

GASTROENTEROLOGY

Environmental factors of celiac disease: Cytotoxicity of hulled wheat species *Triticum monococcum*, *T. turgidum* ssp. *dicoccum* and *T. aestivum* ssp. *spelta*

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Abstract

Background and Aim: In the present paper, the toxicity of prolamines derived from three cereals with a different genome was investigated in human colon cancer Caco-2/TC7 and human myelogenous leukemia K562(S) cells. The purpose of this study was to investigate if species from ancient wheat could be considered as healthy food crops devoid or poor in cytotoxic prolamines for celiac disease.

Methods: Cytotoxicity was measured in terms of inhibition of cell growth, activation of apoptosis, release of nitric oxide (NO), detection of tissue transglutaminase (TG II) and alteration of transepithelial electrical resistance (TEER) on Caco-2/TC7 and K562 (S) cell agglutination. Peptic-tryptic (PT) digest from bread wheat (*T. aestivum* S. Pastore) was used as a positive control.

Results: PT digests of prolamins from spelt wheat (*T. aestivum* ssp. *spelta*) were found to exert toxic effects on Caco-2/TC7 cells and to agglutinate K562(S) cells. Increased amounts of NO and TG II expression were observed in Caco-2/TC7 cells exposed to 1 mg/mL of spelt prolamins, suggesting that spelt wheat can induce cellular mechanisms implicated in the pathogenesis of celiac disease. By contrast, the PT digests from monococcum wheat (*Triticum monococcum*) and farro wheat (*T. turgidum* ssp. *dicoccum*) did not exhibit any negative effects on Caco-2/TC7 and K562(S) cells.

Conclusions: The results have shown a constant and significant toxic effect of spelt wheat which is not shared by the two other ancient cereals. Future studies on celiac intestinal organ cultures are needed to increase the prospects of breeding programs aimed at developing wheat cultivars potentially tolerated by most celiac patients.

Introduction

Celiac disease (CD), an intestinal inflammatory disorder induced by prolamins in susceptible individuals, occurs as a result of the interplay between genetic and environmental factors.¹ The environmental factor that triggers CD is the ingestion of prolamins, the alcohol-soluble fraction of proteins present in the endosperm of cereal grain. Prolamins of common wheat (*Triticum aestivum*) are the main components of gluten, which is responsible for the unique viscoelastic properties of wheat dough.² On the whole, a single cultivar of common wheat contains 65 to 80 different prolamins encoded by genes clustered into 20 loci on six chromosomes, indicating an extremely high level of polymorphism in the prolamins patterns of different genotypes of wheat.³ Feeding trials and *in vitro* or *in vivo* screens based on celiac intestine mucosa found α/β -, γ - and possibly ω -gliadins to be noxious.^{4,5} Moreover, α/β -gliadins, γ -gliadins, HMW and LMW

glutenin subunits were shown to agglutinate human myelogenous leukemia K562(S) cells (an exclusive property of grain proteins harmful to celiac patients),⁶⁻⁸ and to stimulate T cell clones derived from jejunal mucosa or peripheral blood of celiac individuals.⁹⁻¹¹ In addition, a few peptides were shown to elicit a strong and rapid T cell response in nearly all celiac patients, these immunodominant sequences occurring in α - or γ -gliadins.¹² On the contrary, an α -gliadin-derived peptide was found to be weakly immunogenic but very active in triggering mucosal lesions and enterocyte apoptosis.¹³ It was also demonstrated that exposure of different cultured human cell lines to gliadin peptides inhibited cell growth and viability and caused cytoskeleton injuries.^{14,15} On the whole, these findings suggest two distinct effects of prolamins in CD: one is a rapid cytotoxic effect on the intestinal epithelium, the other is an immune response involving T cells that recognize specific prolamins peptides.

