

## **Supplementary Material**

### **Transcriptional landscape of soybean (*Glycine max*) embryonic axes during germination in the presence of paclobutrazol, a gibberellin biosynthesis inhibitor**

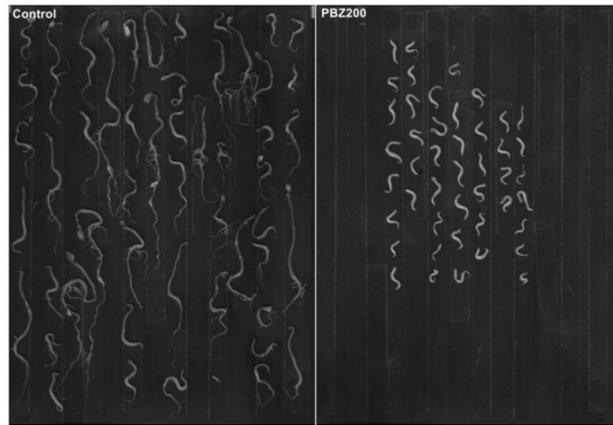
Rajesh K. Gazara<sup>1,#</sup>, Eduardo A. G. de Oliveira<sup>1,#</sup>, Bruno C. Rodrigues<sup>2</sup>, Rodrigo Nunes da Fonseca<sup>2</sup>, Antônia Elenir A. Oliveira<sup>1</sup> and Thiago M. Venancio<sup>1,\*</sup>

<sup>1</sup>Laboratório de Química e Função de Proteínas e Peptídeos, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro; Campos dos Goytacazes, Brazil. <sup>2</sup>Laboratório Integrado de Ciências Morfofuncionais, Núcleo em Ecologia e Desenvolvimento SócioAmbientaI de Macaé (NUPEM), Macaé, Brazil. # These authors contributed equally to this work.

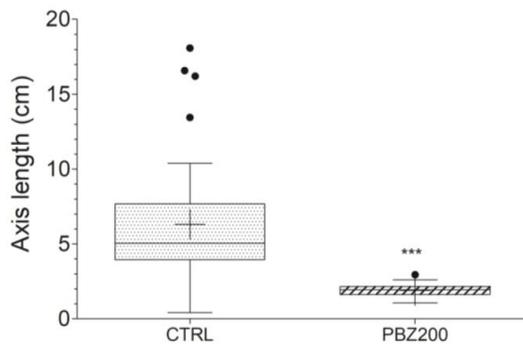
\* Corresponding author

Av. Alberto Lamego 2000 / P5 / 217; Parque Califórnia  
Campos dos Goytacazes, RJ  
Brazil  
CEP: 28013-602  
thiago.venancio@gmail.com

