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A SIMPLE APPROACH TO SELECT FOR TOLERANCE TO HEAT STRESS DURING GRAIN FILLING IN WINTER WHEAT (*Triticum aestivum* L.)

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ABSTRACT

Heat tolerance is widely considered to become in the future a key trait in breeding wheat for increased yields and yield stability in many parts of the world. Success in breeding for heat tolerance is dependent on the efficiency of methods to expose breeding material to higher temperatures in key phenophases. A simple and not expensive approach, based on covering plants in the field with transparent cellophane paper, was tested during 2017 and 2018 at the National Agricultural Research & Development Institute Fundulea, Romania. The adopted system, described in this paper, increased the temperature under cover as compared with surrounding temperature in open air by +1.74 to +3.69ºC on general average, and by +5.55 to +11.83ºC for the average of daily maximum temperatures. As a result, the weight of 1000 kernels (TKW) was reduced by 9% on average over 15 cultivars and two years of testing, with large differences among the tested cultivars, with reductions ranging from less than 5% to more than 20%, on average over the two years. TKW reduction was significantly correlated with anthesis date (r=0.528* to 0.646). Deviations from the regression describing the relationship between anthesis date and TKW reduction allowed the discrimination of the confounding effect of earliness on the performance of cultivars under increased temperatures stress. The classification of cultivars according to the deviations from the regression corresponded quite well to that expected based on previous information and observations.**

Keywords: wheat, heat, climate change, transparent cover, earliness.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is best adapted to cool growing conditions (Shpiler and Blum, 1986) and optimum temperatures for wheat growth and development have been generally considered to be in the range of 15-25°C (Streck, 2005; Wardlaw et al., 1989). A more detailed study revealed that optimum temperatures increase as development progresses, and averaged across cultivars optimum temperatures were <22, 25 and >25°C, for the periods up to terminal spikelet initiation, from terminal spikelet initiation to heading, and from heading to anthesis, respectively. Cultivars also differed substantially in these parameters (Slafer and Rawson, 1995).

Observed temperatures are often higher than optimum for wheat in different phenophases. In Romania this happens most often during the grain filling phase.

The effects of higher temperatures have been estimated by many authors. For each 1°C rise in temperature above the optimum of 15-20°C, Streck (2005) found that grain filling duration was reduced by 2.8 days, and some data indicate a general reduction in yield per ear of 3-4% for each 1°C rise in temperature above a mean of 15°C (Wardlaw et al., 1989). Under controlled conditions, yields decreased by 3 to 5% per 1°C increase above 15°C were reported, while other authors found that 1°C increase of temperatures reduced yield by 6% (Asseng et al., 2015). If grain filling coincides with high temperatures, grain yield can be reduced by up to 28.3% (Mason et al., 2010). A recent study predicted that wheat yields will decline by 4.1% to 6.4% for each global increase of 1°C due to climate change (Liu et al., 2016).

Semenov and Shewry (2011) predicted by modelling that heat stress, not drought, will increase vulnerability of wheat in Europe,

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while Stratonovitch and Semenov (2015) estimated that heat tolerance in wheat is likely to become in the future a key trait in breeding for increased yields and yield stability in southern Europe.

In Romania, high temperatures quite often affect already wheat yields, as illustrated by the fact that grain weight of some wheat cultivars grown in Fundulea – Romania was much smaller, as compared to published data on the same cultivars grown in England. The reduction of grain weight (16% to 32% depending on cultivar) was explained as being mainly a consequence of higher temperatures during grain filling (Mandea and Săulescu, 2018). These effects of higher than optimum temperatures are expected to be amplified by the forecasted climate changes.

Wheat cultivars capable of maintaining high 1000-kernel weight under heat stress appear to possess higher tolerance to hot environments (Reynolds et al., 1994), and therefore maintaining grain weight under heat stress during grain filling can be considered as a measure of heat tolerance (Tyagi et al., 2003; Singha et al., 2006).

