# Transcriptome Analysis of Differentially Expressed Genes at Pre-Meiotic Developmental Stage in Pennisetum Hybrids with Contrasting Modes of Reproduction

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# Transcriptome analysis of differentially expressed genes at pre-meiotic developmental stage in Pennisetum hybrids with contrasting modes of reproduction

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## Introduction

Apomixis is an asexual reproduction through seeds where embryo develops without meiosis and fertilization. It is widely distributed throughout plant kingdom, but is more prevalent in families like Asteraceae, Rosaceae and Poaceae (*Carman*, 1997). This trait is highly desirable for fixing heterosis in F1 hybrids with significant implications for crop improvement (Dwivedi *et al*., 2007). Therefore it is necessary to unravel the molecular and genetic basis of apomixis to tap its potential. Pennisetum is an important genus of the Poaceae family which contains a wide range of species exhibiting wide variability in morphological, molecular, and reproductive traits (Jauhar, 1998). It includes many apomictic wild relatives of cultivated pearl millet (*Pennisetum glaucum*), some of them used extensively for introgression and molecular studies on apomixis, such as *P. squamulatum*, *P. ciliare*, and *P. orientale* (Ozias-Akins and Van Dijk, 2007; Kaushal *et al*., 2010). In order to identify putative genes involved in expression of apomixis, the genes showing differential expression across sexual and apomictic genotypes may be identified and characterized. A variety of methods are available for such molecular differential screening. These include differential display, fingerprinting techniques like cDNA AFLP, Subtractive hybridization, Micro array and Gene Chip technologies. These methods are employed for different purposes based on their convenience, sensitivity, automation and throughput. Texa with contrasting modes of reproduction are resources to identify genes involved in apomixis phenomenon. Broadly, the differentiation in reproduction pathway between apomictic and sexual lines is at three steps *viz*. pre-meiotic (including genes involved in preparing of ovule to enter into apomeiotic pathway), meiotic (genes involved in apomeiosis and embryo-sac development) and post-meiotic (genes involved in embryo-sac maturation and preparing for parthenogenesis). The present study was aimed to carry out a comprehensive transcriptome survey to identify differentially expressed transcripts in ovules of aposporous Pennisetum hybrid during the pre-meiotic stage of apomictic reproduction.

## Materials and Methods

An Obligate aposporous and an obligate sexual hybrid from a pearl millet (sexual, 2n=4x=28) and *P. squamulatum* (apomictic, 2n=8x=56) cross were utilized for this study. Subtractive hybridization and Cloning RNA samples at pre-meiotic stage of embryo-sac development in developing spike were isolated from florets of apomictic and sexual plants of Pennisetum hybrids using Trizol reagent (Invitrogen). Purified mRNA from ovules was used for reverse transcription and the first strand cDNA, thus, prepared was used for suppressive subtractive hybridization with the Clontech PCR Select-cDNA subtraction kit (Clontech Laboratories, Palo Alto, CA). Subtraction was performed, using mRNA from sexual plant as driver and apomictic plant mRNA as tester following the manufacturer's instructions with slight modifications. The primary PCR was performed for 30 cycles (940C, 30 s; 650C, 30 s; 720C, 90 s) and the secondary PCR was performed for 16 cycles (940C, 30 s; 660C, 30 s; 720C, 90 s). Products of the secondary PCR were purified, cloned into pGEMTeasy vector (Promega) and transformed into DH5α *Escherichia coli* competent cells. Automated sequencing using shotgun approach was carried out. The sequenced clones were analyzed and Contigs were made. Before further analysis all the Contigs were analyzed to identify mis-assembly of the sequences. Any such event was rectified manually. The final Contigs were used for further analysis. Consensus sequence of each Contigs was obtained from the high quality region of the Contigs. Further, analysis (BLAST and sequence alignment) of the sequences was done using NCBI software.

## Results and Discussion

Through subtractive hybridization technique, a total of 157 cDNA fragments were identified and cloned into pGEMTeasy vector. The recombinant clones selected by colony PCR and having appropriate insert size were used for DNA sequencing, using shotgun approach (Fig. 1). The sequenced clones on analysis gave Contigs. These Contigs were subjected to NCBI homology search and were found to show significant similarity to various genes and proteins. These transcripts were classified into 10 different categories according to their putative functions. Amongst the identified transcripts, many of them belonged to unknown function followed by those involved in stress response, embryo

development, reproduction signal transduction/stimulus response, metabolic/cellular process, metabolism, cell cycle, growth and differentiation (Fig.2). These differentially expressed genes might be involved in reproduction process, embryo development, signal transduction, cell cycle, growth and differentiation and will be helpful in identifying key genes responsible to regulate apomictic form of reproduction.

