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Effect of extreme heat stress on leaf temperature, TTC reduction, MTS quantification and yield components in four Mediterranean genotypes of *durum* wheat plants

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Abstract

Algeria has not reached self-sufficiency in durum wheat because of several factors such as abiotic stresses including heat stress which strongly affects durum wheat crops, that's why this work was carried out on four Mediterranean genotypes widely grown in Algeria to study their tolerance to this stress and know whether or not we can continue to cultivate them to go towards this much desired self-sufficiency. An experiment was conducted in greenhouse during the winter and spring seasons of 2016-2017. The experiment consisted of two treatments: control and heat stress (45°C) which was applied in the phytotron. Leaf temperature (LT) measurements were made using infrared thermometer. Membrane thermal stability (MTS) assay was done by measuring conductivity before and after autoclaving samples. Triphenyltetrazolium chloride reduction (TTC) were quantified by incubating samples in TTC solution and measuring optical density of each one at 530nm using a spectrophotometer. Measurements were done on the fifth and sixth leaves. At maturity, grain number per ear (GN/E) and weight of ears per pot (WE/P) were recorded. LT values did not reach 30°C in all genotypes. An average jump of 54,06% was recorded in TTC values under heat stress which correlated positively with MTS values (r= 0,536). A high negative correlation was held statistically between TTC, MTS and yield components. The ANOVA analysis made using XLSTAT shows significant but not fatal effect of heat stress on *durum* wheat plants without difference between genotypes studied supporting the hypothesis that Mediterranean genotypes are tolerant to heat stress conditions.

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Introduction

Numerous stress factors affect plants during their lives (Tas and Tas, 2007). These factors are summed up in biotic and abiotic stress which cause changes in physiological functions of the plants (Lichtenhaler, 1996) and may affect their normal growth, development and reproduction (Robert-Seilaniantz *et al.*, 2010).

High temperature and drought stress are common environmental phenomena encounter by wheat and they are often linked under field conditions (Jones and Bradley, 1992).

The extreme conditions of heat stress affect significantly wheat plant (Khanna-Chopra and Selote 2007, Efeoglu and Terzioglu 2009), it prevents plants from exploiting their full genetic potential and cause membrane disorganization and decreased photosynthetic activity (Dong *et al.,* 2011) by causing injuries to photosystem II and damages in thylakoid membrane (Talukder *et al.,* 2014).

Wheat is the most cereal grown in the world (Balouchi, 2010) it is counted among the big cereal crops (Shewry, 2009) providing with more than half of the calories and proteins of one third of the world population (Rajaram, 2001).

Durum wheat is the main crop for food in the Mediterranean basin, but its production is concentrated in the countries of the WANA (West Asia and north Africa), Canada, Mexico, the USA, Argentina, Ethiopia and Mediterranean Europe.

This production is insufficient to face the increasing demand of the population that's why the main objective of research is to improve *durum* wheat productivity for these abiotic stress (Waines,1994), because it's often grown in arid (Tas and Tas, 2007) and hot regions, which have a high potential impact on crop yield (Barnabás *et al.*, 2008) and causes a variety of plant responses and changes in physiological traits (Balouchi, 2010), the response depends on genotype, length and severity of stress and stage of development (Barnabás *et al.*, 2008).

In this study, the effect of heat stress was carried out experimentally to (i) investigate thermotolerance in four Mediterranean genotypes (ii) check if there is a possible genetic variability between the selected genotypes (iii) study the correlation between LT, TTC, MTS and yield, this by measuring leaf temperature (LT), TTC reduction, MTS, grain number per ear and weight of ears per pot.

Materials and methods

This study was conducted during the winter and spring seasons of 2016-2017. *Durum* wheat seeds (Table 1) were supplied by ITGC (Institut Technique des Grandes Cultures-Station El-khroub Algeria).

