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CLONING AND EXPRESSION OF *KALANCHOE XHOUGHTONII* KNOTTED-LIKE GENE (*KXHKN5*)

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In situ hybridization, Kalanchoe xhoughtonii, knox genes, RNA-interference, vegetative vivipary

Vegetative vivipary leads to formation of novel complete plantlets on mature organs. In *K. xhoughtonii* (n=51), a triploid interspecific hybrid between *K. daigremontiana* Hamet & Perrier (n=17) and *K. delagoensis* Ecklon & Zeyher (n=34), viviparous plantlets are formed on leaf margin notches in response to a long day photoperiod and their appearance follow a basipetal fashion. Several well known class 1 *knox* genes, as *Knotted1* (*Kn1*) from maize and *SHOOTMERISTEMLESS* (*STM*), *KNAT1* and *KNAT2* from *A. thaliana*, play an important role in meristem formation and maintenance. Several reports suggest that this class of homeotic genes could be involved in vivipary. In order to identify *knox* genes involved in vegetative vivipary in *K. xhoughtonii* hybrid, leaf tissue was collected before buds formation. Following RNA extraction and cDNA synthesis, semi-nested PCR was performed using anchored oligo-dT primer and degenerated primers designed on homeodomain sequence (Kobayashi et al., 2000). PCR products were cloned and sequenced. To identify full length coding sequence, nested 5' RACE was carried out using whole or digested cDNAs adaptor libraries. Four identified *knotted*-like genes belong to class 2 (*KxhKN1to KxhKN4*) and one to class 1 (*KxhKN5*) and their sequences were submitted to GenBank (NCBI), respectively: EU272787, EU272788, EU272789, EU272790, EU240661. Over-expression and silencing experiments of *KxhKN5* were performed in *K. xhoughtonii* and in eterologous systems, (*Osteospermum ecklonis*). To accomplish over-expression, the complete cDNA sequence of the gene (1161 bp), overdrive by 35S promoter and terminator, was cloned in the binary vector pGreen II (www.pgreen.ac.uk) that contain the NPTII gene, that confer resistance to kanamycin; the derived vector was transferred to *A. tumefaciens*. Post transcriptional gene silencing (PTGS) construct was prepared by cloning in pJM007 (Schattat et al., 2004), a 326 bp fragment of the gene in sense and antisense orientation in the specific cloning sites located at the left and at the right of the PIV2 intron. The silencing cassette was excided from pJM007 and cloned into the binary vector pGreen II NPTII. Following *A. tumefaciens* mediated genetic transformation with either over-expressing or silencing constructs and selection on medium containing kanamycin and cefotaxime to contain bacteria overgrowth, the regenerated shoots were isolated from the leaf explants and separately cultivated on propagation medium to establish plant clones. Some clones were acclimatized in greenhouse. To localize *KxhKN5* mRNA in early fase of *K. xhoughtonii* epiphylly, *in situ* hybridization was performed according to FISH Tag RNA Kit (Invitrogen, Carlsbad - California), with minor modifications.

In vivo, *K. xhoughtonii* plants over-espressing *KxhKN5* usually show a bushy phenotype with entire, rounded basal leaves and deeply palmated apical leaves. In control plants, leaves are lanceolate with dentated margin. Propagules formation is severely reduced in some transgenic plants. Transgenic *O. ecklonis* shows an assortment of phenotypes ranging from plants with small

leaves to plants with short internodes and bracts looking leaves. Extreme phenotypes survive only in vitro and resemble a cushion of dwarf bracteated stems.