Application of TaqMan probes to study the expression kinetics of celiac disease-related toxic epitopes and their presence in the gDNA of contrasted spelt accessions

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INTRODUCTION AND AIMS

Belgian spelt landrace

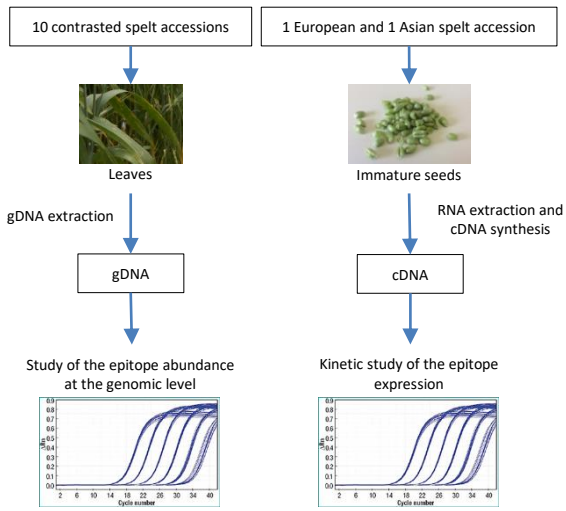
Gluten is the protein fraction found in the seeds of wheat, spelt, barley and rye, which confers visco-elastic properties to the dough. Celiac disease (CD) is an autoimmune disorder where some toxic epitopes (gluten peptides) are recognized by the immune system of CD patients.

In a previous work (Dubois et al. 2017, Plant Methods), TaqMan probes targeting CD-related epitopes found in the α-gliadin gluten protein class were developed for further breeding purpose. Given the multigenic character of the α-gliadin family, a high proportion of the α-gliadin genes are pseudogenes and the TaqMan probes were used to compare the epitope expression levels at the cDNA level.

The objectives of this work are (i) to check whether the application of these probes on gDNA samples, easier and cheaper than on cDNA samples, provides a good estimation of the epitope expression levels, and (ii) to carry out a kinetic study of the epitope expression levels to find out the best stage to study the cDNA epitope content through the application of the developed TaqMan probes.

METHODOLOGY

The presence of these epitopes was analyzed thanks to four TaqMan probes (i) at the genomic level using young leaves of contrasted spelt accessions, and (ii) at the transcriptomic level using spelt immature seeds.



CONCLUSION

In this study, the abundance of sequences encoding for CD-related epitopes was studied in the gDNA of contrasted spelt accessions. The similar trends with results obtained in a previous transcriptomic study with the same accessions might indicate that the developped TaqMan probes could be used as a first approximation on the gDNA, which would represent an easier, faster and cheaper alternative to the use of cDNA, in order to study the epitope content of spelt and bread wheat accessions.

The kinetic study of the epitope expression levels showed different expression patterns between one European and one Asian spelt accessions. Further investigations with more accessions from these origins would be necessary to determine the most appropriate seed stage to study the epitope content. Moreover, it could also provide answers about whether differing epitope expression patterns can be linked up with putative distinct origin of European and Asian spelts.

RESULTS

Study of the epitope abundance in spelt gDNA

The abundance of the four main epitopes involved in CD was studied in the gDNA of 10 contrasted spelt accessions and compared to similar analyses previously carried out in a transcriptomic study.

Interestingly, even if the correlation is not perfect for each accession between gDNA (figure 1a) and cDNA (figure 1b) samples, the general trend is the same. Moreover, the highest and the lowest epitope abundances (denoted by the « + » and « - » symbols in figure 1, respectively) were noticed for the same accessions.

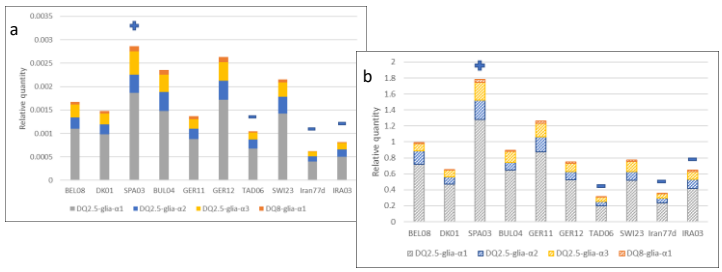


Figure 1. (a) Relative quantification of the four main epitopes involved in CD in the gDNA of 10 contrasted spelt accessions through the application of previously developped TaqMan probes. This results were compared with results obtained in a previous transcriptomic study (b) where the same probes were applied on the same spelt accessions. The « + » and « - » symbols denote accessions that showed respectively the highest and the lowest epitope abundance in both the gDNA and cDNA samples.

Kinetic study of the epitope expression levels

Given the putative distinct origin of European and Asian spelts, this kinetic study was carried out with the same probes using immature seeds from one German (GER11) and one Tajik (TAD06) spelt accessions, harvested 5, 10, 15, 20 and 25 days post-anthesis (DPA).

The results showed different expression patterns according to the accession, the expression peak being reached earlier and with a higher value in the German accession.

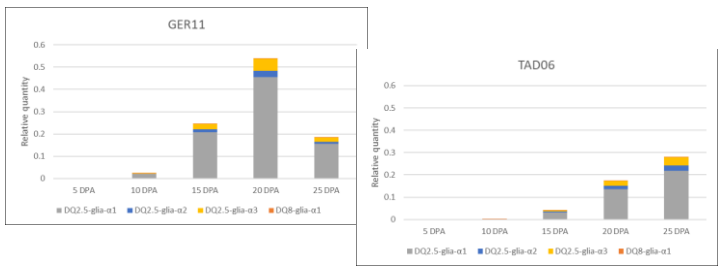


Figure 2. Kinetic study of the epitope expression levels in one German (GER11) and one Tajik (TAD06) spelt accessions. The analysis was carried out by applying TaqMan probes on cDNA samples obtained from immature seeds harvested 5, 10, 15, 20 and 25 DPA.

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