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REGULAR ARTICLE

PROTEIN MAPS OF OAT AND RYE

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ABSTRACT

The aim of our study was to analyze two different species of cereals, oat and rye, in term of content and characteristic of coeliac active proteins. The results showed the wide variability of individual protein fractions and dominance of prolamins with coeliac active polypeptidesthat was also confirmedby electrophoretic profiles of storage proteins by isoelectric focusing and SDS-PAGE. 2-DE protein maps were compared by protein patterns and there were detected the presence of coeliac active polypeptides in both cereals. The content of proteins in oat is smaller than in rye that is revealed also in protein maps.The comparison of protein maps of both cereals showed main differences in protein pattern in the LMW region (<14 kDa), but similarities were found out in the HMW and LMW regions that is evident in gels with a narrow pH range of pH 6-11.

Keywords: oat, rye, coeliac disease, 2-D electrophoresis,proteins maps

INTRODUCTION

Coeliac disease (CD), also known as Coeliac sprue, infantilism, gluten–sensitive enteropathy, is one of the most frequent food intolerances reported worldwide. It may be defined as an inflammatory disease of the upper small intestine (jejunum, duodenum) in

genetically susceptible individuals triggered by ingestion of wheat, barley, rye, triticale, and possibly oats products (**Mowat, 2003**).

Whether oats is harmful remains controversial. Clinical data are now available suggesting that the great majority of Coeliac patients tolerate oats although some concerns remain about the safety of this cereal for Coeliac, oats remains currently on the Codex list of gluten-containing cereals (**Lundin *et al*., 2003**).

The search for the precipitating proteins of CD was mainly performed with wheat and oats (**Shewry *et al*., 2009**). Testing of rye and barley has been rather minimal, the strong similarities of their storage proteins with wheat gluten proteins, however, suggest that they may be capable in causing CD. Recent comparative studies on the intestinal immune response revealed that the gliadins, secalins, and hordeins elicited a similar increase in interferon production in cultured biopsies of CD patients (**Bracken *et al*., 2006**). The amino acid composition of these three prolamin fractions was closely related to both CD toxicity and taxonomy of the cereals.

Though substantial progress has been achieved in understanding the general principles that determine the pathogenesis and treatment of CD, many key questions still remain unanswered. The different types of these proteins are generally characterised by structural domains with sequences rich in glutamin, prolin, and hydrophobic amino acids. Surprisingly, HMW subunits were also toxic; though they have sequences quite different from the other types. A never-ending question is about whether oats are toxic to CD patients; research and regulation has not revealed an answer to this during the last 50 years (**Wieser & Koehler, 2008**).

The objective of our study was to prepare two dimensional protein maps of oat and rye with the aim to comparestudying species and finding out differences between themand subsequently, achieved results use for precise diagnose of allergic patients and assessment of allergen in food.

MATERIAL AND METHODS

Materials

Seeds of rye (cv. OKLON) and oats (cv. VALENTIN) were obtained from the Gene Bank of the Research Institute of Plant Production, Piešťany in the Slovak Republic and were

milled by CU Mill, (Lionhill Company Inc.) to a homogenous flour. Chosen cultivars of oat and rye are the most growing cultivars in the Slovak Republic.

Methods

Samples preparation

Proteins were extracted from the flour by adding 1 ml of buffer {250 µl ditiotreitol (DTT) (28 mg/ml), 12.5 µl IPG buffer (carrier ampholytes) and 237.5 µl ultra-pure water to 2 ml IPG rehydration buffer [7 M urea, 2 Mthiourea, 2 % CHAPS - 3-/(3-cholamidopropyl) dimethylammonio/-1-propanesulfonate]} to 50 mg of flour. The samples were then wheel-mixed for 1 h, RT and then centrifuged 3 min, 9, 000 x g, RT. The protein content of the supernatant (SN) was estimated by Coomassie Plus protein assay (Thermo Scientific, Pierce, UK) (based on the Bradford assay) and samples were stored at -20 ºC until use. The protein content of the oat extract was insufficient, so the Compact-Able™ Protein Assay Preparation Reagent Set (Thermo Scientific, Pierce, UK) was used to precipitate the protein, which was then re-suspended in the extraction buffer as described previously.

