

Understanding the Regulation of Zeins and Non-Zein Genes at Nucleotide and Protein Level for Nutritional Enhancement in Maize

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ARTICLE ID: 82

Introduction

Nutritional quality of maize kernel depends on protein content and composition. Proteins account for 10% of kernel weight. 70% of kernel proteins are storage proteins, of which 60% are prolamins called zeins. Zeins lack lysine, tryptophan and methionine (EAA). However, zein proteins lack two essential amino acids, lysine and tryptophan. Thus, maize cannot provide balanced nutrition to poultry and no ruminant livestock without additional lysin and tryptophan. The 27 kD γ type is one of the most abundant type of zeins. Studying its regulation of zein should help improve seed protein quality. Several transcription qualities, O2, PBF1, OHP1 and OHP2 have been found to regulate the 27 kD γ -zein gene. However, complexity of transcriptional regulation is not fully understood. The importance of the zeins in nutritional point of view is accepted and widely studied in the recent years. However, the nature of expression of genes encoding the zeins and nature of their regulation at the nucleotide level and protein level is rarely taken up. Therefore, studies about their regulation could help in improving nutritional quality of maize grains. This will in turn assists the attempt to alleviate malnutrition among the people consuming maize as a staple food crop.

Classification of zeins:

Based on solubility, ability to form disulfide bonds and molecular mass, zeins are classified into major classes namely, α -zeins (19 and 22 kDa), β -zeins (15 kDa), γ -zeins (50, 27 and 15 kDa), and δ -zeins (10 and 18 kDa). The pictorial representation is presented in figure 1. Zeins are encoded by super family of genes. β -, γ - and δ -zeins are usually encoded by single copy genes. In certain QPM lines, gene encoding 27 kD γ -zein is duplicated. Protein and cDNA analyses indicated that α zeins subclasses are encoded by multiple gene families. 19 kD zeins are encoded by three multimer gene families: z1A, z1B and z1D. While, 22 kD zeins are encoded by family z1C. Together 41 α zein genes are reported. Zein

RNA sequences constitute 50% of endosperm transcripts. α and γ zeins comprise of 30 and 50%, respectively.

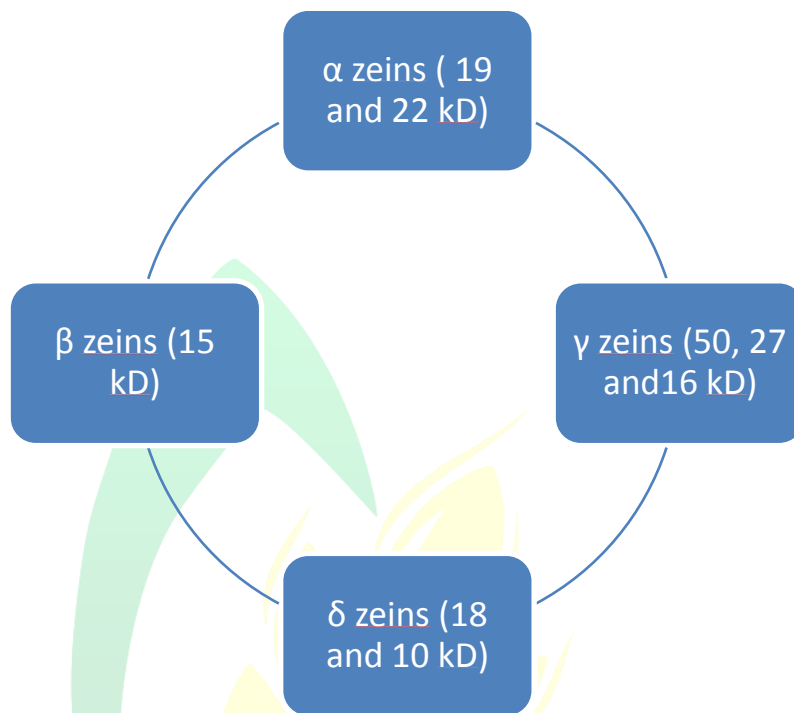


Fig1: Classification of zein genes based on molecular mass

Regulation of zein genes at transcriptional level:

The transcriptional regulation of the zein genes is part of the endosperm regulatory network. TFs.O11 is a central hub of the regulatory network for maize endosperm development and nutrient metabolism and also directly regulates the expression of genes encoding key TFs involved in endosperm development (NKD2 and ZmDOF3) and nutrient metabolism (O2 and PBF1) (Feng et al. 2018). These TFs, O2, O11, and the NKDs regulate both endosperm development and nutrient metabolism, and their regulatory networks are linked via their mutual transcriptional regulation, suggesting that, during kernel maturation, endosperm development and nutrient accumulation are highly coordinated at the transcriptional level through a complex gene regulatory network. In the nucleus, TFs work in combination to regulate the expression of the members in zein gene family. Some of these TFs, e.g., O2, NKDs, PBF1 and O11, also have mutual direct regulation. In the cytoplasm, zeins are translated in RER and then localized in PBs in an ordered manner with the aid of O1, F11, and O10. Hypothetical picture showing the regulation of different zeins genes is shown in fig 2.

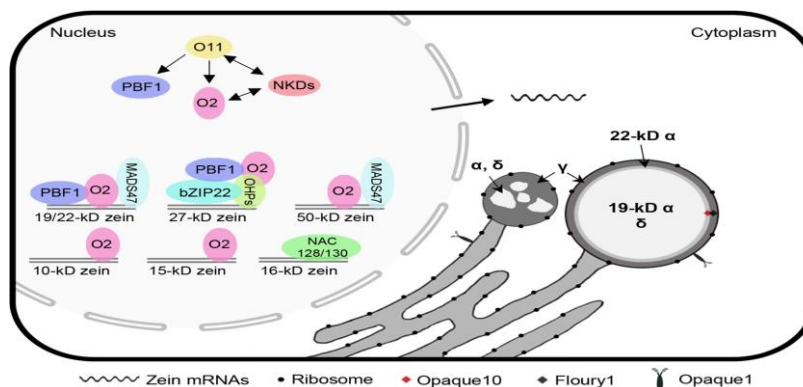


Fig. 2: A schematic diagram showing the transcriptional regulation of zein-coding genes and zein distribution in the protein bodies (picture adopted from Li et al. 2020).

Transcriptional Regulation of the 27-kD γ - and α -Zein Genes

Expression patterns of O2 and OHPs and perhaps is related to the mechanism of PB formation. The expression pattern of TFS and its association with protein body formation is shown in figure 4. The ZmbZIP22 Transcription Factor Regulates 27-kD γ -Zein Gene Transcription during Maize Endosperm Development. Few important questions that need to be investigated are do any other TFs regulate the 27 kD γ -zein and how is the activity of multiple TFs coordinated to regulate the expression of single 27 kD γ -zein gene. 27 kDa γ -zeinis one of the most abundant zeins and crucial for protein body formation. Known TFs which regulates 27 kD γ -zein gene are PBF1, OHPs and o2 which indicates complex transcriptional regulation. Triple mutants of o2, PBF1 and OHPs still producing small amount of 27 kD γ zeins indicates presence of another TFs. It was found thatZmbZIP22 was demonstrated to regulate the regulation the expression of the 27-kD γ -zein gene based on experiment at invitro and invivo. These finding revealed that zmbZIP22 interacts with other TFs(Zhang et al. 2019).

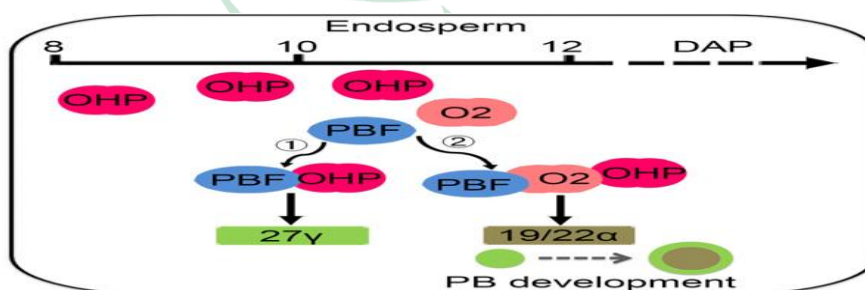


Figure 4. Hypothetical Model Depicting the Transcriptional Regulation of the 27-kD γ - and α -Zein Genes Mediated by O2, PBF, and OHPs (Picture adopted from Zhang et al. 2015).