There is experimental evidence for a natural variation in noxiousness of prolamins from the different cereal species. Rye and barley show some degree of 'toxicity' in CD, whereas rice and maize have generally been considered safe for celiac patients.¹⁶ Furthermore, recent works have provided evidence that oats are tolerated by CD patients, with the only exception being certain individuals possessing peculiar DQ2-restricted T cells.^{17–19} More interestingly, large differences in the harmfulness profile of prolamins have been found in the genus *Triticum*, which contains diploid, tetraploid and hexaploid species. Common (or bread) wheat (*Triticum aestivum*) and its primitive subgroup spelt wheat (*T. aestivum* ssp. *spelta*), which is characterized by brittle spikes and hulled (non-free-threshing) kernels, contain the A, B and D genomes, which accumulated in a single plant as a consequence of natural hybridization between the diploid D-genome wheat *T. tauschii* and the tetraploid AB-genome wheat *T. turgidum*. This latter species originated through spontaneous hybridization between the diploid A-genome *T. urartu* and another B-genome species of wild grass (*T. speloides*). About 10 000 years ago, *T. monococcum*, an A-genome wheat species closely related genetically to *T. urartu* and characterized by hulled kernels, was domesticated in the Fertile Crescent and cultivated for more than 6000 years in the Mediterranean basin and Central Europe to prepare bread and beer. During the Bronze Age, the tetraploid AB-genome species *T. turgidum* ssp. *dicoccum* was the most cultivated wheat in Egypt. This hulled species was the main cereal crop during the Roman period under the Latin name of 'farro', and still survives as a crop in marginal areas of Italy, Turkey and Balkan countries. 'Farro' wheat underwent a mutation which made the separation of the kernel from the chaff easy and originated the free-threshing wheat *T. turgidum* ssp. *durum* (also known as durum wheat) grown in Mediterranean countries for pasta.²⁰

Prolamins from monococcum wheat were found to be unable to agglutinate the K562(S) cells and to trigger lesions in cultured intestinal mucosa from celiac patients.^{21,22} Conversely, gliadin-derived peptides from durum wheat were found to exert much less adverse effects on intestinal mucosa from celiac patients as compared with those from common wheat, whereas a few durum wheat cultivars were not recognized by T-cell clones specific for some immunodominant α -gliadin epitopes.²³ Reduced or no damage was also caused by bread wheat genotypes after removal of some α -, γ - or ω -gliadins.²⁴

Wheat genotypes poor in noxious prolamins are of considerable interest in breeding programs aimed at developing wheat cultivars potentially tolerated by most celiac patients. In the present work, prolamins from three hulled wheat species, that is, monococcum (*T. monococcum*), farro (*T. turgidum* ssp. *dicoccum*) and spelt wheat (*T. aestivum* ssp. *spelta*), which represent different periods of wheat cultivation, were analyzed for their cytotoxicity on K562(S) cells and human colon adenocarcinoma Caco-2/TC7 cells grown *in vitro*.

Method

Plant and chemical materials

Seeds of monococcum wheat (*T. monococcum*, genome A, $2n = 14$), farro wheat (*T. turgidum* ssp. *dicoccum*, genome AB,

$2n = 28$) and spelt wheat (*T. aestivum* ssp. *spelta*, genome ABD, $2n = 42$) from the collection maintained by the Institute of Cereal Research, Rome, Italy, were used in the present study. Common wheat (*T. aestivum*) cv. *S. Pastore* was used as well.

All tissue culture media were obtained from Gibco BRL (Gaithersburg, MD, USA) and staurosporine was obtained from Sigma Chemical (St. Louis, MO, USA).

Extraction and digestion of prolamins

After removing the hull, seeds (100 g) were milled with an experimental mill and added to 400 mL (w/v) of 0.5 M NaCl. The suspension was stirred at 4°C for 1 h and then centrifuged at 2000 g for 15 min at 4°C. Proteins in the supernatant (albumin and globulin) were eliminated. This operation was repeated twice. The pellet was resuspended in 300 mL of 70% (w/v) ethanol, extracted for 1 h at room temperature under stirring and centrifuged at 2000 g for 20 min at 20°C. The supernatant containing prolamins was frozen and freeze-dried. Prolamins were subjected to peptic-tryptic (PT) sequential digestion.²⁵ At the end of the procedure, the PT digest was heated for 30 min at 100°C, lyophilized and stored at -20°C. The protein content of the PT digest was determined according to the method of Lowry.²⁶

