

## Epigenetic Gene Expression Regulation of NSP2, a GRAS Family Transcriptional Factor Controlling Peanut Nodulation

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Cultivated peanut (*Arachis hypogaea* L.) is an allotetraploid species ( $2n = 4x = 40$ , AABB) derived from two wild diploid ancestors *A. duranensis* (AA genome,  $2n = 2x = 20$ ) and *A. ipaensis* (BB genome,  $2n = 2x = 20$ ). As a member of the legume family, peanut has the capacity of nodule formation to perform biological nitrogen fixation through a symbiotic association with rhizobia. Through a map-based cloning approach, we have identified that *Nodulation Signaling Pathway 2* (*AhNSP2-A08* or  $N_a$  and *AhNSP2-B07* or  $N_b$ ) encodes a GRAS family transcriptional factor controlling peanut nodulation. Interestingly, we identified that only the  $N_b$  allele can express in the  $n_a n_a N_b n_b$  Nod+ plant and only the  $n_b$  expressed in the  $n_a n_a N_b n_b$  Nod- plant. The results of backcross indicated that the  $n_a n_a N_b n_b$  plant derived from the combination with  $n_a N_b$  from female gametes and  $n_a n_b$  from male gametes appeared Nod- phenotype. The  $n_a n_a N_b n_b$  plant derived from the combination with  $n_a n_b$  from female gametes and  $n_a N_b$  from male gametes exhibited Nod+ phenotype. Therefore, we hypothesize that the non-Mendelian inheritance pattern of  $N_b$  is under epigenetic control, where DNA methylation alters parental-origin alleles expression. Allele-specific expression of *AhNSP2-B07* in female gametes is suppressed by maternal imprinting. To test the hypothesis, we implemented bisulfite PCR and sequencing to identify the differentially methylated sites between female and male gametes. Specifically, the whole gene sequences including upstream 2000 bp of CDS as potential promoter and the full length CDS (1551bp) of *AhNSP2-B07* were amplified from bisulfite converted ovary and pollen DNA samples from heterozygote plants. The sequencing comparison between the male and female gametes will reveal the epigenetic control of the *AhNSP2-B07* gene expression during peanut nodulation. The results will illustrate the epigenetic mechanism of peanut nodulation, which will provide fundamental knowledge of gene expression regulation in tetraploid peanut with two closely related sub-genomes.