# Seed Oil Content and Fatty Acids Composition of Maize under Heat and Water Stress

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# ABSTRACT

Water deficiency and high temperature has adverse effect on the economic importance of maize crop. A field experiment was conducted during summer 2012 to assess the variation in oil content and fatty acids synthesis with respect to drought and heat tolerance in eight maize genotypes at a farm in El-Omaid village, El Hamam city, Marsa Matroh government. Relative water content (RWC) and cell membrane thermos-stability (CMT) were measured and oil content and fatty acids composition were also estimated. Water deficit and heat caused a significant reduction in RWC of all genotypes studied in contrary to cell membrane injury which was elevated significantly due to stresses. Analysis of maize seeds showed that the oil content decreased significantly. It was found that genotypes have significant effect on oleic, linoleic and arachidic acids, while water stress affected significantly on palmitic, oleic, linoleic, arachidic and homo-gammalinolenic acids. Furthermore, the interaction between genotypes and water stress had a significant effect on both oleic and linoleic. On the other hand, there were an increasing in both ratios of unsaturated/saturated fatty acids (average increased from 3.15 to 3.86) and oleic/Stearic (average increased from 4.89 to 7.73) revealed that unsaturation of fatty acids increased under both stresses. The results indicated that genotype DT62 and DT190 have oil composition less affected under drought and heat stress and it's recommended to be used in the maize improvement programmers in Egypt.

# INTRODUCTION

Maize (*Zea mays* L.) is the third most important cereals after wheat and rice all over the world as well as in Egypt. It is the major cereal grain used for the commercial production of vegetable oil. The parts of corn kernels are endosperm (82%), germ (embryo and scutellum) (12%). 80 to 84% of total kernel oil is present in the germ region followed by 12% in aleurone and 5% in endosperm (Watson *et al.*, 2003). The amount of germ in the kernel and oil in the germ is genetically controlled and vary widely (Becker, 2007). The typical maize kernel, on dry weight basis is composed from 61-78% of starch, 6-12% of proteins, 3.1-5.7% of oil, 1.0-3.0% of sugar and 1.1-3.9% of ash (Watson *et al.*, 2003).

The typical fatty acids profile of a maize kernel contains 57.9% of linoleic acid, <1% of linolenic acid, 25.2% of oleic acid, 11.6% of palmitic acid and 1.8% of

stearic acid (White *et al.*, 2003). Concerning to fatty acids in maize oil, the unsaturated fatty acid is composed of about 80% of linoleic acid which is an indispensable fatty acid. It has the efficiency to reduce cholesterol content, soften blood vessels, enhance the function of cardiovascular system, prevent and improve the arteriosclerosis (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2011).

It is well known that genetic and environmental factors play an important role in the economic importance of a crop and can affect the oil yield and quality of its seed/grain production (Triboi and Triboi-Blondel, 2002). In addition, the combination of drought and heat stress was found to alter physiological processes such as photosynthesis, accumulation of lipids, and transcript expression (Jiang and Huang, 2001; Rizhsky et al., 2004). Some earlier studies have shown that quality of seed composition depends on the type of cultivar, amount of irrigation used for production (Bellaloui and Mengistu, 2008) and period taken to full maturity of cultivars (Zhang et al., 2006). It has been documented that the ratio of oleic /linoleic acid increases under high temperature during seed maturation and, in opposition, it decreases under lower temperature conditions. At elevated temperatures, with high night temperatures, a marked reduction in the percentage of linoleic acid occurs, apparently due to desaturase enzymes that are essential for the conversion of oleic to linoleic acid. It is now well recognized that reduced yields and altered oil composition occur in sunflower crop that matures under high temperature conditions. Whole ranges of metabolic changes that occur due to abnormal temperature cycle adversely affect the biosynthesis of fatty acids in sunflower (Nagarajan and Nagarajan, 2010).

The relative water content (RWC) stated by Slatyer in 1967 is a useful indicator of the state of water balance of a plant essentially because it expresses the absolute amount of water, which the plant requires to reach artificial full saturation. Thus there are a relationship between RWC and water potential (González *et al.*, 2001). It is defined that decrease of relative water content close stomata and also after blocking of stomata will reduce photosynthesis rate (Cornic, 2000). It is reported that high relative water

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content is a resistant mechanism to drought, and that high relative water content is the result of more osmotic regulation or less elasticity of tissue cell wall (Ritchie *et al.*, 1990). The difference of relative water content (RWC) has been reported in durum wheat as 18.6 and 21.8 percent for most resistant and most sensitive genotypes to drought stress in 2 years of experiment. (Merah, 2001). In studies that performed on 4 cultivars of bread wheat, RWC reduced to 43 percent (from 88% to 45%) by moisture stress (Siddique *et al.*, 2000).

