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Molecular Characterization and Construct Designing of Late Phytic Acid Pathway Gene Encoding Inositol Polyphosphate 6-/3-/5- Kinase for Its Ectopic Down Regulation in Developing Soybean Seeds to Generate Low Phytate Grains

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Abstract Phytic acid (PA) is the primary storage form of phosphorus in soybean seeds and a principal factor limiting its bioavailability in both livestock and humans. Concomitant loss of complexed minerals and charged proteins exacerbates the antinutritional impact of phytate and contributes significantly to environmental eutrophication. Thus, reducing the seed phytate level provides an indispensable approach to overcome the nutritional menace associated with soy grain consumption. Inositol polyphosphate 6-/3-/5-kinase (IPK2), a multifunctional kinase catalyzes the formation of inositol pentakisphosphate (IP_5), the immediate substrate of PA biosynthesis. It provides a broad range substrate specificity and is therefore conceived to be the most appropriate target for effective seed phytate reduction. To accomplish the same, we monitored the differential expression profile of *IPK2* gene in the developing seeds of soybean by RT-PCR. The results indicated temporal variations in the expression pattern with the highest transcript levels observed in the 8 to 10 mm size cotyledon. Full length *GmIPK2* gene was amplified from this stage, cloned and sequence characterized (GenBank Accession No. KF297702). Sequence analysis revealed an open reading frame of 840 bp encoding a protein of 279 amino acids. The protein sequence analysis of the predicted *GmIPK2* gene indicated the absence of a signal peptide in the N-terminal region. Further, for targeting *GmIPK2* gene using the dsRNA induced sequence specific degradation mechanism, an intron spliced hairpin (ihp) construct was designed using the sequence information generated. A 355 bp *GmIPK2* gene fragment was amplified from its 3' CDS and

3' UTR with suitable restriction sites and cloned in the sense and the antisense orientation spanning the *GmFAD2-1* intron, thus generating ~1 Kb ihp construct. The expression cassette was further sub-cloned into the binary vector pCWAK containing seed specific vicillin promoter and terminator to generate plasmid pBINIPK2 (ihp) with *bar* gene as selection marker. The recombinant binary vector was thereafter mobilized into *Agrobacterium* through triparental mating and positive colonies were selected by colony PCR using *bar* and *GmIPK2* specific primers. We hypothesize that the transformation vector constructed in this study will thus be capable of inducing seed specific silencing of the endogenously expressed *IPK2* gene and thereby contribute effectively in producing putative low phytate soybean lines.