recorded the higher SLA and lower SLW than other varieties and are more drought susceptible than others. Similar to this finding, Ali et al. (2009) reported that higher specific leaf weight helps for the selection of the genotypes with higher grain yields under water stressed condition. The SLW changes observed in all variety in response to drought stress indicated that the MHQ138 variety is the least drought tolerant variety while, variety Melkassa-4 is more drought tolerant than the variety used in the present study.

# *Relative Water Content (%)*

RWC% is the percentage of the fresh weight(FW), dry weight (DW) and the turgid weight (TW) of the stressed plant. The RWC of the maize variety differs significantly (Figure 1). Mean comparison result showed that RWC of water stress treatment was lowest, while well-watered treatment was recorded with highest value (figure 1). Similar to this finding, Decov et al. (2000) Suggested that RWC of leaf maize declines significantly when leaf is open to drought. And also, Adejare and Umebese (2007) reported the relative water content values of water stress (experimental) treatments were significantly reduced under at flowering stages when compared with that of controlled treatment.

The RWC values of all the varieties were differed under control conditions and experimental condition (figure 1). Under water stress condition the variety Melkassa-4 was recorded the maximum relative water content followed by Melkassa-3. The lowest RWC was recorded in MHQ138 followed by Melkassa-2. It is evident from the present study that the RWC of the six maize varieties decreased significantly under drought stress. The reason behind is Water deficit has exerted a negative effect on RWC.

The possible reason for decreasing RWC in maize variety leaves could be the limitation of soluble sugar content supply caused by water stress (Lawlor and Cornic, 2002). Preservation of higher RWC by the varieties play a role in its tolerance under declining soil moistures mentioned in Shivalli (2000).

RWC is an important indicator of cell hydration and a major indicator of physical strength and growth. RWC was recommended by Balota (1995) as a critical criterion in breeding platforms for selecting drought tolerance in crop plants. According to Colom and Vazzana (2003), drought resistance is indicated by the maintenance of a high RWC during small drought. Melkassa-4 had the greatest RWC, followed by Melkassa-3, for water stress in the current study, whereas MHQ138 had the lowest RWC (figure 1). Shaw et al. (2002) and Ramos et al. (2003) found that the RWC of leaf bean under experimental conditions was lower than the RWC at optimum water conditions, and that a higher RWC is a crucial indicator of water stress tolerance. The relative water content of experimental conditions fell in all varieties in this study; however, this reduction was greater in variety (MHQ138), indicating that this maize variety is drought tolerant, and (Melkassa-4) had a higher RWC. Hassanzadeh et al. (2009) found that sesame grown varieties with high relative water contents are more resistant to water stress than those with lower relative water contents.

# *Chlorophyll Content using SPAD Reading meter*

The data for SPAD readings meter of chlorophyll content of all variety indicated in (Table 2). The mean comparison results showed the water stress had recorded lower chlorophyll content when compared with the controlled treatment which had the highest amount of chlorophyll content. The reason for the decrease of chlorophyll content was that water stress can destroy the chlorophyll synthesis (Lessani and Mojtahedi, 2002). The variety Melkassa-4 was recorded with the maximum SPAD reading and followed by Melkassa-3 While, the variety MHQ138

was recorded with the minimum SPAD value under water stress condition. Similar to this finding, Hassanzadeh et al. (2009) reported that sesame chlorophyll content was higher in control treatment than experimental treatment.



**Figure 1.** Relative water content of maize variety under control and experimental condition. Means assigned with the same later have no significant difference at P < 0.01. Bars represent mean. Where, RWC= Relative water content LSD= List Significance Difference.

**Table 2.** Chlorophyll content reading meter of six maize varieties in well-watered and water stressed condition.

Variety	Chlorophyll content		
	WS	WW	
MH140	50.6bb	82.0ab	
Melkassa-2	48.2bc	82.8abc	
Melkassa-3	60.9bba	83.1aa	
Melkassa-4	65.1ba	84.4aa	
Melkassa-6Q	54 1 bbc	82.0ab	
MHQ138	44.6 <sup>bc</sup>	81.5ac	
$CV\%$	7.39	3.78	
LSD	9.5	5.5	

Means assigned with the same later have no significant difference at  $P < 0.01$ . WW-Well water: WS-Water stress; LSD-List Significance Difference.

According to Hassanzadeh et al. (2009) the reason for lower chlorophyll content under water scarcity is that, water stress in plant cell creates reactive oxygen species that lead to chlorophyll damage. Homayoun et al.(2011) stated that drought resistant cultivars show the maximum chlorophyll content under the water scarcity. Abdellah et al. (2011) recorded the lower chlorophyll content in drought vulnerable wheat variety under water stress of 30 percent field

capacity. In this study, the maize variety Melkassa-4 with higher chlorophyll content is the most stress tolerant, while MHQ138 variety with lower chlorophyll content is stress least tolerant than others under water stressed treatment. Comparable to this study, Ghaffari et al. (2012) specified that the tolerant sunflower line had higher chlorophyll than the susceptible line under water stress condition. The higher chlorophyll content shows that varieties had more efficient mechanisms to protect photosynthesis apparatus than the lower chlorophyll content under drought stress.