The cell membrane thermostability (CMT) assay is an indirect screening technique for heat tolerance and also provides a reliable measure of tissue tolerance to heat and drought stress. The CMT assay has been successfully used to identify heat tolerant and susceptible genotypes in several crop species among of them cereal crops (Tripathy *et al.*, 2000 and Jamal *et al.*, 2014). Under high temperatures, peroxidation of unsaturated fatty acids in membrane lipids is responsible for cell membrane damage (Cui *et al.*, 2006 and Gomathi and Rakkiyapan, 2011). Results of Kebede *et al.* (2012) study showed that heat stress significantly decreased CMT in the corn plants, suggesting that damage to the cell membranes may have been caused by lipid peroxidation.

There is a very little work has been reported about the effect of drought stress on maize kernel oil composition in different Inbred Lines of maize. So, The present work aims at determining the effects of drought stress on physiological processes and seed oil content and fatty acids composition.

#### MATERIALS AND METHODS

A field experiment was conducted during summer 2012 to assess the variation in oil content and fatty acids synthesis with respect to drought tolerance in maize genotypes at a farm in El-Omaid village, El Hamam city, Marsa Matroh government. The soil of experimental site was loamy sand. The hottest day of 2012 was May 22, with a high temperature of 40°C. The hottest month of 2012 was August with an average daily high temperature of 32°C. The longest warm spell was from June 9 to August 31, constituting 84 consecutive days with warmer than average high temperatures. The field experiment was laid out in a randomized completely block design with 8 genotypes; DT235, DT62, DT190, DT79, DT196, DT61 obtained from International Maize and Wheat Improvement Center (CIMMYT) and INB176 and INB209 obtained from Field Crops Research Institute, Agriculture Research Center, Giza, Egypt and two irrigation treatments with three replications. Treatments details (water stress) used for present study are control with normal irrigation period 2 hours per 2 days (75% field capacity) and stressed 1 hour per 2 days (50% field capacity) at the critical point of growth from 45 to 70 days after sowing (DAS).

# **Relative Water Content (RWC)**

The procedure of measuring Relative Water Content (RWC) was carried out as described by Siddique *et al.*, (2000). Relative Water Content was measured using flag leaves after imposing drought condition (60 DAS). RWC was calculated from the equation of:

#### Cell Membrane Thermo-Stability (CMT)

Cell membrane thermo-stability was estimated following the method purposed by Ibrahim and Quick (2001). Membrane thermo-stability was expressed in percentage units as the reciprocal of relative leakage:

# $CMT = (1 - T1/T2) \times 100$

# %Injury=100-CMT

Where T1 is the conductivity reading after heat treatment and T2 is the conductivity reading after autoclaving.

# Lipid content

Crude fat was determined according to Folch method (Folch, 1957). 1 gm of immature grains 20 days after pollination (DAP) was weighed and used for this purpose. The percentage of the crude fat was calculated using the following equation:

% crude fat = 
$$\frac{w2 - w1}{ws} * 100$$

Where:

W1 = Weight of the empty flask

W2 = Weight of the flask + the sample after the evaporation

Ws = Weight of the dried sample

#### **Oil Fatty Acids Composition**

A Hewlett – Packard 5890 gas – chromatography was used complied with a Hewlett – Packard 5989 quadropole mass spectrometer with electron ionization mode [EI] generated at 70 eV. The ion source and quadropole temperature were 230°C and 150°C, respectively, and the filament emission current was 11 LA. Fatty acids were separated on HPS (5% diphenyl, 95% diethyl poly siloxane JWX scientific, Folsom, CA) and Fused silica capillary column (30 m \* 0.25 mm i.d. \*0.25 µm film). The oven temperature was increased from 70° C at a rate of 2°C / min up to 220°C where it was held for 30 minutes. The on – column injector was heated from 20°C to 245°C and 180°C, the detector temperature was 245°C. Helium was the carrier gas 1.1 ml/min. Electron impact mono spectra were recorded in the 40 – 600 amu range at 15 / interval. Injected volumes were 1  $\mu$ l of concentrated extract. Compounds were identified on the basis of linear relation indices on the column and EI mass spectra from the literature. The amount of resulted fatty acids was expressed as  $\mu$ g nhexanol equivalent/g of fresh weight.

