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Presenter Information

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Discovery and functional categorisation of expressed sequence tags from flowers of *Eragrostis curvula* genotypes showing different ploidy levels and reproductive modes

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Keywords *Eragrostis curvula*, expressed sequence tags (ESTs), diplosporous apomixis, ploidy

Introduction Two novel genotypes of weeping lovegrass (*Eragrostis curvula*) - a dihaploid strain obtained *in vitro* from an apomictic cultivar and a tetraploid plant derived from the dihaploid after chromosome duplication – have recently been developed. These materials represent an excellent system for the identification, through transcriptional profiling, of genes involved in diplospory and/or ploidy level gene regulation. The aim of this work was the discovery and functional classification of expressed sequence tags (ESTs) from immature inflorescences of the apomictic *E. curvula* cultivar Tanganyika (2n=4x=40), a dihaploid sexual strain derived from it (2n=2x=20) and a tetraploid sexual strain (2n=4x=40) obtained by colchicine duplication of the dihaploid.

Materials and methods A dihaploid plant was obtained by *in vitro* culture of immature inflorescences of the apomictic *E. curvula* cultivar Tanganyka (2n = 4x = 40) on Murashige and Skoog medium supplemented with 2,4-D and BAP. Duplication of chromosome number of the dihaploid plant with 0.05% colchicine led to the recovery of two tetraploid sexual plants. Inflorescences of Tanganyka, the sexual dihaploid and one of the colchicine-duplicated tetraploid sexual plants were collected at the same developmental stage. Total RNA was extracted from flowers using the RNeasy® total RNA isolation kit (Promega). cDNAs were obtained using a the SMART® PCR synthesis kit, cloned into the pGEM®-T easy vector and used for transformation of XL10-Gold® Ultracompetent *E. coli* cells. Bacterial cultures were performed in 96-well blocks and DNA isolations using a QIAprep®96 turbo Miniprep kit were carried out on a Qiagen Biorobot 9600. 2400 randomly selected cDNA clones from each library were sequenced using a MegaBACE™ DNA sequence analyzer. Same experimental approaches were used for the generation of ESTs from a leaf cDNA library from Tanganyka. Raw EST sequence traces were processed and annotated after the analysis using the XGI pipeline (http://www.ncgr.org/xgi) for automated EST clustering. Processed ESTs were clustered into consensus sequences that were compared using BLASTX against NCBIs non-redundant protein database. Functional categories were assigned by means of gene ontology (GO) annotations.

Results and conclusions A total of 9600 randomly selected cDNA clones from 4 cDNA libraries of *E. curvula* (2400 from 01EC dihaploid flowers, 2400 from 02EC apomictic tetraploid flowers, 2400 from 03EC apomictic tetraploid leaves and 2400 from 04EC sexual tetraploid flowers) were sequenced. Cluster analysis of the corresponding ESTs allowed the identification of 2,218 unique sequences (719 for EC01, 564 for EC02, 589 for EC03 and 346 for EC04). BLASTX analysis revealed no significant similarities at an E-value greater than 10^{-6} for 54% of the non-redundant *Eragrostis* sequences. Functional categories were assigned to 402 out of 1198 annotated sequences. The EST collections from the different cDNA libraries showed a distinctive distribution among different functional categories. Furthermore, the *E. curvula* sequences were compared with those corresponding to differential bands obtained after an extensive transcript profiling of diploid and tetraploid genotypes of the aposporous pasture grass *Paspalum notatum* with different ploidies, in order to identify common patterns in relation to ploidy levels and modes of reproduction.