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ORIGINAL ARTICLE

# Divergent properties of prolamins in wheat and maize

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**Abstract** Cereal grains are an important nutritional source of amino acids for humans and livestock worldwide. Wheat, barley, and oats belong to a different subfamily of the grasses than rice and in addition to maize, millets, sugarcane, and sorghum. All their seeds, however, are largely devoid of free amino acids because they are stored during dormancy in specialized storage proteins. Prolamins, the major class of storage proteins in cereals with preponderance of proline and glutamine, are synthesized at the endoplasmic reticulum during seed development and deposited into subcellular structures of the immature endosperm, the protein bodies. Prolamins have diverged during the evolution of the grass family in their structure and their properties. Here, we used the expression of wheat glutenin-Dx5 in maize to examine its interaction with maize prolamins during endosperm development. Ectopic expression of Dx5 alters protein body morphology in a way that resembles non-vitreous kernel phenotypes, although Dx5 alone does not cause an opaque phenotype. However, if we lower the amount of γ-zeins in Dx5 maize through RNAi, a non-vitreous phenotype emerges and the deformation on the surface of protein bodies is enhanced, indicating that Dx5 requires γ-zeins for its proper subcellular organization in maize.

**Keywords** Cysteines · Dx5 · Endosperm · Suborganelles · Zeins

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**Abbreviations**

| | |
|---|---|
| RNAi | RNA interference |
| DAP | Days after pollination |
| HMW | High-molecular weight |

## Introduction

It is thought that the water-insoluble prolamins arose by tandem gene duplication from water-soluble globulins (Xu and Messing 2009). In the progenitor of wheat, the ancestral prolamin gene gave rise to the High-Molecular Weight (HMW) glutenins, which constitutes the group III prolamins and is unique for the subfamily of Pooideae (wheat, barley, *Brachypodium*). During the evolution of the grasses, prolamin genes were copied and inserted either in tandem or dispersed to different chromosomal locations, which in turn could give rise to further amplifications. Donor copies could either be maintained or lost by unequal crossover (Xu et al. 2012). Genome-wide dispersal gave rise to new groups of prolamins. The largest is group II that includes the Low Molecular Weight (LMW) glutenins, the α-, γ-, and ω-gliadins in wheat and the β- and γ-zeins in maize. Group I represents the youngest prolamins that include the α- and δ-zeins in sorghum and maize, which are unique to the Panicoideae. An interesting aspect of this evolution is the properties of the glutenins and gliadins. For instance, wheat proteins allow flour to make dough required for bread making. While maize flour cannot make this type of dough, maize proteins lack the toxic epitopes causing celiac disease. In wheat, HMW glutenins constitute ~10 % of wheat total storage proteins. They contribute to the extensibility, whereas gliadins contribute to the viscosity of dough (Shewry and Halford 2002). Furthermore,

the HMW 1Dx5 + 1Dy10 glutenins are linked to better baking quality (Shewry et al. 2002). These properties are partly achieved through crosslinking via sulfur bridges. In maize, the β- and γ-zeins are also sulfur rich and evolutionarily closer to wheat HMW glutenin than the α- and δ-zeins are.

The diversification of prolamin seed storage proteins is in part responsible for the different functional properties of cereal products. For example, wheat is able to form a polymer of seed storage proteins called gluten, while maize does not. In spite of the divergence in seed storage proteins, however, prolamins from all species accumulate similar ER-derived protein bodies, suggesting that the basic functional interactions required for protein body formation have been preserved across species. Understanding the roles of different prolamins in protein body formation may allow a rational design of cereal products with improved functionality and the production of foreign proteins (Arcalis et al. 2004).

The cysteine residues in wheat prolamins define protein structure and ultimately flour property. HMW glutenins, linked intermolecularly through disulfide bonds, form the backbone of glutenin aggregates. LMW glutenins could form both inter- and intramolecular sulfide bridges, whereas gliadins mainly form intramolecular bonds with the exception of the ones with unequal number of cysteine residues (Kasarda 1999; Gianibelli et al. 2001). Cysteines are also important in maize protein bodies. Cysteine-rich β- and γ-zeins reside in the peripheral region of protein bodies, while cysteine-poor α-zeins and δ-zeins are in the center (Wu et al. 2010). QTLs raising levels of γ-zeins are believed to contribute to seed hardness in quality protein maize, exhibiting an important physical property for seed architecture. RNAi constructs directed against γ-zeins have nearly normal shaped but underdeveloped protein bodies, and sometimes opaque kernels. βRNAi further distort the shape of the protein bodies and the texture of seeds brought by γRNAi, illustrating the important roles of γ and β zeins in seed development (Wu and Messing 2010).

