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RESEARCH PAPER

Evaluation of canopy temperature depression and relative water content as indicators for drought tolerance in maize (*Zea mays* L.) inbreds

N. Islam[1] · G. Ali[1] · M. A. Wani[1] · M. A. Rather[1] · A. Khan[1] · S. Rafiq[1] · B. Kumar[2] · N. Sunil[3] · K. R. Yatish[3] · F. Rasool[1] · S. Naseer[1] · A. A. Lone[1] · B. A. Lone[1] · Z. A. Dar[1]

Abstract: Drought tolerance is a complex trait involving morphological and biochemical mechanisms. Physiological changes due to moisture stress, which reflect an adaptive mechanism in genotype, are worth measuring for relative assessment of differences. Considering this, the present study was conducted to determine the effect of water stress on canopy temperature depression (CTD) and relative water content (RWC) to evaluate drought tolerance of maize inbreeds. Thirty maize inbreeds were evaluated in greenhouse experiment based on completely randomized design with three replications at two irrigation levels (well-watered and low moisture-stressed). Inbreeds CML-470, DMR-N6, CML-415 and LM-12 had cooler canopies under drought conditions. The difference between CTD under irrigated and drought conditions was small initially (1.20) but increased progressively at 2nd week of stress imposition (1.70) and was highest at 3rd week of stress imposition (2.70) in all maize genotypes. High CTD can be used as a selection criterion to improve tolerance to drought and heat. RWC under drought conditions recorded the highest value in DMR-N6 followed by L-9 and V-335. The rate of RWC in plant with high resistance against drought was higher than others. In other words, plant having higher yields under drought stress should have high RWC. Relative water content can be said to be a good parameter suitable to screening drought-tolerant maize genotypes.

Keywords: Canopy temperature depression · Drought tolerance · Maize inbreeds · Relative water content

Introduction

Maize (*Zea mays* L.) is a common staple food in many countries, especially those in the tropics and subtropics. In terms of acreage and production, maize is the third most popular food grain crop in the world. It grows successfully in low-land, tropical, sub-tropical, and temperate climates around the world as a versatile crop (Elias, 1995). Maize is recognized as the "Queen of Cereals" around the world because it has the highest genetic yield potential of all cereals. Maize production is hampered by a number of abiotic stresses causes in various places of the world. Drought is one of the most prominent factors restricting maize output and productivity among them (Araus *et al*., 2002). It is the most significant constraint to maize production potential realization.

Drought stress has a negative impact on maize output around the world (Feller *et al*., 2014; Wu *et al*., 2017). The drought tolerance in maize is a viable solution to the problem of yield loss induced by drought stress. The drought tolerant lines are being developed by breeders and agronomists, and their properties are being studied. As a result, elucidating the processes of maize response to drought stress and adaptability in order to fulfill rising food demands is critical. Drought stress causes plants to exhibit a variety of morphological, physiological, and biochemical

✉ Z. A. Dar: zahoorpbg@gmail.com

[1]AICRP on Maize, Srinagar Centre (SKUAST-K), Srinagar-190025, J&K, India

[2]ICAR–Indian Institute of Maize Research (IIMR), Unit New Delhi-110012, India

[3]ICAR-IIMR, WNC, Hyderabad, India

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responses, including a deep root system, small leaves, and a range of drought-resistance mechanisms, including reduced water loss, and increased its uptake, reduced transpirational losses, and sustained high leaf tissue water potential, as well as higher chlorophyll (Chl) contents and photosynthetic efficiency (Farooq *et al*., 2009, 2012; Liu *et al*., 2015). On the one hand, drought-tolerant lines must improve their roots' ability to absorb water from the soil. The root system of maize can minimize its investigation of the surface soil and grow deeper soil by reducing the number and increasing the volume of each cortical cell, allowing maize to obtain more water from soil (Chimungu *et al*., 2014). Drought tolerance, on the other hand, can be improved by reducing leaf density and managing stomata opening.