Genetic improvement in performance under heat can be achieved through selection, either directly, for a primary trait (such as grain yield) in a target environment, or indirectly, for a secondary trait related to a higher yield potential and/or to improved behaviour of the crop.

Most approaches for characterising plant response to heat require exposing plants to high temperatures, and several methods, each with advantages and disadvantages, have been proposed. They can be roughly described as:

- methods based on modifying the sowing date, which can in some climates, expose plants to higher temperatures (Khan et al., 2014). This is very convenient for studying many genotypes, even in yield trials, but the environment cannot be precisely controlled, and in winter wheat delaying sowing has a relatively small effect on delaying anthesis and grain filling period.
- methods based on cultivating plants in climate-controlled glasshouses, growth chambers or phytotrons (Yang et al., 2002; Wardlaw et al., 2002; Spiertz et al., 2006). They provide the most exact temperature control, but are most expensive and severely limit the amount of tested material, which reduces its usefulness in breeding. Lack of temperature uniformity in the controlled temperature spaces can also be a problem.
- methods based on enhancing the high temperatures in the field by covering the plants with transparent material. Although this approach depends on solar radiation and does not allow a control of the achieved temperature, it has the advantage of being simple, not expensive and allows large amounts of breeding material to be tested in near natural conditions. The method does not allow increasing night temperature, but these are less frequent in our climate, so we adopted this approach as a method to evaluate the breeding material for response to increased day-time temperature.

This paper presents some results obtained so far by the Wheat breeding team of NARDI Fundulea, using a simple system of enhancing high temperatures in the field, by covering the plants with transparent cellophane paper.

MATERIAL AND METHODS

After testing several solutions, we decided to use supports made of 3 mm thick wire covered by transparent cellophane paper, perforated to facilitate ventilation and avoid problems related to water condensation (the perforation was made with a paper punch). The support had a circular shape (with 25 cm diameter and 40 cm height) and was placed at the level of the spikes by attaching it to a wooden pole, which also ensures a better stability against wind (Figures 1 and 2).

