

CHARACTERIZATION OF PUTATIVE REGULATORS OF *BKn3*, A BARLEY HOMEBOX GENE INVOLVED IN MERISTEMATIC ACTIVITY

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In Barley, the dominant *Hooded* (*K*) phenotype is associated with the duplication of a 305 bp element in intron IV of the *knox* gene *BKn3*. This regulatory element seems to act as an enhancer and causes *Bkn3* ectopic expression in the lemma-awn transition zone, leading to the formation of a new meristem that develops into an epiphylllic flower.

A one hybrid screening aimed at isolating putative regulators of the *BKn3* gene uncovered four different proteins capable of interacting with the 305 bp element (K Intron Binding Proteins, KIBPs).

In order to gain insight into their role in *Bkn3* regulation, recombinant KIBPs have been expressed in different prokaryotic systems and the full length proteins have been purified by affinity chromatography.

In vitro binding of KIBPs to the 305 bp enhancer has been confirmed by ElectroMobilityShiftAssay (EMSA) and the minimal binding sites have been assessed.

As there is no clear association between *KIBP* loci and mutations already mapped and characterised in barley, the *in vivo* function of KIBPs has been investigated in the model species *Oryza sativa*. Screening of insertional mutant collections has yielded stable lines carrying mutations in two *KIBP*-related genes. Thus, the morphogenetic effects of KIBPs loss of function have been investigated in transgenic rice plants; in the first line, a Ds insertion causes defects in tillering and internode elongation, while in the second line, a t-DNA insertion is responsible for lethality in 4 weeks-old seedlings.

In addition, a complementary screening for gain of function (overexpressors) rice plants for the four KIBP genes is under way.