Despite divergence, heterologous storage proteins can be tissue-specifically expressed in related species. For instance, an entire cluster of 22-kDa *kafirin* genes from sorghum, which are orthologous to the 22-kDa α-zein genes, have been introduced into maize, properly expressed with their own regulatory cis-acting elements, and properly processed (Song et al. 2004). It also has been shown that the wheat Dx5 HMW glutenin is expressed in maize endosperm (Sangtong et al. 2002). However, it is unclear how it affects the accumulation of zeins in protein bodies. Still, structural conservation of wheat HMW glutenins and maize β- and γ-zeins led us to hypothesize that these proteins could play similar functional roles in protein body formation. We sought to test this using a genetic complementation approach in which we crossed RNAi maize lines directed against different zein proteins with maize expressing the wheat 1Dx5 HMW glutenin. In α-RNAi lines, the opaque phenotype segregates from Dx5 with the RNAi event 1:1 as expected. In γ-RNAi lines, however, a stronger opaque phenotype is produced in the presence of Dx5. Although Dx5 and the other zeins accumulate at normal levels in the presence of γ-RNAi, normal protein body formation is severely affected as expected for seeds with an opaque phenotype, clearly indicating that the wheat glutenin and the maize prolamin interact in their protein body formation.

## Materials and methods

### Plant material

A *Zea mays* L. transgenic Dx5 event (P2P31-190-4) was from our own collection and has previously been described (Sangtong et al. 2002). It was backcrossed for two generations with inbred B73. The resulting Dx5 heterozygous transgenic plants were selected and used as maternal parents. Transgenic β and γRNAi double mutant (resulting from cross βRNAi/+ × γRNAi) and P6Z1RNAi (αRNAi) plants were also from our own collection and have been previously described (Wu and Messing 2010, 2012b). They were used as paternal parents. Zeins from RNAi lines against different groups of zeins were extracted, and their patterns in 15 % SDS polyacrylamide gel electrophoresis (PAGE) were summarized in Fig. 1.

### Protein extraction

Prolamins including Dx5 were extracted from 50 mg finely grounded mature kernels or 18 DAP endosperm using borate extraction buffer (12.5 mM sodium borate pH 10, 1 % SDS, 2 % β-mercaptoethanol [ME]) to extract total protein followed by precipitation of non-prolamin proteins with 70 % ethanol as described by the Larkins lab protocol

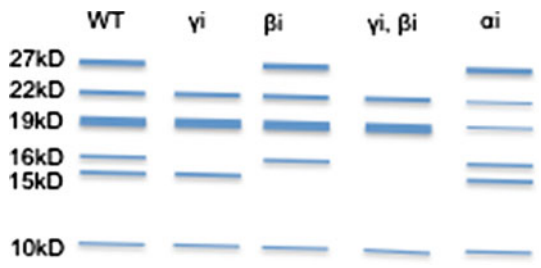


**Fig. 1** RNAi lines against different groups of maize zeins used in this paper. The size and amount of maize zeins in wild-type (WT) maize kernels are used as reference

(http://www.ag.arizona.edu/research/larkinslab/protocols.htm) or directly using 70 % ethanol/2 % ME (Wu et al. 2009). Prolamins were resolved by 15 % SDS-PAGE. Gel preparation and protein loading followed Bio-Rad's Mini-PROTEAN instruction manual (Hercules, CA, USA). SDS-PAGE gels were stained with staining buffer (0.1 % R-250, 40 % ethanol, and 10 % acetic acid) for more than 2 h and destained with 10 % ethanol/7.5 % acetic acid.

### Western blot analysis of Dx5 expression

Specificity of Dx5 antibody and genotyping of Dx5 in selected seeds were done with dot-blot as described (Sangtong et al. 2002), except that endosperm material may not be in equal amount among the 96 well plates. For Western blot, prolamins derived from equal amount of endosperms were diluted 10 times, resolved with SDS-PAGE, and transferred to a PVDF membrane with a Bio-Rad Mini Trans-blot cell. Dx5 was detected using a 1:5,000 dilution of a monoclonal antibody that has previously been described (Barro et al. 1997; Sangtong et al. 2002). Blotting and detection were done with GE Healthcare ECL Western blotting detection system (Piscataway, NJ, USA).

### Transmission electron microscopy and amino acid measurements

Transmission electron microscopy analysis of 18 DAP maize endosperm cells followed published methods (Wu and Messing 2010). Protein content and amino acids in 1 g of corn flour were profiled by New Jersey Field Lab, Inc, Trenton, NJ, USA.