1st Dimension - Isoelectric focussing (IEF): Immobilised pH gradient (IpG) strips (GE Healthcare, Amersham UK), 7 cm, pH 3-11 NL and pH 6-11, were used for the first dimension. Strips were hydrated O/N at 20°C 125 µl rehydration buffer [7 M urea; 2 Mthiourea, 2 % w/v CHAPS; 0.5 M DTT; relevant pH range IPG buffer; 0.001 % w/v bromophenol blue] containing ~ 40µg protein of sample. Focussing was performed at 20 ºC, current 50 µA *per* strip (300V 30 min 0.2 kVh; 1000 V 30 min, 0.3 kVh; 5000 V, 1 h 20 min, 4.0 kVh; 5000 V, 25 min, 2.0 kVh). Focussed IPG strips were stored at -80 ºC until required.

2nd Dimension - SDS PAGE: Focussed IPG strips were equilibrated in tris-acetate equilibration buffer [0.122 M tris-acetate containing 0.5 % w/v SDS; 6 M urea; 3 % w/v glycerol; 52 mM DTT; 0.01% w/v Bromophenol blue]. After 30 min strips were derivatised in the dark with 0.14 Miodoacetamide in equilibration buffer for a further 30 min. Strips were then transferred to 1 mm, 4-12 % Bis-Tris Zoom™ gels for the second dimension. Gels were run at 200 V and 100 W per gel for 35 min using 1 x MES SDS Running Buffer. Gels were fixed O/N in 40 % v/v methanol containing 10 % w/v TCA before staining with SYPRO Ruby Stain(Invitrogen, UK) in the dark O/N. After de-staining O/N with 10 % v/v methanol and 6 % TCA, gels were imaged using a high-resolution molecular imager (PHAROS FX™

Plus, Bio Rad, UK). Imaged gels were returned to de-stain solution and stored in the dark at 4 °C until required.

RESULTS AND DISCUSSION

Barley, wheat and rye are related members of the Tribe Triticeae and consequently share many genetic and biochemical characteristics **(Shewry, 2004/Rev.2006).** Storage proteins ofwheat, rye and barleyaccount for 50 % or more of the total protein in mature grains and have important impacts on their nutritional quality for humans and livestock and on their functional properties in food processing **(Shewry & Halford, 2002)**. The prolamin fractions of wheat, rye, barley and oats are complex mixtures of proteins, which vary in their composition between different genotypes of the same species. The total number of components has not been determined precisely, but analyses by one- and two-dimensional electrophoresis show the presence of at least 50 individual proteins in hexaploid bread wheat and 20-30 in barley and inbred lines of rye. The individual components are classically divided into groups based on their solubility and electrophoretic properties, the groups being given different names in the species (hordeins, secalins, gliadins and avenins + glutenins in barley, rye, wheat and oats, respectively) **(Shewry, 2004/Rev.2006).** Earlier studies of cereal seeds showed that storage proteins dominated 2-DE gels with total protein extracts **(Skylas *et al.*, 2000; Finnie *et al.*, 2004)**; this is also true for all of the cereal gels prepared for this study. Often the storage proteins were the most abundant proteins visible on the gels.

The protein maps of oat and rye were obtained by performing 2-DE over a broad pH range (pH 3-11NL) to obtain total protein profiles. Then, focussing on the gluten fraction, as it is the major trigger for Celiac disease, we separated proteins with a basic pI over a narrow pH range of pH 6-11. Putative identifications of proteins were obtained using current literature.

