Proceedings of the LXIV SIGA Annual Congress
Online, 14/16 September, 2021
ISBN: **978-88-944843-2-8**

Oral Communication Abstract – 1.06

TILLING-BY-SEQUENCING AND GENOME EDITING FOR THE FUNCTIONAL VALIDATION OF CANDIDATE DOMESTICATION GENES IN COMMON BEAN (PHASEOLUS VULGARIS L.)

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TILLING-by-sequencing, Phaseolus vulgaris, domestication genes, genome editing, legumes

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume for human consumption providing up to 15% of total daily calories and 36% of total daily protein in parts of Africa and the Americas. As a legume, it also has a role in sustainable agriculture owing to its ability to fix atmospheric nitrogen.

Wild common bean is organized in two geographically isolated and genetically differentiated wild gene pools (Mesoamerican and Andean) that diverged from a common ancestral wild population more than 100,000 years ago. From these wild gene pools, common bean was independently domesticated in Mexico and in South America nearly 8,000 years ago, and these domestication events were followed by local adaptations resulting in landraces with distinct characteristics (Schmutz et al. 2014). Domestication led to morphological changes in seed and leaf sizes, in the growth habit and photoperiod responses, variation in seed coat color and pattern that distinguish culturally adapted classes of beans. This unique example of parallel domestication is the subject of the PARDOM project that, starting from the Phaseolus replicated experiment, aims at understanding common bean genome evolution and adaptation.

In the framework of the PARDOM project, we are developing TILLING-by-sequencing and genome editing technological platforms for the functional validation of candidate domestication genes in common bean.

For the development of the TILLING-by-seq platform, DNA from seeds of a *P. vulgaris* TILLING

population developed in the Mesoamerican genotype BAT93 (Porch et al. 2009; Cominelli et al. 2018) was extracted. A three-dimensional pooling system of 54 pools, each of 96 samples on average, at resolution of a population of 1728 individuals was used for NGS targeted sequencing based on custom capture probes. For the genotyping, a total of 719 genes of interest were chosen, based on the presence of one or more signals of domestication, differential expression between the Andean genotype and Mesoamerican genotype, known involvement in the phenomenon of shattering, seed development and in the cytokinin hormonal pathway. Among these genes, 27 had a complete CDS sequence coverage, whereas for the others the first 1-3 exons were covered, for a total of approximately 491Mb.

The validation of candidate genes for domestication is currently in progress also via forward genetics, following the identification of target regions in coding sequences for genome editing based on CRISPR/Cas9 technology. Fifteen target candidate domestication genes have been selected, based on the presence of one or more signals of domestication. Current editing approach is directed toward MYB26, encoding a transcription factor involved in pod shattering phenotype. Given the challenges posed by common bean transformation (biolistic transgenesis), the genome editing approach is being simultaneously carried out also on soybean (*Glycine max*) homologous genes.