Curriculum vitae

Xigang Liu

1, EDUCATION:

09/1993-07/1997 B.S. Major: Biology . College of Life Sciences, Hebei Normal University

Academic Degree Paper:

RAPD analysis of Taigu genic male-sterile wheat and Jishi genic male-sterile wheat

09/1998-07/2001 M.S. Major: Molecular and Cell Biology. College of Life Sciences, Hebei Normal University

Academic Degree Paper:

Screening and Functional Analysis of GPA1 or Calmodulin-interaction Protein in *Arabidopsis thaliana* using Yeast Two-hybrid system

09/2001-07/2004 Ph.D Major: Molecular and cell biology .College of Life Sciences, Hebei Normal University

Academic Degree Paper:

Functional Analysis of a Putative G-protein Coupled Receptor Family in *Arabidopsis thaliana*

2, PROFESSIONAL EXPERIENCE:

08/1997-07/1998: Teacher,

Hebei Shenzhou Middle School,

Hebei, China

08/2004-10/2007: Postdoctoral fellow,

National Institute of Biological Sciences (NIBS), Beijing

11/2007- 08/2009: Postdoctoral Research Scholar,

Department of Botany and Plant Sciences

University of California, Riverside

08/2009-06/2011: Visiting Assistant Researcher

Department of Botany and Plant Sciences

University of California, Riverside

07/2011-09/2012: Assistant Specialist, Type III

Department of Botany and Plant Sciences

University of California, Riverside

10/2012-present: P.I., Professor.

The Center for Agricultural Resources Research

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Hebei, China

3, HONORS AND ACADEMIC AWARDS

No.

4, MAJOR RESEARCH INTERESTS，SELECTED RESEARCH PROJECTS

Major research interests:

The work in our lab is focused on two fields: the molecular mechanism of floral meristem maintenance and determinacy in *Arabidopsis* and isolation and functional characterization of grain number genes and dissection of their regulationary networks in wheat.

1), Floral meristem maintenance and determinacy

Research background:

Stem cells are groups of undifferentiating cells which are characterized by the ability to renew themselves and to differentiate into a diverse range of specialized cell types. In higher plants that lack cell migration, stem cells are confined in spatially fixed zones in a microenvironment called the stem cell niche, which displays remarkable longevity and produces primordia of new organs constantly, allowing plants in some species to grow for hundreds of years. The most intensely investigated plant stem cells are located at the tips of shoots and roots, within the zones respectively called shoot apical meristem (SAM) and root apical meristem (RAM). While the RAM forms the underground root system, the SAM continuously produces leaves and stems, forming the aboveground phyllotaxy of the plant. The SAM can also be reprogrammed to produce flowers, ensuring plant reproduction. The balances between cell division for stem cell renewal and

cell differentiation for organ formation are controlled by specific transcription factors and environmental factors.

In Arabidopsis, the shoot apical meristem (SAM) harbors stem cells that produce the entire above-ground structures of a plant in an indeterminate manner. These stem cells are active throughout plant development and continuously produce primordia, which go on to generate various types of lateral organs such as leaves and flowers. A flower originates from a floral meristem that is produced by the SAM after floral transition. A floral meristem harbors stem cells that give rise to all organs found in a flower, sepals, petals, stamens, and carpels. In contrast to the stem cells in the SAM, floral stem cells are determinant in that they generate a precise number of floral organs and then cease to be stem cells. The termination of floral stem cells is concordant with the development of carpel primordia, the last organs to be made from the meristem. As such, floral stem cells provide a good model for studying the mechanisms underlying stem cell termination within developmental contexts.

The temporal termination of floral stem cells involves two key transcription factors, AGAMOUS (AG), a MADS domain protein, and WUSCHEL (WUS), a homeodomain protein. *WUS* is expressed in a few cells underneath the floral stem cells in the floral meristem and signals to the overlying cells to maintain their stem cell identity. By stage 6 of flower development, when the primordia for the final floral organs (carpels) arise, *WUS* expression is shut off, which results in the termination of floral stem cells. The temporally regulated repression of *WUS* expression requires *AG*, which also serves as a key factor in specifying the identities of stamens and carpels. In an ag loss-of-function mutant, stamens and carpels are transformed into petals and sepals, and the floral meristem continues to produce these organs to result in a flowers-within-flower phenotype. Intriguingly, *AG* expression starts at stage 3 in a domain that encompasses that of *WUS*, yet *AG* only shuts off *WUS* expression at stage 6. Because of this, it has been thought that *AG* is not a direct regulator of *WUS*. In fact, *AG* is known to activate the expression of another transcription factor gene, *KNUCKLES* (*KNU*), at around stage 6 in a region that encompasses the *WUS*-expressing cells in the floral meristem, and *KNU* is in turn necessary for the repression of *WUS* expression. Although this nicely explains the temporal lag in the repression of *WUS* by *AG*, the extremely weak and strong defects in floral determinacy exhibited by *knu* and *ag* null mutants, respectively, suggest that *AG* represses *WUS* expression through unknown mechanisms in addition to the activation of *KNU* expression.

