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Comparative transcriptome analysis of embryogenic and non-embryogenic callus of tartary buckwheat

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Motivation and aim: Tartary buckwheat is a nutritionally important crop for human consumption due to the high content of rutin and the absence of gluten protein. Therefore, different conventional breeding strategies are used to improve it to meet the demand of a growing population. However, improvement through conventional breeding is challenging due to cross-incompatibility barriers with wild relatives, which are a key source of beneficial genes for traits such as indeterminate growth habit, thousand-grain weight, and seed shape. But, the availability of modern biological tools such as CRISPR-Cas9 and transgenesis provides an opportunity to improve buckwheat production. However, the success of these molecular tools depends on the availability of efficient *in vitro* regeneration system. Based on previous studies, it is well-established that genetic variability and loss of morphogenic activity in calli during long-term cultivation are common traits in most plant cell cultures, which prevent to develop stable mutant's lines through *in vitro* regeneration. However, under certain experimental conditions (not yet fully understood), Tartary buckwheat can form embryogenic calli that retain their morphogenic potential in culture for decades, making them an excellent model for studying somatic embryogenesis. Therefore, the objective of this study was to conduct a comparative transcriptome analysis of two embryogenic callus (EC) and two descendant non-embryogenic callus (NEC) lines to understand the molecular basis of the long-term embryogenic activity of Tartary buckwheat calli.

Materials and methods: Immature embryos from Tartary buckwheat line K-17 were used to obtain two embryogenic calli (EC), line 1-8 (K1) and line 1-10 (K3), on RX medium containing Gamborg's B_5 mineral salts, hormones and casein hydrolysate. Additionally, two non-embryogenic calluses (NEC) lines, 1-8p (K2) and 1-10p (K4), were derived from K1 and K3 during long-term culture, respectively. Furthermore, these EC and NEC lines were sub-cultured and sampling for RNA-Seq was done at 4 and 11 days. After sequencing, the quality of the sequenced samples and trimming of the poor-quality reads were performed by FastQC and Trimmomatic tools, respectively. Furthermore, the mapping to the reference genome Pinku1 (http://www.mbkbase.org/Pinku1/) was done by HISAT2. After mapping, the count matrix was generated by featureCounts during quantification step. Moreover, the Pearson correlation was calculated to perform the quality control within and between samples. The DESeq2 package was used to conduct differential expression analysis using R software. The enrichment analysis was performed by PlantGSAD (http://systemsbiology.cau.edu.cn/PlantGSEAv2/) software using a customized tool.

Results: In our study, a total of 22,164 and 19,793 DEGs (Differentially Expressed Genes) were observed at 4 and 11 days, respectively, between EC and NEC samples. At 4 days, 5,907 genes were upregulated in EC sample K1 compared to NEC sample K2, while 6,285 genes were upregulated in EC sample K3 compared to NEC sample K4. Similarly, at 11 days, 5,115 and 5,415 genes were upregulated in the samples K1 and K3, respectively, when compared to the samples K2 and K4. Furthermore, at the 4-day time point, 5,409 genes were downregulated in K1 relative to K2, and 4,563 genes were downregulated in K3 relative to K4. At the 11-day time point, 4,816 genes were downregulated in K1 compared to K2, while 4,448 genes were downregulated in K3 compared to K4. Out of total DEGs, around 4,078 genes were common in both 4 and 11 days that potentially involved in the common processes between EC and NEC samples. Some of the key genes were identified as being involved in regulating meristem maintenance and embryo development in our samples. Among these genes, *PDF2, WOX5, YABBY, WOX4, LEC1*, *FUS3*, *ABI3*, and *STM w*ere upregulated in both EC samples compared to NEC samples. On the other hand, an important group of genes, including HDACs (Histone Deacetylases) such as *HDA6* and *HDA19*, as well as *ATRING1b*, *MET1*, *CMT3*, and *EXT*, were identified as upregulated in NEC samples. However, only *HDA6*, *ATRING1b*, *CMT3*, and *EXT* showed expression at both 4 and 11 days in NEC samples. Additionally, genes involved in auxin, ethylene, and gibberellic acid hormone signaling were also identified in EC samples. In EC samples, terms related to glutathione metabolic processes, transcription regulator activity, glutathione transferase activity, oxidoreductase and monooxygenase activity were identified. In contrast, in NEC samples, terms associated with cytoskeleton, cell wall organization, glycosyltransferase and phosphotransferase activity were identified.

Conclusions: In conclusion, our study revealed key genes and GO terms involved in regulating embryogenic activity in Tartary buckwheat. Moreover, in the future, this study will help researchers improve their understanding of the underlying molecular mechanisms of somatic embryogenesis in buckwheat and ultimately enhance *in vitro* regeneration potential of recalcitrant genotypes, enabling the application of modern molecular tools for improving buckwheat.