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Abstract Title: Whole-genome scale analysis of gene expression profiling of Arabidopsis flowering promotion in responding to γ-aminobutyric acid signals

Abstract Text:
γ-aminobutyric acid (GABA) is a four-carbon, nonprotein amino acid. Its regulation role in plant development increasingly triggered much more interesting. Our previous cellular level research indicated that GABA could lead the Ca²⁺ oscillation in tobacco pollen and growing pollen tubes. Further investigation via the patch clamp recording technique and Non-invasive Micro-test Technique (NMT) showed that this inward Ca²⁺ current is resulted from the activation of Ca²⁺ permeable channel in the response to GABA signals. Transcriptional level research demonstrated that GABA could stimulate the gene expression involving ethylene responses and signal pathway components. Recent research indicated that exogenous GABA could promote Arabidopsis early flowering.

To understand the mechanism of GABA modulating early flowering in Arabidopsis, Both the whole genome microarray (Agilent 4×44K oligo array) and microRNA (Agilent miRCURY™ LNA array) expression profiling were conducted. The results showed that GABA can significantly upregulate 895 genes and downregulate 1104 genes (above twofold difference, P≤0.05). In the top 36 genes in flowering transition versus control, 19 genes are the transcriptional factor genes and 8 genes are directly related to circadian rhythms. Previous research clue indicates that circadian rhythms could control the transition from vegetative growth to reproductive growth. RT-qPCR confirmed that exogenous GABA could stimulate the expression of TOCI (TIMING OF CAB-1) and inhibit the expression of CCA1 (CIRCADIAN CLOCK ASSOCIATED-1), which coding transcriptional factors are the central oscillator of circadian rhythms.

MicroRNA is the crucial regulator of plant development. The profiles of microRNA array identified 6 microRNA significantly affected by GABA treatment (above twofold difference, P≤0.05). The target genes of the microRNA were analyzed by on-line software miRBase and PMRD. It is predicted that GABA responding microRNA targets are mRNAs coding SPL transcription factors and leucine-rich repeat transmembrane protein kinase. Previous solid evidence showed that SPL is involved in the expression of three floral meristem identify genes to promote flowering transition and leucine-rich repeat protein could promote flowering.

One of the greatest challenges that modern molecular biology is facing is the understanding of the complex mechanisms regulating gene expression. To analyze the possible common cis-regulatory elements in these genes responding to GABA signal, on-line PlantPAN (Plant Promoter Analysis Navigator) and AtPAN software were employed. The software are used for identifying the co-occurrence of transcription factor binding sites (TFBSs) in a group of gene promoters with distance constraint between two TFBSs, and present graphically the transcription factor binding sites in specific gene promoter regions of interest, which is the basis to reconstructing gene regulatory networks. Promoter regions from -5000bp upstream to +500bp of 33 and 46 down-up/regulated genes of GABA responding microRNA targeting were respectively analyzed. All the genes contained multiple TF binding sequences, such as CCA1 binding sequences AAAAATCT, ARF1 binding sequence TGTCCTC, ATBZIP2 binding sequence ACTCAT, ATWRKY71 binding sequence TGAC, EDF4 (RAV1) binding sequence CAACA and ABI5 (GIA1) binding sequence ACACCTTG. The preliminary analysis displayed that the GABA stimulating genes maybe be related with circadian rhythm, ABA and gibberellin signals to co-regulate flowering phenotype.