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Assessment of Genetic Diversity among Maize Hybrids under Two Different Irrigation Conditions by Using SSR Markers, Drought Indices and Thermal Image

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ABSTRACT

The aim of the study was screened fourteen hybrids of maize; S.C.10, S.C.128, S.C.131, S.C.162, S.C.167, S.C.168, S.C.176, S.C.177, pioneer 3444, T.W.C.321, T.W.C.324, T.W.C.353, T.W.C.360 and T.W.C.368 under normal and drought stress; 100% and 50% of Field Capacity (FC) respectively. A field trial was carried out during two summer seasons 2019 and 2020 on an experimental farm, Faculty of Agriculture, Suez Canal University; Ismailia, Egypt. Several growth, yield physiological traits have been estimated. Indices of drought resistance were calculated for each hybrid; mean productivity, drought susceptibility index, tolerance index, geometric mean productivity and yield stability index (MP, DSI, TOL, GMP, and YSI respectively). Simple sequence repeat analysis of DNA (SSR) was applied to estimate the genetic differentiation among the hybrids. Results showed that corn yields were significantly reduced during drought conditions. Maize hybrids exhibited significant differences for physiological and quantitative traits; plant height, number of leaves, ear height, ear diameter, ear length, RWC and chlorophyll content. In this study the hybrids divided successfully into four groups according to their drought tolerance and yield production by using drought resistance indices. One of these groups; S.C128, S.C162, S.C167 and S.C176 hybrids had the highest yield under both conditions and more adaptive under drought stress. The results of the SSR analysis showed that polymorphism % was ranged from 50% to 67% with an average 58.5%, while polymorphism information content (PIC) values were ranged from 0.32 to 0.57. Genetic similarity coefficient values were ranged from 0.625 to 1 with an average 0.8125. The dendrogram divided the studied hybrids into five main clusters. The study concluded that SSR analysis and drought indices results are quite similar, even though some variations in classes of hybrids regarding tolerance indices. It might be due to grain yield trait was more affected by drought conditions over genetic material. Thermal images were more effective for selecting maize hybrids for drought stress. Consequently; we recommended it to use in plant breeding experiment.

Keywords: Maize hybrids, Drought, Tolerance Indices, SSR, thermal images.

1. Introduction

Maize (*Zea mays* L. 2n=20)) considered an important cereal crop belongs to family Poaceae with producing 7.49 Gg / 0.99 M ha (FAO STAT, 2020). Maize used in human food, animal feed and industrial products. The local production of maize is not sufficient to meet the excessive demand, especially the yellow grains in Egypt (Ali and Abdelaal 2020).

Maize crop needs to high irrigation requirements; so, the crop sensitive to abiotic stresses; salinity, high temperature and drought at growth stages; especially at flowering and pollination stages (Gomaa *et al.,* 2017 and Sah *et al*., 2020). And the plant responses for drought were differed according to; plant age, genotype, properties of soil, drought level and period (Gabr Afaf *et al*., 2018).

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Increasing irrigation intervals or skipping one or more irrigations significantly decreased plant height, number and area of leaves, stem and total dry weight/plant, total chlorophyll, relative water content (RWC), plant height, length and diameter of ear, number and weight of grains/ear, 100 grain weight as well as grain, stover and biological yields/fed, protein and oil % and yields/ fed (Mansourifar *et al*, 2010, Gomaa *et al,* 2017, Mohammed *et al*., 2017 and Li *et al*., 2018 and Ali and Abdelaal, 2020).And addition to grain yield trait was considered a polygenic trait administrated by interaction between environment and genotype so the selection for high grain yield was difficult as a result of low heritability of grain yield and there were positive and significant correlations among grain yield and each of; kernels number/ row and ear length under drought condition (Nigem 1998, Akaogu *et al*., 2017 and Adu, Badu-Apraku *et al*., 2019). Some drought indices were used to evaluate and identify genotypes under normal and drought conditions; Selection index was more effective than direct selection for grain yield of some crops (Grezsiak *et al.,* 2012, Bayoumi *et al.,* 2015, Gabr Afaf *et al*., 2018 and Emam *et a*l., 2022). On the other hand, the using of molecular markers such as simple sequence repeat (SSR) markers present a promising method to identify stress tolerance genes due to the fact that it characterized by its recognition of co-dominancy (Bawa *et al.,* 2015, Elci and Hancer 2015, Younis *et al.,* 2020 and Mdluli *et al.,* 2020).SSR markers were used for doing molecular characterization for twenty-four lines of maize was applied in previous studies (Gazal *et al.,* 2016 and Kamara *et al.,* 2020) Also; hundred maize lines using a set of 32 SSR markers having genome wide coverage, which supports the significantly contributed of SSR markers to identify drought tolerant genotypes (Gazal *et al.,* 2018). Several crops had been characterized according to their response to environmental stresses by using thermal imaging (Munns *et al.,* 2010 and Walter *et al.,* 2012). The study of plant water relations was needed to measure leaf temperature which resulted from several internal and external conditions by thermal infrared (IR) sensing, which used as an indicator of the water loss rate or stomatal opening that result of plant resistance to water stress (Stoll and Jones, 2007 and Bayoumi *et al*., 2016).

The aim of the study is to recommend some hybrids with high productivity, are adaptive and tolerant to drought condition by differentiating among a group of maize hybrids under normal and drought condition through determining agronomic and biochemical traits, physiological characters by thermal camera and genetic differentiation by using SSR markers.

2. Materials and Methods

The aim of the experiment was to study the effect of drought and irrigation (normal 100% and stress water 50% irrigation) on growth, productivity and quality traits of 14 maize genotypes. A field trial was carried out in two summer seasons 2019 and 2020 at experimental farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt., under normal (100% F.C) and stress water (50% F.C) irrigation, the normal condition was irrigated every 10 days, while drought stress condition was irrigated every 20 days, to assess in a randomized complete block design (RCBD)with tow water regimes and 3 replications; each replication includes tow water regimes as a main plot; Each main plot includes 14 hybrids as a sub-plot. Studied maize hybrids were obtained and released by Maize Res. Dep., Field Crops Res. Inst., A.R.C. Egypt, namely: S.C.10, S.C.128, S.C.131, S.C.162, S.C.167, S.C.168, S.C.176, S.C.177 and pioneer 34449 (9 single crosses). Other 5 hybrids (three ways cross) T.W.C.321, T.W.C.324, T.W.C.353, T.W.C.360, T.W.C.3685 presented in Table (1).