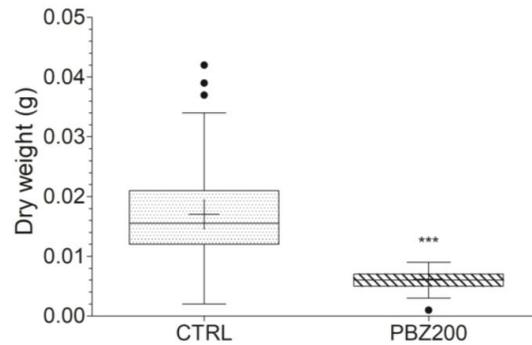
**A)**



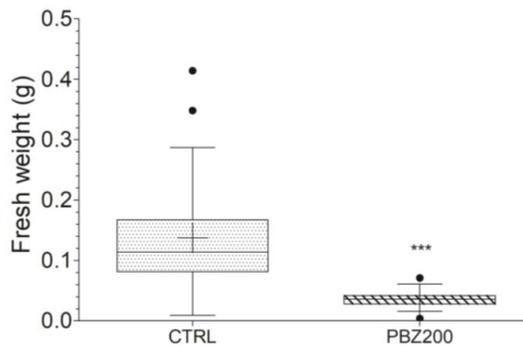
**B)**



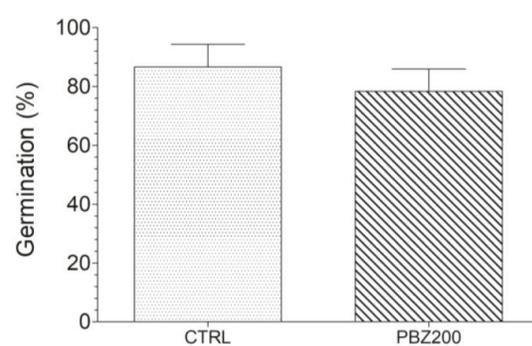
**C)**



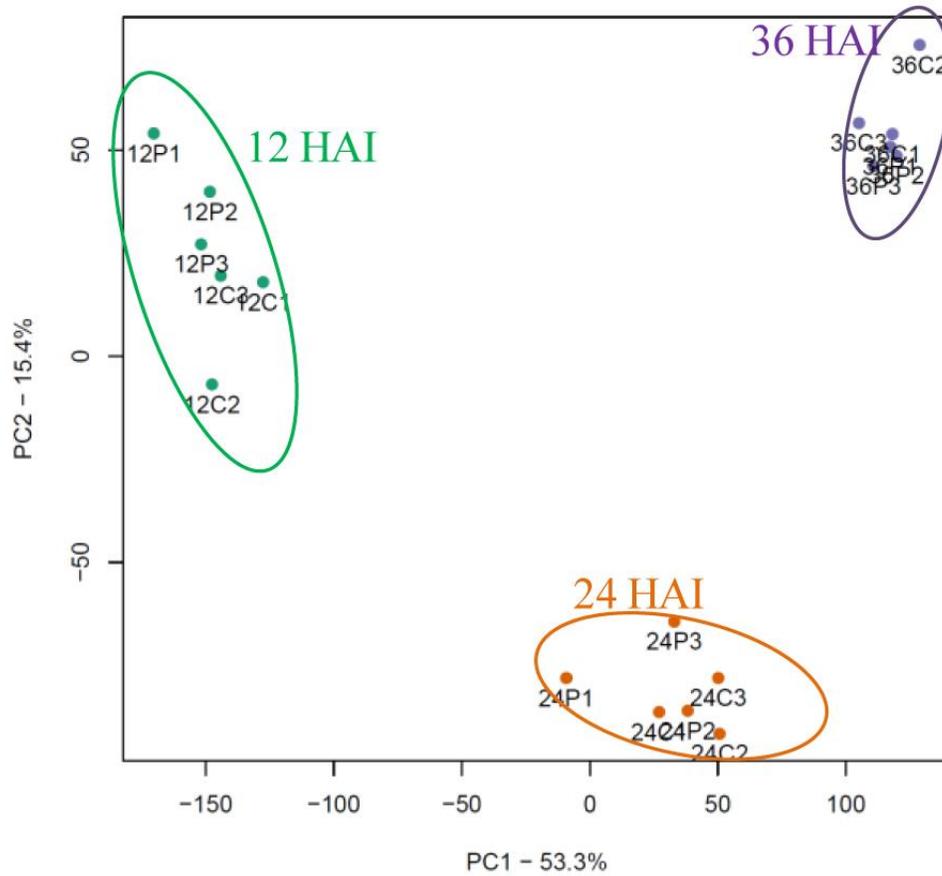
**D)**



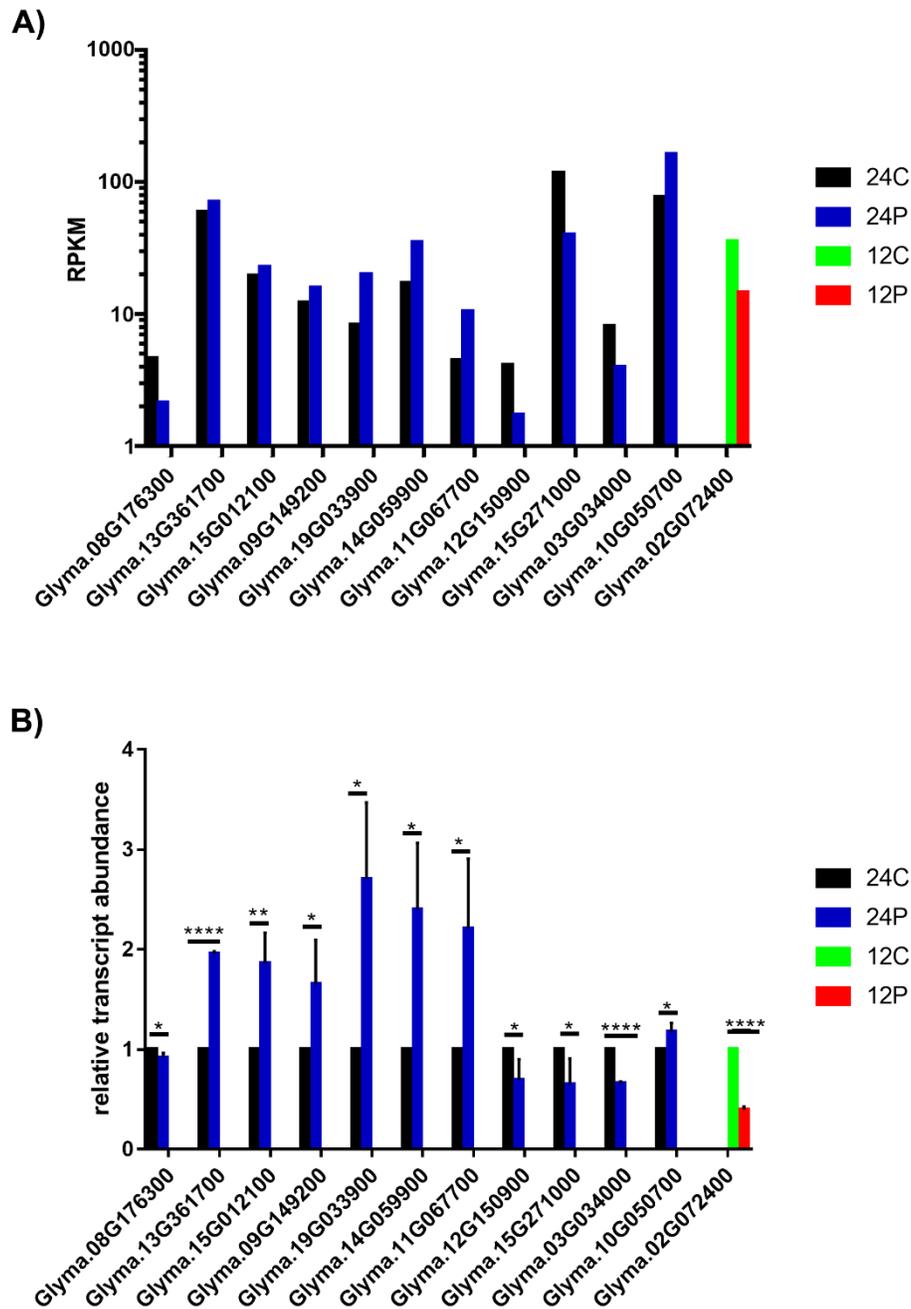
**E)**



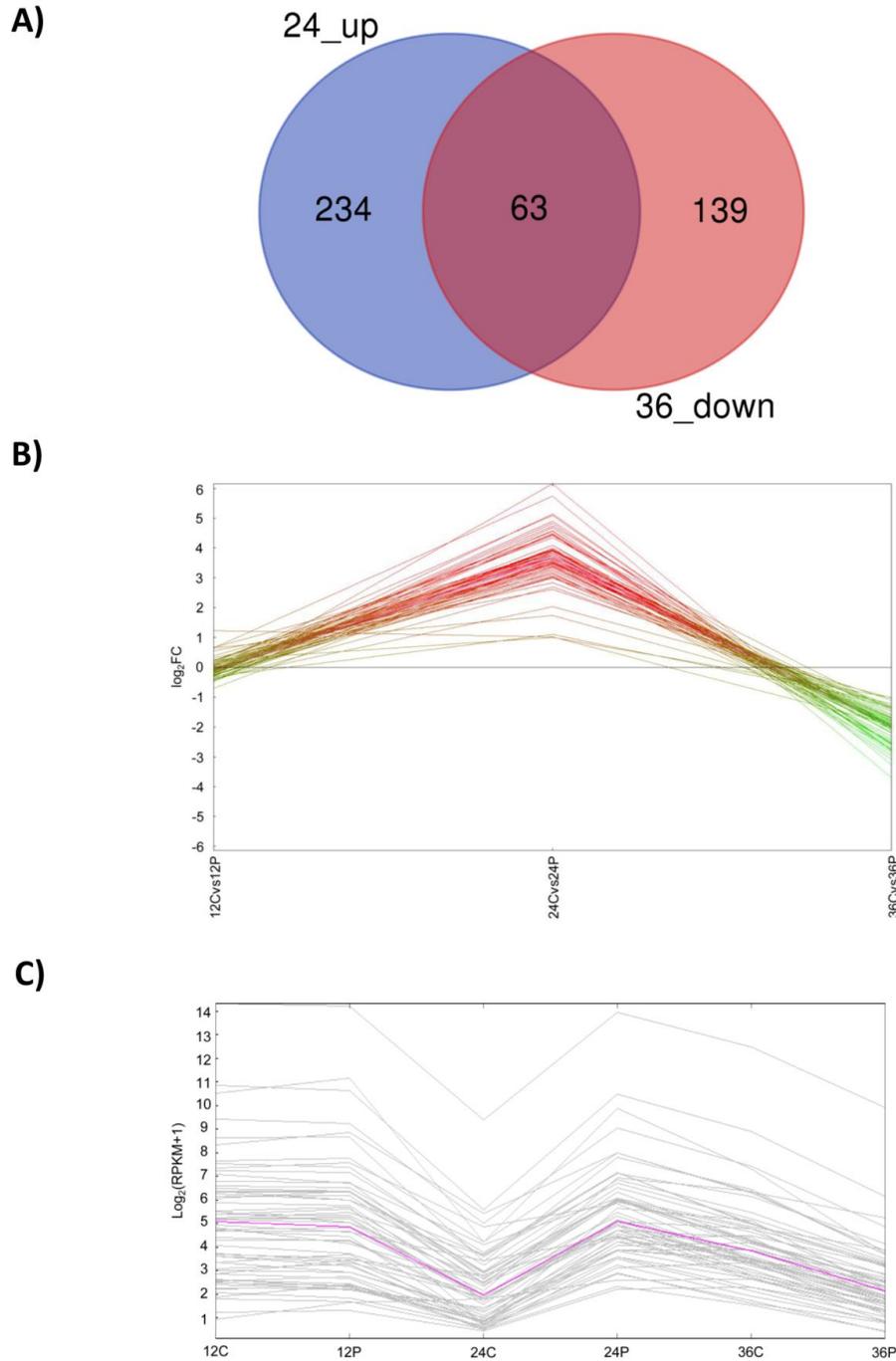
**Supplementary figure S1.** PBZ delays soybean seed germination. Soybean seeds (BRS-284) were allowed to germinate and grow in an incubation chamber under 28°C temperature, and 12/12h photoperiod (dark/light) for 7 days. Seeds were germinated in the presence of 30 ml of sterile water (control) or sterile water with 200  $\mu$ M paclobutrazol (PBZ). **(A)** Photographs of embryonic axis submitted or not to PBZ treatment. **(B)** Embryonic axis length, **(C)** Dry weight, **(D)** Fresh weight, and **(E)** Seed germination. Asterisks above boxplot demonstrate significant difference ( $p < 0.0001$ , Student's T-test).



