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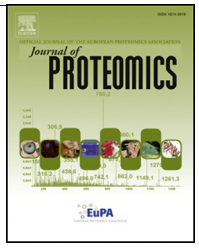

# Affinity chromatography revealed insights into unique functionality of two 14-3-3 protein species in developing maize kernels

Yao Dou[b], Xiangguo Liu[a], Yuejia Yin[a], Siping Han[a], Yang Lu[c], Yang Liu[a], Dongyun Hao[a,b,*]

[a]Institute of Agricultural Biotechnology, Jilin Academy of Agricultural Sciences (JAAS), Changchun, 130124, P. R. China
[b]Key Laboratory for Molecular Enzymology and Engineering of the Ministry of Education, Jilin University, Changchun, 130012, P. R. China
[c]Institute of Plant Protection, Jilin Academy of Agricultural Sciences (JAAS), Changchun, 130124, P. R. China

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ABSTRACT

The 14-3-3 proteins are a group of regulatory proteins of divergent functions in plants. However, little is known about their roles in maize kernel development. Using publically available gene expression profiling data, we found that two 14-3-3 species genes, *zmgf14-4* and *zmgf14-6*, exhibited prominent expression profiles over other 14-3-3 protein genes during maize kernel development. More than 5000 transcripts of these two genes were identified accounting for about 1/10 of the total transcripts of genes correlating to maize kernel development. We constructed a proteomics pipeline based on the affinity chromatography, in combination with 2-DE and LC-MS/MS technologies to identify the specific client proteins of the two proteins for their functional characterization. Consequently, we identified 77 specific client proteins from the developing kernels of the inbred maize B73. More than 60% of the client proteins were commonly affinity-identified by the two 14-3-3 species and are predicted to be implicated in the fundamental functions of metabolism, protein destination & storage. In addition, we found ZmGF14-4 specifically bound to the disease- or defense-relating proteins, whilst ZmGF14-6 tended to interact with the proteins involving metabolism and cell structure. Our findings provide primary insights into the functional roles of 14-3-3 proteins in maize kernel development.

Biological significance
Maize kernel development is a complicated physiological process for its importance in both genetics and cereal breeding. 14-3-3 proteins form a multi-gene family participating in regulations of developmental processes in plants. However, the correlation between this protein family and maize kernel development has hardly been studied. We have for the first time found 12 14-3-3 protein genes from maize genome and studied *in silico* the gene transcription profiling of these genes. Comparative studies revealed that maize kernel development aroused a great number of gene expression, among which 14-3-3 protein genes took a significant proportion. We applied affinity chromatographic approach, in combination with 2-DE and LC-MS/MS, to explore the specific client proteins of two crucial 14-3-3 protein species that exhibit prominent gene expression over other members in the

* *Corresponding author at:* Institute of Agricultural Biotechnology, Jilin Academy of Agricultural Sciences (JAAS), Changchun, Caiyu Street 1363#. Tel.: +86 431 87063195; fax: +86 431 87063080.
E-mail address: dyhao@cjaas.com (D. Hao).

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family during the kernel development. Assessments of the identified client proteins resulted in important information toward understanding the functional mechanism of 14-3-3 protein family in maize kernel development.



## 1. Introduction

Maize kernel development is both an interesting system for plant developmental biology and an important process to meet the human demand for cereal production. This continuous and active developmental process begins when an ovule undergoes double fertilization [1]. After several rounds of continuous division, the zygote differentiates into the embryo and the endosperm at the 6th day after pollination (DAP). Each of these two tissues exchange metabolic intermediates, and both have specific functions in the progression of kernel maturation [2]. Following development of kernels, embryo undergoes continuous cell structure changes for the survival of the species [3]. Whilst, endosperm becomes a predominant tissue accompanying a period of nearly linear accumulation of biomass within the endosperm [4]. Accumulation of storage substances such as starch and proteins in endosperm are achieved through starch anabolism and protein synthesis and transport [5]. Primary metabolism supplies the source of energy for changes in the cellular structure of the embryo and material accumulation in the endosperm. At 37 to 40 DAP, the maize kernel dehydrates and develop towards maturation [6]. In maize kernel development, every constitutive component matures through dynamic and temporal metabolic remodeling [7]. Extensive studies of these processes have been conducted over the last decades and these have provided evidence supporting the notion that regulatory proteins, and their interactions with their client proteins, play important roles in maize kernel development. To mention a few, for instance, Hennen-Bierwagen et al. reported an enzyme complex with a molecular weight of 670 kDa in 15–20 DAP maize endosperm tissue by a co-immunoprecipitation assay combined with mass spectrometry [8,9]. Liu et al. demonstrated that the composition of protein complexes varies between *ae*$^-$ mutants compared and the wild type because of the starch branching enzyme IIb (SBEIIb) [10].

