Improvement of Carotenoid Accumulation in Tomato Fruits Using Forward Genetic and New Breeding Technologies

January 2021

HUNZIKER Johan Francois Jean

Improvement of Carotenoid Accumulation in Tomato Fruits Using Forward Genetic and New Breeding Technologies

A Dissertation Submitted to

the Graduate School of Life and Environmental Sciences,

the University of Tsukuba,

in Partial Fulfilment of the Requirements

for the Degree of Doctor of Philosophy in Agricultural Science

(Doctoral Program in Biosphere Resource Science and Technology)

HUNZIKER Johan Francois Jean

ABSTRACT

Tomato (*Solanum lycopersicum*) is one of the most important crops in the world, as one of the most produced and consumed vegetable. It can be consumed in several forms such as consumed fresh in salad or cooked plate or transformed in purée or soup. It is widely cultivated in the world and appreciated for its health benefit regarding nutrition intake, low calorie and relative facility of culture and adaptation to climate. For this aspect, it has been selected as a plant model for fleshy fruits in Solanaceae family and its genome has been totally sequenced and published in 2012, under an international consortium. Effort on plant breeding are one of the biggest in the world, regarding both basic and applied research, to answer to the request of a world customer.

Even as a plant model, basic research remains slow and costly. Screening of mutant population is necessary for isolating new QTL, which represent time, place, and human cost. Improvement of recent sequencing technology, could help in the improvement of time cost, such as the evolution from a TILLING platform for single gene target, to a system of NGS sequencing, providing the possibility to perform a wide range screening of several genes with only a single sequencing.

However, the insertion of candidate alleles in the elite lines remains slow and costly. Introgression of a mutation by crossing involved the development of analytic platform and places for growing segregant population, and the necessity to insert several traits such as resistance and improvement of yield or metabolite accumulation. However, the development of genome engineering technology such as CRISPR-Cas9 system and the derivative base editing technologies one like Target-AID are an answer for the

improvement of breeding programs. As knockout of genes are not possible systematically, base editing programs such as the Target-AID remain interesting.

In the Chapter 2, we could prove the high efficiency of the Target-AID system to improve the carotenoid accumulation in our edited lines, by getting simultaneously three targets edited in a single generation: *SlDDB1*, *SlDET1* and *SlCYC-B*. It resulted in an efficiency of substitution in the targets selected over than 50%, to almost 99% for some lines. New mutations created resulted in an improvement of total carotenoid content over than 20%, with a phenotype associated on plant sahpe and fruit color. We could so demonstrated the coplementarity of such technology with our EMS mutant bank population for the transfer of several mutations in a single line within a single generation.

In the Chapter 3, we focused our attention on several allelic versions for each targeted gene and their impact on the plant phenotype. As several alleles were generated, only a single weaker version was deeply analyzed for each target. It resulted in an increase of about 50% in carotenoid accumulation, with a stability T_3 generations. Individual mutation effect was evaluated by a generation and observation of the BC_1F_3 population. Plant phenotype was affected by each mutation, with a dwarfism, photomorphism and dark green fruit resulting from the mutation of *SlDDB1* and *SlDET1*, and a lycopene accumulation resulting from both three targets. We could observe the addition of effect on the photomorphogenesis and darkness intensity in green and red stage resulting from the mutation in *SlDDB1* and *SlDET1*, never reported previously at this stage with *hp1* and *hp2* double mutants.

However, such technology is a good complement of an EMS mutant population but, not sufficient due to the limitation of potential targets. Isolation of new genes

responsible in carotenoid accumulation remains important in the understanding of this pathway.

In the Chapter 4, the isolation of the *NRT1* mutant candidate gene showed us the necessity of investigating genetic resources available with mutant banks. The description of a new mutant can help us in the deeper description of the process of fruit ripening and carotenoid accumulation associated with fruit maturation. The isolated candidate mutant showed a putative function of nitrate transporter and/or protein transporter, resulting in an impact on the leaf and the fruit pericarp shape, associated with a change in the β-carotene accumulation, confirmed by complementation experiment. With an accumulation in the *NRT1* mutant almost twice higher compared to the WT, without affecting the lycopene content, such mutant showed a strong interest in an agronomic point of view, as plant were not affect regarding dwarfism. The accumulation of β-carotene without impact on lycopene pool could be confirmed with an overexpression of both lycopene-β-cyclase genes and *SlPSY1*, a bottleneck gene in the carotenoid pathway. As the lycopene amount remained the same, correlated to a *SlCRTISO* expression unchanged, a draw from the xanthophyll might explain such phenotype, with a compensation of lycopene resulting from the higher expression of *SlPSY1* and so higher accumulation of phytoene. However, the mechanism still remains unknown and this mutant open the possibility to connect the nitrate metabolism, protein transport, and the accumulation of carotenoid in fruits during the ripening process.