Table 1: Pedigree of fourteen hybrids of maize

1	**S.C.10**	**Sids7 * Sids63**	**8**	**S.C.177**	**Sakha 10 * Giza 653**
2	S.C.128	Sakha 13 * Sakha 9	9	pioneer 3444	Corteva Pioneer, USA
3	S.C.131	Sakha 9 * Sakha 5	10	T.W.C.321	S.C 21 * Sids7
4	S.C.162	Giza 653* Giza 639	11	T.W.C.324	S.C 24 * Sids7
5	S.C.167	Giza 339* Giza 657	12	T.W.C.353	S.C 162 * Sakha 1
6	S.C.168	Giza 639* Giza 658	13	T.W.C.360	S.C 162 * Sakha 2
7	S.C.176	Sakha 11 * sakha10	14	T.W.C.368	

Each block was consisted of thirty plots; and each plot was consisted of three rows of 21 plants spaced 30 cm apart within the row, while the rows were set 70 cm apart. Properties of the soil and cattle manure (CM) used in the present study in Table (2). Agricultural practices were applied as recommended for the maize crop, according to Ministry of Agriculture Recommendation’s.

Table 2: Properties of the soil and cattle manure (CM) used in the present study

Properties	Soil I	CM
Particle size distribution (%)		
Sand	96.65	-
Silt	2.53	-
Clay	0.82	-
Textural class	Sand	-
Bulk denisity (g cm^{-3})	1.52	-
Soil order	Aridisols	-
Field Capacity (%)	18.2	-
pH	7.68*	7.57**
EC_e (dS m^{-1})***	1.28	12.89
Soluble cations (meq l^{-1})*		
Ca^{2+}	6.11	25.0
Mg^{2+}	4.10	14.5
Na^{+}	2.23	39.0
K^{+}	0.36	50.4
Soluble anions (meq l^{-1})*		
CO_3^{2-}	0.00	0.0
HCO_3^{-}	2.38	40.7
Cl^{-}	5.75	70.3
SO_4^{2-}	4.67	17.9
Organic C (g kg^{-1})	1.41	140.5
Total N (g kg^{-1})	0.16	11.9
Available N (mg kg^{-1})	4.39	126.0
Available P (mg Kg^{-1})	9.32	120.0
Micronutrients (mg Kg^{-1})******		
Fe	0.82	0.15
Mn	1.11	25.2
Zn	0.53	5.71
Cu	0.24	0.24

* In soil-water suspension (1:2.5), ** In CM-water suspension (1:5).
*** In CM and soil saturated extracts, **** In DTPA extract.

2.1. Growth and yield traits:

Plant high, number of leaves and ear traits (height, length and diameter) were measured and recorded. Moreover, at harvest; weight of grain yield (ton/fed.) and 100 grain weight were determined for both water treatments.

2.2. Physiological Traits:

Chlorophyll content: determined by meter readings; SPAD values (502 plus-Minolta, Japan) were taken at the anthesis stage for three times and the reading average was calculated.

Relative water content (RWC): determined according to Schonfeld *et al.* (1988) for combined analysis for both seasons using the following equation:

$$\text{RWC\%} = \frac{\text{Fresh weight - dry weight}}{\text{Turgid weight - dry weight}} \times 100$$

2.3. Tolerance Indices

Relative decrease (RD %): calculated as the ratio of: = (unstressed - stressed)/ unstressed plants (control).

Tolerance indices: mean productivity (MP), yield stability index (YSI) geometric mean productivity (GMP), drought susceptibility index (DSI) and), tolerance (TOL) for water stress were calculated based on grain yield under normal and drought condition were calculated by the following formulas:

According to Gupta et al., (2001) and According to Chaudhuri and Kanemasu (1982)

1. GMP = $\sqrt{Yp * Ys}$
2. MP = Yp + Ys / 2
3. TOL = Y p - Ys
4. Y SI = Ys / YP
5. DSI = (1- Ys / Yp) / DII

where;

Ys = mean yields of a given genotype in stress condition;
Yp = mean yields of a given genotype in normal condition;
DII = Drought intensity index.
The drought intensity index (DII) for each condition was calculated as
DII =1 - Xs / Xp
Ys% = mean yields of a given genotype in stress condition expressed as % of mean under stress condition.

2.4. Molecular analysis

Plant materials: young leaves of maize plants (two-week old) were collected to extract genomic DNA from fourteen maize hybrids; approximately 100 mg of plant tissue was harvested and stored at -80 C
DNA extraction: the Jena bioscience kit was used to extract the DNA from leaves.

Table 3: List of five SSR markers and their sequences retrieved from www.maizegdb.org

Primer	Marker	Forward primer sequence	Reverse primer sequence
Primer 21	Umc2144	CCAGCCCCTATCTATTTGCTTGT	GAATACTATATCACGGTCGGTCGG
Primer 22	Umc2245	GCCCTGTTATTGGAACAGTTTACG	CGTCGTCTTCGACATGTACTTCAC
Primer 23	Umc2383	CATAGACGTGCCCCTTGTCATC	CTCGCAACTGCGCTTCTAGATACT
Primer 16	Umc1664	AATTGTTTACTGCGCTGAAACTCC	CCTCTTTGCCTGTACCGTGTATTC
Primer 14	Umc1424	CCGGCTGCAGGGGTAGTAGTAG	ATGGTCAGGGGCTACGAGGAG

2.5. Polymerase Chain Reaction (PCR)

The isolated DNAs were then amplified using a Polymerase Chain Reaction (PCR) under the following conditions: In a reaction volume of 10 µl. (6.5 l of ddH2O, 5 ngl^{-1} of DNA, 1 mM of primer, and 1.5 l of premix), the DNA was first denaturated at 94 °C for 4 min, followed by 1 min of annealing at 55 °C. and extension for 5 minutes at 72 degrees for of 35 cycles. The PCR results were combined with bromo-phenol blue gel loading dye and then subjected to electrophoresis on a 3.5 percent (w/v) agarose gel for analysis. A 0.5 mgml^{-1} solution of ethidium bromide was used to stain the gels. Gels were analysis to detect the levels of polymorphism,also; band distribution among F1 hybrid and a similarity matrix were determined by the Jaccard index for clustering by the Neighbour-joining algorithm on DARwin 6.5 software. The dendrogram constructed according to the Nei and Li's (1979) similarity matrix which was used to produce the unweighted pair group method with arithmetic mean (UPGMA). Gen. Alex Software was used to analyze the number of effective alleles (Ne) and polymorphic information content (PIC) calculated according to Botstein *et al.* (1980).

2.6. Thermal Image

Thermal images were taken with infrared thermal cameraTi-32 (Fluke Thermography, Germany). The height of the canopy was about one meter, Ti-32 Pro software (Infrared Solutions) used to analyze the images; Plant canopies and leaves emissivity for measurements were set at 0.96 while transmission correction was 85 %. To obtain more accuracy, the span of auto adjusted thermal image is manually set, in addition to the level of the displayed as an important camera feature in order to detect minimum and maximum temperature of the entire display (Wilcox and Makowski, 2014).