The experimentation was carried out in greenhouse. First, we sterilized and did pre-germination of *durum* wheat seeds in Petrie dishes for 48h at room temperature. Then, seedlings were grown in a 2/1 sand/soil mixture under pot-culture conditions, at 25°C and watered with tap water until the 5-6 leaves phase. The experiment consisted in tow treatments: control, by keeping water supply at 25°C and Heat stress, by exposing plants to 45°C for 2 hours/day for 3 days and keeping water supply.

Different plant responses were studied under these treatments. Measurements were done on the fifth and sixth leaves. Three readings were taken for each parameter.

Leaf temperature (LT)

Following the protocol of Silva and *al.*, (2007), leaf temperature was taken on the surface of the attached leaf at a distance of about 2 mm using an infrared thermometer model IL, USA.

The measurements were made respecting the orientation of the leaves with respect to the sun to avoid the shadow effect. The readings were taken from the same leaf side at late morning to early afternoon (Silva and *al.*, 2007).

TTC reduction

TTC reduction was quantified according to the method of Ibrahim and Quick, (2001). Immediately following acclimation, two pieces of 3,5 cm were excised from two leaves from each genotype.

They were rinsed in deionized water then each one was placed in test tube containing 0,1 ml of deionized water. One set of leaves of all genotypes was left at 25°C for 90 min, the second set was placed in a water bath at 49°C for 90 min. following these heat treatments, tissue was incubated for 24h at 25°C in the dark in 10 ml of TTC solution which was added to each leaf in the test tube. After incubation leaves were removed and rinsed in distilled water, placed in separate spectrophotometric tubes containing 2ml of 95% ethanol and left for 24h at 25°C in the dark.

The TTC reduction was quantified using the formula: TTC=(ODh/ODc)*100 where ODh is the mean optical density (530nm) values for the set placed at 49°C for 90 min and ODc is the mean optical density for the set placed at 25°C for 90 min.

MTS test

The cellular membrane thermal stability (MTS) test informs about cellular membrane disruptions caused by heat stress, by estimating the amount of electrolyte leakage from heat stressed tissues using conduct metric technique. In this work, MTS test was done according to the method of Mohammadi and *al.*, (2007) where after acclimation, from each pot three leaves were excised and well washed then placed in test tubes containing 10 ml deionized water.

The control set was left in room temperature while the treatment set wad placed in water bath at 52°C for an hour after what, all tubes were left at room temperature for 24 h then conductivity was measured using a conductivity meter (T1).

All tubes were then autoclaved at 120°C and 0,1MPa for 20 min and left until the next they to measure the conductivity (T2). MTS percentage was calculated using the formula: MTS = (1-T1/T2) * 100.

Yield components

Plants harvested at maturity, and weight of ears per pot and grain number per ear were recorded.

Data of all the parameters were statistically analyzed with XLSTAT computer program. Newman-keuls test was applied, analysis of variance (ANOVA) was carried out and correlation coefficient (r) between different traits was calculated.

Results and discussion

Heat stress effect on LT

It's known that in heat stress conditions, plants with access to water keep their stomates open to reduce their leaf temperature by evapotranspirative cooling (Salvucci and Craft-Brander, 2004). For our LT values, a significant rise is noted under heat stress conditions comparing to the control ($p<0,01$) (Fig. 1).

By comparing genotypes, we find that under both of control or stress conditions the highest LT values are noted in WAHA while the lowest value under control conditions is observed in MBB (16,44°C) but under heat stress CANIZZARA presents the lowest value (20,81°C).Showing the evapotranspiration cooling efficiency, these values have not reached 30°C which was considered by Salvucci and Craft-Brander, (2004) as inhibiting photosynthesis in cotton (Salvucci and Craft-Brander, 2004) and wheat plants (David law and Craft-Brandner, 1999).

Table 1. Studied genotypes chosen for their origins.

