

IMPROVED TOLERANCE TO INCREASED TEMPERATURES DURING GRAIN FILLING IN A WINTER WHEAT (*Triticum aestivum* L.) LINE SELECTED FROM A CROSS INVOLVING *Aegilops speltoides* Tausch

Aurel Giura¹, Gabriela Șerban¹, Matilda Ciucă¹, Daniel Cristina^{1,2},
Alina-Gabriela Turcu¹, Nicolae N. Săulescu^{1*}

¹National Agricultural Research and Development Institute Fundulea, 915200 Fundulea, Călărași County, Romania

²University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd,
District 1, Bucharest, Romania

*Corresponding author. E-mail: n.n.saulescu@gmail.com

ABSTRACT

Progress in breeding for heat tolerance could be enhanced by diversification of gene sources for tolerance to high temperatures. At the National Agricultural Research and Development Institute Fundulea, Romania, *Aegilops speltoides* has been used as a source for introduction of more diversity in the winter wheat (*Triticum aestivum* L.) breeding program. Two of the lines selected from a cross Favorit/*Aegilops speltoides*/3*Favorit were tested for response to enhanced high temperatures induced in the field by covering the plants with transparent cellophane paper. Line G557-6 showed a smaller reduction of grain weight, despite flowering 3-4 days later than the heat susceptible sister line and than most other tested cultivars. Molecular analysis identified three markers located on chromosome 2B, which differentiated G557-6 together with the *Aegilops speltoides* parent from its sister line and from the recurrent parent Favorit. To our knowledge this is the first report of heat tolerance gene(s) transfer from *Aegilops speltoides* to hexaploid wheat, and the identified line might contribute to diversification of gene sources in breeding for heat tolerance. Further studies are necessary to indicate which of the three identified markers could be best for marker assisted selection in crosses with this line.

Keywords: Heat tolerance, common wheat, *Aegilops speltoides*, markers.

INTRODUCTION

Many studies underlined the important effects of high temperatures during grain filling on wheat yield. A general reduction in yield per ear of 3-4% was found for each 1°C rise in temperature above a mean of 15°C (Wardlaw et al., 1989a; 1989b). Under controlled conditions, yields decreased by 3 to 5% per 1°C increase above 15°C, 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). Stratonovitch and Semenov (2015) estimated that heat tolerance in wheat is likely to become a key trait in breeding for increased yield potential and yield stability in

southern Europe in the future. The current perspective of increasing global temperature makes faster progress in breeding for heat tolerance even more necessary, and this is dependent on identification of more diverse gene sources. Breeding for reducing the impact of high temperatures can also include optimising earliness (Rezaei et al., 2015), but improving the inherent tolerance to heat can have more general effects.

Besides sources of heat tolerance available in the *Triticum aestivum* gene pool, especially from regions frequently affected by high temperatures (e.g. India, Australia, Egypt etc), related species could provide chances of diversifying the genetic basis of tolerance to heat stress. Wheat lines derived from synthetic hexaploid wheat involving *Aegilops tauschii* showed better heat adaptation and yield under heat stress (Cossani et al., 2015). On the other hand, based on decrease in grain yield, *Ae. speltoides*

Tausch and *Ae. geniculata* Roth were found as the most tolerant of the tested species (Pradhan et al., 2012).

Aegilops speltoides Tausch, is considered the closest relative of the wheat B genome (Luo et al. 2005), and in hybrids between hexaploid wheat and *Ae. speltoides*, the normal mechanism which prevents pairing between homoeologous chromosomes does not operate, thereby allowing recombination to occur not only between the chromosomes of the three wheat genomes, but also with homoeologues from the *Ae. speltoides* genome (Riley, 1966). Therefore gene transfers to cultivated wheat should not be very difficult. Two powdery mildew resistance genes: *Pm12* (Miller, 1988) and *Pm32* (Lapochkina, 1996), are known to be transferred from *Ae. speltoides*. Experiments confirmed that „the powdery mildew resistance present in a soft red winter wheat that was derived from *Ae. speltoides* is conferred by a single gene, and markers were identified that can be used in breeding programs” (Petersen, 2015). A stem rust resistance *Sr47* was transferred to wheat from *Ae. speltoides* and „homoeologous recombination was induced between chromosomes 2B and 2S by backcrossing to a durum substitution line” to reduce the size of *Ae. speltoides* segments (Klindworth, 2012).

Not so many results were reported about the transfer of heat tolerance genes from *Ae. speltoides* to wheat. Awlachev et al. (2016) reported the transfer and mapping of the heat tolerance component traits of *Aegilops speltoides* in tetraploid wheat *Triticum durum*, and established that chlorophyll and stay-green QTLs on chromosome 2B, acquired thermo-tolerance QTL on 1B and stay-green QTL on chromosome 3B were contributed by *Ae. speltoides*. McGowan (2016) reported the generation of *T. aestivum* x *Ae. speltoides* doubled amphiploids for future use in heat tolerance research, but so far no report is known to us about transfer of heat tolerance from *Ae. speltoides* to hexaploid wheat.