3. Results and Discussions

3.1. Mean performance of maize hybrids under normal and drought conditions.

Growth traits of maize hybrids presented in Tables (4), (5) and (6). Drought caused significant decreasing the plant height, leaves number, Ear height, Ear length and Ear diameter; 13.7, 12.68, 10.49, 24.8 and 10 % respectively comparing with normal condition. This reduction might due to the deficiency of absorbed water and inhibit the photosynthesis process (Bayoumi *et al*., 2002 and Ali and Abdella 2020). Also; Zlatev and Lidon, (2012) showed that reactive oxygen species (ROS)were induced by some types of abiotic stresses causing harmful effects on plant growth through reactions and damage nucleic acids, proteins carbohydrates and lipids namely oxidative stress. In combined season, significant differences were found among studied hybrids under both conditions. High mean values of plant height trait were recorded in plants of three hybrids namely S.C.10, S.C128 and S.C.267 under normal condition and one of them S.C10 and three others T.W.C368, S.C131 and S.C177 under drought condition. These results were in agreement with results obtained by (Gabr Afaf *et al*., 2018) and Gomaa *et al.,* (2017) who reported that S.C10 hybrid had the highest plants while T.W.C.321 had the shortest plants comparing with S.C.162 and S.C.129.

Table 4: Mean values of plant height and leaves number /plant traits for 14 maize hybrids under normal and drought stress conditions in combined analysis.

Hybrids	**pH**		**Mean**	**No. of leaves**		**Mean**
	Normal	**Stress**		**Normal**	**Stress**	
S.C 10	263.33	217.33	240.33	17.67	14.67	16.17
S.C128	260.00	205.00	232.50	18.00	15.33	16.67
S.C 131	220.00	176.00	198.00	16.67	15.33	16.00
S.C 162	224.33	202.67	213.50	17.33	14.33	15.83
S.C 167	253.33	212.67	233.00	17.00	14.00	15.50
S.C 168	242.33	217.00	229.67	16.67	15.00	15.83
S.C 176	233.67	203.33	218.50	16.33	14.00	15.17
S.C 177	251.33	215.00	233.17	16.67	14.33	15.50
S.C 3444	251.67	212.00	231.83	17.33	14.33	15.83
T.W.C 321	201.67	181.67	191.67	17.00	15.33	16.17
T.W.C 324	220.00	195.00	207.50	17.33	15.00	16.17
T.W.C 353	204.00	186.00	195.00	15.67	14.67	15.17
T.W.C 360	208.33	181.00	194.67	16.67	15.33	16.00
T.W.C 368	242.33	222.33	232.33	16.00	14.67	15.33
Mean	234.02	201.93		16.88	14.74	
RD%		13.7%			12.68%	
L.S. D	**G= 4.90**	**& S= 2.59**	**& G*S =3.7**	**G=0.89**	**& S= 0.30**	**& G*S =0.53**

For leaves number traits, T.W.C360, S.C131, S.C168, T.W.C 321 and T.W.C324 had the highest leaves number/ plant under drought condition without any significant difference among them. S.C10 and S.C128 hybrids had the highest mean values under normal condition, but great decreasing happen in this trait for these hybrids under drought condition. Zhu (2016) reported that drought stress was considered the hyperosmotic signal which may be induced increases amount of the abscisic acid hormone (ABA), which enhanced the responses of adaption in plants.

Drought caused significant decreasing of ear length reached 24.81%. Significant differences among all hybrids were reported where; the longest ears were found in S.C10 and S.C168 under both conditions, T.W.C360 under drought condition.

On the other hand, there were insignificant differences among hybrids under normal condition for the ear diameter trait. While, there was a significant difference in responses and values of ear diameter under drought condition. The widest ears were recorded in plants of S.C176, S.C177 and S.C3444 hybrids under both conditions. The lowest mean values of ear height were found in plants of T.W.C360, T.W.324 S.C176 hybrids under normal and drought conditions. While, the highest mean values were found in plants of S.C10, S.C162 and S.C177 hybrids under normal condition and S.C131, S.C167 and S.C168 hybrids under drought condition without significant difference among them.

Table 5: Mean values of ear height and ear length traits for 14 maize hybrids under normal and drought stress conditions in combined analysis

Hybrids	**Ear height (cm)**		**Mean**	**Ear length (cm)**		**Mean**
	Normal	Stress		Normal	Stress	
S.C 10	135.67	119.33	127.50	27.67	21.27	24.47
S.C128	132.33	117.00	124.67	25.30	19.50	22.40
S.C 131	131.33	122.00	126.67	25.23	17.83	21.53
S.C 162	135.33	118.33	126.83	24.40	20.00	22.20
S.C 167	133.33	123.33	128.33	22.70	17.67	20.18
S.C 168	133.67	122.33	128.00	26.60	20.33	23.47
S.C 176	129.33	114.67	122.00	25.50	16.67	21.08
S.C 177	135.33	119.33	127.33	25.70	17.67	21.68
S.C 3444	134.67	118.33	126.50	23.93	16.00	19.97
T.W.C 321	133.00	118.00	125.50	22.67	15.67	19.17
T.W.C 324	128.33	114.67	121.50	23.67	19.00	21.33
T.W.C 353	130.33	117.00	123.67	22.83	18.33	20.58
T.W.C 360	127.00	116.33	121.67	24.37	20.33	22.35
T.W.C 368	132.33	117.00	124.67	22.93	18.00	20.47
Mean	132.29	118.40		24.54	18.45	
RD%		10.49%			24.81%	
L.S.D	G= 3.53	& S=0.98	& G*S = 1.8	G= 1.24	& S=0.49	& G*S = 0.89

Table 6: Mean values of ear diameter, 100 grain weight and grain yield traits for 14 maize hybrids under normal and drought stress conditions in both seasons.