Genotype	Origin
Waha	Algeria
Canizzara	Italy
Wahbi	Algeria
MBB	Algeria

Heat stress effect on cells viability and membrane state

Modifications in membrane functions are caused by high temperature (Barnabás *et al.*, 2008). To study this hypothesis TTC and MTS tests are used to quantify acquired thermo tolerance by evaluating the respirational activity, because it informs about cell viability by quantifying the reduction of TTC salt to formazan by dehydrogenase (Fokar *et al.*, 1998), for the MTS, it's used to estimate the amount of electrolyte leakage from stressed tissues (Mohammadi *et al.*, 2007) caused by decrease of the ability of the plasma lemma to retain solutes (Fokar *et al.*, 1998).

Our results showed that TTC values (Fig. 1) under control conditions ranging from 9,87% in WAHA to 14% in WAHBI while under heat stress conditions all the genotypes have almost the same behavior, we observe a jump in the TTC values which range from 60,66% noted in MBB to 75,91% in WAHA. For the MTS, CANIZZARA exhibits the lowest MTS value under control conditions (22,26%) and MBB the highest (44,78%).

Under heat stress conditions WAHA shows the lowest MTS value and WAHBI the highest.

Table 2. Variance analysis for LT, TTC, MT, GN/E and WE/P under control and heat conditions.

Source	LT	TTC	MTS	GN/E	WE/P
Genotypes (G)	2,341 ns	46,954 ns	51,939 ns	3,206 ns	253171,203 ns
Treatments (T)	143,144*	15746,463***	1618,325*	320,589***	65027960,646***
G × T	0,916 ns	110,508 ns	317,919 ns	30,815 ns	2407002,453 ns

ns: not significant; * p<0,01; ** p<0,001; *** p<0,0001.

The previous hypothesis is confirmed by a significant effect of heat stress by an increase in TTC (p<0,0001) and MTS (p<0,01) values under heat stress conditions. A high positive correlation was shown by the correlation coefficient between TTC and MTS held statistically (r = 0,536) (Table 2) which is in agreement with the results of Fokar *et al.* (1998).

Yield components

The weight of ears per pot (WE/P) has significantly decreased in all genotypes under heat stress (p<0,0001). CANIZZARA showed the highest WE/P value under control conditions (7925,56mg) and the lowest under heat stress (2449,15mg) which indicate the highest loss rate of 69,1%. This rate is 34,88% in WAHBI, 41,67% in WAHA and 42,45% in MBB.

Table 3. Correlation matrix for studied parameters.

	GN/E	WE/P	LT	MTS	TTC
GN/E	1				
WE/P	0,977	1			
LT	-0,410	-0,484	1		
MTS	-0,579	-0,555	0,128	1	
TTC	-0,679	-0,754	0,486	0,536	1

The grain number per ear (GN/E) is highly affected by heat stress (p<0,0001) this is in agreement with the results found by Bahlouli *et al.* (2009). A fall of GN/E values is observed in all genotypes. With no significant genotype effect, WAHBI lost 27,77% of his GN/E, WAHA 35%, MBB 26,32% and 69,57%. Our mean of the loss was 58,8% while Pradhan *et al.*, (2012) found this mean 47% in comparison with control conditions. All these results are in Table 3. A high negative correlation is held statistically between TTC, MTS and yield components (Table 3).

No consistent differences were seen according to genotypes. Probably because of the Mediterranean origin of allstudied genotypes, all acquired tolerance to Mediterranean climate.

