UniTO – PROPOSAL: PhD in Agriculture, Forest and Food Science: Plant Genetics and Breeding

Scientific Project Proposal

Title: CRISPR/Cas9 knock-out of polyphenol oxidase genes for improving the Quality of Eggplant berries (Acronym: CRISQuE)

2(a) State-of-the-art and objectives

Eggplant (Solanum melongena L.) is a crop grown worldwide and ranks third in the genus Solanum after potato and tomato both as regards total production (49M tons, FAOSTAT 2014) and economic importance. China is the world’s leading producer and consumer of eggplant (1-2). Eggplant berries are rich in health-promoting phenolics (3) mostly stored in vacuoles. After cutting phenolics become available to polyphenol oxidase enzymes (PPOs) which catalyze phenolic oxidation and cause browning of fruit flesh to detriment of berry quality for both fresh consumption and industrial transformation. The CRISPR/Cas9 technology has demonstrated high efficiency in the knock-out of genes and it is expected to play a key role in future efforts to improve crop traits. The main objective of the project is to apply the CRISPR/Cas9 system to induce knock-out mutations in PPOs genes, with the goal to limit the browning in eggplant berries. The expected results will enable to: (i) gain a better knowledge of the role of key genes affecting phenolic oxidation, (ii) develop eggplant breeding lines with high content in phenolics, but limited browning.

2(b) Methodology, work plan, team organisation

The prerequisites to address the above mentioned objective are:
(i) An high quality annotated eggplant genome sequence recently developed by an International Consortium, which includes the proponent group (4-5).
(ii) The genome wide identification of PPO genes already tested for expression in eggplant berries.
(iii) The already set up CRISPR/Cas9 based system in eggplant for inducing knock-out of the phytoene desaturase (pds) reporter gene.

Task 1 CRISPR/Cas construct design

CRISPR/Cas constructs for targeting the knock-out of PPO genes will be based on the Golden Braid-gRNA/Cas9 toolbox. This system has been already applied in both tomato (6) and eggplant.

Task 2 Plant stable transformation and regeneration

Leaf and cotyledon explants from in vitro grown eggplants will be infected with Agrobacteria suspension containing CRISPR-Cas binary vector, then transferred to selective medium for callus induction. Regenerated shoots will be transferred on rooting medium.

Task 3 Identification of CRISPR/Cas induced mutation

To test mutation efficiency, genomic DNA will be extracted from leaf tissues and the target region PCR amplified for the presence of mutated fragment estimated with PCR/RE and T7EI assays.

Task 4: Analysis of transformed lines

T0 and T1 mutants will be assayed for content in phenolics (LC-MS), browning intensity (Minolta CM-3600 D spectrophotometer) as well as activity of polyphenol oxydases (spectrophotometer).

The team includes, other than the proponent, three associate professors, two researchers, two technicians. Materials and equipment for the carrying out the planned activities are already available.
References:
5. Barchi et al. 2016. An high quality eggplant genome sequence: a new tool for the analysis of Solanaceae family evolution and for the molecular deciphering of complex traits- 20th EUCARPIA General Congress: Plant Breeding, the Art of Bringing Science to Life. Zurich (Switzerland) 29 Aug – 1 Sep

Contact:

Prof. Sergio Lanteri
DISAFA - Plant Genetics and Breeding
University of Turin
Largo Braccini 2 - 10095 Grugliasco (Torino) - Italy
Tel. +39 011 6708806 - Fax +39 011 2368806
Email: sergio.lanteri@unito.it