CHARACTERIZATION OF PUTATIVE REGULATORS OF \textit{BKn3}, A BARLEY HOMEBOX GENE INVOLVED IN MERISTEMATIC ACTIVITY

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In Barley, the dominant \textit{Hooded (K)} phenotype is associated with the duplication of a 305 bp element in intron IV of the \textit{knox} gene \textit{BKn3}. This regulatory element seems to act as an enhancer and causes \textit{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 \textit{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 \textit{Bkn3} regulation, recombinant KIBPs have been expressed in different prokaryotic systems and the full length proteins have been purified by affinity chromatography.

\textit{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 \textit{KIBP} loci and mutations already mapped and characterised in barley, the \textit{in vivo} function of KIBPs has been investigated in the model species \textit{Oryza sativa}. Screening of insertional mutant collections has yielded stable lines carrying mutations in two \textit{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.