

IDENTIFYING GENE TARGETS FOR THE METABOLIC ENGINEERING OF α -TOMATINE BIOSYNTHESIS IN TOMATO

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α -tomatine, consisting of a tetrasaccharide group attached to the aglycone tomatidine, acts *in planta* as a phytoanticipin against bacteria, fungi and insects. Moreover, it shows interesting pharmacological properties including antibacterial, antifungal, antiviral, antitumoral and anticholesterolemic activities. As concerning the latter property, it is reported that hamsters fed with tomatine exhibited a reduction of plasma LDL cholesterol without changing HDL cholesterol concentration.

Immature tomatoes accumulates tomatine up to 500 mg/kg of fresh fruit weight but this compound is mostly degraded in ripening tomato.

α -tomatine biosynthetic pathway is still weakly understood despite the agrochemical and pharmaceutical importance of this secondary metabolite. Since the pathway of steroids is the starting point of tomatine biosynthesis, molecular cloning of cycloartenol synthase (*CAS1*), (S)-adenosyl methyltransferase (*SMT1*), cycloeucalenol cycloisomerase (*CYCI*) genes was pursued in *S. lycopersicum* by homology-based PCR method or EST clones assembly.

In addition to the 2274-bp full-length clone of *CAS1*, an alternative in-frame splice form of the *CAS1* transcript was found. *CAS1- β* lacks 106 nucleotides and the expression patterns of both forms are being studied in different tissues.

Moreover, using α tomatinase gene isolated from *Septoria lycopersici* as the query in a BLAST analysis, we identified in tomato nucleotide database a beta-D-xylosidase (*LeXyl*) gene putatively involved in tomato catabolic pathway of α -tomatine. Expression patterns of *LeXyl* were achieved performing RT-PCR in different tissues of *S. lycopersicum* and in a biotype with high α -tomatine content in ripe fruits. Overexpression and RNAi using modular Gateway® constructs are being carried out for functional analysis of *LeXyl* gene.

Cloning and characterization of main genes involved in α -tomatine pathway pave the way for metabolic engineering approaches of this valuable compound in tomato.