Modification of barley and wheat spike architecture by gene-specific mutagenesis

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The yield increase of wheat has been almost stagnating since the 1990s and it is expected that in 2050 humans will need the double amount of yield as compared to today. Enhanced sink capacity is one of the means to improve the yield potential of cereals, which can be achieved by an increased number of kernel sites in the spikes. To do so in wheat and barley, two spike architecture genes will be modified by site-directed genome modification using RNA-guided endonucleases. Our first candidate gene is the orthologue of barley *Compositum 2* (*HvCOM2*) in hexaploid bread wheat (*Triticum aestivum* L.), which is represented by the *branched head* (*BH*) locus on chromosomes 2AS, 2BS and 2DS. In two-rowed barley, *com2* causes branching at the basal part of the spike, while in tetraploid wheat, a mutation in the *BH*-locus on chromosome 2A leads to the formation of the branched spikes of 'Miracle-Wheat'. Originating from canonical spikelet meristems, additional spike meristems develop at the basal to central nodes of the rachis, which causes the formation of more spikelets, florets and grains than in the wild-type and thus entails a significantly increased yield. To obtain similar phenotypes in hexaploid wheat, motifs conserved across all three homeoallelic copies of *TaBH* will be targeted by the same guide RNA. Single mutations in the A, B and D genome as well as any possible combinations of those will be generated and homozygous lines will be readily obtained by using haploid technology. The second candidate is the orthologue of rice *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 14* (*OsSPL14*), which, regulated by the microRNA *miR156*, influences spike branching and tillering. For functional analysis in barley, its orthologue *HvSPL14* will be knocked out using site-directed mutagenesis in suitable positions within the coding sequence. In addition, the *miR156*-binding motif will be targeted and individuals with in-frame mutations selected. While conventional site-directed mutagenesis involves cellular DNA repair of Cas9-induced double-strand breaks via the non-homologous end-joining (NHEJ) pathway, in a second approach, the cytidine-residues in the *miR156* site will be converted to thymidine-residues by using a nickase Cas9 (nCas9) or dead Cas9 (dCas9) fused with a cytidine deaminase. In a third approach, the homology-directed repair (HDR) mechanism will be used to cause a predefined point mutation in the *miR156*-binding site. The resultant modification is expected to inhibit binding of *miR156* at *SPL14* leading to deregulation and finally to a branched spike.