MATERIAL AND METHODS

a) Plant material

Ae. speltoides was used at the Cytogenetics Laboratory of the NARDI Fundulea, as donor of foliar disease resistance and stay green. Several lines, including G557-2 and G557-6, were selected from the cross Favorit/*Ae. speltoides*/3*Favorit, where Favorit is an old Romanian winter wheat cultivar selected from the cross Bezostaya1/Odvoş 241, released in 1971. The two lines were selected in F4 based on their meiotic stability, recovery of the wheat plant type of the recurrent parent, and on improved resistance to leaf rust and powdery mildew.

b) Heat tolerance testing

Based on available information about the heat tolerance of *Ae. speltoides* (Pradhan et al., 2012; Awlachev et al., 2016), the lines G557-2 and G557-6 were included in a field test in which day time temperature was enhanced by covering the plants with transparent cellophane paper (Şerban et al., 2019). As maintaining grain weight under heat stress during grain filling is considered a measure of heat tolerance (Tyagi et al., 2003; Singha et al., 2006), 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.

The response to higher temperatures was estimated based on:

- Reduction of TKW under cover as compared with control;
- Heat Susceptibility Index, calculated according to the formula given by Fischer and Maurer (1978):

$$HSI = (1 - YD/YP)/D$$

where:

YD = mean of the genotypes in stress environment;

YP = mean of the genotypes under non-stress environment;

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$D = 1 - (\text{mean YD of all genotypes} / \text{mean YP of all genotypes})$.

c) DNA isolation

Genomic DNA of materials used in this study were extracted from two seeds (except the *Aegilops speltoides* sample from which we cut in small pieces dry spikelets), using the SDS3 method described by Cristina et al. (2017).

d) Molecular assays

In an attempt to estimate the genetic control of different heat tolerance, we analysed the two sister lines, along with the recurrent parent Favorit and the *Aegilops speltoides* sample used as donor, using several markers located on B genome and possibly associated with heat tolerance. Details are given for the three markers, for which polymorphism was detected:

- DNA amplification with **cfa2278** marker was performed in a total volume of 15 μl , including: 1x buffer, 0.4 μM each primer, 0.5 U My Taq Red DNA polymerase (Bioline), 80-100 ng template DNA. PCR conditions: 95°C for 1 min, followed by 40 cycles of 95°C for 15 s, 60°C for 15 s and 72°C for 10 s, subsequently 5 μl of final PCR products (non-denatured) were run on 10% non-denaturing polyacrylamide gel (29:1) at 250V, 17 h (overnight) and 5 μl of final PCR products - denatured were run on 8% polyacrylamide gel (19:1) at 280V, 3 h. The gels were colored using silver staining procedure.

- DNA amplification with functional marker **TaS1** (Zhu et al., 2016) was performed in a total volume of 20 μl , including: 1x buffer DreamTaq Green PCR Master Mix (Thermo Scientific), 0.4 μM each primer, 100-150 ng template DNA. PCR conditions: 95°C for 3 min, followed by 40 cycles of 95°C for 30 s, 56°C for 30 s and 72°C for 1 min, subsequently 5 μl of final PCR products (non-denatured) were run on 10% non-denatured polyacrylamide gel (29:1) at 250V, 17 h (overnight), the gel was colored using silver staining procedure.

- DNA amplification with **Barc167** marker was performed in a total volume of 15 μl , including: 1x buffer, 0.4 μM each primer, 0.5 U My Taq Red DNA polymerase (Bioline), 80-100 ng template DNA. PCR conditions: 95°C for 1 min, followed by 40 cycles of 95°C for 15 s, 50°C for 15 s and 72°C for 10 s, subsequently 12 μl of final PCR products were run on 2% agarose gel, stained with ethidium bromide.

RESULTS AND DISCUSSION

a) Results about heat tolerance

Placing plants under transparent cover induced a temperature increase of +1.74 to +3.69°C for the general average, and of +5.55 to +11.83°C for the average of daily maximum temperatures (Şerban et al., 2019). As a result, TKW was reduced by 5.02 g or about 11% in line G557-2 and only 1.75 g or about 4% in line G557-6, on average over two years of testing (Table 1).

Table 1. Weight of 1000 kernels (g) under natural and enhanced temperatures in two sister lines selected from a cross involving *Ae. speltoides*

Line	Control (open air)		Under transparent cover		Difference under cover – control (g)		Difference under cover – control (%)	
	2017	2018	2017	2018	2017	2018	2017	2018
G557-2	48.5	42.0	43.45	37.0	-5.05	-5.00	-10.41	-11.90
G557-6	42.2	39.0	39.70	38.0	-2.50	-1.00	-5.92	-2.56

Heat Susceptibility Indices calculated on basis of TKW are presented in Table 2.

The superior performance of the line G557-6 as compared with its sister line

G557-2 is remarkable especially because G557-6 flowered 3-4 days later than G557-2, and as such was exposed to higher temperatures, which caused a significant

correlation between TKW reduction and anthesis date (Şerban et al., 2019). In fact, G557-6 showed the largest deviation from the regression of TKW reduction on anthesis

date, being less affected by the enhanced temperatures than predicted by the regression (Şerban et al., 2019).