# *Nitrogen Content*

The mean results showed that the experimental management had the lowest levels of Nitrogen content, while controlled treatment had the highest amount of nitrogen content. The leave Nitrogen content of maize variety recorded in (Table 3) showed that all maize variety differed significantly. Similar to this study, DaMatta et al. (2002) described that nitrogen deficiency occurs when a plant aspects water deficit. Also, Mahieu et al. (2009) informed that there was reduction of nitrogen content under water stress condition in all plant parts. Maximum nitrogen content was observed in Melkassa-4 and lowest was recorded in MHQ138 followed by Melkassa-2. In this study, nitrogen content declined under water scarcity

condition in all variety, but this reduction was higher in least drought tolerant variety. Plants fully flooded plots had higher leaf N concentration than plants from un-watered control (Lu and Han 2010). In this study the maize variety Melkassa-4 with higher nitrogen content which is the most stress tolerant variety, while MHQ138 variety with lower nitrogen content is less stress tolerant than other variety under water stressed treatment. Similar to this study, Lu and Han (2010) revealed that drought tolerant plant has greater nitrogen content than that of drought susceptible plant. De Souza et al. (1997) studied that extreme drought stresses improve leaf deterioration by reducing leaf nitrogen (N) and chlorophyll contents.

**Table 3.** Leaf Nitrogen content of six maize varieties

Variety	Nitrogen content		
	WS	WW	
MH140	2 4bbac	5.5abac	
Melkassa-2	$2.3$ bdc	$5$ 3adc	
Melkassa-3	$2.4$ bbdc	5 <sub>3</sub> abdc	
Melkassa-4	2.5 <sub>bba</sub>	$5$ $4$ aba	
Melkassa-60	2.4 <sub>ba</sub>	5.6 <sup>aa</sup>	
<b>MHQ138</b>	2.2 <sub>bd</sub>	54ad	
$CV\%$	6.43	2.9	
LSD	0.077	0.13	

Means assigned with the same later have no significant difference at P < 0.01. WW-well water, WS-water stress LSD-List Significance Difference

#### *Soluble proteins*

The lower soluble protein content was showed in water stress usage than well-watered treatment which had the maximum amount of soluble protein as mean comparisons results (Table 4). The present study results are consistent with those of Mohammadkhani and Heidari (2008) described that soluble protein content of maize varieties decrease under water stress condition. This is because of that water stress made fluctuations in protein production in maize. Soluble sugar content decrease under water stress condition was due to the reactive oxygen species production as mentioned in Ghaffari et al. (2012).

The soluble protein was maximum in variety Melkassa-4 followed by Melkassa-3 and the least soluble protein content was noted in MHQ138 in water stressed condition. Ghanbari et al. (2007) reported that drought tolerant variety of alfalfa soluble proteins content was higher than in susceptible genotype under water stress which was decreased.

Salekdeh et al. (2002) revealed the significant difference between leaves of fully watered compared to the leaves of cultivars with non-fully watered (control) that showed lower leaf protein contents. The present results are similar with the results of Salekdeh et al. (2002) in which the six varieties showed the variation. Surendar et al. (2013) observed that the tolerant cultivars practiced lesser decrease of soluble protein than the susceptible ones. Our finding is related to finding of Surendar et al. (2013). The results of maize variety Melkassa-4 with higher soluble protein content are the most stress tolerant, while MHQ138 variety with lower soluble protein content is less tolerant than other varieties. In this study, drought reduced the soluble protein content in all varieties, but this decrease was larger in less tolerant variety.





Means assigned with the same later have no significant difference at P < 0.01. WW= well water WS= water stress.

Serraj and Sinclair (2002) revealed that a better production and accumulation of compatible solutes such as soluble protein are the most patent to osmotically regulate them was the key among the responses of plants to abiotic stresses. The maximum proteins content in leaves were recorded at control and decreased with the increased in water stress period in all the variety. Protein synthesizing mechanism will damage by water stress. Increased activity of protease and proteolysis may be was the possible reason for declined protein content under water stress (seems amino acid like proline are less incorporated for protein synthesis during water stress condition).

#### *Soluble Sugar Content*

Under drought stress Soluble sugar content is an important biological indicator of a plants ability to continue life in water scarce conditions. The results of mean showed that the water stress treatment had the

highest levels of soluble sugar and well-watered treatment had the lowest content of soluble sugar (Figure 2). Melkassa-4 recorded with higher soluble sugars in as compared to all other variety used in the present study. However, the variety MH140 and MHQ138 recorded lower sugar content over all other variety used in the present study. This difference in comeback to water strain is also likely to be due to the genetic differences among the variety. This shows that each variety has different ability to synthesis soluble sugar with an increase in drought stress treatment. As the water level decreases soluble sugar content increase (Mohammadkhani and Heidari, 2008). These results indicate that the soluble sugar in the leaves was significantly ( $P < 0.01$ ) increased due to the increase in the level of drought stress.

