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Poster Abstract - G.03

VIVIPAROUS PLANTLETS FORMATION IN *KALANCHOE*: TOOLS FOR MOLECULAR ANALYSIS OF RESPONSIBLE GENES

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The formation of viviparous plantlets is a trait common to several families (*e.g. Graminaceae, Crassulaceae, Liliaceae*). The plantlets are produced over organs such as leaves, roots and flower stalks.

The *Kalanchoe* plantlets develop on leaf margin and are organised with an inverse polarity to that of the mother plant. Inverse polarity was already reported for the extra flower developing on the distal part of the lemma in *Hooded* mutant barley (Müller et al., 1995). The *Hooded* mutation is caused by a duplication involving intron 4 of the gene *Bkn-1* (Müller et al., 1995). Furthermore epiphyllous bud has been observed over *Nicotiana* leaves over-expressing *Bkn-1* e *Bkn-3* genes (Lin & Müller, 2002). These observations show that genes of the *Knotted* family, which control the fate of meristematic cells (Kobayashy et al., 2000), could play a role on the viviparous plantlet formation.

Two strategies have been addressed in order to identify genes involved in bud formation in *Kalanchoe*:

1) Identification of heterologous *Knotted* genes.
2) Construction of a subtracted cDNA library.

Knotted class I genes were identified applying in *Kalanchoe* the methodology used by Kobayashi et al., (2000) in *Pharbitis*. The first step was carried to amplify the 3' region of *Knotted* class I candidate genes by 3' Rapid Amplification of cDNA Ends (RACE) PCR. 3' RACE was carried using as template cDNA obtained through RT-PCR of mRNA, two nested degenerated forward primers, which sequence was based on a conserved domain (homeobox), and a reverse poly-A primer. 4 different genes have been identified. Bioinformatics analysis confirmed their homology to *Knotted* like genes. 5' region is currently under analysis.

The subtracted cDNA library was prepared utilising poly(A)$^+$ RNA extracted from the internal lamina (reference) and from the leaf margin, which contains putative transcripts involved in viviparous plantlets formation. The subtracted and enriched DNA fragments were directly cloned into T/A cloning vector. Screening of the colonies is in progress.