**Supplementary figure S2.** Principal Component Analysis (PCA) of expressed genes under control and PBZ at 12 HAI, 24 HAI and 36 HAI. The first PC (PC1) and second PC (PC2) explained 53.3% and 15.4% of the variances, respectively. Three distinct groups can be observed: 12 HAI (green), 24 HAI (orange) and 36 HAI (purple).



**Supplementary figure S3.** Validation of differential gene expression. **(A)** RNA-Seq expression levels of 12 genes selected for validation using Real-time qPCR. **(B)** Relative transcript abundance of selected genes, obtained by Real-time qPCR. X- and Y- axes represent gene identifiers and RPKM (panel A) or relative transcript abundance (panel B), respectively. Samples are represented in different colors, as follows: 24C, 24 hours after imbibition (HAI) without PBZ (control); 24P, 24 HAI, PBZ-treated; 12C, 12 HAI, control and; 12P, 12 HAI, PBZ-treated. Statistical significance levels were obtained by performing one-tailed T-tests for each gene, as follows: \* ( $p < 0.05$ ), \*\* ( $p < 0.01$ ), and \*\*\*\* (0.0001).



**Supplementary figure S4.** Overlap between 24-up- and 36-down-regulated genes. **(A)** Venn diagram (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) shows 63 overlapping genes between 24 PBZ up- and 36 down-regulated genes. **(B)** Line plot of log<sub>2</sub>(fold change, FC) of the 63 genes at each time point. **(C)** Expression profile of the 63 genes in log<sub>2</sub>(RPKM+1) at each time point. MeV was used to generate graphs B and C. The pink line represents the median gene expression.