K562(S) cell culture and agglutination test

The K562(S) subclone of human myelogenous leukemia origin was cultured as described previously.²⁷ Cells were harvested by centrifugation, washed twice with calcium-free and magnesium-free phosphate-buffered saline solution (PBSS) and resuspended in this buffer at a concentration of 10^6 cells/mL. The cell suspension (25 μ L) was added to each well of a 96-well microtitre plate containing different PT-digest concentrations obtained by serial dilutions (1:1) with PBSS. The final total volume was 100 μ L. After 30 min incubation at room temperature, a drop of cell suspension was analyzed with a microscope to count clumped and single cells. The agglutination test was repeated twice, using control wells as appropriate.

Caco-2/TC7 cell culture

The human colon adenocarcinoma Caco-2/TC7 cells were a kind gift from Dr Rousset at INSERM, Paris, France. Cells were grown in a controlled atmosphere of 5% CO₂ at 37°C in Dulbecco's modified essential medium (DMEM) containing 4.5 g/L glucose, 2 mM L-glutamine, 50 U/mL penicillin, 50 μ g/mL streptomycin, 1% nonessential amino acids, 1% HEPES and 10% heat-inactivated fetal calf serum (FCS), as described previously.²⁸ Experiments were performed using cells between passages 85 and 100.

Caco-2/TC7 cell viability

Caco-2/TC7 cells were seeded at a density of 5×10^3 cells/well in a 96-multiwell plate. Twenty-four hours after seeding, cells were exposed to 1 mg/mL of the PT digest for 48, 72 and 96 h. Cell viability was measured by the neutral red uptake (NRU) assay.²⁹ The absorbance was read on a Novapath microplate reader (Biorad, Hercules, CA, USA) at a wavelength of 540 nm.

Quantification of apoptosis of Caco-2 TC/7 cells

Caco-2 TC/7 cells were seeded in 100 mm plates at a density of 3.2×10^3 cells/cm². On the fifth day after plating, cells were washed with serum-free medium and treated with 1 mg/mL of PT digest for 24 h in complete culture medium with 5% FCS. Staurosporine (1 μ M) was used as a positive control. Apoptosis was evaluated using the In Situ FLICA Pan-Caspase Detection Kit (Chemicon International, Temecula, CA, USA), a fluorescent-based assay for detection of active caspases. The methodology is based on a cell permeable, non-cytotoxic Fluorochrome Inhibitor of Caspase (FLICA), which covalently binds to a reactive cysteine residue on the large subunit of the active caspase heterodimer. The green fluorescent signal produced by FLICA was measured by a 96-well plate reader using an excitation wavelength of 490 nm and an emission wavelength of 520 nm. The fluorescent signal was a direct measure of the amount of active caspases present in the Caco-2 TC/7 cells.

Nitric oxide release by Caco-2/TC7 cells

Caco-2/TC7 cells were plated at a density of 2.5×10^4 cells/well and grown for 7 days. Following 24 h of incubation at 37°C in complete medium w/phenol red in the presence of 1 mg/mL of PT digest, 500 μ L of culture medium was taken from the plates and mixed with an equal volume of Griess reagent (1% sulfanilic acid in 0.5 N HCl and 0.1% N'-1-naphthyl-ethylendiamine-hydrochloride). Absorbance at 540 nm was measured after 30 min of incubation at room temperature in the dark. The combined concentrations of nitric oxide (NO)²⁻ and NO³⁻, the degradation products of the NO in the culture medium, were determined by the Griess reaction.³⁰ The nitrite concentration was determined by reference to a standard curve of sodium nitrite and values were reported as μ M nitrite/mg protein/mL.

