OMICS Group Conferences
Accelerating Scientific Discovery

3rd World Congress on
Biotechnology

September 13-15, 2012 Hyderabad International Convention Centre, Hyderabad, India

Designing and rapid screening of SiRNA molecules for delayed fruit ripening in tomato

Geetha Lakshmi M, Dwarkesh Parihar and Paresh Verma
Bioseed Research India Pvt. Ltd, ICRISAT, India

Tomato (Solanum lycopersicum) being the world's second most essential vegetable fruit, 20 percent of the produce is lost due to post-harvest damage. Post-harvest losses can be reduced by development and application of suitable technology for manipulation of fruit ripening at the molecular level, resulting in extended shelf life of climacteric fruits. RNA interference (RNAi) is a post-transcriptional conserved biological response where 21-23 nt short siRNA molecules mediate the suppression of gene expression in a very homology dependent sequence specific manner. Fruit ripening is modulated by expression of several genes, including ACC synthase (ACS) and ACC oxidase (ACO) responsible for ethylene production and polygalacturonase (PG) is involved in the pectic cell wall degradation. We selected several genes for detailed study of inhibition/slow down of fruit ripening process. The presented work is carried out on the design and effect of specific siRNA molecules targeting ACO gene resulting in inhibition/delayed ripening. The siRNA encoding DNA molecules were synthesized and cloned in pGSA1285 vector in sense and antisense orientation with GUS as spacer for the production of double stranded RNA with hairpin. As a convenient alternative for the stable genetic transformation, we developed an efficient longer duration transient expression methodology for the gene silencing studies. The Agrobacterium tumefaciens LBA4404 strain carrying chimeric vector containing the transgenes were used for tomato fruit pericarp injection method for the functional screening and analysis of SiRNA molecules. Inhibition of ripening around the injection site was observed from 3 days post-injection to 30-35 days of incubation at room temperature. The injection site clearly showed inhibition of natural change of color from green to red pigmentation and the effect was sustained till 40-45 days. This novel method of siRNA designing, rapid screening and longer duration transient expression through fruit injection can be used for high-throughput functional analysis of ripening related plant genes.

Biography

Geetha Lakshmi M has completed her M.Sc (Biotechnology) from Bangalore University, India and has 8 years of experience in the area of molecular biology research. Presently she is responsible for molecular biology research project in one of the premier hybrid seed companies of India and also pursuing doctorate research from Jawaharlal Nehru Technological University, Hyderabad, India.

dwarkesh.parihar@shrirambioseed.com