Hybrids	**Ear diameter (cm)**		**Mean**	**100 grain weight (g)**		**Mean**	**Grain yield (ton/fed)**		**Mean**
	Normal	Stress		Normal	Stress		Normal	Stress	
S.C 10	4.67	3.93	4.30	34.60	24.13	29.37	4.39	3.06	3.73
S.C128	4.40	3.73	4.07	34.73	25.53	30.13	3.93	2.99	3.46
S.C 131	4.63	4.00	4.32	32.43	27.53	29.98	4.17	2.95	3.56
S.C 162	4.40	4.07	4.23	32.23	26.00	29.12	3.62	2.99	3.30
S.C 167	4.40	3.93	4.17	34.57	27.00	30.78	3.95	3.04	3.50
S.C 168	4.33	4.10	4.22	40.43	23.67	32.05	4.04	2.82	3.43
S.C 176	4.73	4.17	4.45	29.37	24.00	26.68	3.74	2.89	3.31
S.C 177	4.67	4.24	4.46	29.40	24.33	26.87	4.21	2.80	3.51
S.C3444	4.50	4.29	4.40	30.47	24.00	27.23	4.21	2.83	3.52
T.W.C321	4.33	4.20	4.27	30.03	23.67	26.85	4.03	2.76	3.40
T.W.C324	4.33	3.97	4.15	30.90	23.00	26.95	3.73	2.67	3.20
T.W.C353	4.37	3.80	4.08	29.13	23.87	26.50	3.13	2.63	2.88
T.W.C360	4.30	3.78	4.04	29.13	22.33	25.73	3.23	2.51	2.87
T.W.C368	4.27	3.65	3.96	30.23	22.67	26.45	3.57	2.48	3.02
Mean	4.45	3.99		31.98	24.41		3.85	2.82	
RD%		10%			23.67%			26.75%	
L.S.D	**G= 0.17 & S= 0.059 & G*S =0.10**			**G= 1.25 & S= 0.51 & G*S = 0.94**			**G=0.15 & S= 0.044 & G*S = 0.08**		

Although drought caused a highly significant decrease in 100 grain weight from 31.98 under normal to 24.41g in combined season, but S.C131 and S.C 167 hybrids under drought condition were recorded the highest mean values. S.C10, S.C 128, S.C177, S.C3444 and S.C167hybrids had higher mean values of grain yield under both conditions respectively. Yadav and Singh, (2010) Pandit *et al.*, (2016) and Gomaa *et al.,* (2017) obtained similar results and reported there were wide variations in quantitative traits among maize genotypes mainly in plant height, ear length, ear height, ear diameter, weight of grain yield/ plant and grain yield and confirmed that there were significant differences among genotypes and water treatments and found that the single crosses had highly significant increasing grain yield over lines and tested populations. Ranatunga *et al*., (2009) confirmed that good knowledge about the environmental effects on quantitative helping to obtain accurate genotype classification.

3.2. Chlorophyll content by SPAD

Chlorophyll content trait showed significant decrease reach to 8.2% under drought condition comparing with normal condition.S.C10 and S.C 167 hybrids were recorded the highest content under normal condition and S.C10 and T.W.C368 hybrids under drought condition. The lowest content of chlorophyll found in plants of T.W.C321 under both conditions (Table.7). Mansourifar *et al*., (2010), Krol (2013) and Younis *et al*., (2017) reported that a water deficiency induced significant decrease in chlorophyll content through several physiological changes such as decreasing the closing of stomata, caused great damage of transpiration, stomatal conductance in leaves and inhibit some of photosynthetic process.

3.3. Relative water content (RWC):

RWC is considered to be a better measure of plant water status than thermodynamic state variables (water potential, turgor potential and solute potential, "Sinclair and Ludlow .1985"). In this study, plant water status under drought conditions was identified by measuring RWC. The data in Table 7 show that under drought stress, the general mean value for RWC (67.86) was 9.8% lower than the control condition (75.27). This may be due to differences in the ability of the hybrids tested to accumulate and osmotically adapt to maintain tissue turgor and hence physiological activities such as expanding root depth to increase water reserves for crops (Siddique et al., 2000). On the other hand, the average values of RWC decreased with drought stress in all the tested hybrids. S.C 10 hybrid had higher RWC value (73.13) while S.C 168 hybrid had lower RWC value (64) under drought stress (Table7). This genotypic variation in RWC values may be recognized to differences in the ability of the variation to absorb more water from the soil and or the ability to control water loss through the stomata's. Similar explanations have been stated in superior hybrids of maize (Farouk *et al.,* 2018 and Ali and Abdelaal (2020). Remarkably, S.C 10 hybrid had the highest value of RWC under stress but it had the second value in the orders under normal condition. The reason could be the plants have capacity to minimize the destructive effects of stress by changing their metabolism to handle with stress.

Table 7: Mean values of SPAD reading of chlorophyll and Relative water content traits for 14 maize hybrids under normal and drought stress conditions in combined analysis

Hybrids	**SPAD**		**Mean**	**RWC**		**Mean**
	Normal	Stress		Normal	Stress	
S.C 10	45.57	42.30	43.93	79.60	73.13	76.37
S.C128	44.87	40.50	42.68	82.33	71.51	76.92
S.C 131	44.20	40.67	42.43	78.10	69.37	73.73
S.C 162	42.00	40.00	41.00	73.70	72.07	72.88
S.C 167	45.27	40.00	42.63	72.31	67.67	69.99
S.C 168	44.33	40.67	42.50	78.50	64.00	71.25
S.C 176	43.73	38.67	41.20	73.37	67.33	70.35
S.C 177	44.60	39.67	42.13	76.33	65.00	70.67
S.C 3444	44.53	39.67	42.10	78.83	69.67	74.25
T.W.C 321	40.60	38.33	39.47	71.90	69.33	70.62
T.W.C 324	42.33	39.33	40.83	71.37	64.67	68.02
T.W.C 353	42.77	40.33	41.55	68.67	65.33	67.00
T.W.C 360	42.50	39.00	40.75	72.34	65.33	68.88
T.W.C 368	43.00	41.00	42.00	76.33	65.60	70.97
Mean	43.59	40.01		75.27	67.86	
RD%		8.21%			9.8%	
L.S.D	**G= 1.08**	**& S= 0.34**	**& G*S = 0.6**	**G= 2.40**	**& S=0. 86**	**& G*S = 1.5**

3.4. Tolerance indices analysis

3.4.1. Mean performance of maize hybrids based on the tolerance indices

This investigation was performed to expose the accurate tolerance indices for testing of hybrids under drought stress; eight drought tolerance indices were valued by means of the grain yield (g) per plant in normal and drought conditions (Table 8). The highest grain yield value (4.39 g) was verified in S.C 10 hybrid followed by S.C 177 and S.C 3444 hybrids (4.21g) under normal condition. Similar trend was showed by S.C 10 hybrid for grain yield value (3.06 g) under drought condition. The lowest grain yield value (3.13 g) was obtained in T.W.C 353 hybrid followed by T.W.C 360 (3.23g) under normal condition and in T.W.C 368 hybrid (2.48 g) and T.W.C 360 hybrid (2.51 g) under drought condition. The variation in the grain yield (g) per plant under normal and drought conditions proposed the existence of important resources for getting drought tolerant hybrids under this study. The findings are matching to those of Abdi et al. (2013).

