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GENETICS OF DOMESTICATION IN COMMON BEAN (PHASEOLUS VULGARIS L.): AN APPROACH FOR THE ANALYSIS OF CANDIDATE GENES VIA TILLING-BY-SEQUENCING

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According to Charles Darwin, domestication can be thought as a great model to study convergent evolution. Among crops, common bean (*Phaseolus vulgaris* L.) represents a unique example of multiple parallel independent domestications: 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, then independently domesticated in Mexico and in South America nearly 8,000 years ago. These processes resulted in morphological changes (*e.g.* seed and leaf sizes, seed coat color, growth habit, photoperiodic responses) that distinguish culturally adapted classes of beans. In addition to the interest emerging from its domestication history, common bean also carries a pivotal agronomic value: it is one of the most important grain legume for human consumption and, as a legume, it also has a role in sustainable

agriculture owing to its ability to fix atmospheric nitrogen.

To better understand the basis of common bean domestication, we developed - in the framework of the PARDOM project - a TILLING-by-sequencing approach for the identification of candidate domestication genes. DNA was extracted from seeds of 1728 M4 individuals of a EMS-mutagenized population developed in the Mesoamerican genotype BAT93 (Porch et al. 2009; Cominelli et al. 2018). Samples were combined using a 3D pooling system and sequenced by Illumina at High Coverage after enriching target regions using custom capture probes. Probes were designed to cover a total of 719 genes of interest (approx. 491 Mb), 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.

Sequencing data were aligned on the BAT93 reference genome and low-frequency variant calling was performed by combining different algorithms (CRISP, NGSEP, GATK Mutect2, VarScan): 226 variants were identified on 132 candidate genes. Data validation on the same DNA samples was performed via PCR and Sanger re-sequencing, that allowed to confirm 84 (64.6%) variants previously found by NGS. For the functional characterization of candidate genes carrying medium or moderate impact variants (*e.g* null mutations), phenotypical analysis of M5 lines is being carried on. The validation of candidate genes for domestication is currently in progress also via genome editing via CRISPR/Cas9 technology, following the identification of target regions in coding sequences.