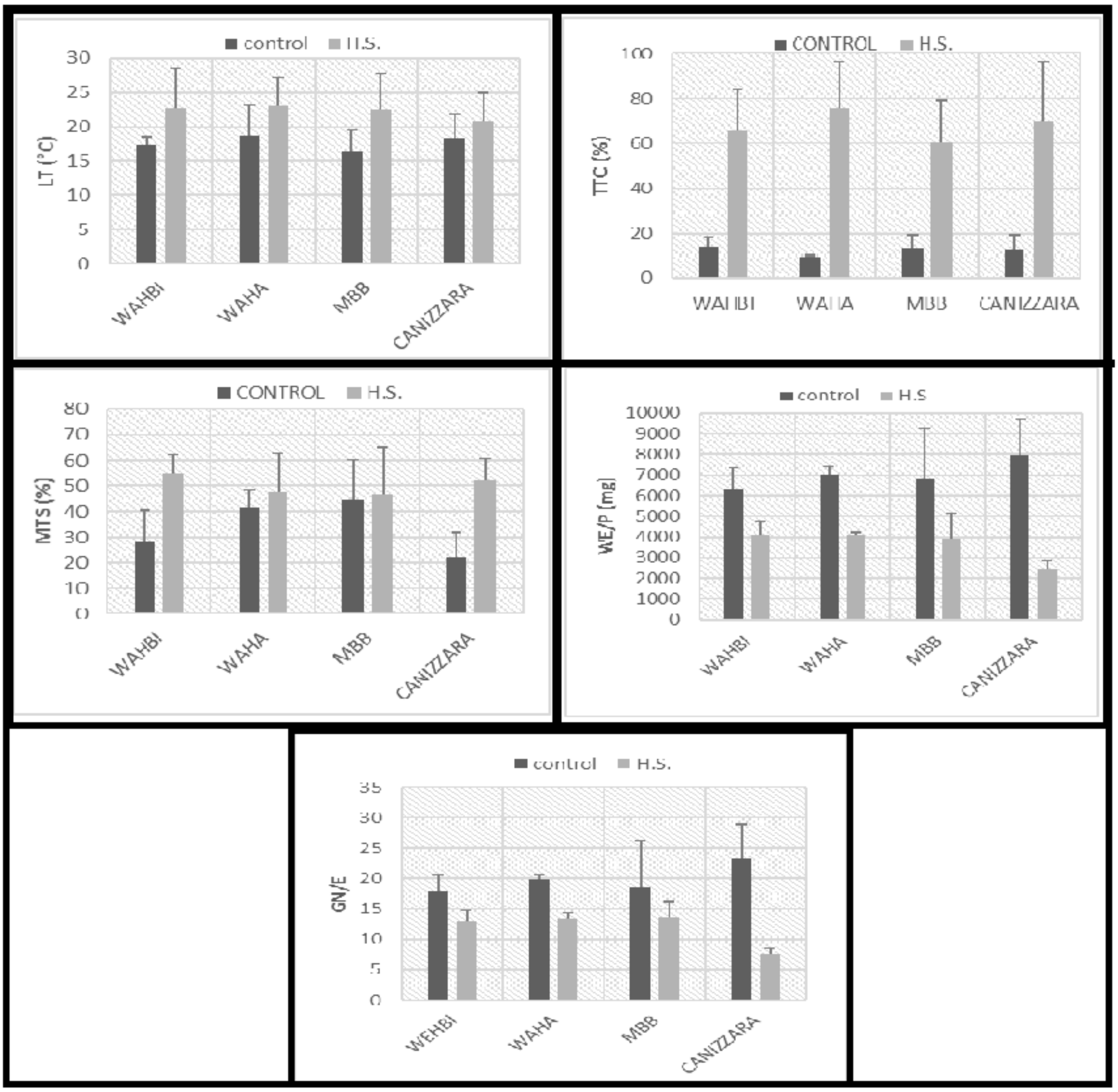

Fig. 1. Effect of heat stress on LT, TTC, MTS, WE/P and GN/E of four *durum* wheat genotypes.

Conclusion

From the obtained results, we can conclude that heat stress affects significantly *durum* wheat plants, it increases LT and membrane permeability, and reduces yield without inhibin photosynthesis and other physiological mechanisms while LT didn't reach the inhibiting values (30°C).

The measured parameters didn't give us an idea on genetic variability, that's why proteomic technics must be used to investigate it. A positive correlation between TTC and MTS, while with yield components TTC and MTS have a negative high correlation. On the basis of these results we can recommend the sowing of Mediterranean genotypes which are more adapted to the climate of the region to increase yields.