MP and GMP were in harmony ranged from 2.87 to 3.73 and, from 2.85 to3.67; respectively. Genotypes with highest GMP and MP values were favored under drought conditions (Farshadfar *et al.* 2013). Consequently, based on these present indices, S.C 10 hybrid showed the highest value, specifying tolerant hybrid whereas T.W.C 360 hybrid was the most sensitive one. Noticeably,
Nine hybrids (single crosses) and two hybrids of three ways cross (T.W.C 321& T.W.C 324) were considered more productive (Table 8).

TOL was measured an efficient capacity to rise yield under drought environment (Fernandez, 1992). The highest TOL values were linked to S.C 177, S.C 3444 and S.C 10 hybrids (1.41, 1.38 and 1.32, respectively). Thus, high amount of TOL is a representation of genotype susceptibility to stress (Parchin et al., 2013). While, T.W.C 353 and S.C 162 with low TOL values (0.51 and 0.63) were considered as tolerant hybrids.

Concerning to the highest YSI values were observed for T.W.C 353 S.C 162 and T.W.C 360 hybrids (0.84, 0.83 and 0.78), respectively. Moreover, the same current hybrids had the lowest values of DII and RD % (0.16&16.17%, 0.17&17.33, and 0.22&22.47), respectively (Table 8). T.W.C 353 S.C 162 and T.W.C 360 hybrids were considered more stress tolerance and stabile. Rosielle and Hamblin (1981) Gabr *et al*., 2018 and Hategekimana *et al*., (2018) recommended that the best option for maize production, yield improvement and yield stability is growing varieties are more tolerant for drought stress and the drought tolerance selection was equal to the selection for lowest decreasing of yield under drought.

Table 8. Tolerance indices and relative decrease of 14 hybrids maize grown under normal and drought stress conditions.

Hybrids	**Grain Yield g/plant**		**MP**	**GMP**	**TOL**	**Ysi**	**DSI**	**DII**	**Ys%**	**RD %**
	Normal	Stress								
S.C 10	4.39	3.06	3.73	3.67	1.32	0.70	1.12	0.30	108.5	30.17
S.C128	3.93	2.99	3.46	3.42	0.94	0.76	0.89	0.24	106.02	23.94
S.C 131	4.17	2.95	3.56	3.51	1.22	0.71	1.08	0.29	104.6	29.23
S.C 162	3.62	2.99	3.30	3.29	0.63	0.83	0.64	0.17	106.02	17.33
S.C 167	3.95	3.04	3.50	3.46	0.92	0.77	0.86	0.23	107.8	23.19
S.C 168	4.04	2.82	3.43	3.38	1.22	0.70	1.12	0.30	100	30.17
S.C 176	3.74	2.89	3.31	3.29	0.85	0.77	0.84	0.23	102.5	22.66
S.C 177	4.21	2.80	3.51	3.43	1.41	0.66	1.24	0.34	99.3	33.54
S.C 3444	4.21	2.83	3.52	3.45	1.38	0.67	1.22	0.33	100.4	32.78
T.W.C 321	4.03	2.76	3.40	3.34	1.27	0.68	1.17	0.32	97.9	31.51
T.W.C 324	3.73	2.67	3.20	3.15	1.06	0.72	1.05	0.28	94.6	28.35
T.W.C 353	3.13	2.63	2.88	2.87	0.51	0.84	0.60	0.16	93.3	16.17
T.W.C 360	3.23	2.51	2.87	2.85	0.73	0.78	0.83	0.22	89	22.47
T.W.C 368	3.57	2.48	3.02	2.97	1.09	0.69	1.13	0.31	87.9	30.56

3.4.2. Hybrids classification:

Many indices offered numerous hybrids as drought tolerant (Table 8). Remarkably, it is confrontational to identify the drought tolerant genotypes based on a single criterion. So that according to Bayuomi et al (2002) that studied hybrids could form into four groups based on mean grain yield percent under stress condition (Ys%) and susceptibility index (Dsi). The first group contained that

S.C128, S.C162, S.C167 and S.c176 hybrids had Ys% over 100 and DSI less than one (< 1). They were higher productive and adaptive under both conditions (Table 8).

The second group consisted of S.C10, S.C313, S.C168 and S.C3444 hybrids that had Ys% over 100 and Dsi more than one (> 1). They were higher productive under both conditions and less adaptive under stress. The third group included that S.C177, T.W.C 321, T.W.C324 and T.W.C 368 hybrids had Ys% below 100 and Dsi more than one (> 1). They were less productive under both conditions and less adaptive under stress. The fourth group contained T.W.C360 and T.W.C353 hybrids that had Ys% below 100 and Dsi less than one (< 1). They were low productive and adaptive under both conditions (Table 8). Ali and Abdelaal (2020) reported that maize genotypes S.C 168 as well as T.W.C 360, T.W.C 352 and T.W.C 368 had the lowest values of relative yield reduction and drought susceptibility index (< 1), indicating that those genotypes were relatively drought tolerant genotypes. Similar results obtained by Khaled *et al* (2013), Habliza and Abdelhalim (2017), Gabr *et al.* (2018) and Ali and Abdelaal (2020) who reported that the genotypes showing DSI values < 1 were found to be more tolerant to drought stress while those had DSI values > 1 were sensitive to drought stress.

3.4.3. Correlation between grain yield and tolerance indices:

The correlation coefficients between grain yield under control and stress conditions and drought tolerance indices were estimated and identified the best drought index. In addition to it could be an acceptable indicator for choosing the most promising genotypes. Table (9) presented that GMP, MP, Dsi, had significantly positive correlation with grain yield under control and stress conditions. According to Blum (1988) indicated the favorable index has positive correlation with yield under stress and non–stress environments. This strong correlation with yield under watered and drought treatments indicated that tolerance indices were adequate parameters to obtain some genotypes had drought tolerant and high yield under both conditions. Therefore, these results might be fruitful for choosing good drought indices. However, yield under control and stress conditions had significantly positive Correlation (0. .647). The reason for positive correlation between yield under control and stress conditions was that the efficiency of water-use remained the same and not varied with change in water availability. Yield under control condition had positive correlation with each of MP (0.961), GMP (0.941), TOL (0.865), DSI (0.735) DII (0.727). These indices were suitable for selection genotypes in non-stress condition. Results were in harmony with Bayoumi *et al.* (2015) who reported that MP considered the mean production under both conditions could be used to select the genotypes. Where, Ysi correlated negatively with TOL, Yp, MP, and GMP (0.970-.727-, 0.511- and 0.454-) respectively.