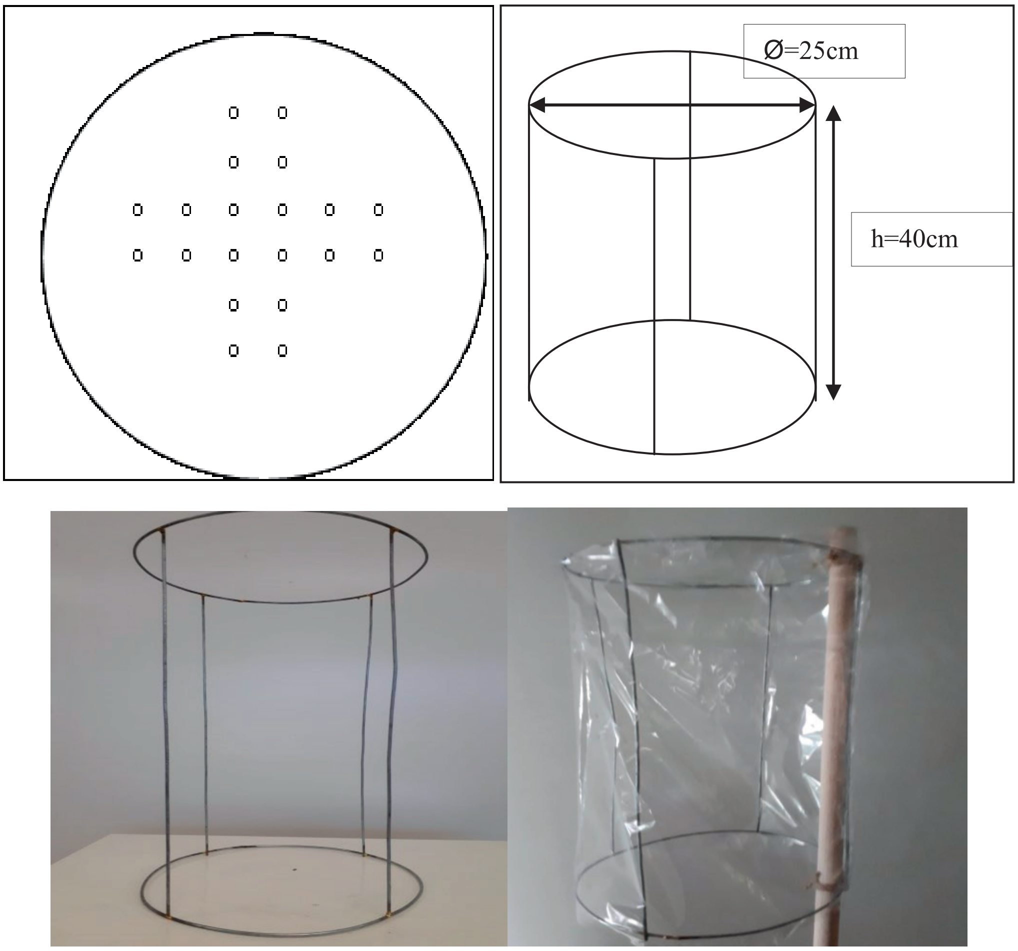

Figure 1. Design of supports and of the transparent cellophane paper perforation

Figure 2. View from the field with installed supports covered with transparent cellophane paper

Temperature was monitored by placing several “Temperature & Humidity Data loggers” (CEM-DT172) at spikes height inside and outside the transparent cover.

The supports with transparent cover were installed 10 days after anthesis, but according to the desired stress intensity, they could be installed earlier or later, and maintained for shorter or longer periods.

At maturity 15 spikes were harvested both from under the transparent cover and from nearby plants left in natural conditions, and weight of grain was measured and divided by the number of grains counted with “Contador seed counter” to calculate TKW.

To estimate the efficiency of the proposed system in evaluating the response to increased temperatures during grain filling, several cultivars were included in the test, namely:

- cultivars mentioned in scientific publications as tolerant to high temperature:
  - Halberd (Mason et al., 2010),
  - Egret (Stone and Nicolas, 1995), for which two seed origins noted (a) and (b) were used,
  - KV RIL 73 – a DH line transgressive for heat tolerance kindly provided by Allan Fritz, selected from the cross Karl/Ventnor (Allan Fritz, personal communication), where Ventnor was the tolerant parent (Talukder et al., 2014);
- cultivars from the NARDI Fundulea breeding program (Izvor, Voinic, Vestitor, 13248G10, G557-2 and G557-6). Vestitor and 13248G10 are lines selected from crosses involving French cultivars, supposedly less tolerant to heat, while G557-2 and G557-6 are lines selected at the Cytogenetics Laboratory of the NARDI Fundulea from a cross involving *A. speltoides.*

Cultivars supposed to have less tolerance to heat, based on their origin from regions with more moderate climate (Capo - from Austria, Rubisko, Apache, Basmati - from France, and Renesansa from Serbia).

RESULTS AND DISCUSSION

Covering the plants with transparent cellophane paper caused increased temperatures as compared with the air temperatures measured in open field. Figure 3 presents the graphs of recorded temperatures measured by data loggers placed under transparent cover and in open air nearby, in 2017 and 2018. One can see that during the day solar radiation induced increased temperatures under cover, while during the night temperatures were practically equal.

On general average, temperatures were higher under cover by +1.74 and +3.69 in 2017 and 2018 respectively, while the average of daily maximum temperatures were higher by +5.55 in 2017 and +11.83 in 2018 (Table 1). Having in mind that the two years when the system was tested were different in cloudiness (data not shown), we anticipate that covering plants with transparent cellophane paper can produce in most years a variable but considerable increase of temperature over the one recorded in the open field.