## Results

### Dx5 accumulation in maize endosperms

In maize, we used a mixture of 70 %/2 % ethanol/ME to extract zeins. This procedure provided a high-resolution of different zein size classes. However, we found that such conditions (2 % ME) were not sufficient for the extraction of HMW glutenin (Fig. 2a). Therefore, we first extracted total proteins with denaturing solvents SDS + ME at high pH (pH 10) to quantitatively remove the glutenin followed by extraction of the zeins as described under "Materials and methods". This procedure was more effective in the

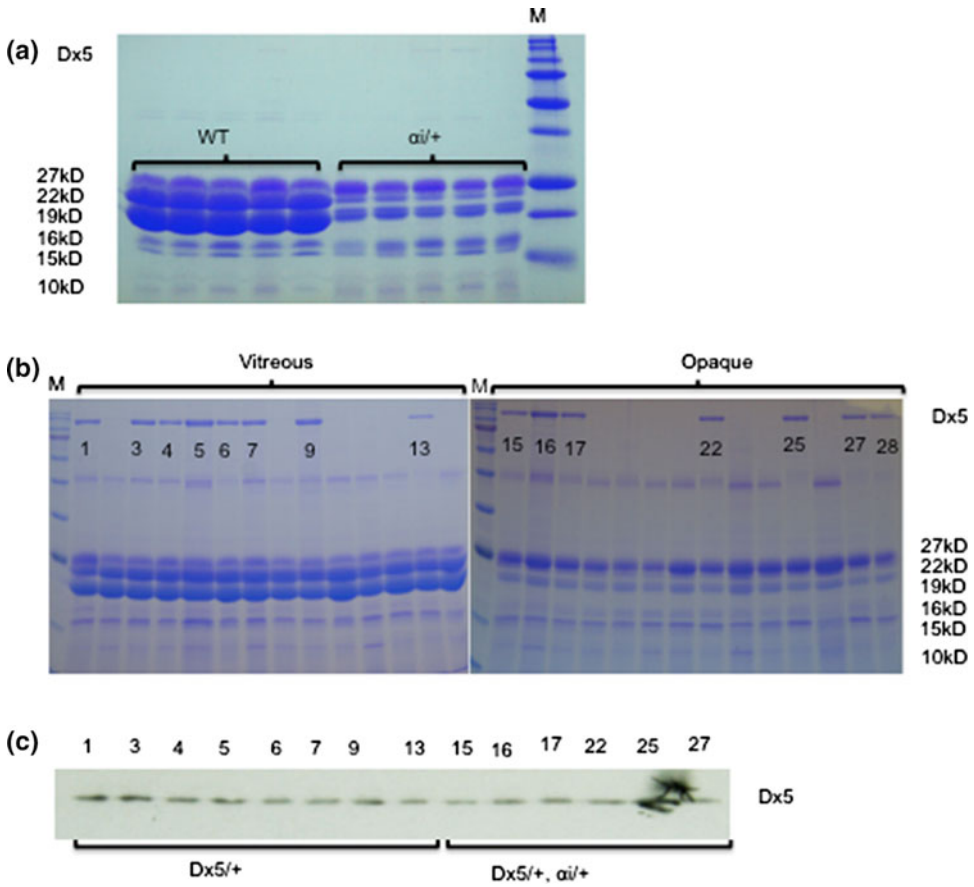


**Fig. 2** Dx5 accumulation in WT and αRNAi mutant seeds detected by SDS-PAGE gel and Western blot. **a** Prolamins extracted with 70% ethanol/2% ME. **b** Dx5 accumulation in WT and αRNAi seeds. Total protein was first extracted with borate buffer and then non-prolamins were precipitated from this extract by addition of 70% ethanol (see "Materials and methods"). The supernatant containing prolamins is dried, dissolved in water, and loaded on the gel. **c** Western blot analysis of Dx5 with anti-Dx5 antibodies in WT and αRNAi flours. Only samples with Dx5 from panel **b** are selected for Western analysis. *M* protein markers from top to bottom being 180, 130, 100, 75, 63, 48, 35, 28, 17 and 10 kDa

fractionation of glutenins and zeins from maize endosperm. Based on size fractionation with SDS-PAGE, Dx5 from our selected transformation event is highly expressed in maize endosperm together with the other zeins. The intensity of the protein band is equal to or greater than that of the 27-kDa γ-zeins, amounting to more than 10 % of total prolamins (Fig. 2b). It has previously been shown that promoters of prolamins from the grass family contain conserved regulatory sequence elements such as the $GCN_4$–like motif and prolamin box, which are recognized by trans-acting factors such as O2 (Opaque2) and PBF (Prolamin-box binding factor) in maize (Wu and Messing 2012a). Because the Dx5 transgene construct included its own promoter and 3′ UTR (Sangtong et al. 2002), the protein expression pattern suggested that the wheat regulatory elements were properly recognized in maize endosperm.

## Protein bodies are affected by the presence of Dx5

Dx5 transgenic kernels are vitreous with normal size and do not differ from the parental non-transgenic lines. However, transmission electron microscopy showed an alteration of morphology of protein bodies in the transgenic kernels. In immature endosperm cells of Dx5 transgenic seeds, the surfaces of the normal-sized protein bodies were distorted and most of them exhibited a budding structure. Furthermore, smaller protein bodies with greater electron density that were surrounded by a fibrillar matrix were attached to larger protein bodies (Fig. 3b). This result is somewhat surprising because so far altered protein bodies have been linked to an opaque phenotype, indicating now that not all protein body deformation cause non-vitreous phenotypes and it is possible that mutations affecting protein body formation have escaped selection.