Table 9. Correlation coefficients between yield under normal, stress drought condition and drought tolerance indices

	Normal	Stress	MP	GMP	TOL	Ysi	DSI	DII
Normal	1	.647*	.961**	.941**	.865**	.727-**	.735**	.727**
Stress		1	.832**	.867**	0.177	0.047	.038-	.047-
MP			1	.998**	.693**	.511-	0.52	0.511
GMP				1	.644*	.454-	0.463	0.454
TOL					1	.970-**	.973**	.970**
Ysi						1	.999-**	1.000-**
DSI							1	.999**
DII								1

*. Correlation is significant at the 0.05 level & **. Correlation is significant at the 0.01 level

3.5. Simple Sequence Repeat (SSR) analysis:

3.5.1. SSR Marker Informative

In this study, microsatellite markers were used for studying the genetic diversity of 14 maize hybrids. 5 primer pairs of microsatellite were used, which had relatively high polymorphism in available literatures. Choice of the marker was based on the way that each 10 chromosomes of maize could cover at least one descriptive marker. Using the primer pairs, genomic DNA was amplified and polymorphism was exhibited among the hybrids (Table 10 and Figure 1).

However, the SSR marker informative was characterized by several parameters such as the number of alleles, percentage of polymorphism and polymorphic information content (PIC) and the effective number of allele (Table 10). The 5 markers produced 11 alleles across the tested maize hybrids.

Six loci only were polymorphic; each marker amplified one polymorphic except SSR marker-Umc1664 generated two polymorphic bands. SSR marker-Umc2383 had one negative band at 140bp. The number of alleles per locus ranged from 2 to 3 alleles with average of 2.2. The low number of alleles might display in part the narrow genetic basis in 14 maize hybrids used in this study. Other explanations might be due to the result by the 'short allele dominance', where, in heterozygotes including a short and a long allele, only the short allele is sufficiently amplified in the PCR reaction (Wattier *et al*., 1998). The variability in the number of alleles per locus may outcome from different locus-specific mutation rates and reveals strong changes in allelic diversity between SSRs loci (Piyusha and Singh, 2018).

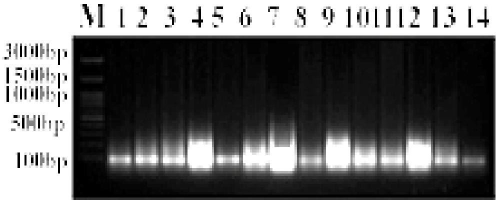
However, the number of alleles noted in this investigation is not the same as what was found in earlier studies of maize using SSR markers (Sserumaga *et al.*, 2014). It is very essential to recognize that the comparisons with the allelic diversities stated by other studies should be measured with attention and take into concern the diverse sample sizes used; in addition, the same mean number of alleles may not display the same amount of variability (Ateş Snmezoğlu and Terzi, 2017).

Effective number of alleles (Ne) is the measure of allelic evenness. In this study, the results presented that the effective number of alleles (Ne) for the polymorphic markers ranged from0.42 for SSR marker-Umc1664 to 0.715 for SSR marker-Umc1424) with average value of 0.567. The total number of effective alleles produced by the 5 SSR loci was 2.835. Table (8) was exhibited that the average of Effective number of alleles was lower (0.567) than observed number of alleles (2.2). Because of low frequencies alleles had slight influence to the effective number of alleles. Agreeing with the selective standard of the microsatellite loci, it ought to have at least four alleles to be measured useful for the assessment of genetic diversity. Bases on this criterion, the 5 SSR loci used in this study were useful for the evaluation of genetic diversity in14 maize hybrids.

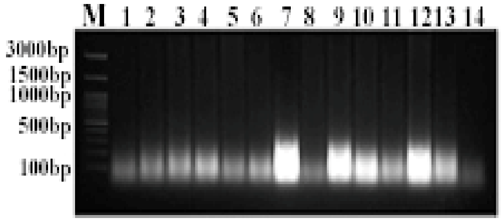
Polymorphism % ranged from 50% to 67%with an average 58.5%. PIC value varied from 0.32 (SSR marker-Umc2144) to 0.57 (SSR marker-Umc1664) with average of 0.435. This lower PIC value may be due to maize hybrids have narrows genetic diversity. On the other hand, the PIC depended on the number of alleles detected and on their distribution frequency. Moreover; PIC was subjective to location of primers in the genome used for study and genotype sensitivity to method used. Hence, PIC values increased proportionally in SSR marker-Umc1664 (0.57) and SSR marker-Umc2383 (0.499) that might be to increase heterozygosity at each locus. While, the lower PIC value (0.32) for SSR marker-Umc2144 might be attributed to the concentration of gene frequencies, which leads to deviance from the condition of maximum information content of a locus. This happens when all alleles have similar frequencies. Gazal *et al*., (2016) explain these values of PIC; closely maize lines had lower values of PIC, while genetically diverse maize lines had high values of PIC. These results were in agreement with Ying *et al.,* (2011) and Hongbo *et al.,* (2012) Nefzaoui *et al*., 2014 and Mdluli *et al* 2020) who reported that markers with PIC values greater than 0.5 are considered polymorphic and informative. Moreover; Semagn *et al*., (2006) illustrated that the SSR markers were characterized with co-dominant nature and high polymorphism.

Table 10: Primer name, total number of bands, Number of effective alleles, number of different bands (monomorphic, polymorphic, and unique), polymorphism percent (%), and the polymorphism information content (PIC) for each SSR primer.

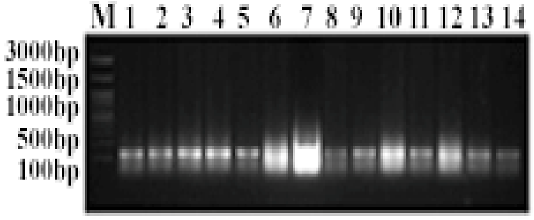
Primer Name	Total Band	Monomorphic Band	Polymorphic band	Unique Band	Polymorphic%	PIC%	Ne
Umc1424	2	1	1	-	50%	0.39	0.715
Umc1664	3	1	2	-	67%	0.57	0.42
Umc2144	2	1	1	-	50%	0.32	0.568
Umc2245	2	1	1	-	50%	0.397	0.618
Umc2383	2	1	1	1	50%	0.499	0.514
Total	11	5	6	1	0.64%	2.176	2.835
Mean	2.2					0.435	0.567



(primer- Umc1424)



(primer-Umc1664)



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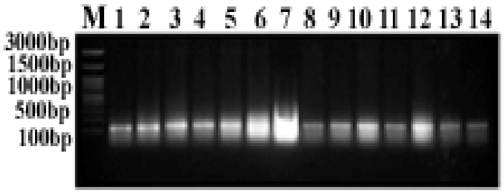
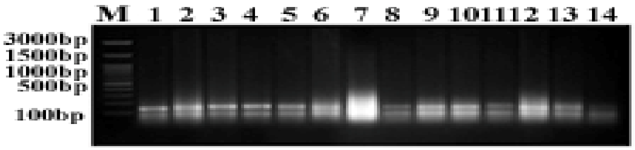


(primer- Umc2245)



(primer- Umc2383)

Fig. 1: Five photos of SSR primers: Umc1424, Umc1664, Umc2144, Umc2245 and Umc2383 respectively each photo contains fourteen hybrids namely; 1: S.C.10, 2: S.C.128, 3: S.C.131, 4: S.C.162, 5: S.C.167, 6: S.C.168, 7: S.C.176, 8: S.C.177, 9: pioneer 3444, 10: T.W.C.321, 11: T.W.C.324, 12: T.W.C.353, 13: T.W.C.360, 14: T.W.C.368)

3.5.2. Genetic Similarity:

Genetic similarity coefficient among studied fourteen hybrids was ranged from 0.63 to 1 with an average similarity of 0.8125 (Table 11).

