Development of molecular markers in order to assess the α-gliadin immunogenic content of an international spelt collection

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Development of molecular markers in order to assess the α-gliadin immunogenic content of an international spelt collection

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INTRODUCTION AND AIMS

Gluten is a protein fraction found in the seeds of bread wheat, spelt and durum wheat which confers important bread-making properties to the dough. Celiac disease (CD) is a health disorder where toxic epitopes (gluten peptides) are recognized by the immune system of CD patients.

Belgian spelt variety « Cosmos »

This study aims to investigate the immunogenic composition of contrasted spelt accessions and to develop molecular markers to quantify it quickly.

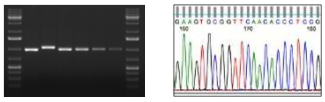
METHODOLOGY

The immunogenic content of 11 contrasted spelt accessions was evaluated by investigating their α-gliadin transcript composition. Molecular markers such as TaqMan probes are currently designed to study the expression levels of the toxic epitopes.

RNA extraction and cDNA synthesis

Total RNA was extracted from **spelt immature seeds** harvested 20 days after flowering.

α-gliadin cloning and sequencing

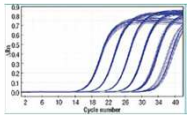


Alpha-gliadin sequences were amplified by PCR, inserted into a cloning vector and sequenced using the Sanger technique.

Study of the epitope composition

The composition in the **four major epitopes** involved in CD was studied in each amplified α-gliadin.

TaqMan probe design



Molecular markers, such as TaqMan probes targeting **intact (toxic) epitopes,** are currently designed.

CONCLUSION

In this study, α-gliadin transcripts from 11 contrasted spelt accessions were successfully cloned and sequenced. According to the genome from which α-gliadins were expressed (A, B or D), high variations were observed in the composition of the four major epitopes involved in celiac disease.

Molecular markers were then designed to target either genome-specific motifs *via* PCR primers or the four epitopes in their intact form *via* TaqMan probes. These markers should be useful to study the immunogenic content of spelt and bread wheat accessions and thus to provide a valuable information to develop safer varieties for CD patients.

RESULTS

Study of the α-gliadin expression profile

For each of the 11 contrasted spelt accessions, about 50 α-gliadin transcripts were cloned and sequenced to analyze their composition in the four major epitopes involved in celiac disease.

High variations in the epitope composition were highlighted according to the genome of origin (A, B and D).

Table 1. Intact form and mutated variants of the DQ2.5-glia-α3 epitope found in the α-gliadin transcripts of 11 contrasted spelt accessions.

Epitope	Amino acid sequence	A genome	B genome	D genome	Total
DQ2.5-glia-α3	FRPQQPYPQ	173	0	93	266
	FPPQQPYPQ	0	149	0	149
	FRQQQPYPQ	60	0	0	60
	FRPQQSYPQ	0	0	13	13
	FRPQKPYPQ	4	0	0	4
	SPPQQPYPQ	0	2	0	2
	FQPQQPYPQ	1	0	0	1
	FPPQQSYPQ	0	1	0	1
	Total	238	152	106	496

Genome-specific primer design

Motifs found in the α-gliadin sequences expressed from only one genome were used to design genome-specific PCR primers.

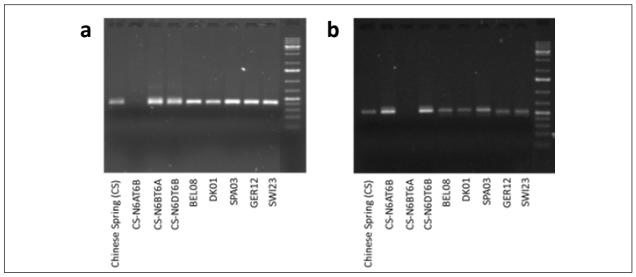


Figure 1. PCR amplifications using primers specific to A-genome (a) and B-genome (b) α-gliadin sequences on genomic DNA of Chinese Spring, on three of its nulli-tetrasomic lines and on five spelt accessions.

TaqMan probe design

In order to reveal the immunogenic potential of spelt or bread wheat accessions, TaqMan probes are currently being designed in view of targeting the intact (toxic) form of the epitopes only.

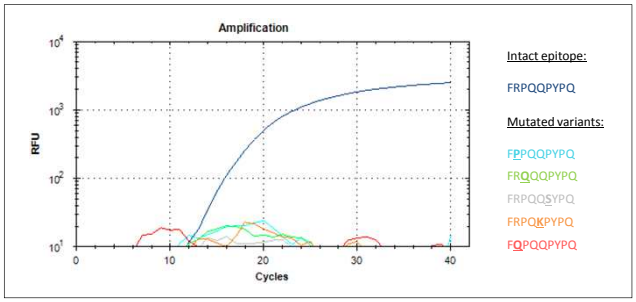


Figure 2. qPCR amplifications with a TaqMan probe using six α-gliadin clones which display the intact form or one of the five mutated variants of the DQ2.5-glia-α3 epitope, respectively.

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