Protein maps of Oat

In the total protein profile from the oat proteins were resolved between 6-116,0kDa and focussed over the range of pI 5-9. A distinct train of abundant proteins was present at approximately 36-55,0kDa (~38,000 Da); pI 5-8 on both the pH 3-11 gel (figure 1i) and pH 6-11 gel (figure 1ii). These highly abundant proteins probably corresponded to gluten proteins/ avenins (**Klose *et al.*, 2009**). The major storage proteins of oats are avenins and globulins. The

avenins are a highly polymorphic group with M_rs 20-30,0kDa and a minor group M_rs 30-40,0 kDa. The oat globulins consist of 12S species, with M_rs 330,0kDa comprising six subunit of about M_rs 55,0 kDa comprising M_rs 33,0 and M_rs 23,0 kDa chains linked by S-S bonds. It is likely these are involved as allergens in oat. In the study of **Varjonen *et al.* (1994)** oat allergic sera cross-reacted with proteins from wheat, rye and barley. A 66 kDa allergen was found in oat, but its sequence and protein family is still not known. It could be the group of proteins highlighted at approximately pI 6.5-8 and M_rs 66,0kDa (figure 1v). Two abundant spots located in the basic region of the pH 3-11 gel (figure 1iii) and 6-11 gel (figure 1iv), were observed at approximately 14,0 kDa, pI 7.

Rocher *et al.* (1992) identified three major avenins, which react with coeliac sera and that they called oat "coeliac immunoreactive proteins" (CIP), as well as one alpha amylase inhibitor from oat endosperm, which showed a significant homology (60-80 % of identity) with the alpha amylase inhibitor from ragi, as well as with the two probable alpha amylase inhibitor from rice and barley. Avenin-A (CIP-1), which belongs to the gliadin/glutenin family and causes allergic reaction in human and are one of the cause of the Coeliac disease. It has theoretical pI 4.25 and M_rs 4, 393 kDa, as well as Avenin-F (CIP-2), which has theoretical pI 3.45 and M_rs 5, 213 kDa and Avenin-E (CIP-3) with theoretical pI 7.44 and M_rs 21, 0 kDa, it could be highlighted protein spots with pI ranging from 7 to 8 and Mrs 15-25,0 kDa (figure 1vi). Avenin-A and Avenin-F are not visible on our gels. We could not identify protein spots and compare our results very properly due to lack of information because there has been very little research done for oats, especially 2-D electrophoresis.