Plant 14-3-3 proteins are regulatory proteins of this kind. They usually form a highly conserved protein family of divergent regulatory functions in cellular physiological processes such as cell signal transduction, cell cycle regulation, cell apoptosis, and transmembrane transportation [11–13]. Their broad functionalities are typically attributed to the interactions between abundant ligands and divergent species [14]. For this reason, 14-3-3 proteins have, over the last decade, attracted extensive attention examining their functions in various plant physiological processes, including seed development. Many 14-3-3 protein species in this family have been reported in plants such as *Arabidopsis* [15], rice [16], tomato [17], and other plants [18]. In *Arabidopsis*, seven different 14-3-3 protein species were identified; their average expression enrichment accounted for approximately 1% the proteome of seeds at nine days after flowering [19]. Swatek et al. reported in an interactome analysis of *Arabidopsis* 14-3-3 proteins that 14-3-3 species χ and ε exhibit diverse binding specificities to their client proteins functioning in glycolysis, proteolysis, and other cellular processes [20]. These discoveries suggest that 14-3-3 proteins and their interactions with the client proteins are synergistically involved in seed development. However, 14-3-3 proteins have hardly been studied in maize [21] in comparison with model plants such as *Arabidopsis* and rice. Indeed, a genome-wide study on the functional mechanism of 14-3-3 proteins in maize kernel development has not been conducted.

We are interested in maize kernel (seed) development because it is an important physiological process related directly to the ultimate trait, i.e. yield. Following the development of functional genomics, with the help of the advantages of the next generation sequencing (NGS) technologies in recent years, efforts have been made to improve the understanding of the mechanism of metabolic remodeling during seed development. Several large-scale proteomics studies have been conducted that sought to clarify the metabolic networks active in the process of seed filling in *Glycine max, Brassica napus, Arabidopsis thaliana,* and *Ricinus communis* [19,22–24]. In these four parallel proteomics studies, in addition to other important findings, a common observation was that 14-3-3 proteins were abundantly expressed during seed development, implicating that 14-3-3 proteins likely participate actively in this sophisticated metabolic remodeling process. We studied the literature of maize 14-3-3 proteins [21,25], together with the genome data of maize inbred line B73 [26], and noticed that there were in total twelve 14-3-3 proteins species documented in the maize genome. Bioinformatic analysis, using a well developed gene function prediction program for maize [27,28], revealed that two 14-3-3 protein species genes, *zmgf*14-4 and *zmgf*14-6 (their proteins are designated as be ZmGF14-4 and ZmGF14-6 in this work respectively), were predominantly expressed during seed filling (Fig. 1). We analyzed the total available transcripts of the maize genome and discovered that a great number of functional genes are correlated to the prominent expression of *zmgf*14-4 and *zmgf*14-6. This encouraged us to adopt ZmGF14-4 and ZmGF14-6 as bait proteins to capture their client proteins in an entire maize kernel developing course of 6 to 37 DAP. We refined the initial protein samples using an affinity chromatography technology prior to protein identification by LC-MS/MS. This strategy allowed us to capture a number of specific 14-3-3 client proteins. The functional interpretation of these client proteins revealed important evidence for understanding the functional mechanism of 14-3-3 proteins in maize kernel development.

## 2. Materials and methods

### 2.1. Plant materials

Maize inbred line B73 cultivated and self-pollinated at the Gongzhuling experimental field of the Jilin Academy of Agricultural Sciences (Jilin province, PRC) was used in this study.



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