Transepithelial electrical resistance of Caco-2/TC7 cells

Transepithelial electrical resistance (TEER) of Caco-2/TC7 cell monolayers was measured using a Millicell ERS device (Millipore, Bedford, MA, USA). Cells were seeded at a density of 4.4×10^4 cells/cm² on polycarbonate inserts (0.45 μ m pore diameter, 0.9 cm² area) and left to differentiate for 19 days. Culture medium was regularly changed three times a week. TEER was expressed as Ohms $\times$ cm², subtracting from each point the TEER value of the filter alone. TEER data were recorded at room temperature 4 h after treatment with 1 mg of PT digest.

Gel electrophoresis and western blotting

After 24 h incubation with 1 mg/mL of PT digest, Caco-2/TC7 cells were added to a buffer containing 150 mM NaCl, 0.01% sodium dodecylsulfate (SDS) and 1% Triton X-100 and then centrifuged for 5 min at 4°C. The supernatant was stored at -70°C as whole cell protein lysate. The protein concentration was measured according to Lowry *et al.* SDS-polyacrylamide gel electrophoresis (PAGE) was carried out on 4% stacking and 7.5% resolving gel with low range molecular weight standards. Equal amounts of

protein (30 μ g) were loaded in each lane with loading buffer containing 0.1 Tris (pH 6.8), 20% glycerol, 10% mercaptoethanol, 4% SDS and 0.2% Bromophenol Blue. Samples were heated at 100°C for 5 min before gel loading. Following electrophoresis, the proteins were transferred to a PVDF membrane (Biorad). Membranes were blocked overnight with 5% non-fat milk in TBST (100 mM NaCl, 5 mM KCl, 100 mM Tris-HCl, pH 7.4 and 0.05% Tween 20). Membranes were probed with transglutaminase II antibody (mouse, clone CUB 7402) diluted in the blocking solution at room temperature for 1 h and then washed three times in TBST, for 5 min each. Secondary antibody (goat-antimouse, conjugated to horse-radish peroxidase HRP; Biorad) was diluted at 1:3000 in the blocking solution, added to membranes for 1 h at room temperature and then washed three times in TBST, for 5 min each. Proteins on membranes were revealed by the chemiluminescence detection kit (Biorad), according to manufacturer's instructions. Intensities of protein bands on blots were evaluated using the Biorad Chemi-Doc densitometer. Membranes were stripped and reprobed with β -actin antibody to verify equal loading of proteins.

Statistical analysis

Each experiment was run in duplicate and repeated three times. Data expressed as mean $\pm$ SD were analyzed using the two-sided Student's *t*-test for unpaired variables ($P \leq 0.01$).

Results

Agglutination of K562(S) cells

Agglutination of K562(S) cells was shown to be an exclusive property of PT-digested prolamins from bread wheat, rye and barley.⁶ In the present study, the minimal agglutinating concentration (MAC) of the PT digest from spelt wheat required to agglutinate 100% of K562(S) cells was 73 mg/L, a value very similar to that observed in bread wheat *vs. S. Pastore*.^{7,8} On the contrary, the PT digests from monococcum wheat and dicoccum (farro) wheat did not show any agglutination activity, even when tested at a concentration as high as 5000 mg/L.

Effect on viability of prolamins on the Caco-2/TC7 cells

Over the past decade, the Caco-2 cell culture model has been confirmed as a valuable *in vitro* system to assess intestinal epithelial permeation properties and physiological effects of several chemical compounds. Here, the effects of prolamins digests from hulled wheat species on Caco-2/TC7 cells were measured in terms of inhibition of cell growth, activation of cell suicide (apoptosis), NO release, alteration of TEER and induced expression of tissue transglutaminase.