## Dx5 does not alter segregation of the αRNAi opaque phenotype

Maize seeds containing the Dx5 protein are vitreous, while seeds bearing αRNAi, in which both 22- and 19-kDa α-zeins are knocked down, are opaque. Indeed, seeds from a cross between heterozygous Dx5/+ and α-RNAi/+ segregate into opaque and vitreous kernels 1:1. As shown in the left panel of Fig. 2b, randomly selected vitreous kernels contains normal deposition of α-zeins, and opaque kernels have greatly reduced α-zeins, with or without Dx5. On the other hand, Dx5 does not show any differences between normal and α-RNAi kernels in a protein gel (Fig. 2b). We selected protein samples that contain Dx5, and further tested those with Dx5 antibodies and found no differences in blot intensities between samples of normal α-zeins and samples with reduced α-zeins (Fig. 2c). Furthermore, the presence of Dx5 in maize endosperm did not seem to influence the deposition of other zeins either (Fig. 2b). Therefore, we can conclude that Dx5 does not diminish the filling of protein bodies with alpha zeins and that the opaque phenotype is dominant.

## γ-Zeins are required for Dx5 organization in the perimeter of protein bodies

A different result emerges when Dx5 heterozygous plants (Dx5/+ as female) were crossed with βRNAi/+, γRNAi/+ plants. They showed a 1:3 ratio of the opaque and vitreous kernels (Fig. 4a). Further analysis of the genotypes of the opaque and vitreous seeds revealed that almost all opaque seeds (45 out of 46) contain Dx5, while about half of the vitreous kernels contain the Dx5 protein (Fig. 4b). Moreover, all tested opaque kernels are γRNAi/+ mutants, with some of them βRNAi/+ and γRNAi/+ double mutant (Fig. 4c). Five exceptions exist among the tested vitreous kernels where the coexistence of Dx5 and γRNAi does not give a complete opaque phenotype (Fig. 4d), but rather contains a small region of vitreous endosperm after detailed dissection. A variable penetration of non-vitreousness from the central to the peripheral endosperm region has also been observed in the original studies of βRNAi/+ × γRNAi/+ crosses (Wu and Messing 2010). In both cases, the variable penetration indicates that there could be a threshold level of residual γ-zeins left in some cases where knock-down by RNAi is less severe.

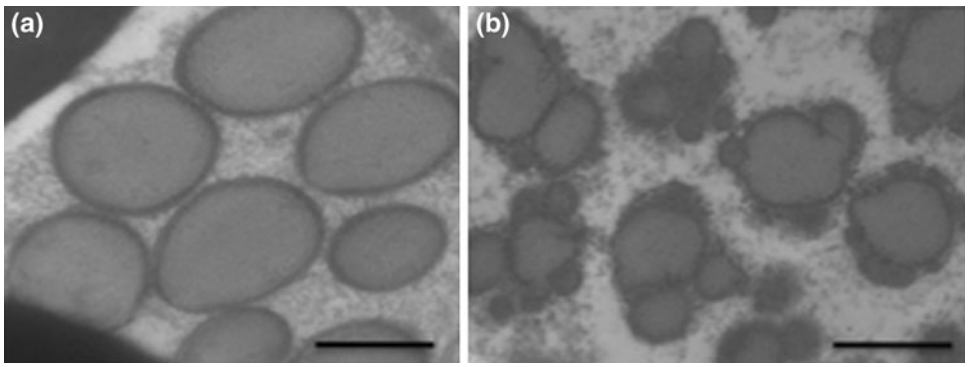


**Fig. 3** Transmission electron micrographs of protein bodies of 18 DAP endosperm cells from WT (**a**) and Dx5/+ (**b**). *Bar* = 1 μm