Table 2. Heat susceptibility indices in two sister lines selected from a cross involving *Ae. speltoides*

Line	Heat susceptibility index		
	2017	2018	Average
G557-2	1.24	1.61	1.42
G557-6	0.71	0.35	0.53

b) Results of molecular analysis

In our molecular studies the markers *cfa2278* and *TaS1* (both with localization on the 2B chromosome) showed polymorphism between parental forms and between the two sister lines (G557-2 and G557-6).

Electrophoretic patterns obtained with *cfa2278* (Figure 1) revealed similar bands in *Ae. speltoides* and G557-6 line (heat tolerant line). The marker *cfa2278* was also described by Bhusal et al. (2017) as associated with longer grain filling duration under heat induced by late sowing, in 397 recombinant inbred lines derived from a cross between HD2808 (heat tolerant) and HUW510 (heat susceptible), this suggests that one or more genes influencing heat tolerance, and more or less similar with genes present in heat tolerant Indian wheat, were transferred from *A. speltoides*

to chromosome 2B of line G557-6.

Similar bands in *Ae. speltoides* and G557-6 line, but different from G557-2 and the recurrent parent Favorit were also obtained with *TaS1* (Figure 2). Considering that *TaS1* marker is functional marker for *Tabas1-B1* gene (Zhu et al., 2016) for 2-Cys peroxiredoxin, *BAS1*, with important role in protecting chlorophyll from abiotic stresses, this result suggests that the heat tolerance of the line G557-6 could be due to the protection of chlorophyll, based on peroxidases.

The molecular analysis with the *barc167* marker showed no PCR product for the samples *Ae. speltoides* and G557-6. This result suggests that for the *barc167* marker there is a different sequence in *Ae. speltoides* and G557-6, highlighting another similarity between *Ae. speltoides* and this line.

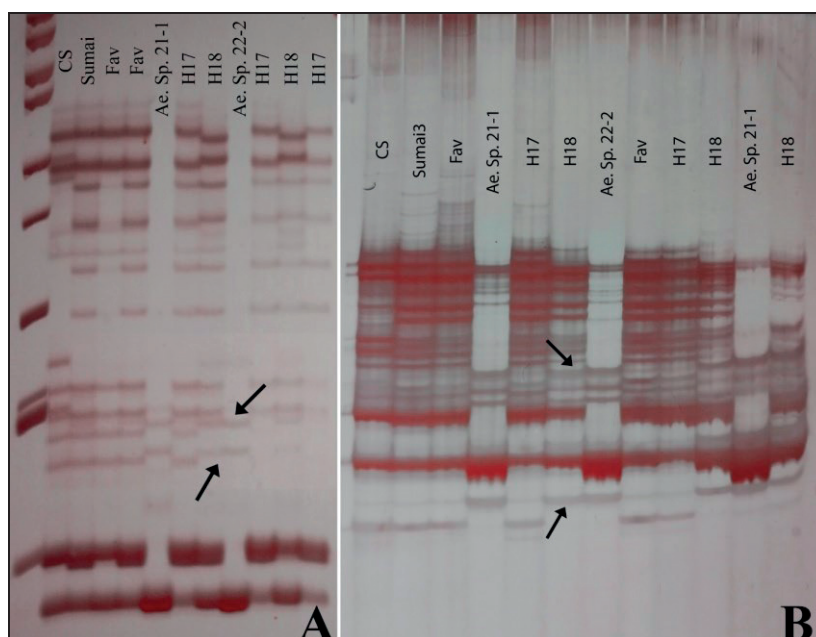


Figure 1. Electrophoretic patterns obtained with *cfa2278* marker

A) Non-denatured products; B) Denatured products.

H17 = G557-2; H18 = G557-6.

[The arrows show the similar DNA bands between G557-6 and *Ae. speltoides*.]

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Figure 2. Electrophoretic patterns obtained with functional marker TaS1
H17 = G557-2; H18= G557-6
[The arrow shows the similar DNA band between G557-6 and *Ae. speltoides*.]

It is interesting to note that Awlachev et al. (2016) also identified in *Triticum durum* progenies from crosses with *Ae. speltoides*, QTLs for chlorophyll and stay-green under heat QTLs chromosome 2B, contributed by *Ae. speltoides*.

CONCLUSIONS

A line G557-6 selected from a cross Favorit/*Aegilops speltoides*//3*Favorit, tested for response to enhanced high temperatures induced in the field by covering the plants with transparent cellophane paper, showed a smaller reduction of grain weight, despite flowering 3-4 days later than a sister line and than most other tested cultivars.

Molecular analysis identified three markers located on chromosome 2B, which differentiated G557-6 together with the *Aegilops speltoides* parent from its heat non-tolerant sister line and from the recurrent parent Favorit.

The identified line might be a contribution to the diversification of gene sources in breeding for heat tolerance. Further studies are necessary to indicate which of the three identified markers could be best for marker assisted selection in crosses with this line.

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