A comprehensive analysis of gene expression and genomic alterations in a newly formed autotetraploid of *Paspalum notatum*

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Introduction The proportion of angiosperms that have experienced one or more episodes of chromosome doubling is estimated to be of the order of 50-70%. This prominence of polyploidy probably implies some adaptive significance. The emergence of novel phenotypes could allow polyploids to enter new niches or enhance their chances of being selected for use in agriculture. Although the causes of novel variation in polyploids are not well understood, they could involve changes in gene expression through dosage-regulation, altered regulatory interactions and rapid genetic/epigenetic changes. The objective of this work was to carry out an extensive transcript profiling and genome analysis of a diploid genotype of the pasture grass *Paspalum notatum* and its tetraploid derivative obtained after colchicine treatment.

Materials and methods Several different clonal individuals of a *Paspalum notatum* diploid genotype (C4-2x, 2n = 2x = 20) and its newly synthesized autotetraploid derivative (C4-4x, 2n = 4x = 40) (Quarin *et al*., 2001) were studied. Total RNA was obtained from flowers (at a particular developmental stage) or leaves. Differential display experiments were conducted under the general protocol reported by Liang and Pardee (1992). Differential expression was validated by reverse-Northern blot. Sequencing was done by Macrogen Inc., Korea. RAPDs reactions were conducted according to the method of Williams *et al*. (1990) and electrophoresed in polyacrylamide gels.

Results Differential display banding patterns generated from flowers of the diploid and tetraploid lines were compared in duplicated tests. From a total of 9617 bands scored, 129 (1.34%) were polymorphic between both lines. The isolated clones were subjected to reverse-Northern validation to discard false positives. Sixty four (64) were confirmed to represent up- or down-regulated genes in the autotetraploid. Sequencing showed that 42 of them were homologous to 26 different genes of known function, involved in processes related to DNA repair, protein trafficking, regulation of transcription, proteolysis, protein folding, carbohydrate and lipid metabolism and signal transduction The remaining 22 clones represented novel sequences. We also analysed the expression of around 2000 transcripts from leaves and found alterations in the profile of expression of 10 transcripts. Six of them were novel sequences, while the rest corresponded to an rRNA sequence and two mRNA of uncharacterized function. RAPD analysis was used to compare the genomic structure of both lines. Genome fingerprinting assays using 565 markers revealed the presence of a significant rate of polymorphisms (9.2%), that were mainly revealed by 4 particular decamers. Twelve polymorphic bands were isolated from the polyacrylamide gels, cloned and sequenced. Five of the isolated bands showed significant homology to a protein of uncharacterized function containing a SIS (sugar isomerase) binding domain. Two clones showed homology with a family of retroelements and a methyltransferase domain, respectively. The rest of the clones showed no significant similarities in the data banks.

Conclusions The results of the present study show that a change in the number of genomic complements modifies the expression of several genes in flowers and leaves of *Paspalum notatum*. Several general pathways (like carbohydrate and lipid metabolism, regulation of chromatine structure, ubiquitination and signal transduction) might be affected. We detected genomic modifications associated with polyploidization. Alterations in the structure of genomic sectors codifying retrotransposons and methyltransferases could be related to the well-documented epigenetic modifications occurring in recently formed polyploids.

References

Liang, P. and Pardee, A.B. (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science* 257: 967-971.

Quarin, C.L., Espinoza, F., Martínez, E.J., Pessino, S.C.and Bovo, O.A. (2001) A rise of ploidy level induces the expression of apomixis in *Paspalum notatum*. *Sex Plant. Reprod.* 13: 243-249.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6531-6535.