Table 1. Average temperatures, enhanced by transparent cover and in open field (control)

Year	Treatments			Average of all temperatures			Average of maximum temperatures		
2017	Control			23.18			37.99		
		Enhanced			24.92			42.54	
			Difference			**+1.74**			**+5.55**
2018	Control			23.85			32.09		
		Enhanced			27.54			43.92	
			Difference			**+3.69**			**+11.83**

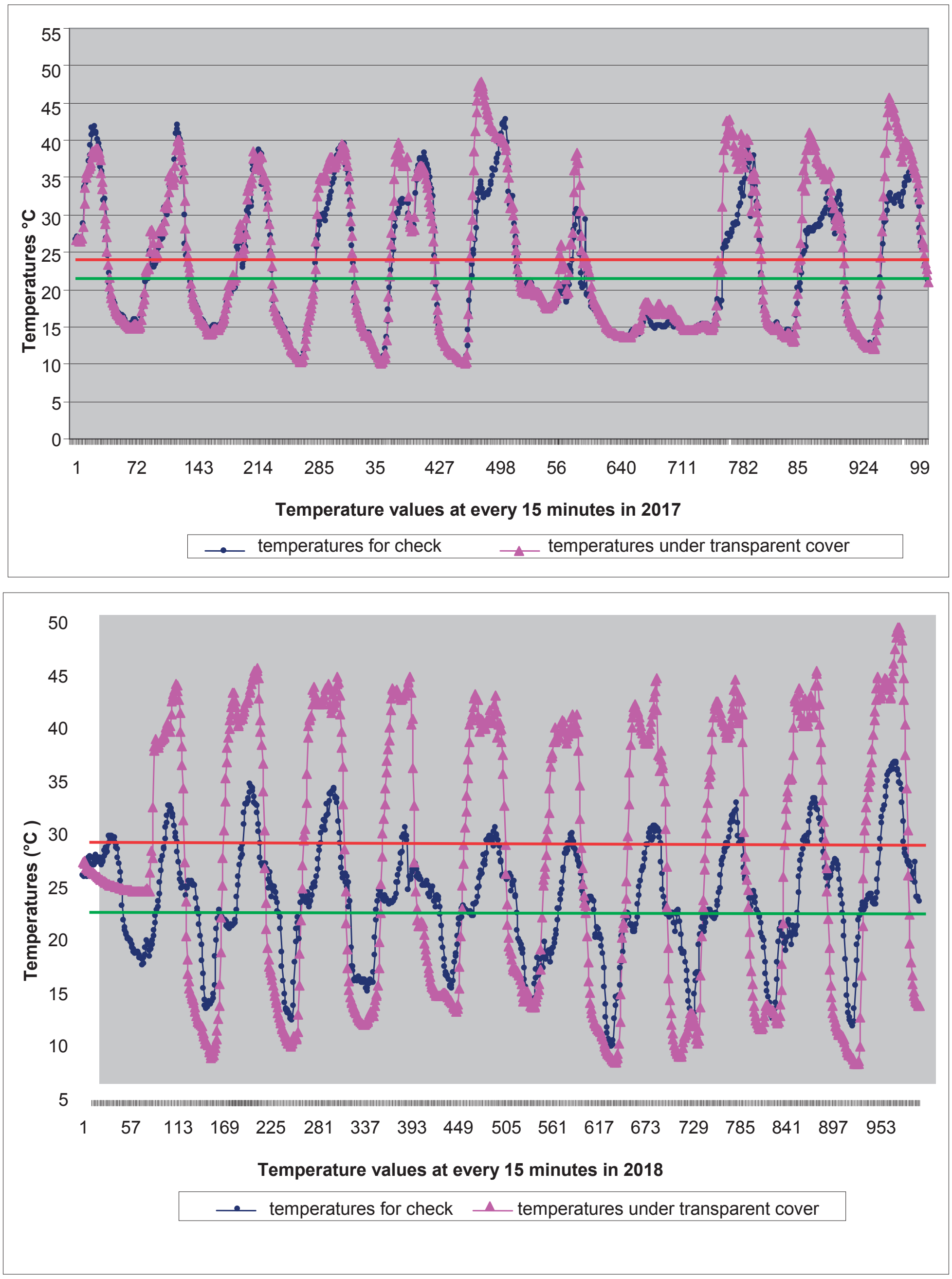

Figure 3. Temperatures recorded by data loggers under transparent cellophane paper cover and in open air in 2017 and 2018

The increased temperatures under cover produced a significant decrease of thousand kernels weight (Table 2). Average TKW of all 15 tested cultivars was reduced by 10% in 2017 and 8% in 2018, with large differences between cultivars (Table 3).