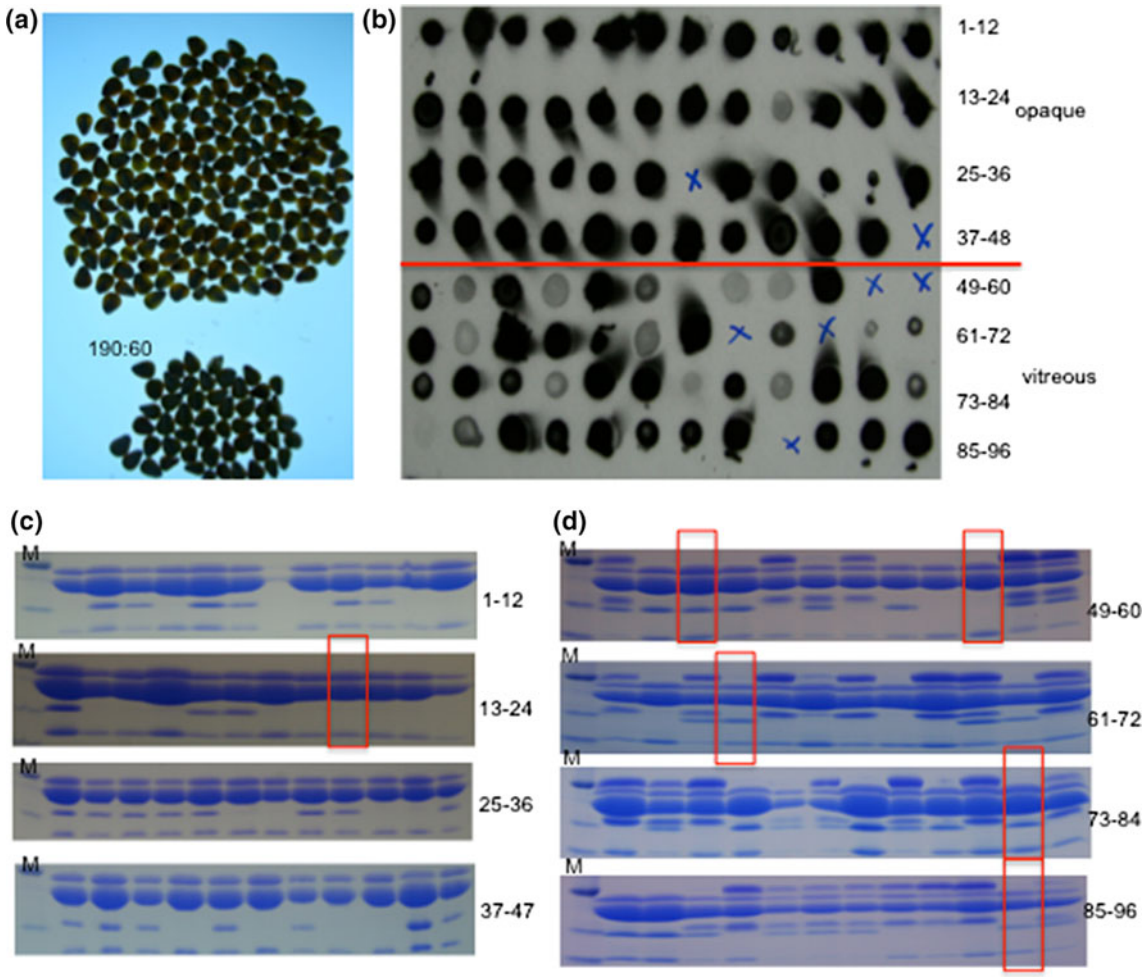


**Fig. 4** Genetic segregation of opaque and vitreous seeds resulting from Dx5/+ × βRNAi/+, γRNAi/+ crosses. **a** Vitreous and opaque seeds from one ear of Dx5/+ × βRNAi/+, γRNAi/+. **b** Dx5 presence/absence in randomly selected 48 opaque and 48 vitreous seeds. Protein samples lost from broken wells were marked as *blue*

*crosses*. **c** Genotyping of β and γ zeins in 48 opaque seeds. The *red frame* indicates seeds containing only γ/β-RNAi that are opaque. **d** Genotyping of β and γ zeins in 48 vitreous seeds. The *red frames* indicate seeds containing both Dx5 and γRNAi with partially non-vitreous phenotype. *M* protein markers as in Fig. 2

In the resulting seeds from the Dx5/+ × βRNAi/+, γRNAi/+ cross, protein gels with equal load among samples showed that β- and γ-zeins did not affect the deposition of Dx5 in maize (Fig. 5). Protein staining of Dx5 from lanes 1, 6, 8, 11 lacking β- and γ-zeins showed no intensity differences from that of the other lanes (Fig. 5). Based on these observations, altered α or β/γ zeins did not affect the expression of Dx5 in maize, and Dx5 in maize did not influence the amount of zeins that are deposited into maize endosperm.

Therefore, it appears that Dx5 requires γ zeins to produce seeds with a vitreous phenotype. In kernels with Dx5 and reduced γ zeins, protein bodies were not single structures, but further accumulated into bigger clumps containing tens of different sized protein bodies (Fig. 6c). The clumps further expand with some irregular shaped protein bodies in the absence of β-zeins; smaller, electron-dense protein bodies were interspersed among larger, electron-light protein bodies (Fig. 6b, d). It appears that the loss of β/γ zeins causes a greater effect of Dx5 glutenins on protein body morphology.

