Mannose signaling in *Arabidopsis thaliana*

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INTRODUCTION

Sugars act as energetic source and structural componenets as well as important regulators of various processes associated with plant growth and development. Understanding how the pathways are distinct or can converge to a common set of regulated genes is crucial to determine the function of sugar as signaling molecule and their role in plant development and responses to environmental cues. Earlier studies in our laboratory suggested that in *Arabidopsis thaliana* mannose, a hexose structurally similar to glucose, acts as a signal independent of glucose in controlling gene expression. Mannose is the precursor of vitamin C and is also required for cell wall assembly and glycosylation of proteins and was shown to efficiently inhibit seed germination. Despite the apparent importance of mannose in plant cells, almost no information related to its signaling and/or regulatory network is available.

RESULTS

To evaluate the importance of control of genes expression by mannose in *Arabidopsis thaliana* (ecotype Col-0) and in order to obtain clues about mannose-related signaling pathway, RNA profiles changes associated with mannose availability were analyzed using DNA microarray technology (platform CATMA, http://www.catma.org). From these genomic data, a set of 45 genes specifically or preferentially regulated by mannose using qRT-PCR were validated and this collection of genes was classified into 3 categories according to the direction of the regulation (induction versus repression) and specificity (restricted to mannose versus mannose preferred with respect to glucose or mannitol) (Figure 1).

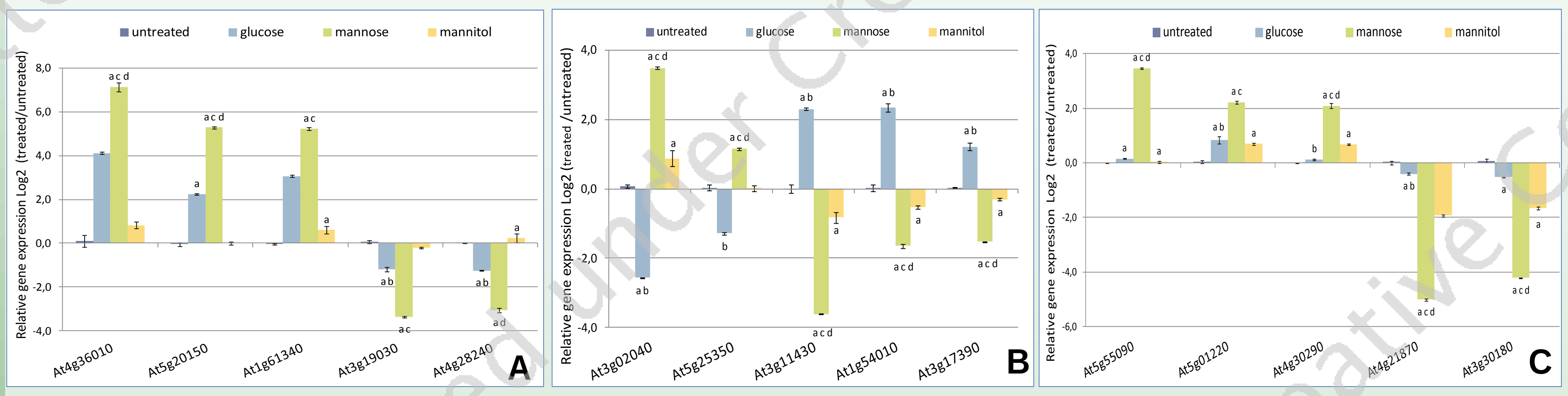

Figure 1. Quantitative analysis by qRT-PCR gene expression: **A.** Sampled genes regulated similarly by mannose and glucose but with stronger regulation by mannose; **B.** Sampled genes regulated by glucose and mannose in the opposite direction and **C.** Sampled genes regulated only by mannose. Seedlings of ecotype Columbia (Col-0) were treated with 2% glucose, 2% mannose and 2% mannitol for 2 hours. The relative abundance of transcripts of each gene was normalized using the gene as endogenous control *Pdf2* and relative expression was normalized using the expression levels of untreated seedlings. Significant differences were established by Student's *t* test; P< 0.05.

The 45 genes were classified according to *Gene Ontology* criteria and a 5.5 fold enrichment for genes in response to stress, 4.5 times for genes involved in the response to abiotic and biotic stimuli, 3 times for proteins with hydrolase activity and transport activity and genes with extracellular location, as well as a 2-fold enrichment for genes located in the plastid, cell wall and chloroplast were identified (Figure 2).

