conferenceseries.com SciTechnol

Sun-Hwa Ha, J Plant Physiol Pathol 2017, 5:5
DOI: 10.4172/2329-955X-C1-012

3rd Global Summit on

Plant Science

August 07-09, 2017 | Rome, Italy

The polycistronic expression using non-mammalian viral 2a sequences for carotenoid metabolic engineering in rice plants

Sun-Hwa Ha
Kyung Hee University, South Korea

The β-carotene biofortified rice showing golden color phenotype has been developed via bicistronic expression using a 2A sequence from foot and mouth disease virus (FMDV) that was known for a mammalian pathogenic virus. As a substitute for this FMDV-2A (F2A, 20aa), 2A peptides of non-mammalian virus-origins, T2A (20aa) from Thosea asigna virus and I2A2 (30aa) from Infectious myonecrosis virus, were chosen considering of the reported cleavage efficiency. Their synthetic DNA sequences using rice codon usage were in-frame embedded between two synthetic genes for β-carotene production to generate two recombinant genes, stPTAC (stPsy-T2A-stCrtI) and stPIAC (stPsy-I2A2-stCrtI), respectively. Both transgenic rice plants displayed golden color in seed endosperms. Three independent lines being verified the single copy-insertion of T-DNA were selected for transgene analysis, respectively. Total amounts of carotenoids in stPTAC were an average of 1.66μg/g, which were 2.1-fold higher value than stPIAC seeds (0.81μg/g). Gene expression patterns of stPsy and stCrtI were similarly showed the highest level among stPTAC and the lowest level among stPIAC lines. Immunoblot analysis showed higher expression of proteins in stPTAC than stPIAC lines. None of large recombinant proteins was detected as a linked form of stPSY and stCRTI by T2A or I2A2 unlike F2A, demonsterating both T2A and I2A2 peptides are good system to simultaneously express two genes with better efficiency than a F2A peptide in plant system. In addition, unusually high zeaxanthin content in stPTAC line1 were identified by the integration of T-DNA into 1st intron region of genomic DNA encoding zeaxanthin epoxidase (ZEP). Expression of ZEP gene was completely suppressed in seeds and leaves and its knock-out increased the levels of total carotenoids (2.1-fold) as well as zeaxanthin (13-fold) in β-carotene biofortified stPTAC rice seeds. Therewith, polycistronic expression of carotenogenic genes successfully produced zeaxanthin and astaxanthin.

Biography

Sun-Hwa Ha has her expertise in plant metabolic engineering. Her longterm research interests are pathway engineering of specialized metabolites of importance to human health including terpenoids (carotenoids) and phenolics (anthocyanins), two major colored pigment groups that have quite different physical and chemical properties. To alter plant metabolism by genetic engineering of more than one gene at once, she has successfully built two bicistronic expression systems involving the ribosome skipping 2A sequence from the foot-and-mouth disease virus and the internal ribosome entry site sequence from the crucifer-infecting tobamovirus using Golden Rice phenotype to the biosynthesis of carotenoids in rice endosperm (Ha et al. 2010). This approach has been being improved the use of the synthetic gene and novel non-mammalian viral 2A sequences. Furthermore, polycistronic expression of two, three and four genes has been proved by the de novo synthesis of zeaxanthin and astaxanthin in plant system of rice endosperms. Her system might be effective as tools for multigene stacking in crop biotechnology.

sunhwa@khu.ac.kr

Notes: