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Genome organization and non-colinear distribution of the knob-associated sequences in maize

Fei Yang[1+], Yanhao Xu[2+], Jing Huang[1], Lijia Li[1*]

[1]State Key Laboratory of Hybrid Rice, College of Life Sciences, Wuhan University, Wuhan, 430072, Hubei, China
[2]Engineering Research Center of Wetland Agriculture in the Middle Reaches of the Yangtze River, Ministry of Education, College of Agriculture, Yangtze University, Jingzhou 434025, Hubei, China
[+]These authors are equally contributed to this work.

***Corresponding author: ljli@whu.edu.cn**

Abstract

The maize (*Zea mays* L.) genome contains abundant of repetitive elements. In this study, fluorescence *in situ* hybridization (FISH) was used to reveal the distribution of the knob-associated 180-bp repeats and TR-1 elements among eleven maize inbred lines. Non-colinear distribution of the knob-associated sequences was detected, and a distinct FISH pattern for each maize inbred line was constructed. This result confirmed that FISH using the knob-associated sequences as probes may be a rapid method to identify maize germplasm resources. The 180-bp repeats were more numerous and widely distributed than the TR-1 elements in the maize genome, which provided cytogenetical evidence for the hypothesis that the TR-1 elements were derived from the 180-bp repeats. The knob-associated sequences were concentrated in the satellite of chromosome 6. The signals of the TR-1 elements were readily detected in the satellites of all the maize inbred lines, and the signals of 180-bp repeats were detected in the satellites of six maize inbred lines. This finding indicated that the TR-1 elements tend to accumulate in regions of exceedingly low recombination.

Keywords: Chromosome; fluorescence *in situ* hybridization (FISH); knob-associated sequences; maize; satellites.
Abbreviations: FISH-fluorescence *in situ* hybridization; NOR-Nucleolus organizer region.

Introduction

One of the most prominent features of maize chromosomes is the large blocks of heterochromatin referred to as knobs (McClintock, 1929). Previous studies have revealed that the locations, numbers, sizes and frequencies of knobs varied among maize stocks (Brown, 1949; Dennis and Peacock, 1984; Kato, 1976; McClintock et al., 1981) and related taxa (Albert et al., 2010; Dennis and Peacock, 1984; Kato and Lopez, 1990). The polymorphism of knobs distribution provided important information in maize evolution (Albert et al., 2010; Kato et al., 2004; Kato, 1976; McClintock et al., 1981). At the molecular level, knob are composed primarily of the 180-bp repeats and TR-1 elements (350-bp repeats). These repeats comprise up to approximately 8% of the maize genome (Buckler et al., 1999) and are organized in tandem arrays (Ananiev et al., 1998a; Buckler et al., 1999). It has been revealed that individual knobs could either be composed exclusively of the 180-bp or TR-1 elements or contain a mixture of both (Ananiev et al., 1998b; Kato et al., 2004). Most of the 180-bp repeats and TR-1 elements were located on visible knobs. However, the 180-bp repeats and TR-1 elements have also been detected in knobless maize inbred lines (Adawy et al., 2004). Moreover, a few 180-bp repeats existed in some euchromatic sites and the termini of chromosome arms (Lamb et al., 2007; Viotti et al., 1985). The further investigations have found that the stretches of TR-1 elements formed fold-back segments that were integrated into tandem arrays of the 180-bp repeats indicating that knobs may have some properties of transposable elements (Ananiev et al., 1998b). In addition, an invariant 27-bp sequence was identified by analyzing sequences of the 180-bp repeats cloned from various species, including *Zea diploperennis* Iltis, Doebley & Guzman. sp. nov. and *Tripsacum dactyloides* L.. These findings suggested that strong selection pressure was existed on that sequence and indicated that knobs might have a functional role (Dennis and Peacock, 1984). Both the TR-1 elements and 180-bp repeats exhibit meiotic drive, while, the two types of neocentromeres have visibly different cytological phenotypes at meiotic anaphase (Hiatt et al., 2002; Kanizay et al., 2013). The TR-1elements and 180-bp repeats are confirmed to operate in competition with each other (Kanizay et al., 2013). All these data implied that the molecular evolution and genome organization of the knob-associated sequences are very complicated. More research should be performed to clarify this issue.

Numerous researches have been performed to investigate the genetic polymorphism and variation of maize germplasms used in China (Zhang et al., 2013). The chromosomal distributions of the knob-associated sequences provided unique cytogenetical diversity in maize evolution (Albert et al., 2010; Kato et al., 2004; Kato, 1976; McClintock et al., 1981). However, little information is available about the chromosomal distributions of the knob-associated sequences among maize germplasms used in China.

In this study, FISH was used to investigate the distribution and frequency of 180-bp repeats and TR-1 elements in the chromosomes of 11 maize germplasms widely used in China (Liu, 2002). A distinct cytogenetical pattern of the knob-associated sequences was constructed for each inbred lines. The genome organization and evolution of the knob-associated sequences were analyzed and compared with

Table 1. The distribution of the the knob-associated sequences.

Variety	No. of 180-bp repeat signals	No. of TR-1 element signals	No. of colocalized signals	No. of solo signals	
				180-bp	TR-1
Mo17	4	2	2	2	0
S7913	4	8	2	2	6
8n191	6	6	2	4	4
J51	6	6	2	4	4
Hz85	8	4	2	6	2
78599-2	8	6	2	6	4
C7-2	8	8	4	4	4
P995	8	2	2	6	0
P926	10	8	6	4	2
Cml75	10	4	2	8	2
Hz4	16	8	8	8	0
Total	88	62	34	54	28

previous works. These results might be an important complementation of previous works (Kato et al., 2004; Lamb et al., 2007), which could provide insight into molecular evolution and genome organization of the knob-associated sequences.

Results and discussion

The non-colinearity of the knob-associated sequences is revealed by FISH

The hybridization signals of the 180-bp repeats and TR-1 elements varied in position and intensity among the 11 maize inbred lines (Fig. 1). The signals of the knob-associated sequences were all located on the termini or sub-termini of chromosomes, similar to the results reported by Lamb et al., (2007). In different maize inbred lines, the number of 180-bp repeats ranged from 4 to 16, and that of the TR-1 elements ranged from 2 to 8. The number of signals of 180-bp repeats that co-localized with signals of TR-1 elements ranged from 2 to 8 (Table 1). The distribution patterns of the knob-associated sequences differed between 8n191 and J51 (Fig. 1), although the number of knob-associated signals was the same in these two lines (Table 1). Thus, each maize inbred line had a distinct pattern of 180-bp repeats and TR-1 elements (Fig. 1).

Mo17 is an exotic germplasm introduced to China and is one of the parent of hybrid maize Zhongdan 2 with the largest cultivated area in China in 1980s (Tong, 2001). HuangZao4 is an elite Chinese landrace and one of the most widely used maize germplasms in China (Liu, 2002; Tong, 2001). The other nine maize germplasms are backbone inbred lines in south China (Liu, 2002). In this study, no identical distribution patterns of the knob-associated sequences were detected among the eleven maize inbred lines, which displayed high level polymorphism of the knob-associated sequences in maize genome. Similar phenomena were also reported in other maize germplasm resources (Dover, 1982; Kato et al., 2004; Sadder and Weber, 2001). Both the distribution and sequences of tandemly repeated DNAs showed polymorphisms among different lines of a species (Heslop-Harrison, 2000). The distinct chromosomal distributions of the knob-associated sequences observed in this study confirmed that FISH using the knob-associated sequences as probes may be a rapid method to identify maize germplasm resources.

Brunner et al. (2005) reported that the genes and repeated sequences diverged in the allelic chromosomal regions between maize lines B73 and Mo17, which indicated non-colinearities in different maize inbred lines. The divergence of the knob-associated sequences on the allelic chromosomes among the eleven maize inbred lines (Fig. 1) demonstrated the non-colinearity of highly tandem repeats in maize. The non-colinearity of the knob-associated sequences in different lines may result from chromosomal rearrangement due to highly tandem repeat character. Maize knobs may be considered to be complex megatransposons (Ananiev et al., 1998b). Thus, the non-colinearity of the knob-associated sequences may result from molecular rearrangements and transpositions of all or parts of the knob regions.

The knob-associated sequences are mainly localized at the sub-terminus of chromosomes and are clustered in the satellite on chromosome 6

The distribution of the knob-associated sequences varied among the chromosomes (Fig. 2). No signals of the knob-associated sequences were found on chromosomes 1 and 10. On chromosomes 3 and 5, only the 180-bp repeats were detected. On chromosomes 4 and 9, no co-localized signals were detected. All three patterns of signals were detected in chromosomes 2, 6, 7, and 8. Higher frequencies of the knob-associated sequences were detected on chromosomes 6, 7, and 8 than on other chromosomes. The co-localized signals of the knob-associated sequences were primarily distributed on chromosomes 6 and 8 (Fig. 2).

The chromosome with the most knob-associated sequences was chromosome 6 (Fig. 2). The TR-1 elements were observed in the satellite of all eleven maize inbred lines, and the 180-bp repeats were found in the satellite of six maize inbred lines (Fig. 1). Our results showed that the knob-associated sequences were clustered in the satellite on chromosome 6. The satellite of chromosome 6 is a preferred location for the knob-associated sequences. Maize chromosome knobs were confirmed to suppress local recombination (Ghaffari et al., 2012). The above findings indicated that the TR-1 elements tend to accumulate in regions of exceedingly low recombination.

Little is known about the composition, origin and function of the satellite, although the satellite occurs frequently in the chromosomes of almost all eukaryotes. In maize, the 5.8/18/25s rDNA (NOR) was located in the secondary constriction on chromosome 6 (Li and Arumuganathan, 2001). Previous studies have detected the signals of the 180-bp repeats and TR-1 elements at the chromomere flanking the NOR on the satellite in pachytene (Ananiev et al., 1998a; Chen et al., 2000). The rDNAs and NORs are interested

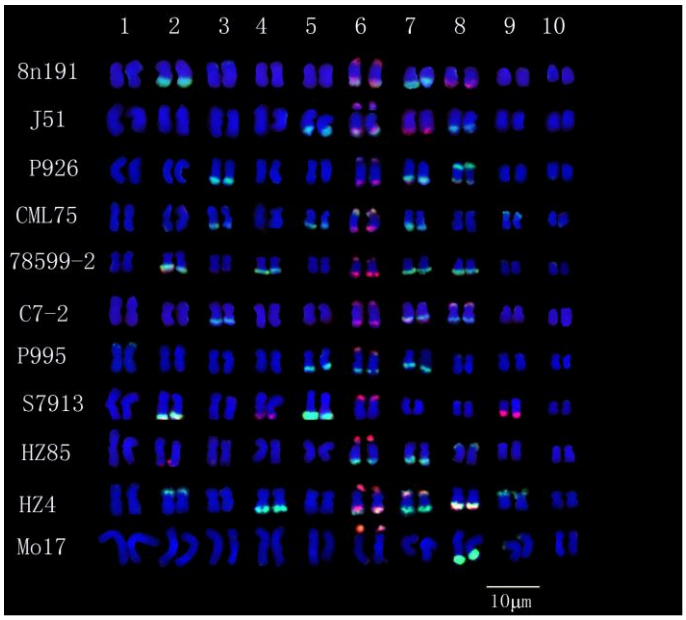

Fig 1. The somatic karyotypes of 11 maize inbred lines probed with the knob-associated sequences. The DAPI stained chromosomes are shown in blue, the FITC probed 180-bp repeats in green, and the Cy3 probed TR-1 elements in red. The hybridization signals of the 180-bp repeats and TR-1 elements varied in position and intensity among the eleven inbred lines. The signals of the knob-associated sequences were all located on the termini or sub-termini of chromosomes. A total of forty-four pairs of 180-bp repeat signals and thirty-one pairs of TR-1 element signals were detected. Among these signals, twenty-seven pairs were detected with the probe for the180-bp repeat, fourteen pairs were detected with the probe for the TR-1 element, and seventeen pairs were detected with both probes (Scale bar, 10 μm).