Viability of Caco-2/TC7 cells, as measured by the neutral red uptake (NRU) assay, was significantly ($P \leq 0.01$) inhibited by 1 mg/mL of PT digest from spelt wheat as compared with untreated control cells and very similar to the results obtained with *vs. S. Pastore*. This negative effect was evident after 48 h of treatment and was still relevant after 72 h of exposure to prolamins

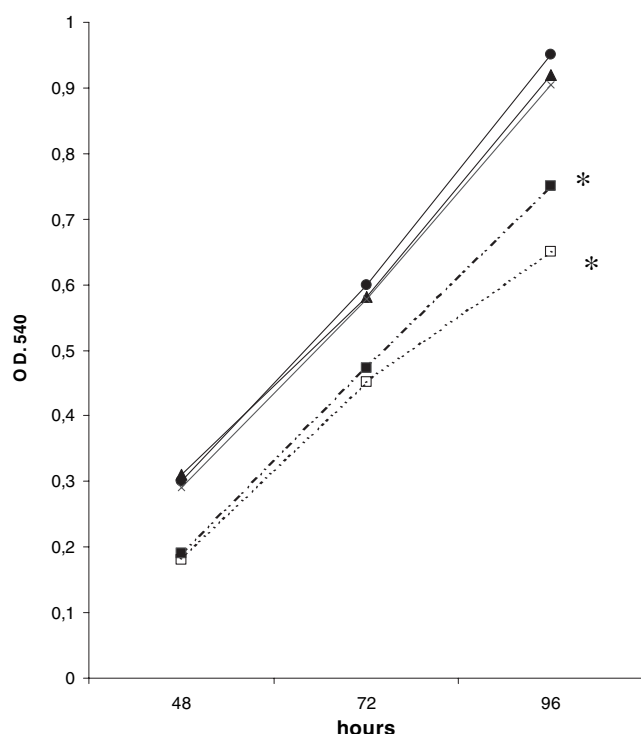


Figure 1 Neutral red uptake by Caco-2/TC7 cells after 48, 72 and 96 h of exposure to 1 mg/mL of peptic-tryptic (PT)-digested prolamins from monococcum wheat (*Triticum monococcum*), dicoccum (farro) wheat (*T. turgidum* ssp. *dicoccum*) and spelt wheat (*T. aestivum* ssp. *spelta*). Data are the mean of three experiments performed in duplicate. * $P \leq 0.01$ in respect to untreated (control) cells. (—●—), ctr; (—■—), spelt; (—▲—), monococcum; (—□—), dicoccum; (—●—), *S. Pastore*.

(Fig. 1). During the entire time of exposure to prolamins, NRU by Caco-2/TC7 cells treated with PT digests from monococcum or dicoccum wheat was not significantly different from that of control cells.

Activation of caspases

In previous studies it was reported that PT digest of wheat gliadin *vs.* *S. Pastore* was able to activate the apoptosis of intestinal Caco-2 cells via an autocrine mechanism involving the Fas-Fas ligand pathway.³¹ The central component of this process is a cascade of proteolytic enzymes called caspases, which are synthesized as precursors that undergo proteolytic maturation to form an active tetramer responsible for proteolytic cleavages leading to cell death.³²

In this experiment, apoptosis was evaluated in terms of the amount of active caspases present in the Caco-2/TC7 cells treated with 1 mg/mL of PT-digested prolamins for 24 h, as determined by a fluorometric detector and expressed as RFU. The Caco-2/TC7 cells treated with prolamins from spelt wheat exhibited a significant ($P \leq 0.01$) increase in active caspases as compared with untreated cells. On the contrary, the PT digests from monococcum or dicoccum wheat caused a small but not significant increase in caspase activity with respect to control cells (Fig. 2).