Table 11: values of genetic similarity coefficient among fourteen maize hybrids.

Hybrids	**S.C 10**	**S.C 128**	**S.C 131**	**S.C 162**	**S.C 167**	**S.C 168**	**S.C 176**	**S.C 177**	**S.C 3444**	**T.W.C 321**	**T.W.C 324**	**T.W.C 353**	**T.W.C 360**	**T.W.C 368**
S.C 10	1.00													
S.C128	0.92	1.00												
S.C 131	0.92	1.00	1.00											
S.C 162	0.86	0.93	0.93	1.00										
S.C 167	0.92	0.86	0.86	0.80	1.00									
S.C 168	0.86	0.80	0.80	0.75	0.93	1.00								
S.C 176	0.75	0.82	0.82	0.78	0.82	0.89	1.00							
S.C 177	0.92	1.00	1.00	0.93	0.86	0.80	0.82	1.00						
S.C 3444	0.75	0.82	0.82	0.89	0.82	0.78	0.90	0.82	1.00					
T.W.C 321	0.80	0.88	0.88	0.82	0.88	0.94	0.95	0.88	0.84	1.00				
T.W.C 324	0.92	1.00	1.00	0.93	0.86	0.80	0.82	1.00	0.82	0.88	1.00			
T.W.C 353	0.71	0.78	0.78	0.84	0.78	0.84	0.95	0.78	0.95	0.90	0.78	1.00		
T.W.C 360	1.00	0.92	0.92	0.86	0.92	0.86	0.75	0.92	0.75	0.80	0.92	0.71	1.00	
T.W.C 368	0.91	0.83	0.83	0.77	0.83	0.77	**0.67**	0.83	0.67	0.71	0.83	0.63	0.91	1.00

The highest value of similarity (1) found between S.C10 and T.W.C360 hybrids and between S.C128 and each of S.C131, S.C177 and T.W.C324 hybrids and between S.C131 hybrid and S.C177 and T.W.C324 hybrids. While the lowest value (0.63) was found between T.W.C353 and T.W.C386 hybrids. These results showed wide range and high values of similarity contrasting to Roy *et al*., (2015) who found the wide range of similarity (0.22-0.87) but low values; might due to needing to increase the number of SSR primers to cover the whole genome to appear the accurate variability among related hybrids.

3.4.3. Cluster Analysis:

The dendrogram of fourteen studied hybrids constructed according to The Nei & Li's similarity matrix (Figure 2). The studied hybrids were divided into five main clusters (A, B, C, D, and E). Cluster (A) had three hybrids; separated into two sub clusters; one of them had PIONEER3444 hybrid only. The second sub cluster had T.W.C.353 and S.C.176. Second Cluster (B) had two hybrids; T.W.C.321 and S.C.168. Third Cluster (C) had T.W.C.368 only. Fourth Cluster (D) had two sub clusters; S.C.162 hybrid found only in one. While the second sub cluster had T.W.C.324, S.C.177, S.C.131 and S.C.128 hybrids. The fifth cluster (E) had two sub clusters; S.C.167 hybrid in one and the second had T.W.C.360, and S.C.10 hybrids. From these clusters which illustrated the relationship and distance among different hybrids to direct any hybrid for a suitable breeding program. Because of SSRs markers were randomly scattered throughout the genome flanking vital regions might be coding or non-coding regions related with a specific trait (Henry 2001). These results were in harmony with Gazal *et al.*, (2016), Kamara *et al.,* (2020) and Acharjee *et al* (2021) who reported that there was greet correlation between the morphological characters of the landraces and their pattern of clustering based on SSR molecular analysis that was found to be effective in identifying suitable landraces as a promising parent for a future breeding program of maize and rice respectively.

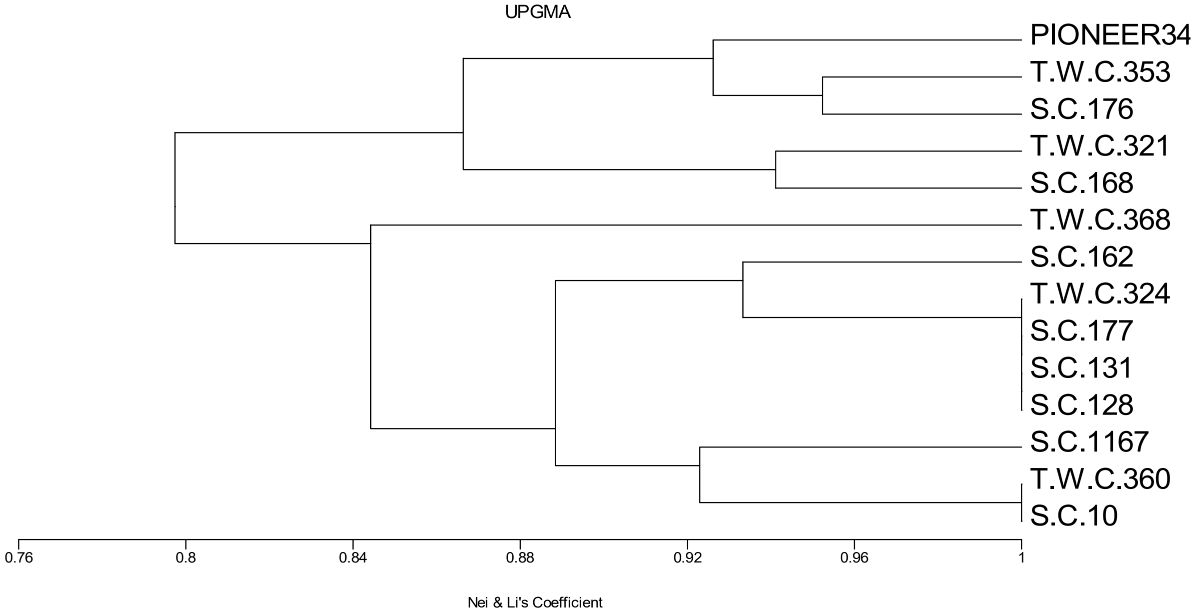


Fig. 2. The dendrogram of fourteen studied hybrids of Maize constructed based on average of genetic distance among them by Unweighted pair group method of arithmetic averages (UPGMA**).**