Soluble sugar is key one among important functional indicators of plants capability to survive under drought stress. The rise of simple sugars (glucose and fructose) could be supplementary with a proliferation in the leaf cell wall plasticity and membrane turgidity (Trouverie et al., 2003).

The soluble sugars (accountable for protecting the cells during drought) increased in the leaves to other organs in plant under water stress (Anjorin et al., 2016). The data of soluble sugars indicated significant differences among the varieties (Table 8).



**Figure 2.** Soluble sugar content of maize variety under both treatments condition (well-watered and water stress). Means assigned with the same later have no significant difference at P < 0.01. Bars represent mean. Where, SS= soluble sugar.

# *Proline content*

The proline content results revealed significant differences among the variety (figure 3). These results showed that the leaves proline content was significantly increased due to the rise in the content of drought pressure. The differences in the responses to drought stress among the six selected maize variety showed that each variety has different ability to synthesis proline with an increase in drought strain treatment. Aminatun et al. (2013) reported that drought-susceptible had lower proline contents than drought-tolerant maize cultivars ones. According to this study, in Melkassa-4 higher proline content was recorded over all other variety and is more drought tolerant than other variety. The minimum proline accumulation was recorded in MHQ138 and it is less drought tolerant than other variety. This difference in response is also likely to be due to the genetic differences among the variety. Also, Sharada and Naik (2011) reported that the cultivars that had higher concentrations of proline were drought-tolerant.

Proline content increase under water stress condition could be used as a marker of drought tolerance (Naser et al., 2010). According to this statement maize variety Melkasa-4 is more drought tolerant than other variety and MHQ138 variety is less drought tolerant than others (Figure 3). Verbruggen and Hermans (2008) revealed that the the increase of Proline content of plant is to play adaptive characters in plant stress tolerance. The higher record of proline during drought stress condition encouraged the proline content as a parameter of selection for stress tolerance (Jaleel et al., 2007).



**Figure 3.** Proline concentration of leaf under both treatments (well-watered and water stressed) condition. Means with the same later have no significant difference at  $P < 0.01$ .

#### *Correlation analysis*

In order to analyze how each parameter interrelated to a given condition, correlation analyses were performed for each water level. Based on the correlation value, we can conclude that there is a negative correlation between watering treatment and proline, soluble sugars, chlorophyll content and relative water content parameters, while SLA, SLW, nitrogen content and protein content which showed positive correlation. As water level increase, the content of proline, soluble sugars, chlorophyll content and relative water value decrease, while the SLA, SLW, nitrogen and protein content values increase. As water level decrease, the content of proline, soluble sugars, chlorophyll content and relative water value increase, while the SLA, SLW, nitrogen and protein content values decrease.





**Key:** PROT= protein, PROL= proline SLA= specific leaf area, SLW= specific leaf weight, SS= soluble sugar, N= nitrogen, CHLO= chlorophyll, RWC= relative water content.







Note: SLA= specific leaf area, SLW= specific leaf weight, SS= soluble sugar, N= nitrogen, CHLO= chlorophyll, RWC= relative water content.

# **CONCLUSIONS**

Drought tolerant maize variety sustains their progress (growth) and development under drought stress, while the maize varieties that are susceptible to drought may be lack progress and development and drought condition and facing severe yield reduction. In the present study all, maize varieties are not equally affected or show variability to drought stress. The differences exist was due to the ability of different maize varieties to tolerate water stressed conditions. In the drought stress condition, highest level of proline content, soluble sugar content, N content, Protein content, SLW, RWC content, Chlorophyll content and lower SLA was obtained in the maize variety Melkasa-4, while for lower proline content, SS content, N content, Protein content, SLW, RWC content, Chlorophyll content and higher SLA at the same drought stress treatment, the lowest level was obtained in the MHQ138. This assessment mainly evaluates the maize varieties to determine the SLA, SLW, proline, soluble sugar, nitrogen, protein, chlorophyll and relative water content to identify the most drought tolerant and least drought tolerant variety. According to the evaluation work, it was found out that Melkasa-4 which is most drought tolerant one among the varieties that used in the research study and MHQ138 is the least drought tolerant one among the varieties that used in the research study. Therefore, higher proline content, SS content, N content, Protein content, SLW, RWC content, Chlorophyll content and lower SLA can help in selection of most drought tolerant maize variety. Among the parameters SLA, proline, soluble sugar and chlorophyll contents are the best parameters to identify the most drought tolerant maize varieties. Further work in the future has to be done on identification of the gene that code for increased accumulation of metabolites under drought stress at molecular level which would assist rapid development of maize variety and further investigation on the resistance mechanisms in maize variety at the molecular level is required. Since the current research was done under controlled condition, the consistency of observed traits should be studied under field condition.

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# **CONFLICT OF INTERESTS**

No potential conflict of interest was reported by authors.

# **CONTRIBUTIONS OF AUTHOR**

The experiment, collection of data, data analysis, and the write-up of the manuscript was carried out by Dale Abo. Leta Tullu led the project, helped with the design of the trial, and supervised the study. The cosupervisor of this study was Mulugeta Kebede. The final manuscript read and approved all authors.

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