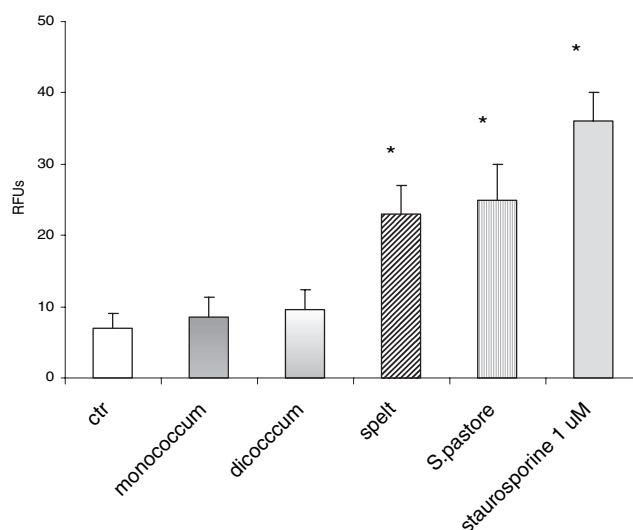


Figure 2 Apoptosis of Caco-2/TC7 cells treated with monococcum-peptic-tryptic (PT), dicoccum-PT, spelt-PT and wheat PT evaluated as relative fluorescence unit (RFU) of active caspases. Data are the mean of three experiments performed in duplicate. * $P \leq 0.01$ in respect to untreated (control) cells.

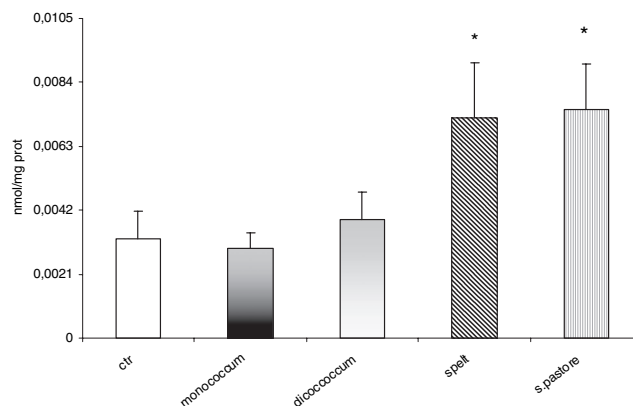


Figure 3 Nitric oxide (NO) concentration (μ M nitrite/mg protein/mL) in the culture medium of Caco-2/TC7 cells after 24 h of exposure to 1 mg/mL of peptic-tryptic (PT)-digested prolamins from monococcum, dicoccum and spelt wheat. Data are the mean of three experiments performed in duplicate. * $P \leq 0.01$ in respect to untreated (control) cells.

Release of nitric oxide in the medium of Caco-2/TC7 cells

Previous studies have shown that the Caco-2 cells produce NO in the presence of noxious stimuli.³³ When digested prolamins (1 mg/mL) from spelt wheat were administered to the Caco-2/TC7 cells for 24 h, a significant ($P \leq 0.01$) amount of NO was revealed in the culture medium as compared with that of control cells (Fig. 3). This result is in accordance with previous indications that the Caco-2/TC7 cells produce NO after exposure to gliadin digests from bread wheat.³⁴

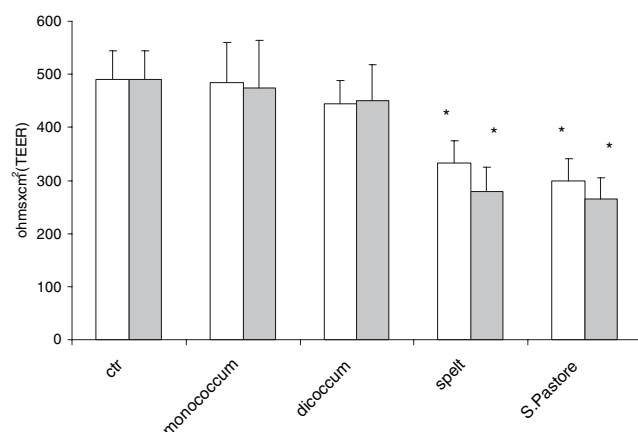


Figure 4 Trans epithelial electrical resistance (TEER) of Caco-2/TC7 cells after 4 h exposure to 1 mg/mL of monococcum, dicoccum, spelt and wheat bread *S. Pastore* prolamins administered from either apical (AP) or basolateral (BL) side. Data are the mean of three experiments performed in duplicate. * $P \leq 0.01$ in respect to untreated (control) cells. (□, AP; (■) BL.