Lower leaf or canopy temperatures imply increased potential to absorb soil moisture or sustain a better water status for the plant. The degree to which transpiration cools leaves under a severe environmental load, leading to greater stomatal conductance and net photosynthesis, is shown by canopy temperature in relation to air temperature (Araus *et al*., 2008). Because of the large natural variation in crops and its link with yield under both stress and non-stress circumstances, canopy temperature depression has emerged as a viable substitute (Sofi *et al*., 2019). Relative water content (RWC) properly reflects cell volume and reveals the balance between absorbed and consumed water through transpiration, making it a useful indicator of plant water status in terms of the physiological consequences of cellular water shortage. For carbon fixation, maize uses the NADP-malic enzyme-dependent C4-dicarboxylic acid pathway. The C4 route, on the other hand, offers no special ability to endure low leaf water potential, which was previously utilized as a reliable predictor of drought-tolerant triticale and maize genotypes (Grzesiak *et al*., 2006).

Materials and methods

The present study was conducted at the green house facility of the Division of Genetics and Plant Breeding, Faculty of Agriculture Wadura, SKUAST-K Sopore. Thirty inbreds of maize *viz.,* L-1, L-2, L-9, L-18, L-6, L-10, L-8, HKI- 101, CML-129, HKi-1015-W8, CML-470, L-72, CML-488, CML-167, LM-14, DMR-N6, CML-135, CML-415, LM-12, CML-139, CML-425, CML-286, CML-474, V-338, V-5, V-412, V-351, V-405, V-400, V-335 were evaluated in well-watered and water-deficit conditions.

Canopy temperature was measured using a hand-held infrared thermometer (Fluke 68 Max, Fluke Corporation USA) at five inches from leaf surface inclined at 45 degrees in three stages *viz.*, two-, three- and four-week intervals, after imposition of stress between 10 am-2 pm. Three readings per replications were recorded and averaged out. Canopy temperature depression was calculated as difference of air temperature and canopy temperature.

$$CTD = AT - CT$$

Where,
CTD = Canopy temperature depression
AT = Air temperature
CT = Canopy temperature

Relative water content (RWC) estimates the current hydration of leaf tissue to its maximum potential hydration of each genotype was measured at two weeks after stress imposition from top most fully expanded leaves. Relative water content was measured at noon (between 11.00-1.00 noon). Four leaves were taken from each replication from both water stressed and well-watered treatments and were weighed immediately to obtain their fresh weight. Then the leaves were submerged in distilled water in beakers till saturation for 6 h, after which the turgid weight was measured. Afterwards the leaves were subjected to drying at 60°C for 72 h to determine their dry weights. From these data sets *viz.*, FW, TW and DW, RWC was calculated as follows and expressed as percentage (Barr and Weatherley, 1962).

$$RWC\ (\%) = \frac{FW - DW}{TW - DW} \times 100$$

Where,
RWC = Relative water content
FW = Fresh weight
DW = Dry weight
TW = Turgour weight

Results

Thirty maize inbred lines were evaluated to study the effect on CTD under drought and irrigated conditions in green house conditions and the comparative performance of genotypes for CTD under drought and irrigated conditions is presented in Table 1. Observations were taken at two, three and four weeks after stress (WAS).

Table 1. Canopy temperature depression across three growth stages under irrigated and drought conditions in maize genotypes

