

## Functional characterization of candidate genes in barley: transgenic plants and grown cultivars

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Barley (*Hordeum vulgare* L.) is one of the major and most distributed crops in the world. Currently it is becoming a novel cereal model plant representing a number of small-grain cereal species. While the barley genome is similar to that of other cereals, it is amenable to explorations of molecular genetics through its true diploidy.

The first *Agrobacterium*-mediated barley transformation reports was published in 1997 by Tingay and co-workers, using the variety called Golden Promise. The method that we established here was developed for this model cultivar and it's based upon protocols by Trifonova et al. (2001) and Kumlehn (IPK, Gatersleben, unpublished).

The selected genes we used for the plant transformations can be divided into the following three subgroups:

1. The regulators of the cell division cycle: *MsCDKB2;1* (a *Medicago sativa* cyclin dependent kinase which plays a central role in regulation of the cell cycle, in particular in the G2/M phase transition and in mitosis). In previous studies it revealed that the overproduction of the *MsCDKB2;1* resulted in significant changes in agronomically important parameters in transgenic rice (Lendvai et al., unpublished). Other genes of this group, like OsPP2A B'' regulatory subunit, OsRBRI2, OsRBRI5 are previously identified interactors of rice retinoblastoma-related protein, OsRBR1. Since cell cycle regulatory functions of the retinoblastoma proteins are primarily modulated by changing their phosphorylation status, *in planta* studies of the OsRBR1 interaction partner, the OsPP2A protein phosphatase B'' regulatory subunit is particularly important from this viewpoint.

2. The 'oxidative stress-defense genes'. First transformation from this group of genes were made by the alfalfa aldo-keto reductase, *MsALR*. This enzyme plays important role in detoxification of the reactive aldehydes issued during oxidative stress, and helps the recovery of the plants (Oberschall et al. 2000). In order to accumulate protective enzymes in different subcellular compartments we constructed a vector for chloroplast targeting of protective enzymes using the transit peptide encoding region of the barley Rubisco LSU gene.

3. The genes involved in grain size determination (*GW2*, *GIF1*). Loss of *GW2* function increased grain width, weight and yield (Song et al. 2007) Antisense approach results increased grain size, even with constitutive expression of gene fragment in transgenic rice. We have identified and cloned the homologous gene from barley, a specific fragment of it was used for the generation of *HvGW2* antisense plants. *GIF1* (*GRAIN INCOMPLETE FILLING 1*) gene that encodes a cell-wall invertase required for carbon partitioning during early grain-filling (Wang et al. 2008). *GIF1* is responsible for grain weight reduction, ectopic expression of the cultivated *GIF1* gene with the 35S or rice Waxy promoter resulted in smaller grains, whereas over-expression of *GIF1* driven by its native promoter increased grain production. These findings, suggest that *GIF1* is a potential domestication gene and that such a domestication-selected gene can be used for further crop improvement.

Establishing a reliable barley transformation technology is very important for the functional characterization of candidate genes and the produced transgenic lines are subject for further studies.

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## Molecular basis of the blood-brain barrier function

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One of the most important functions of the mammalian blood-brain barrier (BBB) is to restrict the free movement of different substances between blood and neural tissue, and it plays a key role in the homeostasis of the central nervous system. The principal components of the BBB are the cerebral endothelial cells that form a continuous monolayer and are interconnected with tight junctions and adherens