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Exploitation of the *Thinopyrum* genus to enhance durum wheat heat tolerance: chromosomally engineered recombinant lines perform remarkably under stress application at flowering time

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Introduction

Durum wheat (DW) is one of the major staple crops in the Mediterranean basin. In recent decades, climate changes, among which high temperature (T) stress, with ever more severe and frequently occurring day/night extremes, are alarmingly affecting its yield performance. With the aim to improve DW tolerance to heat stress (HS), incorporation of chromosomal segments derived from wild gene pools, naturally adapted to stressful environments, is a promising strategy.

Materials and Methods

Here, four DW-*Thinopyrum* spp. recombinant lines (RLs) were tested for their tolerance to a controlled heat application at anthesis. RLs included two primary types (R112+, R5+), carriers of a differently sized *Th. ponticum* 7el$_1$L segment on DW 7AL arm (28% and 23% of 7AL, respectively), and two corresponding secondary RLs (R69-9/R112+, R69-9/R5+), with a *Th. elongatum* 7EL segment distally inserted ("nested") into the 7el$_1$L ones (**Fig. 1**). Their non-carrier sib lines ("-") and the ICARDA cv. Margherita were included as genetic and heat tolerant control, respectively.

HS started when the main culm of each plant reached anthesis, and lasted for 3 days, with a 37/27°C day/night T regime. A set of unstressed plants remained at the optimum T of 22/18°C.

In order to analyse plant physiological changes during HS progression, chlorophyll content (SPAD) and efficiency (*Fv/Fm*) together with stomatal conductance (SC) were measured on flag leaves (FL) during HS. At the end of the third day, relative water content (RWC, FL-1) and proline accumulation (PRO, FL) were analysed. Finally, yield-related traits were measured at harvesting, at the main culm and whole plant levels (**Fig. 2**).

Fig. 1

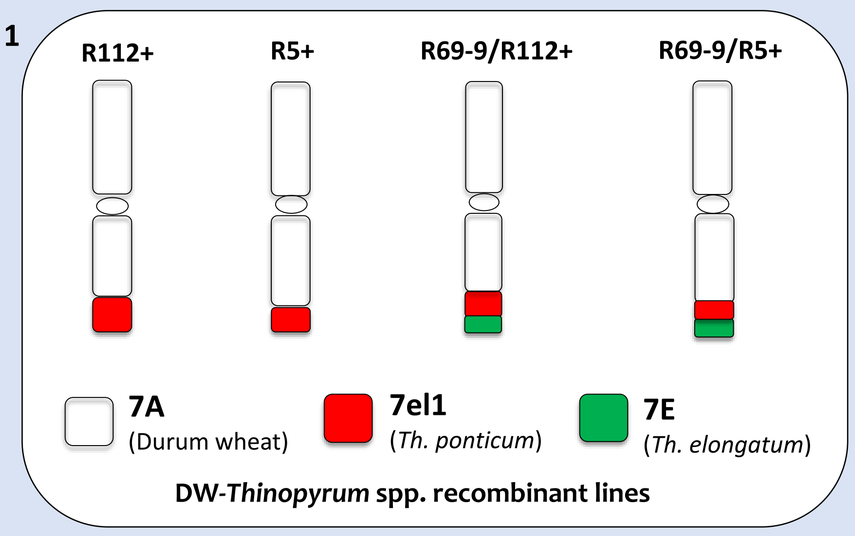
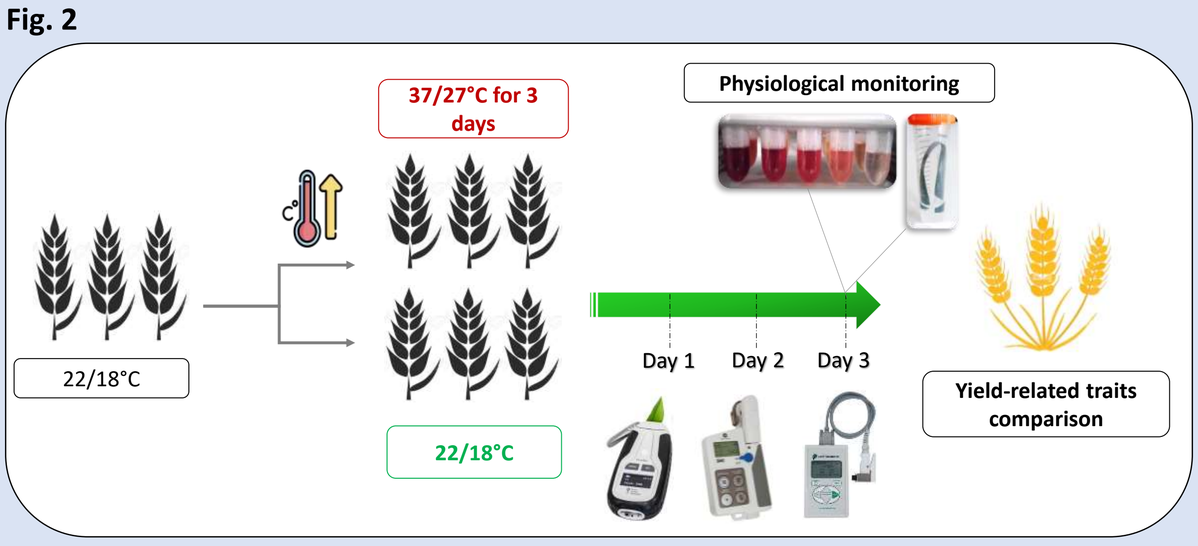