Inbreds	Irrigated			Sub Mean	Drought			Sub Mean	Factor Mean		Mean across irrigated conditions	Mean across drought conditions	Change under drought
	CTD2	CTD3	CTD4		CTD2	CTD3	CTD4		Inbreds	Stages	Im	Dm	Im-Dm
L-1	3.60	2.50	1.70	2.60	2.90	0.30	-1.20	0.67	1.63	2.90	2.60	0.67	1.93
L-2	2.60	2.00	1.50	2.00	2.30	0.80	-1.50	0.53	1.27	1.60	2.00	0.53	1.47
L-9	3.20	3.00	2.20	2.80	2.10	0.70	-1.90	0.30	1.55	-0.35	2.80	0.30	2.50
L-18	2.60	1.50	1.30	1.80	1.70	1.10	-1.20	0.53	1.16		1.80	0.53	1.27
L-6	4.00	3.20	2.30	3.20	2.70	-0.40	-2.30	0.00	1.58		3.20	0.00	3.20
L-10	2.70	2.10	0.30	1.70	1.60	0.70	-1.90	0.13	0.91		1.70	0.13	1.57
L-8	4.20	3.00	1.70	3.00	2.90	0.60	-1.40	0.70	1.83		3.00	0.70	2.30
HKI-101	4.02	3.60	1.50	3.00	2.70	0.90	-2.20	0.47	1.74		3.00	0.47	2.53
CML-129	3.30	2.20	0.60	2.00	1.90	1.20	-1.60	0.50	1.26		2.00	0.50	1.50
HKI-1015-W8	2.70	1.90	0.70	1.80	1.60	0.60	-2.30	-0.03	0.87		1.80	-0.03	1.83
CML-470	4.40	3.70	0.80	3.00	3.60	1.70	-1.30	1.33	2.15		3.00	1.33	1.67
L-72	3.80	2.40	0.40	2.20	2.50	0.70	-1.80	0.47	1.33		2.20	0.47	1.73
CML-488	4.40	3.60	1.20	3.10	2.40	1.60	-2.00	0.67	1.87		3.10	0.67	2.43
CML-167	3.30	2.60	1.20	2.40	1.30	0.70	-2.60	-0.20	1.08		2.40	-0.20	2.60
LM-14	2.70	2.30	1.60	2.20	1.80	0.50	-0.60	0.57	1.38		2.20	0.57	1.63
DMR-N6	3.60	2.50	1.20	2.40	2.30	1.50	-0.80	1.00	1.71		2.40	1.00	1.40
CML-135	2.70	2.00	0.50	1.70	1.70	0.70	-2.10	0.10	0.91		1.70	0.10	1.60
CML-415	4.80	2.60	0.30	2.60	2.70	1.00	-1.40	0.77	1.67		2.60	0.77	1.83
LM-12	3.20	2.30	0.70	2.10	2.00	0.50	-0.20	0.77	1.42		2.10	0.77	1.33
CML-139	4.50	3.50	1.20	3.10	2.60	-1.30	-1.70	-0.13	1.47		3.10	-0.13	3.23
CML-425	2.30	1.40	0.50	1.40	1.50	0.70	-2.60	-0.13	0.63		1.40	-0.13	1.53
CML-286	4.80	2.80	0.80	2.80	2.60	0.50	-1.20	0.63	1.71		2.80	0.63	2.17
CML-474	3.20	2.40	0.50	2.00	2.30	0.30	-3.00	-0.13	0.94		2.00	-0.13	2.13
V-338	3.20	2.20	0.30	1.90	2.40	0.60	-2.40	0.20	1.05		1.90	0.20	1.70
CML-451	3.70	2.90	1.00	2.50	2.90	0.20	-1.70	0.47	1.49		2.50	0.47	2.03
V-412	2.40	1.30	0.60	1.40	1.60	0.60	-1.30	0.30	0.86		1.40	0.30	1.10
V-351	4.00	2.80	1.00	2.60	2.80	0.60	-2.40	0.33	1.46		2.60	0.33	2.27
V-405	3.80	2.10	0.70	2.20	2.10	0.40	-0.80	0.57	1.38		2.20	0.57	1.63
V-400	3.70	2.50	0.50	2.20	2.50	0.70	-2.50	0.23	1.22		2.20	0.23	1.97
V-335	2.80	1.50	0.40	1.60	1.60	0.50	-2.00	0.03	0.80		1.60	0.03	1.57
Mean	**3.50**	**2.50**	**1.00**	**2.30**	**2.30**	**0.64**	**-1.70**	**0.39**			**2.30**	**0.39**	**1.92**

CD (p<0.05) Regimes = 0.02; Stages = 0.03; Genotypes = 0.10; Regimes*Stages =0.04; Regimes*Genotypes = 0.15; Stage*Genotypes = 0.18; Regimes*Stages*Genotypes = 0.26

CTD at S1 under irrigated conditions had mean value of 3.50 with highest value recorded in CML-415 and CML-286 (4.80) followed by CML-139 (4.50) and CML-470 and CML-488 (4.40) and was lowest in CML-425 (2.30) while as under drought it had mean value of 2.30 with highest value recorded in CML-470 (3.60) followed by L-1, L-8, CML-451 (2.90) and V-351 (2.80) and was lowest in CML-167 (1.30).