## Dx5 alters amino acid composition in maize kernels

Because sulfur flows through cysteine to methionine, removal of the cysteine sink shifts sulfur to the methionine sink (Wu et al. 2012). The opposite is also true, increasing the methionine sink depresses the cysteine sink. Consistent with such balance, one could test whether raising the cysteine sink through the ectopic expression of a cysteine-rich protein such as the wheat Dx5 would reduce the methionine sink or the expression of the δ-zeins. On the other hand, Dx5 contains only five cysteine residues, which with respect to the overall molarity of cysteine is probably

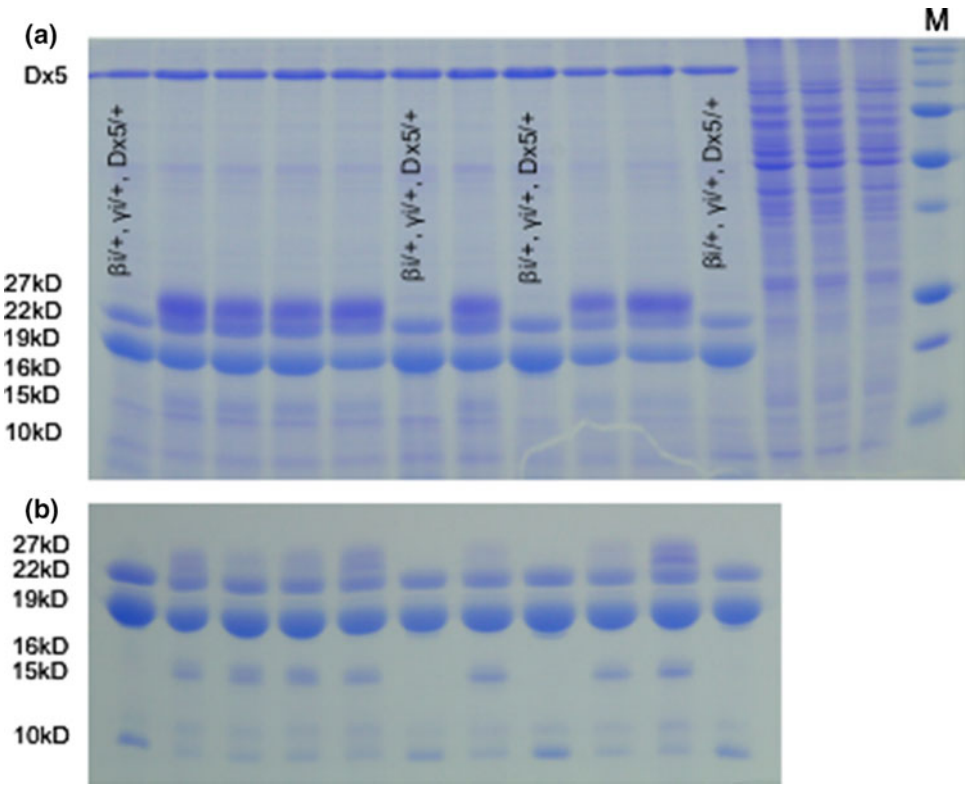


**Fig. 5** Dx5 accumulation in WT and β/γ RNAi mutant seeds. **a** Dx5 accumulation patterns in WT and β/γRNAi seeds. *Lane* 1–11, prolamin patterns; *lane* 12–14, non-prolamin proteins after prolamin extraction for *lane* 1–3. Prolamins are extracted under borate buffer and dissolved by 70% ethanol. **b** Zein segregation patterns. Flour samples are same as in **a**, zeins are extracted with 70 % ethanol/2 % ME