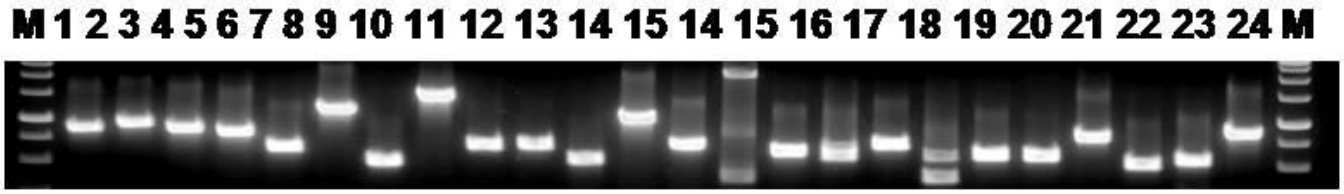


**Fig. 1:** Colony PCR of 24 randomly selected differentially expressed transcripts at pre-meiotic developmental stage in Pennisetum hybrids

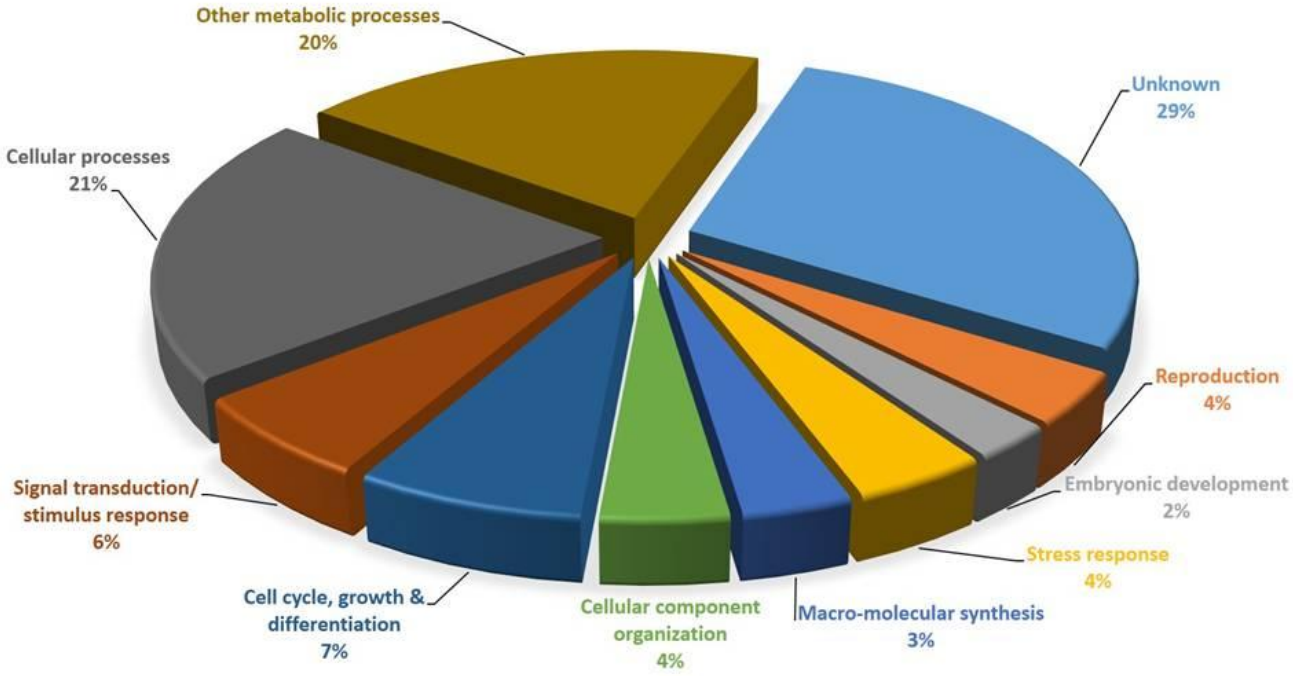


**Fig. 2:** Functional classification of 157 differentially expressed transcripts at pre-meiotic developmental stage in Pennisetum hybrids

## Conclusion

To identify genes involved in apomixis a comparative transcriptome analysis of differentially expressed genes in ovules of aposporous Pennisetum hybrid during the pre-meiotic stage of apomictic reproduction was investigated. These differentially expressed transcripts were recovered by suppression subtractive hybridization might be involved in complex network of gene governing reproduction process, and will be helpful in identifying key genes responsible to regulate apomictic form of reproduction.

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