

## **HEAT TOLERANCE RESPONSE OF CHROMOSOMALLY ENGINEERED DURUM WHEAT- THINOPYRUM PONTICUM RECOMBINANT LINES: ANALYSIS OF MORPHO- PHYSIOLOGICAL PARAMETERS, YIELD-RELATED TRAITS AND MIRNA EXPRESSION UNDER CONTROLLED STRESS CONDITIONS**

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Global warming is one of the most threatening challenges for the agricultural sector. In the Mediterranean basin, heat waves and high temperature peaks heavily affect fertility and production of major crops like durum wheat (DW). Introgression of novel genes from wild relatives could be a good strategy to increase DW heat tolerance and yield stability. We tested the response to heat stress (HS) application of DW-Thinopyrum ponticum recombinant lines (RLs), carrying (+) on their 7AL arms a differently sized 7el1L segment transferred from the wild species, besides non-carrier sib lines (-) and the ICARDA cv. Margherita (heat-tolerant control). Comparative analyses highlighted morphological, physiological and molecular differences in HS response associated with presence of the wild segment(s). To identify early tolerance indicators, the reaction of 14 days-old seedlings to 2h HS at 42°C was evaluated. Differently from all other lines, the R112+ RL (28%-long 7el1L segment) had shoot dry biomass unchanged, slightly decreased root biomass and increased relative water content (RWC), whereas Margherita had all shoot-related parameters and RWC heavily penalized, but a minimal decrease of root dry biomass. R112+ was also very efficient in proline accumulation, particularly 24h after HS, when it maintained a high osmolyte concentration, which instead returned to pre-HS values in Margherita and R112-. This suggests the possible presence

of genes/QTLs preventing proline degradation in the R112+ 7el1L segment. The positive behaviour of R112+ was confirmed upon HS application onto adults plants at anthesis, a critical stage for HS effects on yield. Following HS for 3 consecutive days (2h: 22°C to 38°C, 2h: 38°C, 2h: 38°C to 22°C) R112+ revealed remarkable stability of many yield-related parameters, including No. grains/spike, No. grains/plant and grain yield/plant (GYP) in comparison with R112-, the other RLs and Margherita. The latter showed conspicuous reduction of spike and plant fertility traits, though maintaining a fairly good GYP thanks to a high grain weight. Physiological traits (photosynthesis-related, RWC, temperature) indicated a very efficient flag-leaf and hence the presence of genes/mechanisms linked to the R112+ 7el1L segment able to reduce the negative impact of HS. To elucidate the genetic mechanisms underlying the differential reaction to HS associated with the R112+ 7el1L segment, the expression of 7 microRNAs (miRNAs) was quantified in seedlings by droplet digital PCR. The results demonstrate that despite all miRNAs were similarly up/down regulated in R112+ and R112- immediately after the HS, the modulation was significantly lower in R112+. Moreover, R112+ was able to restore physiological miRNA levels in a shorter time than R112-, regaining comparable levels to those of unstressed seedlings 2h after HS.

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