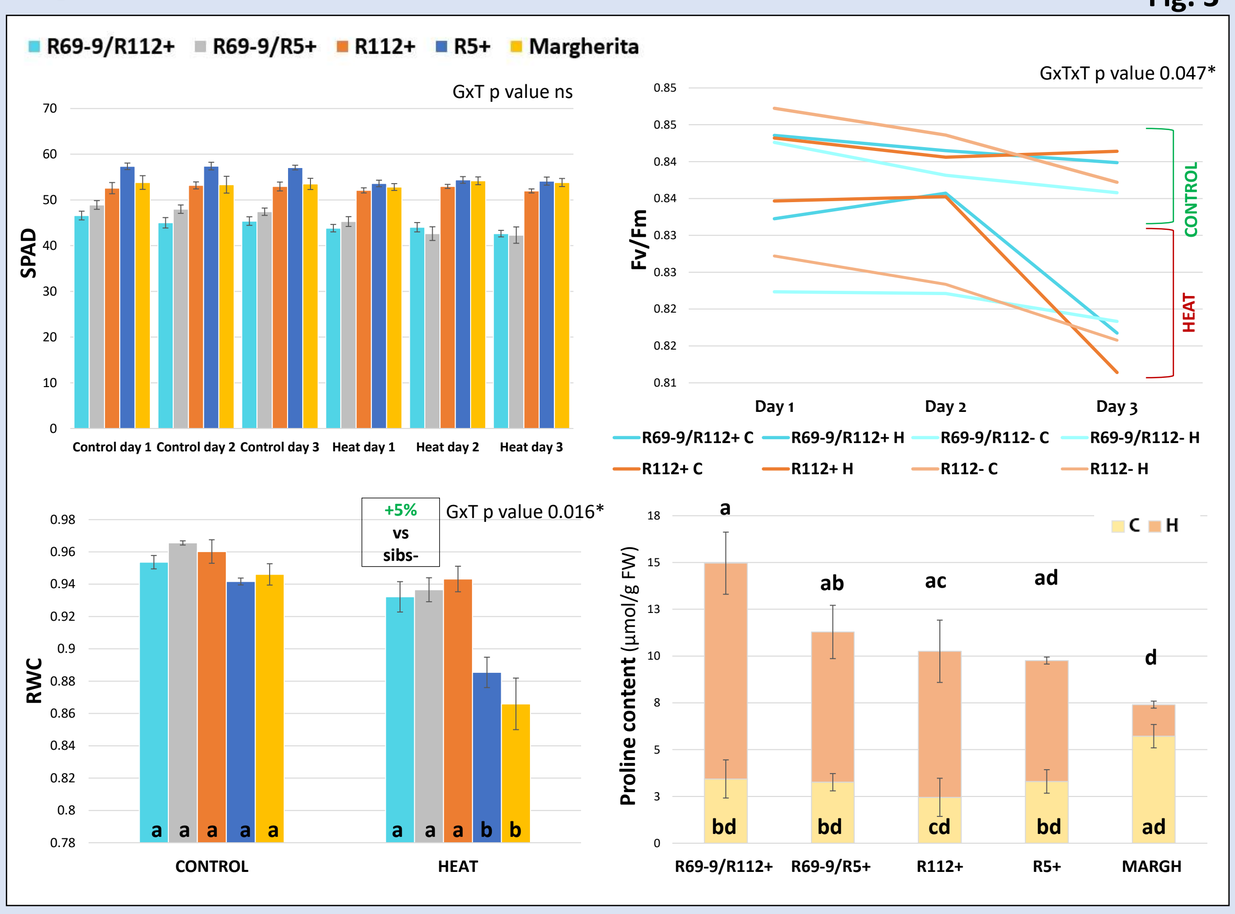
Fig. 2



Results

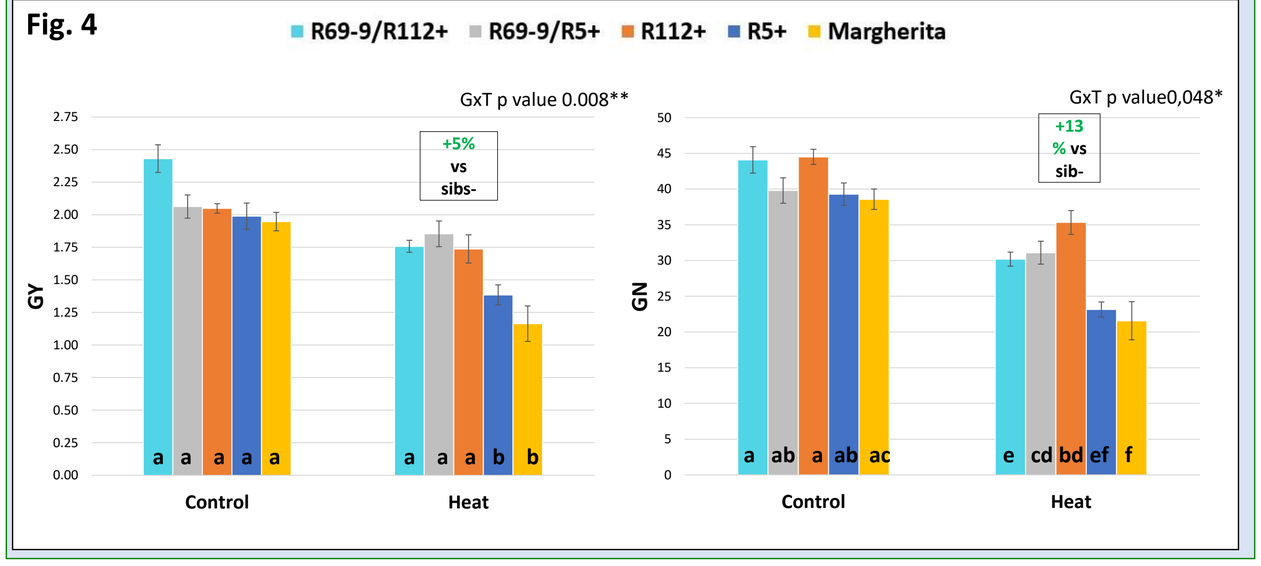
FL physiology was highly modulated by HS and different responses to stress were detected among the genotypes (**Fig. 3**). R112+, R5+ and Margherita showed higher SPAD values in both control and HS conditions, with only minor perturbations during HS progression. As for *Fv/Fm* measurements, R112 types showed a strong stability in photochemical efficiency of PSII during the first 2 days of HS, confirming previous observations on the positive relation existing between the 28%-long 7el$_1$L segment and photosynthetic efficiency under stress. SC reduction stood around 70% for RLs and 85% for Margherita, the latter having exhibited higher SC in control condition. However, after 3 days of HS, all genotypes reached comparable values (*data not shown)*. The best stress-responding genotypes for maintenance of RWC turned out to be R112+, R69-9/R112+ and R69-9/R5+, with both the secondary RLs showing about 5% higher values than their corresponding "-" sibs. All the RLs+ also displayed an increase of PRO content after HS, with R69-9/R112+ accumulating the maximum osmolyte amount. Furthermore, both R112+ and R5+ had a superior PRO content than their "-" sibs (+240% and +110%, respectively), which is indicative of an effect of their *Th. ponticum* segments in PRO accumulation under severe heat conditions.

Fig. 3



Yield-related traits confirmed the results of physiological analyses, showing an overall good performance under HS of R69-9/R112+, R69-9/R5+ and R112+, all being able to maintain good grain yield (GY), mainly due to grain number (GN), of main culm and whole plant (**Fig. 4**). As a whole, the 3 RLs+ yielded on average 50% more than Margherita after HS.

Fig. 4



Conclusions

Targeted chromosomal segments derived from wild species of *Thinopyrum* genus, inserted into DW via chromosome engineering, provide a substantial tolerance to severe heat stress applied at flowering time. Above all, the RLs R112+ (carrying a 28%-long *Th. ponticum* segment), as well as R69-9/R5+ and R69-9/R112+ (*Th. ponticum* + *Th. elongatum*) showed an outstanding yield performance under HS, especially ascribable to grain number preservation. PRO accumulation, RWC and, for R112 types, maintenance of photochemical efficiency of PSII around anthesis, turned out to be the main physiological traits involved in thermotolerance acquisition under HS.