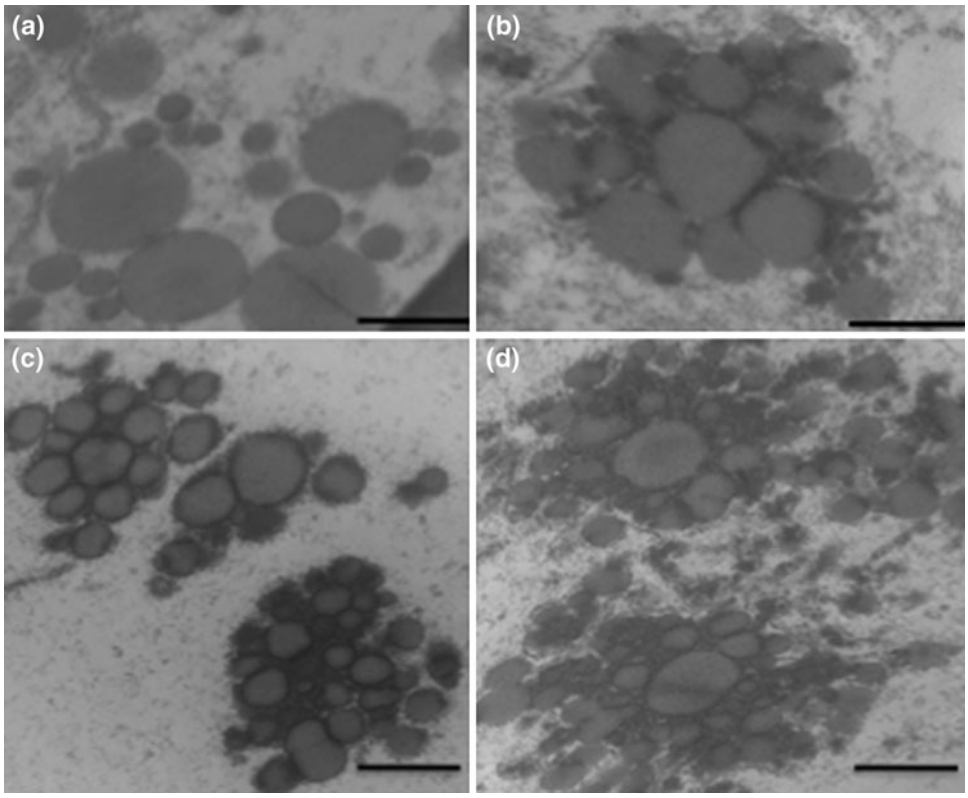


**Fig. 6** Transmission electron micrographs of protein bodies of 18 DAP endosperm cells. **a** βRNAi/+, γRNAi/+. **b** Dx5/+, βRNAi/+, γRNAi/+. **c** Dx5/+, γRNAi/+. **d** Dx5/+, βRNAi/+, γRNAi/+. *Bar* = 1 μm

too low to cause a shift in sulfur balance. But other amino acids of Dx5 might create a shift. As with maize zeins, the 848 amino acids-long Dx5 protein is rich in glutamine and proline, but also very rich in glycine. To determine whether the expression of the Dx5 glutenin in maize changes the overall amino acid composition of the seed, we analyzed

protein composition of wild-type, αRNAi and β/γ RNAi kernels with or without the expression of Dx5. Consistent with the protein level in B73 as previously reported, all six types of kernels contain 12.3–13.8 % total proteins in the endosperm. Indeed, Dx5 raised the relative levels of proline, glycine, and glutamine (here measured as glutamic acid) as expected, although the change of proline is more obvious when either α or β/γ zeins are absent. Dx5 also increased the levels of arginine and isoleucine and reduced the levels of alanine and phenylalanine. However, most of the other amino acids including cysteine were not or were slightly influenced by the expression of Dx5 (Table 1). Therefore, expression of Dx5 had minimal influence on the sulfur balance.

## Discussion

Our results validate previous evolutionary studies that placed wheat glutenins at the root of the diversification of prolamins in the grasses. It does not interact with α-zeins which emerged with the subfamily of the Panicoideae, but it does interact with the older β/γ zeins which would be analogous to the interaction of glutenins and gliadins. Indeed, β/γ zeins and gliadins both belong to the group II prolamins. These classes of prolamins differ from the younger ones, group I, in their effects on formation of protein bodies and non-vitreous phenotypes. Such an effect is the strongest with the withdrawal of γ-zeins, but can be enhanced with the additional withdrawal of β-zeins (Wu and Messing 2010).

The morphology of the protein bodies of Dx5 transgenic endosperm is similar to that in wheat or barley, in a way that protein bodies are interspersed with a fibrillar matrix (Shewry and Halford 2002; Arcalis et al. 2004). Beyond normal zein protein bodies, smaller protein bodies occur with the presence of Dx5. The grouped protein bodies in Dx5/− kernels expand to larger groups with the loss of γ zeins, and further accumulate into a complex mass with different sized protein bodies and matrix (Fig. 5). Until today, the aggregated protein body morphology has been observed in the floury-2 mutant (Lopes et al. 1994), in Z1CRNAi/+ (knock-down of 22-kDa α-zeins), βRNAi/+, and γRNAi/+ triple mutant, and in Mo2/+, o2/o2, γRNAi/+, and βRNAi/+ (Wu et al. 2010). Unlike the above genotypes, Dx5/+, βRNAi/+, and γRNAi/+ seeds have normal α-zein deposition (Fig. 5b), suggesting a different mechanism of aggregation from the above mutants. Floury-2 prevents processing of the signal peptide and thereby distorts the membrane of protein bodies in a dosage-dependent manner. In that case, a single amino acid substitution renders an α-zein resistant to a signal peptidase. Dx5 appears to be properly synthesized and processed, but accumulates like γ-zein on the surface region of the protein bodies. Perhaps, because of its size, it causes a deformation of the protein body membrane. Moreover, it is believed that cysteines of Dx5 interact with cysteines from other HMW glutenins via intermolecular