CTD at S2 under irrigated conditions had mean value of 2.50 with highest value recorded in CML-470 (3.70) followed by HKI-101, CML-488 (3.60) and CML-139 (3.50) and was lowest in V-412 (1.30) while as under drought conditions it had mean value of 0.64 with highest value recorded in CML-470 (1.70) followed by CML-488 (1.60) and DMR-N6 (1.50) and was lowest in CML-139 (-1.30).

Table 2. ANOVA for CTD in maize inbreeds lines

Source of variation	DF	CTD
Genotype	29	2.377
Water regime	1	71.983
Stages	2	117.373
Genotype × Regime	29	0.911
Genotype × Stage	58	0.931
Regime × Stage	2	77.378
Genotype × Regime × Stage	58	0.638
Error	360	0.265

Significant at 5% level

CTD at S3 under irrigated conditions had mean value of 1.0 with highest value recorded in L-6 (2.3) followed by L-9 (2.2) and L-1, L-8 (1.7) and was lowest in L-10, CML-415, V-338 (0.3) while as under drought conditions it had mean value of -1.7 with highest value recorded in LM-12 (-0.2) followed by LM-14 (-0.6) and DMR-N6, V-405 (-0.8) and was lowest in CML-167, CML-425 (-2.6).

Analysis of variance for canopy temperature depression across three growth stages under irrigated and drought conditions is presented in Table 2 which shows that mean square due to genotypes was significant for CTD. Thirty maize inbreeds genotypes were evaluated to study the effect on relative water content under irrigated and drought conditions in green house and the comparative performance of genotypes for RWC under irrigated and drought conditions is presented in Table 3. Readings were taken at two weeks after stress (WAS).

Under irrigated conditions, RWC had a mean value of 60.85 with highest value in V-412 (85.7) followed by L-1 (78.5) and CML-135, V-351 (78.4) and lowest in V-405 (25.1) followed by DMR-N6 (42). Under drought conditions it had a mean value of 48.31 with highest value in DMR-N6 (78.2) followed by L-9 (77.7) and V-335 (67.7) and lowest in HKI-1015-W8 (22.8) followed by L-8 and CML-129 (30.2). Per cent reduction under drought was highest in CML-129 (36.6) followed by V-400 (34.6) and V-351 (34.1) and lowest in V-405 (-36.4) followed by DMR-N6 (-36.2).

Analysis of variance for relative water content under irrigated and drought conditions is presented in Table 4 which shows that mean square due to genotypes, water regime and interaction genotype × water regime was significant.

Table 3. Mean performance of maize inbreeds for relative water content

Inbreeds	Relative water content (%)		
	Irrigated	Drought	Percent change
L-1	78.50	54.50	24.00
L-2	72.70	38.70	34.00
L-9	71.90	77.70	-5.80
L-18	60.50	49.40	11.10
L-6	57.20	50.00	7.20
L-10	51.30	33.30	18.00
L-8	54.80	30.20	24.60
HKI-101	62.10	35.40	26.70
CML-129	66.80	30.20	36.60
HKI-1015-W8	52.90	22.80	30.10
CML-470	61.50	50.40	11.10
L-72	60.00	70.60	-10.60
CML-488	68.30	38.70	29.60
CML-167	43.70	65.20	-21.50
LM-14	75.60	43.50	32.10
DMR-N6	42.00	78.20	-36.20
CML-135	78.40	46.60	31.80
CML-415	62.50	42.30	20.20
LM-12	69.80	46.60	23.20
CML-139	48.30	65.50	-17.20
CML-425	61.50	51.50	10.00
CML-286	48.90	31.00	17.90
CML-474	55.50	40.40	15.10
V-338	51.90	48.00	3.90
CML-451	55.50	39.30	16.20
V-412	85.70	600	25.70
V-351	78.40	44.30	34.10
V-405	25.10	61.50	-36.40
V-400	70.40	35.80	34.60
V-335	53.80	67.70	-13.90
Mean	60.85	48.31	12.54