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References

Bahlouli F, Bouzerzour H, Benmahammed A. 2009. Etude des mécanismes de stabilité du rendement grain de quelques génotypes de blé dur (triticum durum desf.) Sous climat semi-aride. Annales de la Faculté des Sciences et Sciences de l'Ingénieur **1(3)**, 9-19.

Barnabás B, Jäger K, Fehér A. 2008. The effect of drought and heat stress on reproductive processes in cereals. Plant, Cell and Environment **31,** 11–38. http://dx.doi.org/10.1111/j.1365-3040.2007.01727.x

David Law R, Crafts-Brandner SJ.1999. Inhibition and Acclimation of Photosynthesis to Heat Stress Is Closely Correlated with Activation of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase. Plant Physiology **120**, 173–181.

Dong J, Wang X, Wang K, Wang Z, Gao H. 2012. Isolation and characterization of a gene encoding an ethylene responsive factor protein from Ceratoides arborescens. Molecular biology reports **39(2),** 1349-1357. http://dx.doi.org/10.1007/s11033-011-0869-0

Efeoglu B, Terzioglu S. 2009. Photosynthetic responses of two wheat varieties to high temperature. Eur Asian Journal of Biosciences**3**, 97-106. http://dx.doi.org/10.5053/ejobios.2009.3.0.13.

Fokar M, Nguyen Henry T, Blum A. 1998. Heat tolerance in spring wheat. I. Estimating cellular thermotolerance and its heritability. Euphytica**104,** 1–8.

Ibrahim AMH, Quick JS. 2001.Heritability of heat tolerance in winter and spring wheat. Crop science **41**, 1401-1405.

Jones PD, Bradley RS.1992. Climatic variations over last 500 years. In, climate since A.D. 1500. Ed. R.S. Bradely and P.D. Jones, London, 665 p.

Khanna-Chopra R, Selote Devarshi S. 2007. Acclimation to drought stress generates oxidative stress tolerance in drought-resistant than susceptible wheat cultivar under field conditions. Environmental and Experimental Botany **60**, 276–283.

Mohammadi V, Bihamta MR, Zali AA. 2007. Evaluation of screening techniques for heat tolerance in wheat.Pakistan journal of biological sciences **10(6)**, 887-892.

Pradhan GP, Prasad PV, Vara FAK, Kirkham MB, Gill BS. 2012. Effects of drought and high temperature stress on synthetic hexaploid wheat. Functional Plant Biology **39**, 190–198.

Robert-Seilaniantz A, Bari R, Jones JDG. 2010. A Biotic or Abiotic Stress ? Abiotic Stress Adaptation in Plants: Physiological, Molecular and Genomic Foundation, Ed. A. Pareek, S.K. Sopory, H.J. Bohnert and Govindjee, 103–122 p. http://dx.doi.org/10.1007/978-90-481-3112-9_6

Salvucci ME, Crafts-Brandner SJ. 2004. Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. PhysiologiaPlantarum**120**: 179–186.

Shewry PR. 2009.Wheat. Journal of Experimental Botany **60(6)**, 1537–1553.

Silva MDA, Jifon JL, Da Silva JAG, Sharma V. 2007. Use of physiological parameters as fast tools to screen for drought tolerance in sugarcane.Brazilian Journal of Plant Physiology**19(3),**193-201.

Talukder SK, Babar MA, Vijayalakshmi K, Poland J, Vara Prasad PV, Bowden R, Fritz A. 2014. Mapping QTL for the traits associated with heattolerance in wheat (Triticum aestivum L.). BMC Genetics **15(97),** 1-13. http://dx.doi.org/1471-2156/15/97

Tas S, Tas B. 2007. Some physiological responses of drought stress in wheat genotypes with different ploidity in turkiye. World journal of agricultural sciences **3(2),** 178-183.