NO concentration in the culture medium of Caco-2/TC7 cells exposed to prolamins from monococcum or dicoccum wheat did not differ significantly from that measured in the culture medium of control cells.

Trans epithelial electrical resistance

Epithelial permeability plays a key role in the intestinal function, allowing selective processes of absorption and secretion of orally ingested molecules. Barrier integrity of differentiated Caco-2/TC7 cell monolayers grown on polycarbonate filters and exposed to prolamins digests was assessed by TEER measurements. The Caco-2/TC7 cells treated with spelt prolamins for 4 h from either the apical or the basolateral side of the cell monolayer showed a significant ($P \leq 0.01$) decrease in TEER values with respect to control cells (Fig. 4). Interestingly, basolateral exposition to spelt prolamins caused a higher decrease in TEER compared with apical exposition. On the contrary, exposure to prolamins from monococcum and dicoccum wheat did not affect the TEER values of the Caco-2/TC7 cells.

Western blot analysis

In the SDS-PAGE pattern of proteins extracted from Caco-2/TC7 cells incubated for 24 h with different prolamines, the expression level of transglutaminase II (75 kDa) revealed by immunoblotting was increased in cells exposed to spelt and wheat bread *vs.* Spastore (Fig. 5, lane 4–5) with respect to the control (lane 1); this was less evident in the case of monococcum and dicoccum wheat (lane 2–3).

Discussion

ABD-genome spelt wheat (*T. aestivum* ssp. *spelta*) probably arose through cultivation around 10 000 years ago as a consequence of a

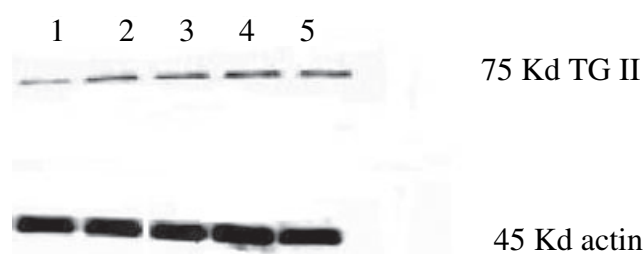


Figure 5 Reactions of proteins extracted from Caco-2/TC7 cells, fractionated by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted against transglutaminase II (75 KD) antibodies. Before protein extraction, the Caco-2/TC7 cells were submitted to 24 h exposition to peptic-tryptic (PT) digests of (2) monococcum (3) dicoccum (4) spelt, and (5) bread wheat *S. Pastore*. Lane 1 shows the protein extract from untreated cells (5 A). β -actin (45 KD) is shown for equal protein loading.

natural hybridization event involving tetraploid AB-genome farro wheat (*T. turgidum* spp. *dicoccum*) and wild diploid grass *T. (Aegilops) tauschii*, which contributed genome D to the novel hexaploid wheat species.³⁵ It is becoming increasingly popular as a healthy food crop because it is characterized by a high protein content and a good vitreous kernel texture. Furthermore, it is claimed to be suitable for individuals who are intolerant to bread wheat flour. However, no experimental data have been published to support this conclusion. In contrast, the results obtained here strongly suggest that spelt wheat exerts toxic effects on K562(S) cells, its toxicity being similar to that of bread wheat. As mentioned above, proteins or peptides from bread wheat, rye and barley, which are harmful to celiac patients, were found to possess the exclusive property of causing agglutination of K562(S) cells.^{6,8,27}

It has been demonstrated that under standard culture conditions Caco-2 cells differentiate to exhibit morphophysiological and biochemical features similar to those of the intestinal mucosa *in vivo*.³⁶ Therefore, Caco-2 cell culture has been adopted as a major tool in many preclinical screening programs to obtain experimental data on physicochemical modifications induced by different chemical entities on intestinal epithelial cells. When administered to Caco-2/TC7 cell culture, spelt prolamins confirmed their cytotoxicity, measured in terms of inhibition of cell growth and activation of apoptosis, comparable to that of prolamins extracted from bread wheat.³¹

