

THE INTRA-GENIC OVEREXPRESSION OF THE TONNEAU1B GENE INCREASES GRAIN LENGTH IN DURUM WHEAT

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Durum wheat (*T. turgidum* ssp. *durum*) is one of the most cultivated crops in the Mediterranean basin, being semolina used as an ingredient for the production of numerous foods typical of the Mediterranean Diet. One of the main objectives of the Agenda 2030 for Sustainable Development is Food Security creating a world free of hunger by 2030. Unfortunately, the number of people going hungry and suffering from food insecurity had been gradually rising between 2014 and the onset of the COVID-19 pandemic. The COVID-19 crisis and the war in Ukraine have pushed those rising rates even higher and have also exacerbated all forms of malnutrition, particularly in children. Although wheat production has increased by over 70 q/ha in the last decades, it is yet not enough for the future needs of a population in constant growth.

Wheat yield is a complex quantitative trait, controlled by numerous genes and by a strong environmental influence. Several studies identified genes associated to yield, such as *Gw2*, *Gw7*, *DEP1* and *GS1*. In this work, we investigated the role of *Tonneau1* (*Ton1*), a gene encoding a protein present in the two isoforms *TON1a* and *TON1b*, which share 85% amino acid identity.

TON1 is a component of the TTP (TON1-TRM-PP2A) protein complex, involved in preprophase band formation and the control of cell division in plants. To date, there are no studies regarding the role of the Ton1 gene in rice and wheat, but the overexpression, by tandem duplication at the Grain Weight 7 (GW7) locus (whose protein product interacts directly with FASS /TON2 and TON1 during the formation of the TTP complex) caused an increase in seed size in the longitudinal direction.

Here an intragenic construct using the CRE-LOX system was used to over-express Ton1b in durum wheat kernel crops. Molecular screening by PCR led to the identification of eight intragenic lines. The main traits related to yield were evaluated. The number of kernels and 100 kernel's weight were increased in the intragenic lines; in contrast, the number of spikelets was decreased compared to the controls (untransformed Svevo and null segregant lines). Further, the evaluation of grain size through SMARTGRAIN software highlighted a significant difference in grain length between the obtained lines and controls, in favour of the first. Finally, gene expression analysis by Real-Time PCR is in progress to evaluate: i) Ton1b transcription level in the intragenic lines compared to WT; ii) the correlation of the transcription level between Ton1b and linked genes (GW2, Ton2, Cen1 and Lf).

This approach allows to obtain intragenic durum wheat lines avoiding alien DNA insertion. The molecular analysis will give a deeper understanding of the molecular role of Ton1b and how its over-expression affects the transcription of related genes.