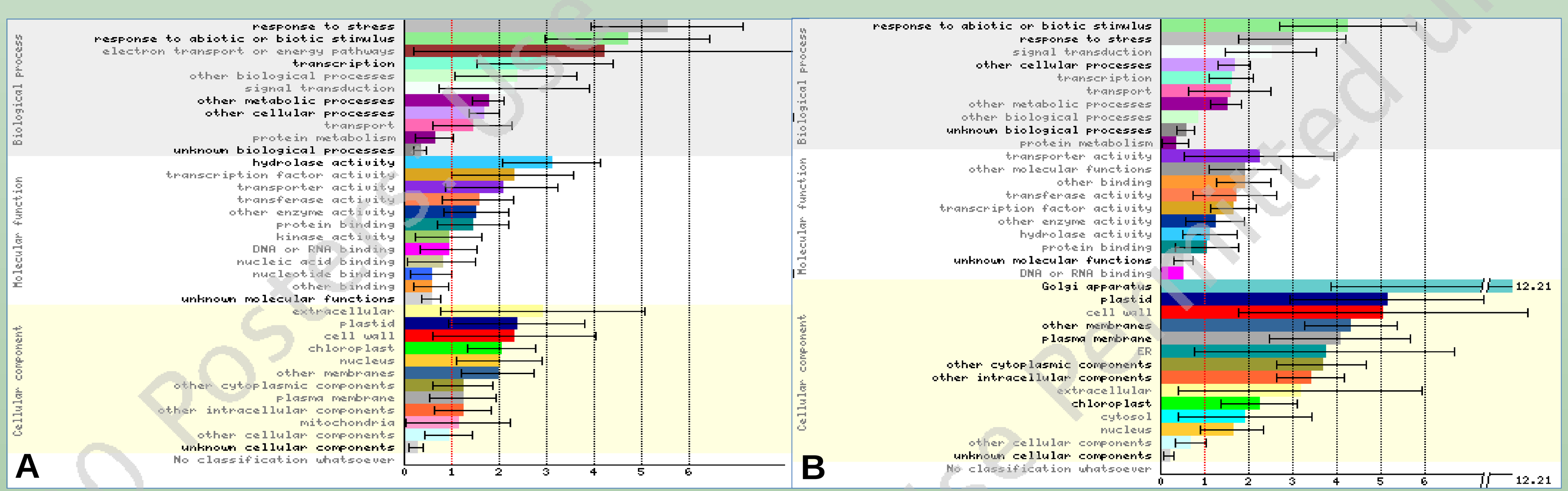

Figure 2. The graphs represent the percentage of the genes enriched set of genes upregulated (A) and negative (B) of mannose in our experiment according to the criteria *Gene Ontology* (GO, htpp://www.bar. utoronto.ca/) based on estimating the total number of genes of Arabidopsis, whereas p-value ≤ 0.05.

Considering the possibility of metabolic interconversions of mannose into glucose, we compared the kinetics (0, ½ h, 1h and 2h) of regulation by mannose 2% versus glucose 2% and mannitol 2% (Figure 3).

