Available online at www.sciencedirect.com

ScienceDirect

Journal of Genetics and Genomics 42 (2015) 639−649

JGG

ORIGINAL RESEARCH

Competitive Expression of Endogenous Wheat CENH3 May Lead to Suppression of Alien *ZmCENH3* in Transgenic Wheat × Maize Hybrids

Wei Chen [a,1], Qilin Zhu [b,1], Haiyan Wang [a], Jin Xiao [a], Liping Xing [a], Peidu Chen [a], Weiwei Jin [b,*], Xiu-E. Wang [a,*]

[a] *State Key Laboratory of Crop Genetics and Germplasm Enhancement, Cytogenetics Institute, Nanjing Agricultural University/JCIC-MCP, Nanjing 210095, China*

[b] *National Maize Improvmental Center, China Agricultural University, Beijing 100193, China*

Received 21 January 2015; revised 15 April 2015; accepted 6 May 2015
Available online 10 August 2015

ABSTRACT

Uniparental chromosome elimination in wheat × maize hybrid embryos is widely used in double haploid production of wheat. Several explanations have been proposed for this phenomenon, one of which is that the lack of cross-species CENH3 incorporation may act as a barrier to interspecies hybridization. However, it is unknown if this mechanism applies universally. To study the role of CENH3 in maize chromosome elimination of wheat × maize hybrid embryos, maize *ZmCENH3* and wheat *αTaCENH3-B* driven by the constitutive *CaMV35S* promoter were transformed into wheat variety Yangmai 158. Five transgenic lines for *ZmCENH3* and six transgenic lines for *αTaCENH3-B* were identified. RT-PCR analysis showed that the transgene could be transcribed at a low level in all *ZmCENH3* transgenic lines, whereas transcription of endogenous wheat *CENH3* was significantly up-regulated. Interestingly, the expression levels of both wheat *CENH3* and *ZmCENH3* in the *ZmCENH3* transgenic wheat × maize hybrid embryos were higher than those in the non-transformed Yangmai 158 × maize hybrid embryos. This indicates that the alien *ZmCENH3* in wheat may induce competitive expression of endogenous wheat *CENH3*, leading to suppression of *ZmCENH3* over-expression. Eliminations of maize chromosomes in hybrid embryos of *ZmCENH3* transgenic wheat × maize and Yangmai 158 × maize were compared by observations on micronuclei presence, by marker analysis using maize SSRs (simple sequence repeats), and by FISH (fluorescence *in situ* hybridization) using 45S rDNA as a probe. The results indicate that maize chromosome elimination events in the two crosses are not significantly different. Fusion protein ZmCENH3-YFP could not be detected in *ZmCENH3* transgenic wheat by either Western blotting or immnunostaining, whereas accumulation and loading of the αTaCENH3-B-GFP fusion protein was normal in *αTaCENH3-B* transgenic lines. As ZmCENH3-YFP did not accumulate after AM114 treatment, we speculate that low levels of ZmCENH3 protein in transgenic wheat may be one of the factors that lead to failure of suppression of maize chromatin elimination in *ZmCENH3* transgenic wheat × maize hybrids.

KEYWORDS: CENH3; Transgenic wheat; Uniparental chromosome elimination; *Triticum aestivum*; *Zea mays*

INTRODUCTION

Uniparental chromosome elimination was first found in human × hamster hybrid cell lines in 1967 (Weiss and Green, 1967). In plants, this phenomenon was reported in many wide crosses, such as *Hordeum vulgare* × *H. bulbosum* and crosses between wheat and *H. bulbosum*, *Zea mays*, *Pennisetum glaucum*, *Sorghum bicolor*, and *Imperata cylindrica* (Falk and Kasha, 1983; Zenkteler and Nitzsche, 1984; Laurie and Bennett, 1988a; Laurie and Reymondie, 1991; Matzk and Mahn, 1994; O'donoughue and Bennett, 1994; Chaudhary et al., 2013). In crop improvement, uniparental chromosome elimination offers a rapid means to produce completely

* Corresponding authors. Tel: +86 25 8439 5308, fax: +86 25 8439 5344.
E-mail addresses: xiuew@njau.edu.cn (X.-E. Wang), weiweijin@cau.edu.cn (W. Jin).

[1] These authors contributed equally to this article.

http://dx.doi.org/10.1016/j.jgg.2015.05.006

homozygous lines. The wheat × maize system is now extensively used in double haploid (DH) breeding programs because: i) DH lines can be produced efficiently from all genotypes, ii) DH lines represent a random sample of the parental gametes, and iii) DH embryogenesis is genetically normal and stable (Laurie and Bennett, 1988b; Suenaga and Nakajima, 1989; Inagaki and Tahir, 1990; Sadasivaiah et al., 1999).

