

Expression of Wheat Glutenin Genes in Maize Endosperm*



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Background

Wheat storage proteins are the base for the rheological properties of wheat flour [1] and its superior quality in bread making. People susceptible to some of these proteins can develop celiac disease [2], an inflammatory condition of the gastrointestinal tract. Approximately 0.3-0.8% of the European and North American population is affected by the celiac condition [3].

Maize flour is a food source that contains starch yet does not induce celiac disease symptoms. It has also been demonstrated that maize kernels are a well suited system for the commercial, high level expression of transgenes [4].

We created maize plants expressing in the endosperm the x-type High Molecular Weight (HMW) glutenin subunit 1Dx5, the y-type HMW glutenin subunit 1Dy10 as well as the Low Molecular Weight (LMW) glutenin subunit a3 [5] in order to produce glutenin subunits, which after purification would be suitable for toxicity tests in celiac patients. This will help to study the toxicity of glutenin subunits, and to find possible toxic epitopes within these proteins. The glutenin expressing maize lines would also be used to test whether the combined expression of the different glutenin subunits would

lead to gluten-like structures in maize dough, which are likely to improve the baking properties of maize flour.

Conclusions

The HMW subunits 1Dx5, 1Dy10 and the LMW subunit a3 were successfully expressed in maize endosperm. So far crosses of different maize lines have led to plants expressing two different subunits. These lines will help to create plants which combine all three glutenin proteins in the endosperm. Flour obtained from these plants will be tested for gluten like structures, rheological features and for celiac toxicity.

Theoretically, toxic epitopes, once identified, could be removed from the glutenin proteins. By the combined expression of those 'unarmed' wheat glutenins in maize endosperm, a maize flour with gluten-like structures, and hence strongly enhanced baking properties, could feasibly be created. This flour could then be used for the production of 'gluten free' bread.

References

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Results

To achieve a high level of transgene expression in the endosperm, we chose a HMW glutenin promoter from *Triticum aestivum* cv. 'Florida' [6], which we found highly active in maize endosperm. For termination we chose the endogenous 27 kD γ -zein terminator (**Figure 1**). Maize was transformed by the biolistic method [7].

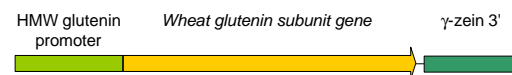


Figure 1

Basic expression construct used for transformation of maize. The expression of the different wheat glutenin subunits is driven by the wheat HMW glutenin promoter. Transcription is terminated by the maize 27 kD γ -zein terminator.

We regenerated maize lines, harbouring full length expression cassettes and screened these for the presence of the respective wheat storage proteins. A SDS-PAGE of endosperm proteins from maize lines expressing the different glutenin proteins is shown in **Figure 2**. Reversed phase HPLC profiles enabled us to estimate the relative amount of recombinant gluten protein. The 1Dx5 subunit constituted up to 4.9% of the extracted storage protein fraction, the 1Dy10 subunit 7.1%, and the LMW subunit a3 6.7%.

In order to confirm the correct processing of the signal sequence in recombinant 1Dy10, the sequence of the six n-terminal amino acid residues was determined. It matched the n-terminus of the native 1Dy10 from wheat, as did the molecular mass of the recombinant protein, measured by MALDI-TOF MS.

Lines expressing single glutenin subunits are crossed for the combined expression of glutenin genes in the endosperm. Extracts from these endosperms are shown in **Figure 2** (lanes 5 and 6).

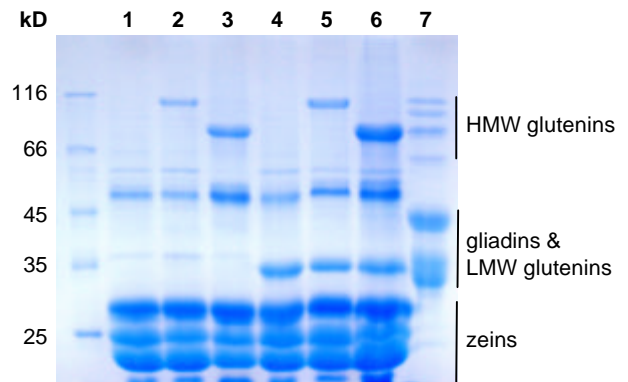


Figure 2

Recombinant wheat glutenins in maize endosperm. SDS-PAGE of 2-propanol extracts of reduced endosperm proteins (28 dap) from non-transgenic (1), 1Dx5 expressing (2), 1Dy10 expressing (3) and LMW a3 expressing (4) maize plants. Lanes 5 and 6 show extracts from endosperms originating from crosses of lines expressing either 1Dx5 and LMW a3 or 1Dy10 and LMW a3, respectively. For comparison: proteins extracted from endosperm of wheat cv. 'Cheyenne' (7), the origin of the transferred HMW glutenin genes.