Table 2. ANOVA for the TKW reduction under enhanced heat during grain filling

Source of variation	SS	df	MS	F	F crit
Cultivars	962.88	14	68.78	4.30**	2.48
Years	13.64	1	13.64	0.85	4.60
Error	223.88	14	15.99		
Total	1200.40	29			

Table 3. Percentage difference between TKW of plants exposed to enhanced temperatures and control exposed to natural conditions

No.	Cultivars	2017	2018	Average
1	Halberd	-2.68	2.70	0.01
2	Egret (a)	-0.36	2.67	1.15
3	K-V-RIL 73	0.43	4.44	2.44
4	Egret (b)	4.19	3.95	4.07
5	G557-6	5.92	2.56	4.24
6	13248G10	9.03	9.38	9.20
7	Apache	11.82	6.67	9.24
8	Izvor	12.44	6.33	9.39
9	Voinic	9.55	10.20	9.88
10	Basmati	9.31	12.82	11.06
11	G557-2	10.41	11.90	11.16
12	Renesansa	16.32	10.98	13.65
13	Vestitor	17.60	14.10	15.85
14	Rubisko	24.41	7.50	15.95
15	Capo	19.09	21.05	20.07
Average		10.03	8.48	9.16

In both years, the smallest TKW reductions were recorded in the cultivars already described by other authors as heat tolerant. Australian cultivar Halberd and one of the seed sources of Egret showed no TKW reduction in 2017 and only 2.7% reduction in 2018, while the other seed source of Egret and the line KV RIL 73 showed in both years TKW reductions of less than 5%. This suggests that the simple approach used by us was able to detect the superior performance of cultivars mentioned in other studies as heat tolerant. An average TKW reduction of less than 5% was also recorded in one of the Fundulea lines selected from a cross involving *A. speltoides*.

It is obvious that the inherent plant response to high temperatures during grain filling is always confounded with differences in earliness. Although the cages with plastic cover could be installed at different times, according to the anthesis date of each cultivar, for reasons of convenience we decided to install them concomitantly for all cultivars, to closer mimic what happens naturally. In an attempt to discriminate the confounding effect of differences in earliness, we used regression to correct the data according to anthesis date.

TKW reduction was significantly correlated with the anthesis date in both years and on average (r=0.528* in 2017, r=0.646** in 2018 and r=0.595* on average over both years). Figure 4 shows the relationship between the TKW reduction under enhanced heat averaged over 2017 and 2018 and average anthesis date. On average, the TKW reduction was higher by 1.2% for each day of later flowering.