Despite the successful application of wheat × maize hybridization, the underlying mechanism of maize chromosome elimination remains unclear. Several hypotheses for uniparental chromosome elimination have been proposed for different cross combinations, and include asynchronous cell cycles (Michel, 2000), asynchronous nucleoprotein synthesis (Bennett et al., 1976; Laurie and Bennett, 1989), spatial separation of genomes during interphase and metaphase leading to budding extrusions (Schwarzacher et al., 1987; Linde-Laursen and von Bothmer, 1999), and degradation of alien chromosomes by host-specific nucleases (Davies, 1974). In hybrid embryo cells of common wheat × *Z. mays*, *H. vulgare* × *H. bulbosum*, and *H. vulgare* × *Secale cereale*, paternal chromosomes cannot move to the poles during anaphase due to failure of attachment of their centromeres to the spindle fibers, indicating that specific inactivity of uniparental centromere may be a leading cause for chromosome elimination (Schwarzacher et al., 1987; Kim et al., 2002; Mochida et al., 2004).

It is well known that centromere function depends on CENH3 (centromere-specific histone H3 variant) (Earnshaw and Rothfield, 1985; Talbert et al., 2002), which differs from the conserved conventional histone H3 due to its highly divergent N-terminal tail. CENH3 is prerequisite for correct assembly of kinetochore components, and is also the link between centromeric DNA and the proteinaceous kinetochore (Blower et al., 2002). In active centromeres, CENH3 forms the centromere-specific nucleosomes instead of canonical histone H3 (Malik and Henikoff, 2001; Talbert et al., 2002). In *Drosophila* and mouse, loss-of-function of CENH3 leads to mis-placement of all detected kinetochore proteins and results in disturbed mitosis (Howman et al., 2000; Blower and Karpen, 2001). Homozygous *CENH3* null mutants in mice are embryo-lethal, whereas heterozygous mutants are normal and fertile (Howman et al., 2000). In *Arabidopsis thaliana*, *CENH3* RNAi transformants have dwarf stature and reduced fertility (Ravi et al., 2010). In *Drosophila* and *Arabidopsis*, in particular, it was found that CENH3 is sufficient for centromere formation through the CENH3-GFP-LacI repressor and LacO operator systems (Mendiburo et al., 2011; Teo et al., 2013). CENH3 is the suggested assembly site for the kinetochore complex and is absolutely essential for centromere function. Sanei et al. (2011) found that abnormal chromosome segregation and elimination in *H. vulgare* × *H. bulbosum* hybrid embryos were due to loss of CENH3 protein and centromeric inactivation. This provided direct evidence for the association of CENH3 and chromosome elimination. All these results show that CENH3 has deterministic roles in centromere function and may also be involved in the process of chromosome elimination by influencing centromeric activity.

To investigate whether expression of ZmCENH3 can influence the maize chromosome elimination in wheat × maize hybrid embryos, we transformed *ZmCENH3* from maize into wheat. We found that *ZmCENH3* was transcribed at a low level, but ZmCENH3 protein could not be detected in the transgenic wheat. However, endogenous wheat *CENH3* transcription was highly up-regulated, but the level or rate of maize chromosome elimination in *ZmCENH3* transgenic wheat × maize was not significantly different from that in non-transformed Yangmai 158 × maize. These findings provide evidence that CENH3 has a role in maize chromosome elimination, perhaps by failure of ZmCENH3 translation, loading, or instability in the wheat background.

RESULTS

Transiently expressed ZmCENH3-YFP was localized in nucleus of onion epidermal cells

To investigate the subcellular localization of ZmCENH3-YFP, the vector *pCAMBLA3301-ZmCENH3-YFP* was particle bombarded into onion epidermal cells by a GeneGun. YFP signals were ubiquitously distributed when *pCAMBLA3301-YFP* was transiently expressed (Fig. 1A−C), whereas transiently expressed fusion ZmCENH3-YFP was present only in the nucleus. This indicated that ZmCENH3 was localized and expressed in the nucleus (Fig. 1D−F).

ZmCENH3 was transcribed at low levels in transgenic wheat, and led to competitive up-regulation of endogenous wheat *CENH3*

The expression vectors *pCAMBLA3301-ZmCENH3-YFP* and *pZY101-αTaCENH3-B-GFP* were transformed into calli of immature embryos of wheat variety Yangmai 158 by particle bombardment. Totals of 426 and 189 independent T_0 transgenic plants with *ZmCENH3* and *αTaCENH3-B*, respectively, were obtained. PCR analyses using specific primer pairs for *ZmCENH3* and herbicide gene *Bar* as selective marker gene in *ZmCENH3* transgenic wheat showed that specific bands for both genes were amplified in five plants (Fig. 2A and B). The identities of these five positive transgenic plants were confirmed by GUS staining of root tips (Fig. 2C). The positive *ZmCENH3* transgenic lines and Yangmai 158 were analyzed by semi-quantitative RT-PCR (semi-qRT-PCR), and an additional 405 bp specific amplicon was amplified only in the transgenic lines (Fig. S1A). Subsequent sequencing of these amplicons showed that it was the same as *ZmCENH3* (Fig. S1B), indicating that the transformed *ZmCENH3* was transcribed in the wheat genetic background.

Quantitative RT-PCR (qRT-PCR) was performed to investigate the expression of alien *ZmCENH3* and endogenous wheat *CENH3* in the *ZmCENH3* transgenic lines. The results showed that even though *ZmCENH3* was driven by the constitutive *CaMV35S* promoter, its expression level in the