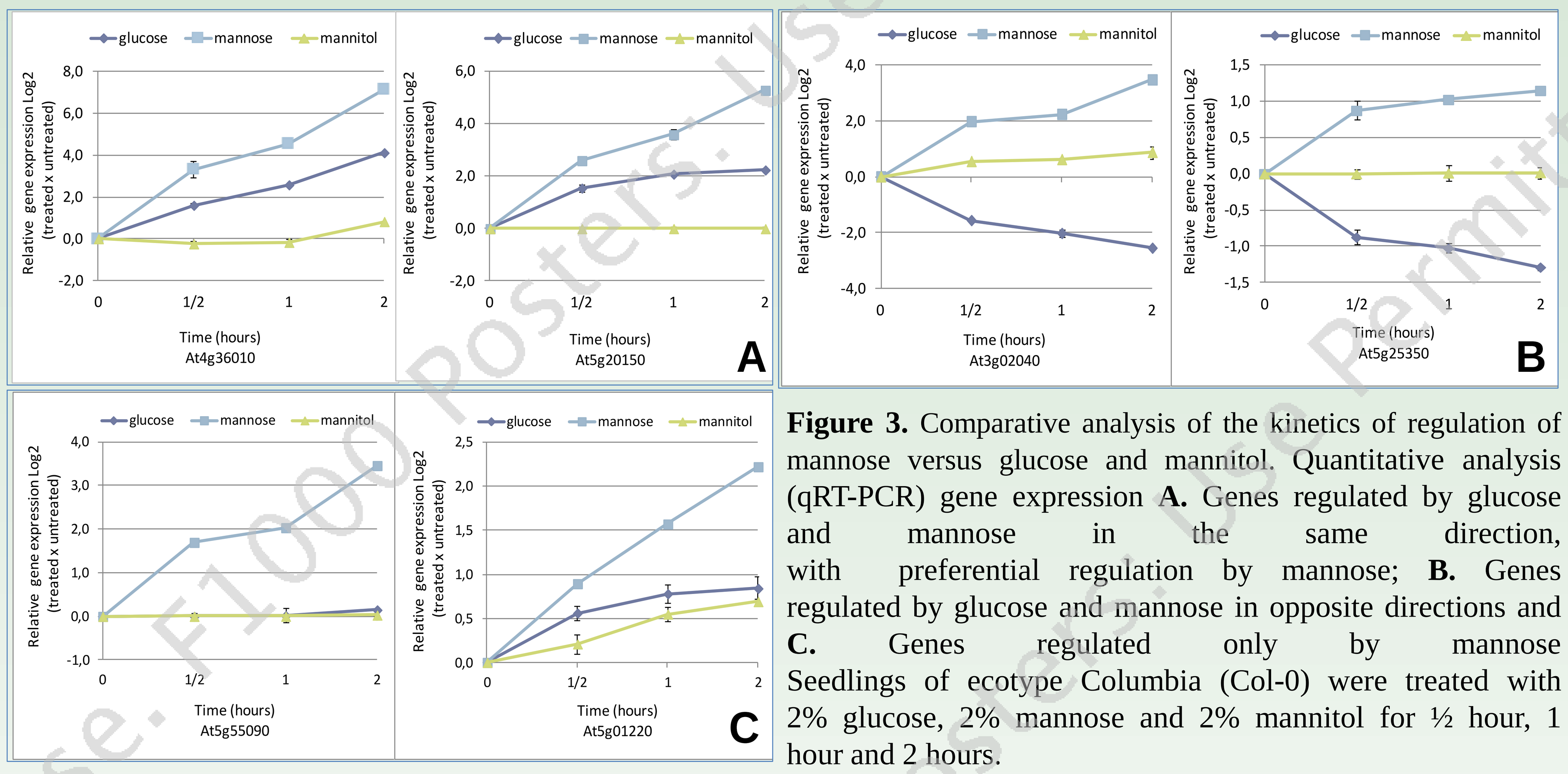

Figure 3. Comparative analysis of the kinetics of regulation of mannose versus glucose and mannitol. Quantitative analysis (qRT-PCR) gene expression **A.** Genes regulated by glucose and mannose in the same direction, with preferential regulation by mannose; **B.** Genes regulated by glucose and mannose in opposite directions and **C.** Genes regulated only by mannose Seedlings of ecotype Columbia (Col-0) were treated with 2% glucose, 2% mannose and 2% mannitol for ½ hour, 1 hour and 2 hours.

Due to the interconversion of mannose 6-phosphate to fructose 6-phosphate, we evaluated the degree of overlap between the regulation of mannose and fructose (Figure 4).

