**Engineering Wheat Genotypes Compatible for Gluten Sensitive, Allergenic and Intolerant Individuals**

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**INTRODUCTION**

Wheat supplies about 20% of the total food calories consumed worldwide, feeds approximately half of the global demand for dietary proteins, and is a national staple in many countries [1]. In the United States, the per capita consumption of wheat exceeds that of any other single food grain. Besides being a major source of energy and nutrition it is also a major cause of frequent diet-induced health issues especially celiac disease, gluten sensitivity, food allergies, obesity, diabetes mellitus, cardiovascular disorder, and colorectal cancer [2-5]. Gluten-intake in the sensitive individuals can elicit various reactions, which in combination with their respective genetic constitutions lead to diverse symptoms from gastrointestinal or neurological to fatal [4,6]. These symptoms are broadly classified into gluten intolerance, sensitivity and allergy. Gluten intolerance includes celiac disease, which is one of the most common food-born enteropathies in humans, occurring in the US with an incidence of 1 in 100 [5]. The gluten-sensitivity is very wide spread and in combination with celiac disease and gluten allergy affect >7.5% of the US population (US Census Bureau as of 8/22/11). Recent advances in the understanding of the disease onset in schizophrenia and dermatitis herpetoformis (the celiac disease of skin) unveiled that gluten peptides are also elicitors for these disorders as was earlier reported for the gluten sensitivity and celiac disease [5,7]. Due to adaption of gluten-rich dietary pattern and improvements in disease diagnostics the celiac disease is increasingly being diagnosed in apparently ‘celiac-free’ areas of the world [5,8-13].

In view of the magnitude of problem we undertook a multipronged approach to develop a natural dietary therapy for the gluten-induced disorders collectively referred as ‘gluten syndrome’. The three approaches undertaken in this direction are: i) epigenetic elimination of immunogenic prolamins [namely low molecular weight (LMW) glutenins and gliadins] using transgenic and non-transgenic approaches, ii) post transcriptional silencing of immunogenic prolamins via RNA interference, and iii) post translational detoxification of prolamins by expression of ‘glutenases’ in wheat grains (the enzymes were selected such that they will degrade prolamins in the human gut only after consumption).

The earlier research performed on the high-lysine barley mutant Rísø 1508 (lys3a) revealed two-types of transcriptional regulation for the prolamin genes in the barley endosperm: i) the genes whose transcription depend on demethylation of their promoters in the endosperm, such as LMW glutenins and gliadins, and ii) the genes whose transcription solely depend on expression of specific transcription factors, such as high molecular weight (HMW) glutenins. Based on the functional similarity between the barley Lys3 and Arabidopsis DEMETER genes, we undertook cloning of barley and wheat DEMETER homoeologues. These single mutants identified in **DEMETER** homoeologues are currently being pyramided to obtain double mutants in Kronos and triple mutants in the Express backgrounds. These single mutants identified in **DEMETER** homoeologues are currently being pyramided to obtain double mutants in Kronos and triple mutants in the Express backgrounds. The sequence information was also used to design one hairpin RNA and three artificial micro RNA constructs to silence wheat **DEMETER** homoeologues. Transformations using the above four RNAi constructs resulted in identification of a total of 191 mutants in the Express and 77 mutants in the Kronos backgrounds. These single mutants identified in **DEMETER** homoeologues are currently being pyramided to obtain double mutants in Kronos and triple mutants in the Express backgrounds. The sequence information was also used to design one hairpin RNA and three artificial micro RNA constructs to silence wheat **DEMETER** homoeologues. Transformations using the above four RNAi constructs resulted in identification of a total of 191 mutants in the Express and 77 mutants in the Kronos backgrounds. These single mutants identified in **DEMETER** homoeologues are currently being pyramided to obtain double mutants in Kronos and triple mutants in the Express backgrounds.

For the post-transcriptional silencing of the immunogenic prolamins a chimeric hairpin construct was assembled by putting together the conserved target sequences identified for different gliadin groups and the LMW glutenins. The hairpin was
provided with the endosperm specific HMW glutenin promoter, and introduced into the wheat genome by biolistic approach or via microspore electroporation based method. The T\textsubscript{s} grains of the selected transformants are currently being examined for their prolamin content, and the screen has so far resulted in the identification of genotypes showing significantly reduced accumulation of gliadins.

For the post-translational detoxification of prolamins a combination of barley cysteine endoprotease B2 (EP-B2) and a \textit{Flavobacterium meningosepticum} prolyl endopeptidase (Fm-PEP) was introduced into the wheat genome using the biolistic approach. The genes encoding for Fm-PEP and EP-B2 were provided with the endosperm specific HMW glutenin promoter. Integration of these genes in the wheat genome was confirmed at the T\textsubscript{0} and T\textsubscript{1} generations, and the transformants showing gene integrations are currently being investigated for the expression of enzymes and their efficiency to degrade prolamins in simulated gastro-intestinal conditions. To ensure survival of these enzymes during the baking process a site-directed mutagenesis approach was followed to introduce mutations in the first and the seventh blades of the β-propeller domain of the Fm-PEP and at the junction of left and right-domains in the EP-B2. Three mutations in the Fm-PEP showed thermostability at 90°C and retained their activity after the heat shock. Characterization of the EP-B2 mutants is currently underway.

In a nutshell our results show a great potential in obtaining non-immunogenic wheat cultivars, which will have a broad impact on the quality of lives of a vast majority of the wheat consumers.

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