# **RESULTS AND DISCUSSION**

# **Relative Water Content (RWC)**

The results of the mean squares for Relative water content (RWC) character presented in Table (1) showed that there were highly significant differences among the two irrigation treatments and the eight maize inbreds. The interaction "treatment\*inbred" was also highly significant.

The Relative water content (RWC) means for the inbred lines were ranged from 81.36 for DT190 to 43.48 for INB209 (Table 1 and figure 1). The best inbred for the relative water content (RWC) in the stressed treatment was DT190 (71.21) followed by DT62 (62.48). These cultivars were significantly different. Whereas the lowest relative water content for the stressed treatment was obtained from INB209 (43.48) and it was significantly different from INB176 (48.84). According to these results, it can be suggested that DT190, DT62 are relatively resistant to drought, but INB209 and INB176 are sensitive.

Results of Siddique *et al.*, (2000) was in agreement with the results of this study who concluded that, exposure of plants to drought led to noticeable decreases in relative water content (RWC) with concurrent increase in leaf temperature. Results of another studies showed that, identification of resistant and sensitive genotypes was firstly based on the relative water content (RWC) measurement and showed that *Triticum* and *Aegilops* seedlings differing in their response to drought stress at the physiological and molecular levels (Rampino *et al.*, 2006). Moreover results by Li-Ping *et al.* (2006) indicated that drought stress relied on drought intensity and duration, with more severe drought stress creating more serious effects on maize. Compared with well-watered conditions, during the silking and blister stages moderate stress did not significantly change the relative water content (RWC); however, severe stress significantly was decreased (P < 0.01) the leaf RWC.

Chen *et al.*, (2012) evaluated maize inbred lines by selection for drought and heat stress tolerance under field conditions in 2009 and 2010 and identified several inbred lines that showed high tolerance to drought. Tolerant inbred lines (Tx205, C2A554-4, and B76) were able to maintain relatively high leaf relative water content when subjected to drought stress, while sensitive lines (B73 and C273A) showed a rapid reduction in leaf relative water content at very early stage of drought. The tolerant lines also showed significantly greater ability to maintain vegetative growth and alleviate damage to reproductive tissues under drought conditions compared to the sensitive lines.

#### Cell membrane thermostability

Analysis of variance of CMT of maize genotypes under study conditions showed that there are highly significant differences among the two irrigation treatments and the eight maize inbreds. The interaction "treatment\*inbred" was also highly significant. Tables 2 and figure 2 showed that plants under stressed conditions were injured more higher than well-watered plants. The highest values of cell membrane injury under Normal conditions were for INB 176 (46.67) and DT 196 (45.45). Conversely, the lowest injury resulted from plants grown in the same conditions were 29.69 and 35.00 for DT 62 and DT 235 respectively. On the other hand under stress, the highest records for cell membrane injury were 75.63 and 74.07 for inbred lines INB 209 and DT 61 respectively, while the lowest were 44.00 and 45.16 for DT 190 and DT 62 respectively (see figure 2 also).

Table1. Relative water	content of	maize inbred lines	under normal an	d stressed irrigation.
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Inbread line	Irrigation	treatment	Maar
	Normal	Stress	Mean
DT235	55.13*	61.94	58.54
DT62	67.02	62.48	64.75
DT190	81.36	71.21	76.29
DT79	61.60	59.48	60.54
DT196	52.16	50.92	51.54
DT61	52.28	50.49	51.39
INB176	54.00	48.84	51.42
INB209	61.66	43.84	52.75
Mean	60.65	56.15	

L.S.D. <sub>0.05</sub> (Inbred Lines) = 4.69 L.S.D. <sub>0.05</sub> (Irrigation Treatments) = 2.34

L.S.D. <sub>0.05</sub> (Inbred Lines\*Irrigation Treatments) = 6.63 \* Mean of 5 replicates

Turk used T turks	Irrigation	treatment	%	Injury
Inbred Lines	Normal	Stress	Normal	Stressed
DT235	$65.00^{*}$	50.00	35.00	50.00
DT62	70.31	54.84	29.69	45.16
DT190	62.96	56.00	37.04	44.00
DT79	60.87	45.00	39.13	55.00
DT196	54.55	34.48	45.45	65.52
DT61	57.89	25.93	42.11	74.07
INB176	53.33	27.27	46.67	72.73
INB209	56.90	24.37	43.10	75.63

Table 2. Cell membrane thermostability of maize inbred lines under Normal and stressed conditions