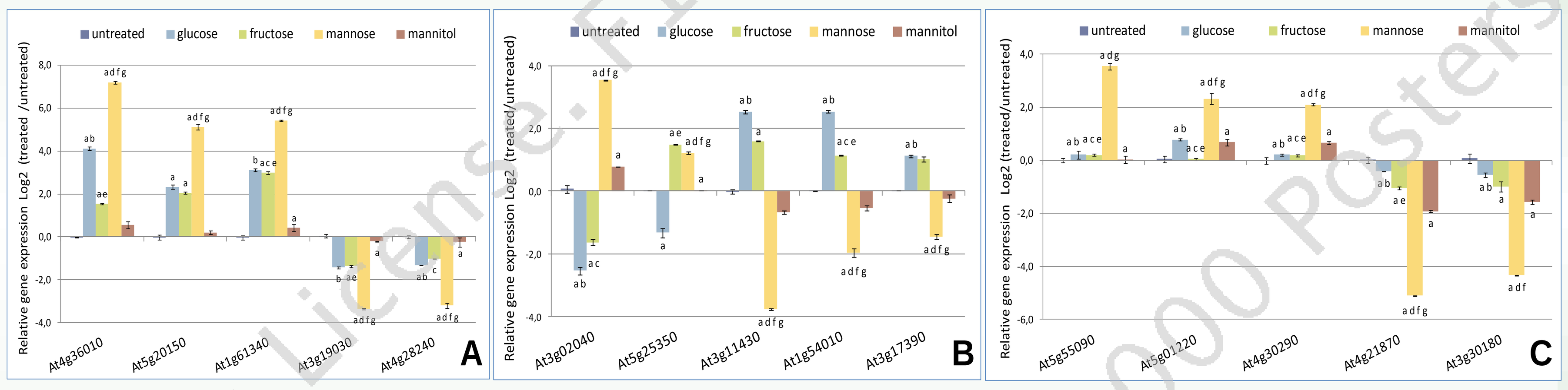

Figure 4. Comparative analysis of expression (qRT-PCR) of genes in response to glucose, fructose, mannose and mannitol. **A**. Genes regulated by glucose and mannose in the same direction, with preferential regulation by mannose; **B.** Genes regulated by glucose and mannose in opposite directions and **C.** Genes regulated only by mannose. Seedlings of ecotype Columbia (Col-0) were treated with 2% glucose, 2% fructose, 2% mannose and 2% mannitol for 2 hours.

We also analyzed the involvement of HXK1, a glucose sensor in the regulation by mannose by analyzing gene expression in the in null mutant to HXK1 (*gin2-1*) (Figure 5).

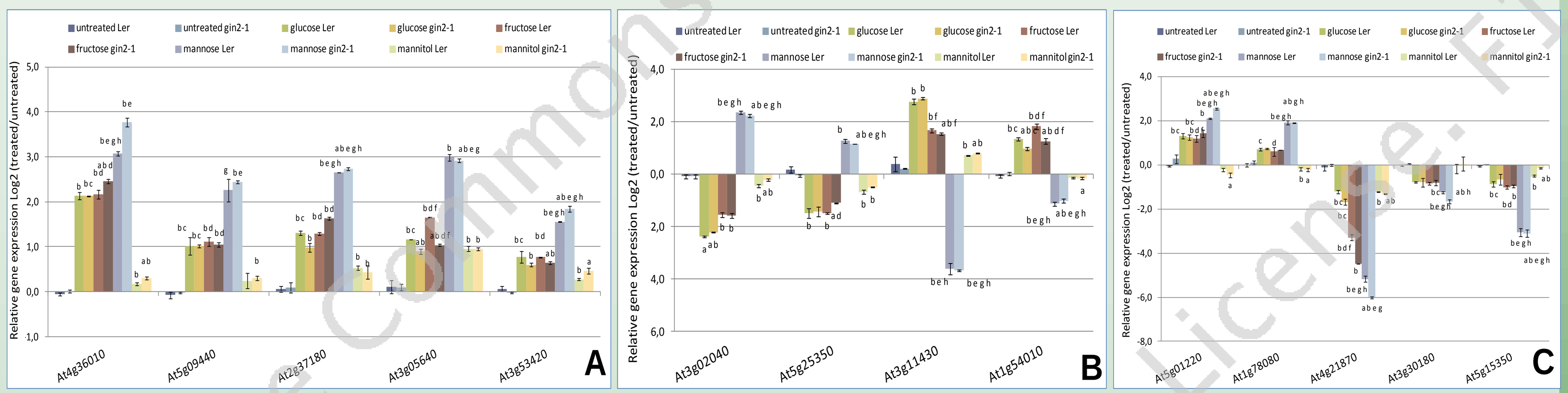

Figura 5. Involvement in regulating HXK1 mediated mannose. Quantitative analysis by qRT-PCR gene expression: **A.** Genes regulated similarly by mannose and glucose but with stronger regulation by mannose; **B.** Genes regulated by glucose and mannose in the same direction and **C.** Genes regulated only by mannose. Used seedlings of ecotype Ler and mutant *gin2-1*, 6 days after stratification, grown in liquid medium MS/50, mild agitation and constant light. Seedlings were treated with 2% glucose or 2% fructose or 2% mannose or 2% mannitol for 2 hours.

CONCLUSIONS

-Identified 45 genes specifically or preferably regulated by mannose;

-The adjustment for preferential regulation by mannose and mannose and glucose in opposite directions remain constant between ½ and 2 hours of treatment, confirming the notion of specific regulation by mannose;

-Showed that fructose or glucose could not replace mannose and that mannose-mediated regulation is independent of the glucose sensor HXK1;

-Verified that mannose-induced regulations were most likely not due to an indirect effect of ATP depletion or any energetic stress (data not shown).