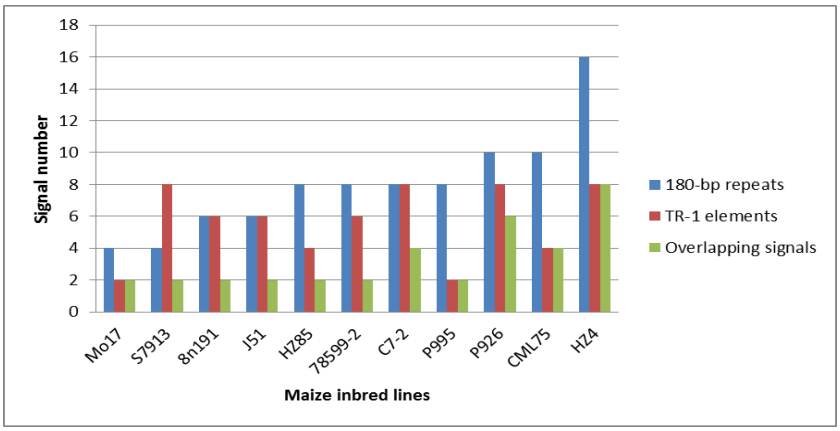

Fig 2. The distribution of the knob-associated sequences on 11 maize inbred lines.

because of their important functions. In contrast, the satellite has been overlooked. One interesting observation was that two types of tandem repeats (rDNA and the knob-associated sequences) were tightly linked in the short arm of chromosome 6. This phenomenon was also observed in pachytene (Wang et al., 2006). These data may indicate that different tandem repeats have the tendency to be clustered; however, more studies are needed to analyze the coincidences or relationships of rDNA and the knob-associated sequences.

The knobs-associated sequences are not necessary for the formation of knobs on maize chromosomes

In all of the maize inbred lines, a total of forty-four pairs of 180-bp repeat signals and thirty-one pairs of TR-1 element signals were detected (Table 1). Among these signals, twenty-seven pairs were detected with the probe for the180-bp repeat. Fourteen pairs were detected with the probe for the TR-1 elements, and seventeen pairs were detected with both probes (Table 1). Among the 58 loci, 76% of the sites contained the 180-bp repeats, while 53% of the sites contained the TR-1 elements (Table 1). Approximately 39% (17 of 44) of the loci detected by the 180-bp repeat probe co-localized with the TR-1 elements, whereas 55% (17 of 31) of the loci detected by the probe for the TR-1 elements co-localized with the 180-bp repeats (Table 1). This indicated that the distribution of the 180-bp repeats was more varied than that of the TR-1 elements in maize chromosomes. In this study, the 180-bp repeats were more numerous and more widely distributed than the TR-1 elements in the maize genome. This finding can be explained by the hypothesis that TR-1 elements have evolved from the 180-bp repeats as a result of duplication and subsequent divergence (Ananiev et al., 1998b; Hsu et al., 2003). Our research provided some cytogentical evidence for the hypothesis that the TR-1 elements were derived from the 180-bp repeats.

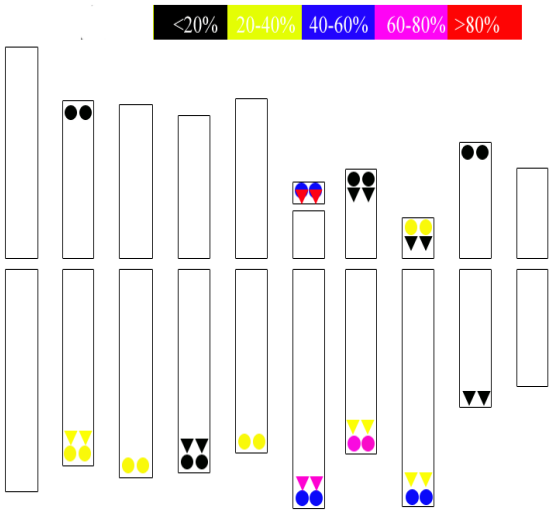

Fig 3. Scheme of *Zea mays* L. with the knob-associated sequences signals. Circles represent the signals of 180-bp repeats and triangles the signals of TR-1 elements. The different frequency distributions of the signals have different colors.