The aim of our project is to identify players that regulate the temporal program of floral stem cells, we performed an ethyl methanesulfonate (EMS) mutagenesis in the *ag-10* background which is a

weaker *ag* allele mutant showing mildly defective in floral stem cell termination. *ag-10* flowers generate a full complement of floral organs like wild type, but one to a few siliques on an *ag-10* plant are short and bulged with additional floral organs inside, reflecting a defect in stem cell termination. In the *ag-10* mutagenesis screen, mutations that enhanced the *ag-10* mild stem cell defects were isolated based on the presence of bulged siliques throughout the plant.

Major research achievements

① Based on the EMS mutagenesis screening and molecular mechanism analysis, we found that AG directly represses *WUS* expression by binding to the *WUS* locus and recruiting, directly or indirectly, the Polycomb group (PcG) proteins that methylate histone H3 lysine 27 at *WUS*. Our studies show that PcG is recruited to a key stem cell regulator by a transcription factor to result in the temporally precise termination of floral stem cells.

② By the EMS mutagenesis screening and molecular mechanism analysis, we uncovered a role for *TOP1α*, a DNA topoisomerase, in Polycomb Group (PcG) protein-mediated histone 3 lysine 27 trimethylation (H3K27me3) at, and transcriptional repression of, the stem cell maintenance gene *WUS*. We demonstrated that H3K27me3 deposition at other PcG targets also requires *TOP1α*.

③ By chemical treatment and molecular mechanism analysis, we found that treatment of plants with camptothecin, a *TOP1α* inhibitor, or loss of function in *TOP1α*, led to the de-repression of RdDM target loci, which was accompanied by loss of H3K9me2 or DNA methylation. The role of *TOP1α* in RdDM could be attributed to its promotion of Pol V, but not Pol IV, transcription to generate long noncoding RNAs.

Current research and future directions

① Based on the EMS mutagenesis screening and map based cloning combination of molecular mechanism analysis, we found that a mutation in *Auxin Response Factor 3*(*ARF3*) enhanced *ag-10* floral meristem determinacy defect. *WUS* is epistatic to *ARF3* and *AG*. *ARF3* is a target gene of *AP2* and negatively regulated by *AP2*.

② Based on the EMS mutagenesis screening and map based cloning, we found that one mutant of *FHY3* could enhance *ag-10* floral meristem determinacy defect. Functional analysis and molecular mechanism study are ongoing.

Since *ARF3* is one of auxin response factor which functions in auxin signaling pathway to regulate plant development. Meanwhile, *ARF3* regulates cytokinin biosynthesis, which subsequently

affects the induction of the shoot apical meristem (SAM) via auxin and cytokinin signaling. *ARF3* maybe serves as a node to integrate the function of transcription factor and phytohormone in floral meristem determinacy.

FHY3 functions in far-red signaling pathway regulating many plant developmental processes. Functional analysis of *FHY3* in floral meristem determinacy will uncover the role of light signal in stem cell maintenance and differentiation.

2), Isolation and functional characterization of grain number genes and dissection of their regulationary networks in wheat

Research background

Grain number per plant, which is affected by environment easily and displays quantitative trait with continuous variation, is crucial for determining the yield potential of crops. To date, many genes and QTLs involved in the panicle development were isolated or mapped in rice, such as the major QTLs involved grain number, *GN1*, and panicle architecture gene, *DEL1*. All those genes will contribute to the breeding improvement in rice due to their function in positive regulation for grain number productivity.

Panicle development and formation in grass family are highly dependent on the timing of various meristems maintenance and specification which are controlled by many coordinated expression of multiple genes. Mutations in these genes will destroy the determinacy and indeterminacy developmental process resulting in the changes of inflorescent architecture. In rice, reduced expression of CKX2 in *gn1*, a cytokinin oxidase which was encoded by *GN1*, caused the accumulation of cytokinin in the inflorescent meristem and then enhanced inflorescent meristem activity, resulting in increased number of panicle branching and spikelet. The mutations in *IPA1/OsSPL14* gene which is the miRNA165 target gene and controls the rice plant architecture, inhibit the RNA degradation by miR165 resulting in high abundance of *IPA1/OsSPL14* product which lead to the reduction of tillering number and increased the number of painicle branch and spikelet. Thus, prolonged inflorescent meristem activity results in increased productive organs, including more panicle branches number, more spikelet number as well as larger panicle. In addition, the timing of transition from one type of meristem to another type of meristem is also a crucial factor to determine the inflorescent architecture. *APO2/RFL* in rice is a homolog of *LFY* in *Arabidopsis* which controls the transition from vegetative stage to reproductive stage, the *apo2* mutant displayed the precocious conversion of meristem from inflorescence to spikelet mode, which was opposite to that of mutant *lyf* in *Arabidopsis* with the repression of conversion from