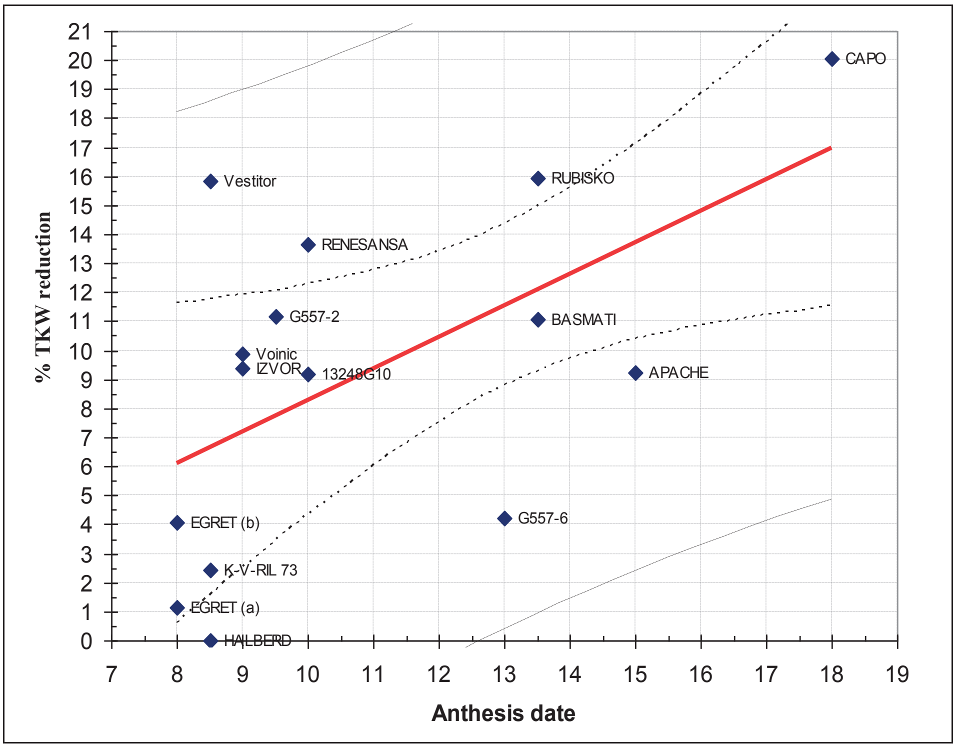

Figure 4. Relationship between average anthesis date and average TKW reduction under enhanced heat (2017-2018)

Although the correlation was significant, several cultivars showed important deviations from the regression (Table 4). All cultivars included in the study based on previous references about their heat tolerance showed negative deviations from the regression on anthesis date, meaning that, besides being among the earliest cultivars included in the study, they had smaller TKW reductions than expected based on the regression.

Table 4. Deviations from the regression between anthesis date and percentage TKW reduction under enhanced heat during grain filling

Cultivar	2017	2018	Average
G557-6	-7.08	-8.53	-7.74
Halberd	-7.47	-2.81	-6.53
Egret (a)	-6.57	-2.84	-4.78
K-V-RIL 73	-7.14	-1.07	-4.10
Apache	-2.09	-2.56	-2.74
Egret (b)	-2.48	-1.56	-1.86
Basmati	-5.50	3.59	-1.52
13248G10	0.55	0.15	0.85
Capo	0.66	2.53	2.05
Izvor	4.87	-1.04	2.25
Voinic	2.88	0.97	2.74
Rubisko	9.60	-1.73	3.37
G557-2	1.93	4.53	3.41
Renesansa	7.84	1.75	5.30
Vestitor	10.03	8.59	9.31

The line G557-6, selected from a cross involving *A. speltoides,* showed the largest negative deviation from the regression, being able to maintain a high TKW after being exposed to high temperature, despite flowering 4-5 days later than the early cultivars.

On the other hand, cultivars like Renesansa, previously noticed by us as showing faster senescence in a year with higher temperatures, Rubisko, a French cultivar, Vestitor, selected from a cross with a French cultivar, and Capo, an Austrian cultivar, showed positive deviations from the regression i.e. higher TKW reductions than expected based on their earliness.

CONCLUSIONS

A system to increase temperatures by covering plants with transparent cellophane paper provided temperatures higher under cover than surrounding air temperatures, by +1.74 and +3.69ºC for the general average, and by +5.55 to +11.83ºC for the average of daily maximum temperatures, in 2017 and 2018 respectively.

As a result, TKW was reduced by 9% on average over 15 cultivars and two years of testing.

Large differences were found among the tested cultivars, in which TKW were reduced from less than 5% to more than 20% on average over the two years.

As expected, the TKW reduction was significantly correlated with anthesis date (r = 0.528* to 0.646**), but several cultivars showed important deviations from the regression describing the relationship between anthesis date and TKW reduction.

Deviations from the regression anthesis – TKW were in agreement with the behaviour of cultivars, as expected based on information from previous studies and observations.

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