3.5. Effect of drought stress on maize plant hybrids temperature (Canopy temperature)

The image analysis (Figure 3) was used to determine the differences of the average temperature for each hybrid to differentiate among fourteen maize hybrids under normal and stress conditions. Results were reported differences among normal, stress and hybrids (Table 12). Regarding drought stress, the average canopy temperature increased from 35.8 °C in normal stress to 39.8 °C in drought stress. In general, there was no wide variation in average temperature of canopy in maize hybrids under normal condition. While there was a wide range in temperature of canopy hybrids under drought stress, from 37.1 °C in hybrid S.C 162 to 42.6 °C in hybrid S.C 177. It is worth mentioning that hybrid S.C 162 were higher productive and adaptive under normal and drought stress condition. So, we can say that thermal images were effective for selecting maize hybrids for drought stress based on the absolute canopy temperatures. Similar results had been reported by Stoll and Jones, (2007), Munns *et al.,* (2010) and Walter *et al.,* (2012).

Table 12: Average temperatures (°C) and stress indices for maize hybrids under drought stress.

Hybrids	Normal	Stress	Mean
S.C 10	35.2	41.1	38.2
S.C128	36.3	38.2	37.3
S.C 131	35.8	41.5	38.7
S.C 162	35.8	37.1	36.5
S.C 167	36.2	38.6	37.4
S.C 168	35.6	40.1	37.9
S.C 176	35.1	37.9	36.5
S.C 177	36.2	42.6	39.4
S.C 3444	35.1	40.6	37.9
T.W.C 321	36.1	39.5	37.8
T.W.C 324	35.5	42.1	38.8
T.W.C 353	36.2	38.2	37.2
T.W.C 360	35.5	39.2	37.4
T.W.C 368	35.5	40.8	38.2
Mean	35.7	39.8	

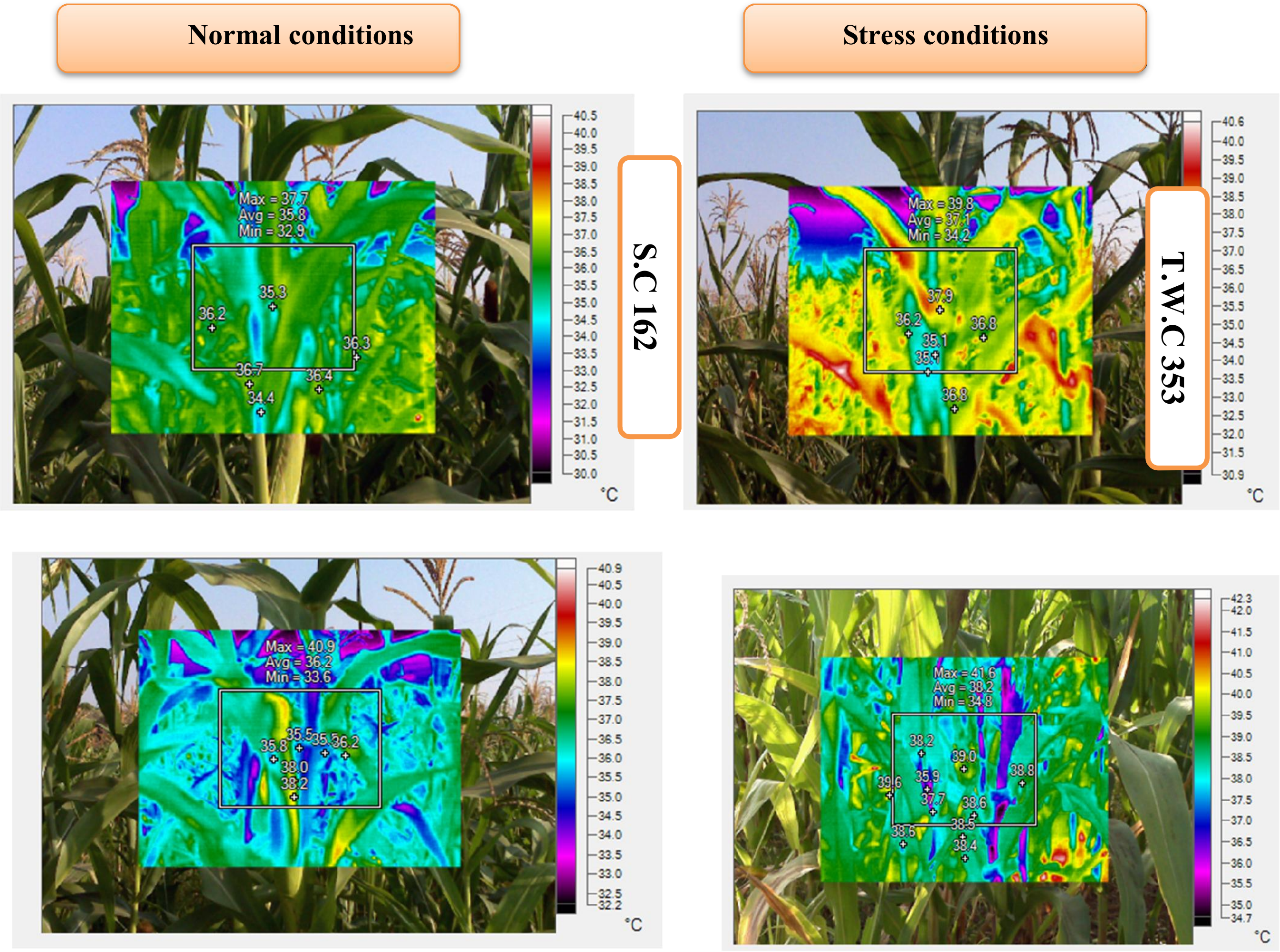


Fig. 3: Example Infrared thermal images for hybrids maize (S.C 162 and T.W.C 353) when analyzing the images under normal and drought stress condition.

4. Conclusion

This study succeeded to assess the extent of genetic variation among 14 hybrids maize grown using different selection methods under normal and drought conditions. The use tolerance indices and SSR markers were effective in the current study to estimate the genetic diversity among fourteen hybrids and distinguished superior hybrids; S.C128, S.C162, S.C167 and S.C176 had high yield and high adaptive to drought tolerance. These single crosses were recommended for commercial production under normal and drought conditions and it can be crossed with other superior hybrids to produce three- or four-way hybrids for potential use. Thermal images were more effective for selecting maize hybrids for drought stress.

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