Identification of differentially expressed transcripts associated to apomixis in *Brachiaria* using cDNA microarrays

Eduardo Gorrón[1, 2], Diana Bernal[1], Silvia Restrepo[2] & Joe Tohme[1*]

[1] International Center for Tropical Agriculture (CIAT), A.A. 6713, Cali, Colombia

[2] Universidad de los Andes, Carrera 1 No 18A-10, Bogotá, Colombia

* Corresponding author, j.tohme@cgiar.org - eduardogor@gmail.com

INTRODUCTION

Apomixis is a trait which confers to flowering plants the ability to produce seeds by asexual mechanisms. One of its most studied forms is gametophytic apomixis, in which a diploid embryo sac develops parthenogenetically (without meiosis) to form a viable embryo. The evidence for genetic basis of this phenomenon is crushing, and suggests concrete chromosome regions to be responsible of it. But molecular mapping to locate it has failed, and different studies, using techniques based in differential-display PCR (DD-PCR), had not been able to find the corresponding candidate genes. One reason for these results may be the use of techniques which only shows great genetic differences, but not fine molecular changes in gene expression. In this study, we used a combination of substractive libraries, mRNA amplification and cDNA microarrays, to analyze transcriptomic profiles associated to apomixis in the forage grass *Brachiaria*.

METHODOLOGY

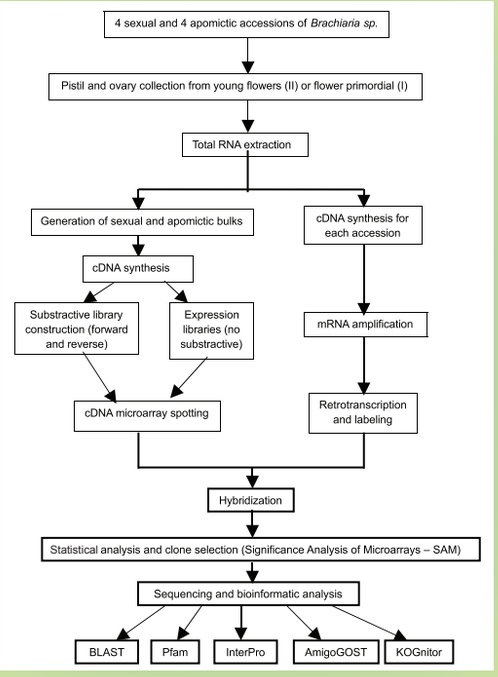

Figure 1: Summary of methods employed in this study.

Libraries employed	Number of clones
Differencial Substraction Chain (DSC) (foward and reverse)	1584
Supression subtractive hybridization (SSH) (foward and reverse)	1584
Mirror oriented selection (MOS) (foward and reverse)	1584
Stage I expression libraries (no substractive)	1584
Stage II expression libraries (no substractive)	1584
DSC bands	7 x 7 = 49
Amplicons from degenerate primers (designed from sequences reported in previous works as apomixis-related)	15 x 5 = 75
Total (Without negative controls)	8044

Table 1: Design of cDNA microarrays

RESULTS

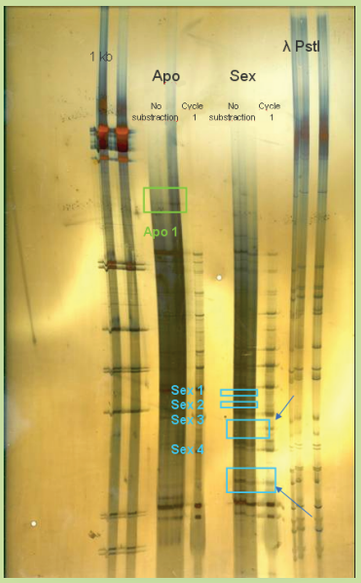

Figure 2: Substraction by DSC in both senses visualized in polyacrylamide gel. Some bands typical of one sample or the other can be seen, as shown. 1 and 3: 1 kb ladder; 4.6: Apomictic tester before substraction; 7: Apomictic tester after substraction; 8-10: Sexual tester before substraction; 11: Sexual tester after substraction; 12, 13 and 15: λ DNA after digestion with *PstI*.

Gene Function	Gene expression in apomictic ovaries (related to sexual)	Signal quotient (Sex/Apo)
Putative function identified (al least 3 bioinformatic programs)		
DSC bands		
Ribosomal protein RPS15A	↓	1,460
Microtubule-associated protein	↑	0,816
Libraries		
Rad51 recombinase family (homologue to meiotic DMC1)	↓	1,241
RPS15A	↓	1,282
Ubiquitin-ligase E2 (meiosis-associated)	↓	1,485
Alpha-tubulin	↓	1,291
Microtubule-associated protein (related to meiosis in *S. cerevisiae*)	↑	0,785
Chorismate synthase (Polyploid stage related)	↓	1,167
Histone H3 (Possibly related to meiosis)	↓	1,379
Putative function not clearly identified		
DSC bands		
Protein with putative EB-1 domain (assigned by Pfam)	↓	1,232
Transcription factor NAC (*Arabidopsis*) (poor related)	↓	1,365
Calmoduline	↓	1,400
Unknown (3)	↓	1,275 (average)
Libraries		
Puroindoline related	↓	1,214 (average)
	↓	1,242 (average)
EST from maize endosperm	↓	1,259 (average)
Senescence related (Poor related to transcription factors) (4)	↓	1,339
Zinc-finger protein	↓	1,389
epl2 (interaction with RNA pol II)	↓	1,256
H1 histone	↓	1,334 (average)
Unknown (6)		

Table 2: Differential genes found in cDNA microarray analysis

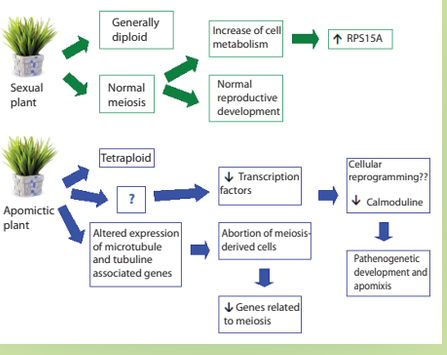

Figure 3: Possible model which explains the results obtained in microarray analysis. Sexual plants are usually diploid, and apomictic, polyploid, explaining differences in chorismate synthase and puroindoline genes. In sexual plants, meiosis occurs normally, which implies a slightly increased cellular metabolism. Alterations in cytoeskeleton proteins may help to induce meiosis abortion, reducing its related proteins. Changes in patterns of transcription factors and possible alterations in cell signaling may be the cause of cell reprogramming and parthenogenetic development.

DISCUSSION

Meiosis-associated protein expression was reduced in apomictic plants. This is an expected result, yet in apomixis meiotic-derived cells degenerates or meiosis is aborted. We found genes possibly related to abortion of meiosis, as shown in figure 3. The finding of these sequences (including the DMC1 related) gives validity to our results. Transcription factors retrieved (especially NAC related) must be considered as the possible master element of apomixis. Their confirmation by real-time PCR is in course, in order to make further work with them. The combination of substractive libraries and mRNA amplification for microarray analysis showed to be effective to find differentially expressed genes for this trait. The low difference in gene expression may explain the difficulties to reveal them in previous studies and shows the potential of microarrays to analyze this kind of phenomenon. This approach could be applied in other kind of studies in which researchers have only very low starting sample material and/or expression differences are not well resolved with other techniques.

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