inflorescent meristem to floral meristem. *TAWI* in rice encodes a protein belonging to ALOG family. Increased expression of *TAWI* was detected in inflorescent and branching meristem of the dominant gain-of-function mutant *taw1-d*, in which the activity of the inflorescence meristem is prolonged and spikelet specification is delayed, resulting in prolonged panicle branch and increased numbers of spikeletets. In contrast, the reduced expression or functional lose of *TAW1* caused precocious inflorescent meristem abortion and spikelet formation, resulting in smaller panicle. *RCN1* and *RCN2* in rice, the homolog of *TFL1* in Arabidopsis, are negative regulators in conversion from inflorescent branching meristem to floral meristem. Over-expression of them yielded more panicle branches and dense inflorescent architecture.

In wheat, lots of QTLs of grain numbers have been located by using different segregation populations. However, up to now, none of these QTLs was cloned because of a lack access to complete genome sequences owning to the large genome and hexaploid nature of wheat. Taken advantage of the great progress in Next Generation Sequencing technology and Bioinformatics , the whole genome sequences references of A and D ancestor of wheat and partial *ChineseSpring* genomic sequences have been released, which will greatly push ahead the gene cloning and functional analysis in wheat.

Grain number per plant in wheat is the final result of inflorescent development, the process of inflorescent development is distinct by several clear stages, including single bridge, double bridge and flower meristem stages et al.. Up to now, it has been rare reported on genes cloning and functional characterization in the process of inflorescent development in wheat. On the other hand, genes which originated by vertical descent from a single gene of the last common ancestor, so called the orthologous genes tend to be conferred similar developmental functions in different species. The work in our lab is focused on the isolation of wheat genes involved in the panicle development from particular stage and meristem by Comparative Genomics combination of Bioinformatics skills followed by transgenic plant analysis. Meanwhile, we are also performing RNA-seq comparable analysis for several particular developmental stages of unique wheat accessions (more grain number and panicle branching) . Based on the two strategies, our aim is to clone several important genes controling the inflorescent development in wheat, dissect their functions and interaction network, which will facilitate better understanding of mechanism of wheat panicle development and formation, and provide target genes and theoretical support for wheat breeding improvement.

Major research achievements

① We have isolated full length cDNA of six genes involved in the inflorescence development

from wheat by homologous cloning and tissue specific expression analysis

② We have completed over expression constructions of the six genes driven by 35S promoter, respectively, and site-directed mutation constructions based on the *CRIPS-CAS* system.

③ We have successfully built exogenous gene transformation system in Brachypodium distachyon using Agrobacterium-mediated transformation of compact embryogenic calli derived from immature embryos. The efficiency of transformation reach to about 20%.

Current research and future directions

① We plan to continue to clone more wheat genes involved in the inflorescent development and crop yield, and followed by Brachypodium distachyon transformation to assess their function.

② The tissue specific expression of target genes will be further confirmed by *in situ* hybridization analysis.

③ We will collect the particular meristem at different developmental stages from Brachypodium distachyon and wheat followed by RNA-seq and bioinformatics analysis to mine genes expressing particularly in different meristems. .

④ In order to explore the interaction network, we will carry out genetic analysis for transgenetic plants and biochemical assays.

5, FUNDING AND LABORATORY PERSONNEL

Funding

Project	Resource	Time	funding（ten thousand RMB）
“The hundred talents program ”	Chinese Academy of sciences	2013-2015	200
Crop high yield and plant abiotic stress response research	IGDB of CAS	2013- 2015	50

Laboratory personnel

Name	Professional title	Degree	Time	Comment
Lin Guo	Assistant Scientist	Ph.D	2013-present	Research staff
Meichen Zhao	Assistant Scientist	Ph.D	2013-present	

Furong Li	Engineer	Master	2013-present	
Shuang Jia		Master	2013-present	Technician
Lin Li	Postdoctor		2013-present	Postdoctor
Xiuwei Cao	Master		2012-2015	Graduate student
Yongpeng Li	Docdor		2013-2016	

6, SELECTED PUBLICATIONS, PATENTS GRANTED , VARIETIES OBTAINED

No.

7, EDITIONAL DUTIES

No.

8, CONFERENCE ORGANIZATION

No.