Based on the presence of FISH signals of the knob-associated sequences on each chromosome, the scheme of the maize karyotypes was constructed (Fig. 3). Locations of the knob-associated sequences on 10 chromosomes were marked, and their occurrence frequencies in eleven maize inbred lines were calculated (Fig. 3). The different colors denote the different frequencies of the signals on the chromosomes (Fig. 3). Buckler et al. (1999) and Kato (1976) summarized 23 knob heterochromatin sites on 1S, 1L (2 sites), 2S, 2L, 3S, 3L (3 sites), 4S, 4L, 5S, 5L, 6L (3 sites), 7L (2 sites), 8L (2 sites), 9L, 10L (2 sites) across all 10 maize chromosomes. In our study, only 13 different positions of the knob-associated sequences were detected. Although different materials used in the two investigations should be considered, the comparison between previous researches (Buckler et al., 1999; Kato, 1976) and this research (Fig. 3) also indicated a complex relationship between knobs and the knob-associated sequences. No knob-associated sequences were detected in the knob sites of 1L, 1S, 3S, 4S, 5S and 10L. The absence of the knob-associated sequences in knob sites may depend on the materials used in this study. However, the knob-associated sequences were detected on 6S, 7S and 8S without corresponding knob sites. The knob-associated sequences have been reported on chromosomes of knobless lines (Adawy et al., 2004). Retroelements belonging to the RE10, RE5, PREM2, and Zeon families were isolated from knob regions (Ananiev et al., 1998a). All these data implied that the presence of the knob-associated sequences on chromosomes is not the only requirement for forming knobs. Recently, the epigenetic modification of the 180-bp repeats and the TR-1 elements and their expression were modified by cold treatment (Hu et al., 2012), which indicated a complex role for the knob-associated sequences in maize.

Materials and methods

Plant materials

Eleven standard maize (*Zea mays* L.) inbred lines were used in this study, including 8n191, J51, P926, Cml75, 78599-2, C7-2, P995, HuangZao4, Mo17, Hz85, and S7913. Seeds were provided by the Institute of Crop Genetics and Breeding, Huazhong Agricultural University, China.

Chromosome preparation and FISH

Mitotic chromosome preparation was performed using the routine protoplast technique as described (Yang et al., 2010). Root tips were harvested when they were 0.5-1 cm long from seedlings grown on moist filter papers in a culture tank. The excised root tips were pretreated in water supersaturated with α-bromonaphthalene in the dark at room temperature for 3 h and then fixed in 100% ethanol/acetic acid (3:1, v/v) at 4℃ overnight.

A dimer of the 180-bp repeats, ZmKR180-2, and a monomer of the TR-1 elements, ZmKR350-1, were provided by Dr. Rachel Wang of the University of California, Berkeley (Chen et al., 2000). ZmKR180-2 and ZmKR350-1 were labeled with digoxigenin-11-dUTP (Roche Molecular Biochemical, USA) or biotin-14-dATP (Roche Molecular Biochemical, USA), respectively, using a nick translation method.

FISH was carried out using the procedure described as Li and Arumuganathan (2001). The hybridization mixture (40 µl) contained 50% deionized formamide, 10% sodium dextran sulfate, 2×SSC, 1 mg/ml salmon sperm DNA and 30 ng of probe (denatured at 75℃ for 5 min, and then chilled on ice for 5 min). Hybridization reactions were incubated overnight at 37℃. Biotin-labeled probes were detected by a three-step detection/amplification system consisting of stretavidin-Cy3 (Vector Laboratories, Burlingame, USA), biotinylated anti-streptavidin (Vector Laboratories, Burlingame, USA) and strepavidin-Cy3. Digoxigenin-labeled probes were detected with sheep anti-digoxigenin-FITC (Roche Molecular Biochemical) and amplified with rabbit anti-sheep-FITC (Vector Laboratories, Burlingame, CA, USA). For each step of the immune reaction, slides were placed in a wet chamber at 37℃ for 1 h and then washed with PBS 3 times for 5 min. Chromosomes were counterstained with 1 µg/ml DAPI (4', 6-diamidino-2-phenylin-dole) in Vectashield (Vector Laboratories, Burlingame, USA).

Image captures and analyses

Chromosomes and signals were visualized using an Olympus BX60 fluorescence microscope system (equipped with a Metamorph 4.6.3, Universal Imaging Crop.). Gray-scale images were captured with a cooled CCD camera, Sensys 1401E, using the appropriate filter for each fluorescent channel and processed with Photoshop 8.0.1 software.

Conclusions

Our results revealed the genome organization and non-colinear distribution of the knob-associated sequences in maize by FISH. The distinct FISH pattern for each maize inbred line provided a cytological illustration of maize genome diversity. The distribution of the knob-associated sequences was not random in maize chromosomes, and the sequences may have site preferences. Obvious discrepancies between the sites of knobs and the knob-associated sequences were detected. Chromosome 6 and the satellite were found to contain clusters of the knob-associated sequences.

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