L. S.  $D_{.0.05}$  (Inbred Lines) = 2.53 L. S.  $D_{.0.05}$  (Irrigation Treatments) = 1.26

L. S.  $D_{.0.05}$  (Inbred Lines\*Irrigation treatments) = 3.57

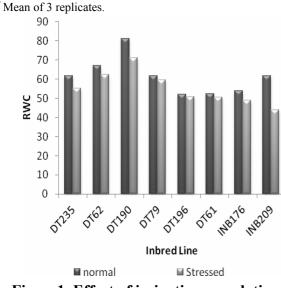
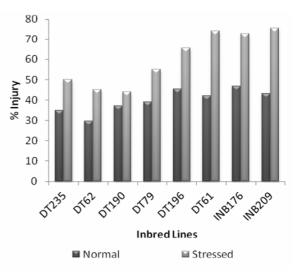


Figure 1. Effect of irrigation on relative water content of eight maize inbred lines

Cell membrane thermostability was reduced because of change in secondary and tertiary structure due to higher electrolyte losses (Wahid *et al.*, 2007), since high temperature increases kinetic energy of molecules across membranes which results in lossening of membranes either because of increase in unsaturated fatty acids or due to protein denaturation (Savchenko *et al.*, 2002).

However, inbred lines DT 190 and DT 62 can be considered as drought tolerant these results were in accordance with Ali *et al.* (2011) who illustrated that a decreasing trend in CMT in maize plants in response to increasing drought levels is due to damage to membrane structure caused by drought stress, which resulted in solutes leakage from cell hence CMT was reduced. Moussa and Abdel-Aziz, (2008) Maize genotypes Giza 2 (drought tolerant) and Trihybrid 321 (drought



# Figure 2. Effect of irrigation on cell membrane thermostability of eight maize inbred lines

sensitive) were sown in the small pots under laboratory condition. The results revealed that tolerant maize genotype; Giza 2 maintained a higher membrane stability index under water-stress conditions.

### **Oil Content and Fatty Acids Composition**

Analysis of variance showed that water deficit and heat stressed and genotype affected both kernel oil content and fatty acids composition in different ways. It was clear that genotypes have significant effect on oleic, linoleic and arachidic acids, while water stress affected significantly on palmitic, oleic, linoleic, arachidic and homo-gamma-linolenic acids. The interaction between genotypes and water stress had a significant effect on both oleic and linoleic as shown in Tables 3 and 4. Oleic and linoleic acids concentrations were the main constituents that were affected by water stress. Neither genotypes nor water stress had any significant effect on stearic acid.

Table 3. Fatty acids composition	of oil	from	eight	maize	inbred	lines	germs	under	normal
and stressed irrigation									

Fatty Acid	Inbred Line (A)		reatment (B)	– Mean (A)	L. S. D. <sub>0.05</sub>
Palmitic	DT235	<u>Normal</u> 14.17 <sup>*</sup>	Stress 12.28	13.23	A = NS
ammuc					A = NS B = 1.18
	DT62	12.19	11.68	11.94 12.31	AB = NS
	DT190 DT79	12.33 14.85	12.29 14.29	12.51 14.57	AD = NS
	DT196	14.80	14.14	14.37	
	DT 190 DT 61	15.03	12.80	13.92	
	INB176	13.83	11.52	12.68	
	INB170 INB209	13.62	10.71	12.08	
	Mean (B)	13.85	12.46	12.17	
Stearic	DT235	3.74	3.56	3.65	A = NS
nearre	DT62	4.66	4.15	4.41	A = NS B = NS
	DT190	5.22	3.62	4.42	AB = NS
	DT190 DT79	3.50	3.10	3.30	AD NO
	DT196	5.45	4.37	4.91	
	DT190 DT61	5.39	4.73	5.06	
	INB176	7.36	5.85	6.61	
	INB170 INB209	6.11	5.14	5.63	
	Mean (B)	5.18	4.32	5.05	
Dleic	DT235	24.00	35.85	29.93	A = 2.36
	DT62	25.35	38.32	31.84	B = 1.18
	DT190	27.11	35.17	31.14	AB = 3.33
	DT79	17.70	18.59	18.15	11D 5.55
	DT196	24.99	28.28	26.64	
	DT61	25.67	32.30	28.99	
	INB176	28.98	38.24	33.61	
	INB209	22.47	35.78	29.13	
	Mean	24.53	32.82	27.15	
Linoleic	DT235	36.49	30.83	33.66	A = 2.36
	DT62	50.58	45.97	48.28	B = 1.18
	DT190	34.80	31.74	33.27	AB = 3.33
	DT79	30.07	31.68	30.88	112 0.00
	DT196	35.59	34.16	34.88	
	DT61	30.84	25.48	28.16	
	INB176	33.09	31.00	32.05	
	INB209	35.22	29.38	32.30	
	Mean (B)	35.84	32.53	52.50	
Arachidic	DT235	0.61	0.47	0.54	A = 0.12
	DT62	0.35	0.35	0.35	B = 0.06
	DT190	0.32	0.21	0.27	AB = NS
	DT79	0.34	0.27	0.31	
	DT196	0.48	0.30	0.39	
	DT61	0.40	0.31	0.37	
	INB176	0.42	0.21	0.32	
	INB209	0.43	0.25	0.34	
	Mean (B)	0.42	0.30	0.01	
Iomo-	DT235	0.22	0.13	0.18	A = NS
Gamma-	DT62	0.37	0.13	0.25	B = 0.06
Linolenic	DT190	0.18	0.12	0.15	AB = NS
	DT79	0.18	0.12	0.15	
	DT196	0.17	0.12	0.16	
	DT61	0.14	0.12	0.13	
	INB176	0.17	0.12	0.15	
			0.10	V.1/	