Spelt prolamins also induced high levels of NO in the culture media of Caco-2/TC7 cells. In this context it is noteworthy that NO has been demonstrated to play a pivotal role in the pathogenesis of the histological features of celiac disease.³⁷ Another remarkable finding was that PT digests from spelt prolamins decreased trans epithelial permeability in differentiated Caco-2/TC7 cells cultivated in semipermeable filters measured after 4 h of treatment while monococcum and dicoccum digests did not show the same effect. Previously, it was demonstrated that gliadin alters barrier function of epithelial cells almost immediately by decreasing trans epithelial resistance and increasing permeability to small molecules, causing a reorganization of actin filaments and altered expression of the tight junction proteins occludin, claudin-3 and claudin-4, the TJ-associated protein ZO-1 and the adherens junc-

tion protein E-cadherin.^{38–40} Our experiments confirm that spelt and bread wheat do exert a similar disturbing effect on epithelial permeability.

An increased amount of tissue transglutaminase (tTG) was revealed by western blot in the protein extracts of Caco-2/TC7 cells treated with spelt prolamins as compared with untreated control cells. It is well recognized that tTG is increased in the intestinal mucosa of celiac individuals, tTG molecules being engaged to form complexes with gliadins and other substrates in CD intestinal mucosa⁴¹ and to enhance gliadin recognition by T cells from celiac patients.⁴² More recently, surface transglutaminase II has been shown to modulate actin rearrangement and apoptosis induced by α -gliadin-derived peptide p31–43 in human colonic adenocarcinoma T84 cells.⁴³

The results obtained are in agreement with the fact that spelt and bread wheat are hexaploid subspecies of the same species *T. aestivum*, while monococcum and dicoccum are, respectively, diploid and tetraploid, and therefore phylogenetically distinct.²⁰

Earlier, only minor differences have been found in the amino acid sequences of prolamins between spelt and bread wheat.⁴⁴ In addition, toxic effects on the small bowel mucosa have been observed in a celiac patient after the addition of spelt wheat to a gluten-free diet, leading to the conclusion that spelt wheat is not a safe grain for celiac patients.⁴⁵ Our findings vividly reinforce this conclusion, providing experimental data on the cytotoxicity of spelt prolamins against intestinal epithelial cells. In contrast, prolamins from diploid monococcum wheat (*T. monococcum*) did not show any negative effects on K562(S) or Caco-2/TC7 cells. This finding supports early observations that monococcum wheat is devoid or low in cytotoxic sequences able to agglutinate the K562(S) cells and trigger lesions in cultured intestinal mucosa from celiac patients.^{21,22} A number of accessions of *T. monococcum* have also been found to possess low amounts of T-cell stimulatory sequences from α - or γ -gliadins.²³ Recently,²⁴ two out of four cultivated dicoccum accessions analyzed did not react against T-cell clones specific for some immunodominant α -gliadin epitopes, and displayed only marginal responses when exposed to polyclonal T-cell lines derived from intestinal biopsy specimens of five adult celiac patients.

The accession of farro wheat analyzed here did not exhibit any cytotoxic effect as well. This implies that the ancient hulled wheat species *T. turgidum* ssp. *dicoccum* likely contains genotypes with reduced amounts of toxic prolamins, if any. Future studies will be designed to determine if the distinct differences in Caco-2/TC7 cell responses to the three primitive hulled wheat species analyzed here are due to the presence or absence of the D genome in their nuclei.

It is noteworthy that alcohol-soluble peptides able to prevent agglutination of K562(S) cells by toxic prolamins also occur in monococcum wheat,²¹ suggesting that inactivity of prolamins from monococcum and dicoccum wheat against intestinal cells could be partly due to the presence of protective sequences in these species.

In conclusion, prolamins of spelt wheat were found to alter the physical barrier properties of Caco-2/TC7 cells and to induce cellular mechanisms (NO and tTG overproduction) implicated in the pathogenesis of CD. The occurrence of monococcum and dicoccum accessions devoid or poor in cytotoxic prolamins greatly increases their prospects as healthy food crops.

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