CD (P< 0.05) Genotypes = 1.830
Water regime = 0.473
Genotype × Water regime = 2.588

**Significant at 1% level and * Significant at 5% level

Table 4. Analysis of variance of maize inbreds for relative water content

Source of variation	DF	RWC (%)
Genotype	1	484.413**
Water regime	29	7,077.425**
Genotype × Water regime	29	659.201**
Error	120	2.557

**Significant at 1% level and * Significant at 5% level

Discussion

The current investigation found a significant variation in the CTD of irrigated and stressed maize inbreeds during three weeks. In all maize genotypes, the difference between CTD under irrigated and drought conditions was initially moderate (1.20), but rose gradually during the 2nd week of stress imposition (1.70) and was maximum at the 3rd week of stress imposition (2.70). As CTD represents an entire, integrated physiological response to drought and high temperature, so it can be used to monitor plant water status (Idso, 1982). CTD can be utilized as a selection criterion to improve drought and heat resistance. Talebi (2011) and Guendouz *et al*. 2012 obtained similar results in durum wheat, as did Kumar *et al*. (2016) in chickpea, and Dar *et al*. (2018) in maize.

As a result of deeper roots, cooler canopy temperatures have been linked to enhanced plant availability to water (Lopes and Reynolds, 2010). These researchers discovered that genotypes with cooler canopy temperatures yielded 30 per cent more and had a 40 per cent increase in root dry weight at 60-120 cm. CTD is used in practical applications such as plant response to environmental stress (Howell *et al*., 1986), irrigation scheduling (Wanjura *et al*., 1995; Evett *et al*., 1996), cultivar comparison for water use (Hatfield *et al*., 1987; Pinter *et al*., 1990) and heat (Reynolds *et al*., 1998) and drought tolerance (Reynolds *et al*., 1998) (Royo *et al*., 2002). Drought stressed plants displayed higher canopy temperatures than well-watered plants (Siddique *et al*., 2000). High CTD has been used as a selection criterion to improve tolerance to drought and heat (Amani *et al*., 1996; Ayeneh *et al*., 2002). The genotypes with negative values of CTD suggest that these genotypes are very sensitive to water stress. CTD is used a tool for predicting performance (Reynolds *et al*., 1998; Ayeneh *et al*., 2002). So, in the present evaluation inbreeds like CML-470, DMR-N6, CML-415 and LM-12 depicting low canopy temperature can be considered to be performing better under drought stresses.

V-412 had the highest RWC while V-405 had the lowest in irrigated conditions. Under drought conditions, however, DMR-N6 had the highest RWC value, whereas HKI-1015-W8 had the lowest. Under drought, CML-129 had the highest per cent decline in RWC, whereas V-405 had the lowest. RWC levels are higher in drought-tolerant plants than in others. In other words, plants with high RWC should have higher yields under drought stress. Drought-tolerant maize cultivars can be identified using relative water content as screening criteria. RWC was higher under irrigated conditions than drought, according to Velicevici *et al*. (2012). Leaf RWC is one of the most accurate growth/biochemical markers for determining the severity of stress (Alizade, 2002). The rate of RWC in drought-resistant plants is higher than in other plants. In other words, plants with high RWC should have higher yields under drought stress). According to Ludlow (1990), RWC is an alternative measure of plant water status that reflects metabolic activity in tissues and lethal leaf water status. Chen and colleagues (1990) determined that under water stress, water potential and relative water content dropped, and that relative water content in leaves reduced significantly in drought susceptible varieties compared to resistant varieties. According to Guang-hua Yin *et al*. (2012), relative water content varied significantly depending on soil water content and maize variety, but not with interactions.

RWC values that are higher under well-watered and water-stressed conditions imply that plant tissues can absorb and hold more water than tissues with low RWC values (Rosales *et al*., 2012). As a result, relative water content might be considered a useful measure for identifying drought-tolerant maize genotypes. Drought tolerance may be associated with genotypes that have a high RWC in this study.

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