Proceedings of the 50th Italian Society of Agricultural Genetics Annual Congress
Ischia, Italy – 10/14 September, 2006
ISBN **88-900622-7-4**

Poster Abstract – D.23

ADVANCES ON THE CHARACTERIZATION OF PHYTIC ACID PATHWAY IN *PHASEOLUS VULGARIS* (L.)

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common bean, IPK1, MIPS, primary metabolism, seed quality

Phytic acid (inositol hexakisphosphate, $InsP_6$) is an ubiquitous component in plant seeds where is regarded as the major storage form of phosphorus. $InsP_6$ is usually considered an antinutrient component since it can form complexes with proteins and minerals, reducing their digestive availability. Indeed, minerals when bound to $InsP_6$ are hardly or not absorbed in the intestine and are largely excreted, resulting in iron and zinc deficiencies, especially in developing countries where food is mainly seed-based. Therefore, there is a strong interest to develop seeds with a reduced content in phytate. However, the manipulation of phytic acid content in the seed requires knowledge on the key enzymes involved in its biosynthetic pathway.

The first step in the phytic acid biosynthesis is the conversion of D-glucose-6P to D-myo-inositol-1P ($InsP_1$) by the isomerase myo-inositol phosphate synthase (MIPS). After this well characterised step several kinases, many of which still need to be identified, catalyse the sequential addition of phosphate units to the $InsP_1$, to produce $InsP_2$, $InsP_3$, $InsP_4$, $InsP_5$ and $InsP_6$. Recently, several papers have reported that in the later steps of *Arabidopsis thaliana* phytic acid pathway at least two kinases are involved: Inositol-(1,3,4,5,6)-pentakisphosphate 2 kinase (Ipk1) and Inositol-(1,4,5) trisphosphate 3- kinase (Ipk2).

Our group is interested in reducing phytic acid content in bean seeds and strategies of forward genetics (Doria et al. 2006; BIC, vol. 49: p.153-154) and reverse genetics by TILLING, have been undertaken. In a previous work we already isolated and characterised the MIPS gene (Fileppi et al. 2004; BIC vol.47: p. 189-190) and now we are focusing on the middle part of the biosynthetic pathway. To this purpose our goal is to identify and isolate in *P. vulgaris* the orthologous of Ipk1 and Ipk2 genes of *A. thaliana.* The proteins coded by these genes have been used as queries against the plant genome and soybean EST databases. After tBLASTx searches, several genomic and cDNA sequences with high similarity to the *Arabidopsis* Ipk1 and Ipk2 sequences were identified. Multiple sequence alignment revealed, among all the putative kinases analysed, three very high conserved regions. Then, several pairs of PCR primers were designed along these conserved regions. PCR amplifications on *P. vulgaris* genomic DNA and seed cDNA were obtained only for Ipk1, which primers produced abundant and specific PCR products of the expected size. Preliminary sequencing data suggests that the amplified products indeed code for Ipk1. Southern blot analysis indicates that probably only one Ipk1 gene is present in the *P. vulgaris* genome, while expression analyses in different bean tissues and during seed development are in progress.