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TOWARDS THE METABOLIC ENGINEERING OF PHYTIC ACID METABOLISM IN SOYBEAN BY THE CRISPR/CAS9 SYSTEM

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Phytic acid (PA) is the major form of phosphorus storage in seeds (up to 85% of total phosphorus) and in other plant organs, and it's involved in the regulation of different cellular processes. From a nutritional point of view, it is considered an antinutritional factor because its presence reduces micronutrient bioavailability from the diet. In recent decades, the development of low phytic acid (lpa) mutants has been an important objective for nutritional seed quality improvement and different lpa mutants have been isolated and characterized so far in different species, mainly cereals and legumes. Different lpa mutations affect phytic acid biosynthetic genes or genes coding for different transporters. In particular, PA reduction could be achieved by modifying inorganic phosphorus (Pi) availability for PA synthesis through mutations in transporters involved in Pi loading and organ/intracellular distribution (SULFATE TRANSPORTER 3;3 - SULTR3;3). The aim of this work is to evaluate the possibility to manipulate phytic acid biosynthesis/accumulation in soybean (Glycine max L.) seeds through precise genome editing techniques based on the CRISPR/Cas9 approach. The application of CRISPR/Cas9-based mutagenesis for knocking out the GmSULTR3;3a (Glyma.07G218900) and GmSULTR3;3b (Glyma.20G017100) genes, two putative soybean sulfate transporters involved in PA biosynthesis, will allow us to increase our knowledge in relation to the accumulation of these compounds and their possible role in plant growth and reproduction.

To this purpose a CRISPR/Cas9 toolkit was developed based on a modified pEn-

chimera Gateway-Entry vector coupled with the pDECas9 vector, which contains the Cas9 and the phosphinothricin acetyltransferase (bar) selectable marker gene that confers resistance to L-phosphinotricin (glufosinate). The modified pEn-chimera carried besides the AtU6-26 promoter and sgRNA backbone also contained a GmU6 promoter and sgRNA backbone in order to allow multiple gRNA editing. The final construct, named pDE109, contained 3 gRNAs (gRNA79, gRNA119, gRNA216) directed towards the Glyma.07G218900 (gRNA79, gRNA119) and Glyma.20G017100 (gRNA79, gRNA216) soybean genes coding for GmSULTR3;3.

The CRISPR/Cas9 construct pDE109 was introduced into EHA105 disarmed A. tumefaciens strain for plant transformation. Experiments of A. tumefaciens-mediated transformation in Glycine max (cv. Jack) were carried out and several independent plant lines were regenerated. The presence of the transgenes in different putative transformants was demonstrated by PCR analyses. Molecular analyses are currently under way in order to identify CRISPR/Cas9-induced mutations in the target GmSULTR3;3 genes. One editing event has been already identified in the Glyma.20G017100 (gRNA79) consisting in a in frame 3bp deletion (nts 367-369) and a synonymous G to A base change at nt 365 of the first exon.