	Mean	0.20	0.13	
* Mean of 3 replicates	NS = Difference	s are not significan	t at $p = 0.05$	

Table 4. Fat content percentage,	usaturated fatty	acids/saturated fatt	y acids ratio,
oleic/stearic ratio and linoleic/oleic	ratio of eight ma	lize inbred lines unde	er normal and
stressed irrigation			

Inbred L	ine	% Fat content	% Fat content reduction	UFA:SFA	Oleic:Stearic	Linoleic:Oleic
DT235	Ν	1.51	50.99	3.28	6.42	1.52
D1255	S	0.74		4.10	10.07	0.86
DT62	Ν	1.02	6.86	4.44	5.44	2.00
D102	S	0.95		5.22	9.23	1.20
DT190	Ν	1.12	52.68	3.47	5.19	1.28
D1190	S	0.53		4.16	9.72	0.90
DT70	Ν	0.81	8.64	2.57	5.06	1.70
DT79	S	0.74		2.85	6.00	1.70
DT104	Ν	0.77	31.17	2.93	4.59	1.42
DT196 S	S	0.53		3.33	6.47	1.21
	Ν	0.77	12.99	2.72	4.76	1.20
DT61	S	0.67		3.25	6.83	0.79
ND17(	Ν	0.74	9.46	2.88	3.94	1.14
INB176	S	0.67		3.95	6.54	0.81
NID200	Ν	0.95	22.11	2.87	3.68	1.57
INB209	S	0.74		4.05	6.96	0.82

As shown in Table 4, the total oil content in all genotypes subjected to water constraint declined significantly (P < 0.05) as compared to control plants. The lowest genotype in oil content loss was DT62 (6.86%) while DT190 was the most genotype which lost its oil content by about 52.68%. Drought stress significantly reduced the seed oil content of two maize cultivars, however, the more drought induced reduction in seed oil contents was observed in cv.Agaiti-2002 (drought tolerant) though this cultivar had higher seed oil content under non-stress conditions, (Ali et al., 2009). In addition, Zhong et al., (2011) and Orhun and Korkut, (2011) observed a direct relationship between oil content and unsaturated fatty acids (oleic, linoleic and linolenic) in Bermuda grass and maize, but saturated fatty acids (palmitic, stearic) have showed non-significant correlations with oil content.

There is also an increasing in both ratios of unsaturated/saturated fatty acids (average increased from 3.15 to 3.86) and oleic/Stearic (average increased from 4.89 to 7.73) revealed that unsaturation of fatty acids increased under drought stress (table 5). These results are similar to those obtained in a previous work by Flagella *at al.* (2002) and Ali *et al.* (2010) and who showed that contents of oleic acid in the maize kernel oil increased due to water stress with a subsequent decrease in linoleic acid caused an increased oleic/linoleic ratio of kernel oil from drought stressed plants. Also these results are in agreement with those obtained by Bellaloui *et al.* (2013) who indicated that both water stress and high temperature altered seed oil

composition of soybean by increasing oleic acid and decreasing linoleic and linolenic acid concentrations. Severe water stress or high temperature resulted in higher palmitic acid and lower stearic acid.

# CONCLUSION

From the previous results, it can be concluded that drought and heat might be considered as the most visible factors which affected the physiology and oil composition of maize. Oil content, degree of unsaturation and fatty acid composition are the most affected parameters by water deficit and heat. Maize genotypes were differed in their response to drought and heat. DT62 and DT190 from the eight studied genotypes are the less oil composition affected under drought and heat stress and it's recommended to be used in the improvement breeding programmers in Egypt.

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