**Table 1** Protein and amino acid composition analysis of Dx5/+, different RNAi against zeins, and their combinations

| Amino acids | Dx5 protein | WT | Dx5/+ | αRNAi/+ | Dx5/+, αRNAi/+ | βRNAi/+, γRNAi/+ | Dx5/+, βRNAi/+, γRNAi/+ |
|---|---|---|---|---|---|---|---|
| Methionine | 0.36 | 1.95 | 1.87 | 1.52 | 1.67 | 1.95 | 2.29 |
| Cystine | 0.48 | 2.33 | 2.48 | 1.72 | 1.90 | 1.32 | 1.37 |
| Lysine | 0.84 | 2.47 | 2.98 | 3.87 | 3.52 | 2.54 | 4.17 |
| Phenylalanine | 0.36 | 4.67 | 4.19 | 2.68 | 2.51 | 5.18 | 4.23 |
| Leucine | 4.92 | 12.78 | 11.32 | 5.07 | 5.25 | 13.16 | 9.90 |
| Isoleucine | 0.48 | 1.70 | 2.11 | 1.30 | 1.42 | 1.95 | 1.77 |
| Threonine | 3.00 | 2.80 | 3.30 | 3.35 | 3.41 | 2.81 | 2.95 |
| Valine | 2.52 | 3.01 | 3.52 | 3.16 | 3.20 | 3.00 | 3.35 |
| Histidine | 0.48 | 1.69 | 1.95 | 1.92 | 1.92 | 1.68 | 2.11 |
| Arginine | 1.44 | 2.88 | 3.27 | 3.54 | 3.67 | 3.15 | 4.35 |
| Glycine | 19.21 | 2.55 | 3.61 | 3.12 | 4.20 | 2.55 | 4.27 |
| Aspartic acid | 0.48 | 6.61 | 6.83 | 12.89 | 9.99 | 6.89 | 6.18 |
| Serine | 5.76 | 5.10 | 5.05 | 4.22 | 4.39 | 5.30 | 4.80 |
| Glutamic acid | 36.61 | 18.95 | 21.40 | 21.55 | 23.68 | 17.92 | 19.94 |
| Proline | 12.97 | 9.12 | 9.27 | 6.45 | 8.09 | 7.87 | 10.55 |
| Hydroxy proline | | 0.10 | 0.25 | 0.32 | 0.27 | 0.23 | 0.33 |
| Alanine | 3.48 | 8.44 | 7.48 | 7.84 | 7.24 | 7.59 | 6.59 |
| Tyrosine | 5.52 | 3.14 | 3.34 | 2.64 | 2.91 | 2.99 | 3.44 |
| % cp | | 13.85 | 13.24 | 12.88 | 12.49 | 13.72 | 12.28 |

% cp, total measured protein; amino acid concentrations are the percentage of amino acid in total protein

disulfide bonds. Dx5 has an extra cysteine comparing to other HMW glutenins, and this cysteine introduces branching to produce gluten polymers. Possibly, the extra cysteine could interact with cysteines of cysteine-rich zeins such as the γ-zeins. Still, this by itself has a very mild effect on kernel appearance. First, Dx5 and all other zeins accumulate at normal levels (Fig. 2). Second, despite the Dx5 effect on the protein body surface, it does not cause a non-vitreous phenotype such as the mutant α-zein in the floury-2 mutant. However, the Dx5 effect changes dramatically when the support of γ-zeins to the formation of the surface of protein bodies is withdrawn.

In maize, the 27-kDa γ-zein accumulates very early during endosperm development, and it has been suggested that together with 50-kDa γ-zeins, 27-kDa γ-zeins allow the nucleation and early growth of protein bodies (Wu et al. 2010). Cysteines in non-repetitive domains of 27-kDa γ zeins also form intramolecular bonds; the other cysteines at the N-terminal end could be interacting with other molecules (Ems-McClung et al. 2002). In the absence of cysteine, no interaction between 27-kDa zeins and α- or δ-zeins could be demonstrated in a yeast two-hybrid system (Kim et al. 2002). This lack of interaction has been suggested to beneficially prevent uncontrolled aggregation, thereby permitting the separation of protein bodies in maize (Wu et al. 2010).

It is possible that Dx5 can cause an aberrant protein body initiation, whereby multiple protein bodies are initiated in one region of the ER and they all develop together in proximity to each other, but that the presence of γ-zeins moderates this aberrant protein body initiation. If cysteine-rich 27-, 16-, and 15-kDa γ and β-zeins in the outer regions of protein bodies block the polymerization of Dx5, one would expect that a reduction of those proteins in the respective RNAi lines would further distort the formation of protein bodies. This is consistent with the protein body clumps in the combined transgenic lines (Fig. 6b–d). Vitreous kernels rely on the proper deposition as well as distribution of zeins in maize kernels. It has been proposed that sub-aleurone cells contain more protein bodies than central endosperm cells that contain more starch granules. Protein bodies are interspersed among starch granules. In Dx5/−, γ RNAi kernels, rather than being dispersed between starch granules, protein bodies would clump together and lead to uneven dispersion between starch granules, which then could explain the opaque phenotype.

## Conclusion

Wheat Dx5 and maize β/γ-zeins are evolutionarily related. Moreover, the cysteins in both Dx5 and β/γ-zeins are important in the development of protein bodies. When expressed in maize, Dx5 accumulates to a high-level in endosperm, independently from other zeins. However, wheat Dx5 in maize does not complement the function of γ-zeins in protein bodies, but requires them for its organization in protein bodies. This interaction does not affect α-zein deposition, consistent with the divergence of prolamin during speciation.

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