Proceedings of the LX SIGA Annual Congress
Catania, Italy – 13/16 September, 2016
ISBN **978-88-904570-6-7**

Poster Communication Abstract – 3.07

SOMATIC EMBRYOGENESIS IN ZUCCHINI (*CUCURBITA PEPO* L.): *IN VITRO* INDUCTION AND BIOINFORMATIC SEARCH FOR CANDIDATE GENE SEQUENCES INVOLVED IN CELL DIFFERENTIATION

CHIAIESE P., DI DONATO A., PEIRCE S., ERCOLANO M.R., FILIPPONE E.

Department of Agricultural Sciences, University of Naples "Federico II", Via Università 100, 80055 Portici (Italy)

Cucurbita pepo, somatic embryogenesis, regeneration, bioinformatics, candidate genes

The New Biotechnology Techniques, such as genome editing and cisgenesis, are mainly based on *in vitro* plant regeneration from manipulated cells, preferably *via* somatic embryogenesis. However, regeneration is still based on protocols set up on empirical observations. Our research is aiming to establish a reliable regeneration protocol for local zucchini (*Cucurbita pepo* L.) cultivars and to elucidate the genetic bases of this important biological phenomenon in this species. To set up a reliable and efficient regeneration protocol for two *C. pepo* genotypes (cvs. San Pasquale and Bianca di Trieste) we have assayed three types of growth regulators (BAP, at 4.4 or 8.8 μM; Thidiazuron (TDZ), at 2.3 or 4.6 μM; 2,4-D, at 4.5 or 11.3 μM) added to the culture medium (MS salt, 3% sucrose, 0.8% Plant Agar, pH 5.8) and three types of explants: cotyledonary leaf explants from ungerminated seeds or CLEUS; cotyledons or hypocotyls from 10-day old plants. Cultures were incubated at 25°C under 16 h light : 8 h darkness photoperiod. Every 30 days explants were transferred onto fresh medium. Explants treated with BAP or TDZ at all concentrations produced an abundant white callus. However, neither regeneration nor somatic embryogenesis was observed and, after few more weeks of culture, all explants became necrotic. In presence of 2,4-D, explants produced a profuse white callus and differentiated roots. After about 5 months of culture, all calli showed a brown-yellowish colour; however, a few of them showed green structures in their inner part, similar to somatic embryos. They were then placed on fresh medium added with 2,4-D and some embryo-like structures were formed on their surface. Another set of experiments is now in course to verify the production of somatic embryos and/or adventitious shoots and to evaluate the expression of BBM-like and SERK-like genes. Regarding the bioinformatics approach to find out *C. pepo* candidate genes involved into regeneration and/or somatic embryogenesis, an in house bioinformatics pipeline was developed with the aim of assessing SERK (Somatic Embryogenesis Receptor Kinase) and BBM (Baby Boom) gene family in zucchini. A BLASTn analysis against *C. pepo* transcripts (cucursbigene.upv.es/db/transcriptome_v3) was conducted in order to identify sequences similar to SERK and BBM proteins. Transcripts found were screened using InterProscan (www.ebi.ac.uk/interpro) for the presence of the SERK and BBM typical protein domains. SERK and BBM transcripts were also mapped on *C. pepo* scaffolds in order to discover *C. pepo* corresponding loci (cucurbigene.upv.es/genome-v3.2/). A subsequent *ab-initio* gene prediction on *C. pepo* candidate scaffolds was performed by Augustus web interface (bioinf.uni-greifswald.de/augustus). SERK and BBM putative proteins were separately aligned using MUSCLE software (www.ebi.ac.uk/Tools/msa/muscle/) with reference sequences. The phylogenetic relationships of aligned proteins were inferred separately with MEGA 6 using the maximum

likelihood method based on the WAG model. The phylogenetic analysis allowed to identify 2 putative SERK and 2 putative BBM *C. pepo* genes.