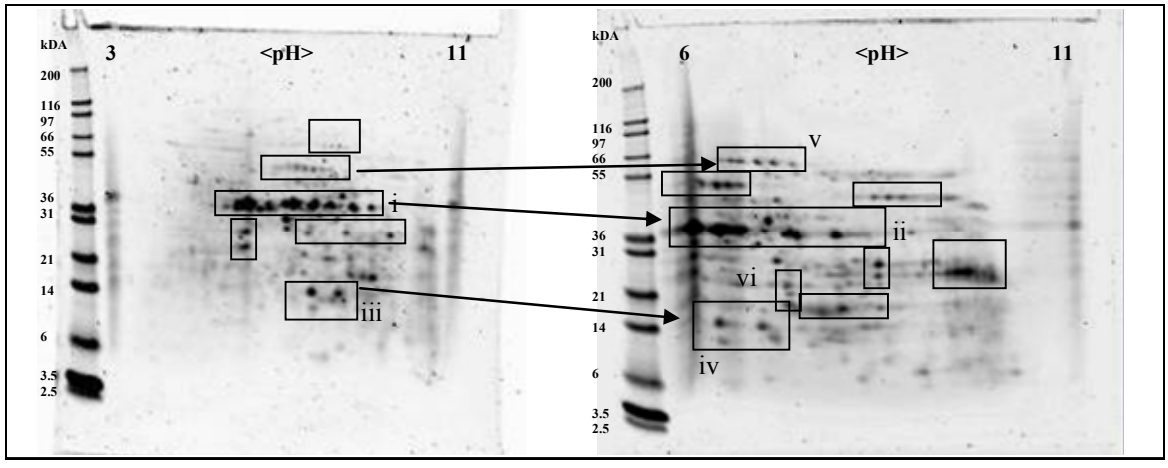

Figure 1 Protein maps of oat (cv. Valentin**),** MWM – molecular weight marker

Protein maps of Rye

In the2-DE protein map of ryewas observed a higher number of abundant groups visible over a broad range (~ 6-200,0kDa, pI 4-9). Highly abundant proteins visible at approximately 55,0kDa, pI 6-9 (figure 2i) and 40,0 kDa, pI 6-9 (figure 2ii) were observed as over-lapping spots forming an isoelectric streak. These proteins were running at the correct Mr and pI observed for HMW and MWM subunits of glutenin **(Gellrich *et al*., 2003)**. **Rocher *et al.* (1995)** identified γ- and ω-type secalins together with two low molecular mass glycoproteins as the major coeliac immunoreactive proteins from a chloroform/methanol soluble extract from rye endosperm. One ω-type secalins of 40 kDa (ω1-40); three γ-type secalins one of 70 kDa (γ-70) and two of 35 kDa (γ-35); as well as two low molecular mass glycoproteins of 15 and 18 kDa, all exhibit coeliac serum antigenicity. Coeliac immunogenic secalins, γ-70, γ-35 and ω1-40 indicated molecular masses of 71,457, 32,240 and 39, 117 kDa respectively. There is absence of information about rye proteins, especially of those obtained by 2-D electrophoresis as well as by oat proteins.

When proteins were separated over a narrow pH range of pH 6-11, we found some proteins that were at similar pI and Mr to protein spots separated on wheat 2-DE gels, including isoelectric streaks of protein spots in the region of 55,0 kDa, pI 6-7 (figure 2a) and in the region of 45-50,0 kDa, pI 6-8 (figure 2b).

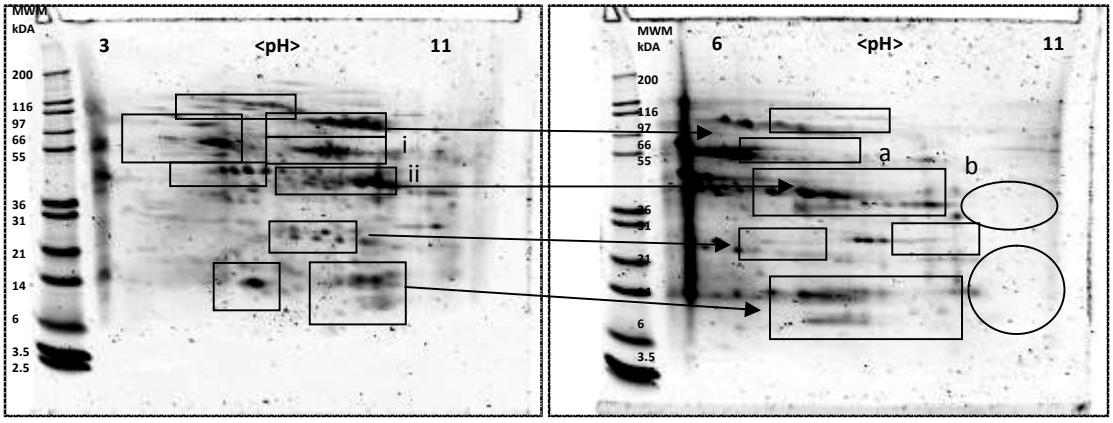

Figure 2 Protein maps of rye (cv. Oklon),),MWM – molecular weight marker

Klose *et al.* (2009) observed the same protein spot areas of approximately 15,0; 30-35, 0 and 40-45,0kDa, these were obtained after the extracting the oat proteins (Osborne fraction), followed by using 2-DE.

CONCLUSION

Our work deal with comparing of oat and rye proteins, finding out differences between them by 2-DE. First, 2-DE - gels with pH 3-11 were run, that were followed by 2-DE - gels with pH 3-11. The chemical properties of rye proteins were very similar to oat. On the other hand, the proteins map of oat was quite different compare to the protein map of rye. This showed differences between species that also are related to differences in functional properties. The two dimensional protein maps can be beneficial in many ways. It could be used, for example, for precise diagnose of allergic patients and assessment of allergen in food.

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