

# Identification of Zinc Binding Proteins of the Wheat Seed

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## INTRODUCTION

Zinc content of the wheat seed has relevance for crop production and human health. It was shown that seeds containing higher amounts of micronutrients, especially Zn, germinated better and were resistant to soil-driven pathogens. Furthermore, in developing countries where the diet is largely based on cereals, increased Zn content in the wheat kernel is important for those who suffer Zn deficiency.

Studies on mechanisms related to Zn uptake and accumulation in the shoot and seed showed an existing correlation between Zn and protein content. It appears that some proteins, mostly abundant in the embryo and the aleurone layer, may be sinks for Zn. The literature on Zn-binding proteins in wheat seeds is limited (Shewry 2002).

## METHODS

A durum wheat genotype (*Triticum turgidum*, cv. Balcali-2000) was grown at different Zn and N application rates (0.5 to 24 mg Zn kg<sup>-1</sup> soil, 75-675 mg N kg<sup>-1</sup> soil) to have a wide range of seed Zn and N (protein) concentrations. Seeds were collected at harvest and used for extraction of proteins and polyacrylamide gel electrophoresis analysis.

Total proteins or protein fractions were isolated from seeds using published techniques including total protein extraction for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis (Fido 2004), Osborne fractionation (Fido 2004), gliadin and glutenin extraction (Singh 1991) and native extraction. Extractions were carried out with whole seeds and separately from dissected embryo and endosperm sections. Extracts were analysed using SDS and native polyacrylamide gel electrophoresis, and the bands were identified according to the protein profiles published for different genotypes. Further analyses were carried out on SDS protein profiles transferred to polyvinylidene fluoride (PVDF) membranes for detection of Zn binding. Transfer membranes were incubated in buffers containing Zn (Mazen 1988, Schiff 1988) and were later stained using metal indicators like dithizone and pyridylazonaphthol for visualization of the protein bands in which Zn was bound. The method was verified by various control experiments.

## RESULTS AND DISCUSSION

The Zn accumulation in seeds was proportional to the Zn and N application during growth. Phosphorus accumulation was independent of Zn application, but high N concentrations resulted in a drop of accumulated P. Nitrogen content in the seeds increased with N application. At N applications higher than 225 mg kg<sup>-1</sup> soil, increasing Zn concentration affected N accumulation positively, but the effect was negative at 75 mg N kg<sup>-1</sup>.

Comparisons between extractions from seeds with different Zn and N content did not show significant differences. SDS-PAGE protein profiles for total extracts of whole seed, embryo and endosperm sections are shown in Fig. 1. (a). As indicated by lane 2 and 3, embryo and endosperm protein profiles show significant differences. High and low molecular weight subunits of glutenins and gliadins were identified by comparing extracts acquired by different methods (Fig. 2). Visualization of Zn binding to proteins is shown in Fig. 1. (b). Zinc binding was detected on prolamin superfamily proteins between 60 kDa and 30 kDa.

According to the results from experiments with metal chelates and reducing agents as our detection method, we found that Zn binds to cysteine in prolamins.

Currently, several tests are conducted to identify Zn-binding proteins or subunits in seeds differing in Zn and N concentrations. The results obtained will be presented at the conference.

Fig. 1.

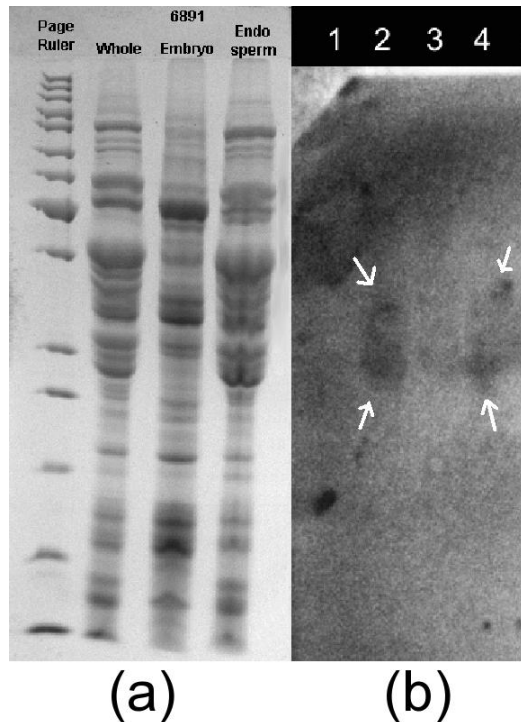
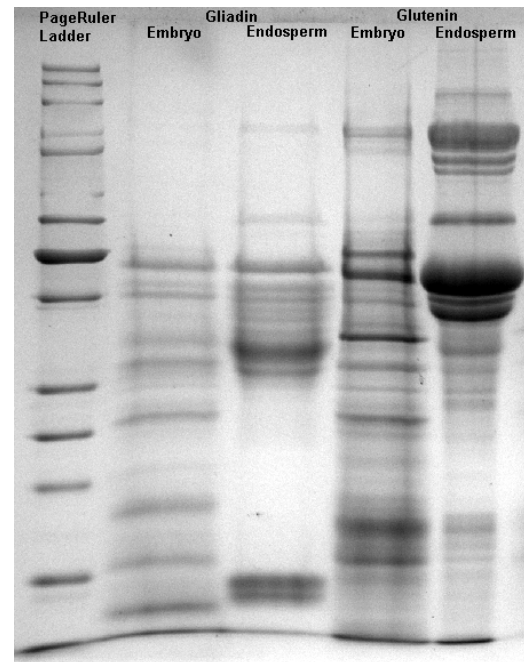


Fig. 2.



**Fig. 1. (a) SDS-PAGE analysis of total protein extractions from whole seed, embryo and endosperm. Lane1: PageRuler protein ladder; Lane2: whole seed; Lane3: embryo; Lane4: endosperm. (b) Detection of Zn bound proteins. A replica of the gel in (a) was used to detect Zn bound proteins. Stained regions are shown with white arrows. Staining in lane 3 is minimal when compared to lane 2 and 4.**

**Fig. 2. Gliadin and glutenin extraction from embryo and endosperm sections. Lane1: PageRuler protein ladder; Lane2: embryo gliadin extract; Lane3: endosperm gliadin extract; Lane3: embryo glutenin extract; Lane4: endosperm glutenin extract.**

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