Biosynthesis pathway of essential amino acids in zein and non zein fractions and its regulation:

Metabolic pathway of methionine synthesis is depicted in Figure 6. In depth understanding of the metabolic steps and branch point is crucial to regulation the accumulation of a substance or products. Essential amino acids such as lysine which is a non-zein and methionine, a zein residue are synthesized through this biosynthetic pathway. This shows the complex regulation of the synthesis of these essential amino acids at the nucleotide level. Expression of 10 Kda δ -zein is the prime contributor towards kernel Met accumulation is attributed to differential expression of *zps10/22* (now *Dzs10*) (Chromosome 9L) trans-regulated by *dzr1* (Chromosome 4S) (Chauduri and Messing, 1994)

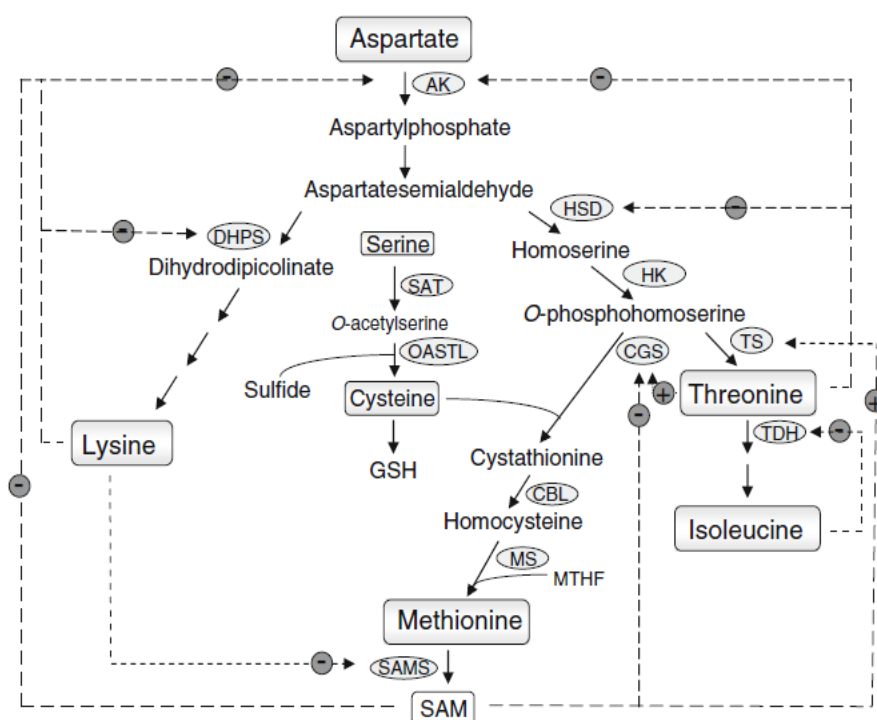


Fig. 6: The aspartate family of amino acids and the biosynthesis pathways leading to methionine and cysteine in higher plants. Only some of the enzymes and metabolites are specified. Dashed arrows with a “minus” sign represent either feedback inhibition loops or repression of gene expression. The dashed and dotted arrow with the “plus” sign represents the stimulation of gene expression or enzyme activity. AK Asp kinase; DHPS dihydrodipicolinate synthase; HSD homoserine dehydrogenase; HK homoserine kinase; TS threonine synthase; TDH threonine dehydratase; OPH O-phosphohomoserine; CGS cystathionine c-synthase (marked in red); CBL cystathionine b-lyase; MS methionine



synthase; SAT serine acetyltransferase; OPH O-phosphohomoserine; OASTL O-acetylserine (thiol)lyase; GSH glutathione; SAM S-adenosylmethionine; SAMSSAM synthase (Figure adopted from Amir, 2008).

Conclusion:

Endosperm development and nutrient accumulation are highly coordinated at transcriptional level through complex regulatory network. Studies on zein gene regulation could help in improving nutritional quality of maize kernels. Methionine enriched maize hybrid to avoid using synthetic methionine in feeds. An efficient venture to alleviate protein energy malnutrition. Uncharacterized TFs regulating zein biosynthesis. Possibility of tissue specific methylation and other modifications are under investigation. Methionine could be enhanced in QPM background for more nutritious maize variety. In depth study on protein rebalancing for better understanding of zein gene regulation

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