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Title: Characterization of satellite CentC repeats from heterochromatic regions on the long arm of maize B chromosome

Article Type: Original Research

Keywords: B-chromosome; centromere; CentC; CL-repeat; heterochromatic regions; B-10L translocation.

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Order of Authors: Shu-Fen Peng, Ph.D;Ya-Ming Cheng, Ph.D

Abstract: The B-chromosome of maize contains an A-chromosome centromere-specific satellite CentC repeat in its centromere region (CENB) and at multiple locations in its distal heterochromatic regions (BDHs). Because CentC is highly repetitive, it is a challenge to study CentC sequences within individual centromeres or chromosome regions. The combined structure of CentC and a BDH-specific CL-repeat has allowed us to isolate CentC sequences from BDHs. In the study described herein, we have used a PCR method to amplify 13 CL-CentC variant products that were specifically mapped to A-centromeres (CENAs), the CENB, and BDHs via the tertiary trisomes and hypoploids of five B-10L translocations. Cloning and sequence analyses of these CL-CentC products have revealed a local CentC homogenization within the three CentC-containing regions. Phylogenetic analysis has indicated that the CentC sequences of BDHs are more closely related to those of CENAs in comparison to that of the CENB. Furthermore, the CentC monomers that are within the CENB are more diverse than those within BDHs and CENAs. These results shed light on the evolution of CentC repeats on the B-chromosome and provide a better understanding of B-chromosome evolution.

Response to Reviewers:
Dear Dr. Hans de Jong:
We have completed our revision on the manuscript (no. CHRO211) entitled "Characterization of satellite CentC repeats from heterochromatic regions on the long arm of maize B chromosome". All modified texts in the revised manuscript were in red. Our point-by-point responses were listed as below.

Sincerely yours,
Ya-ming Cheng

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Comment:
These results are interesting for an understanding of the evolution of centromere repeats and how they associate with CENH3 or not. Interestingly, A CentC and BL CentC are more similar but it is A

CentC and BCEN CentC that associate with CENH3. This further illustrates the standing conundrum of what determines whether CentC binds to CENH3.
Response:
We fully agree the reviewer's perspective.

Response to Reviewer #2

Comment:
The current version of the manuscript is not very reader friendly and requires some improvement regarding its style and grammar.
Response:
The manuscript has been revised the best we can and we hope the further improvement can be done by the editing manager of the journal.

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Please consider a different title. I would recommend:
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Abstract:
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The first sentence in the Abstract has been revised as suggestion.

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Results, page 8
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Line 7, As shown in Fig. 3a, ...FISH signals were concentrated on the mitotic CENB and CENAs, indicating that the products contain CentC sequences..
FISH does not confirm CentC-sequence similarity. Only the chromosomal position of the sequence was determined. Only of sequencing of the PCR products it is possible to conclude on the sequence similarity. Please change the sentence accordingly.
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Comment:
Discussion, page 11, between lines 9 and 16.
Remove the paragraph:
In eukaryotic cells, centromeres mediates.... in Oryza sativa (Cheng et al. 2009).
Response:
The paragraph has been removed from the Discussion. And the References have been revised accordingly.

Comment:
In order to shorten the manuscript please show figures 2 and 6 as supplementary figures.
Response:
Figures 2 and 6 are now showed as supplementary figures, and the text is revised according to the redeployment of the two figures.

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There is a potential for sampling bias that might skew the results with only 10 or so monomers from each of the three locations. Using CentC adjacent to CL and B centromere repeats might also be a cause of bias, but it is difficult to know. The authors should at least discuss this. Of course, it could mean the cluster results could change if other copies of CentC were sampled. This might change the central interpretations of the work.
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Characterization of satellite CentC repeats from heterochromatic regions on the long arm of maize B chromosome

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E-mail: ymcheng@dragon.nchu.edu.tw

Running title: Characterization of CentC repeats on maize B long arm

Keywords: B-chromosome; centromere; CentC; CL-repeat; heterochromatic regions; B-10L translocation.

Abbreviations

CENB

B-chromosome centromere

CENAs

A-chromosome centromeres

BDHs

Distal heterochromatic regions of the B-chromosome

BS

B short arm

CK

Centromeric knob

PE

Proximal euchromatic region

DH

Distal heterochromatic region

DE

Distal euchromatic tip

FISH

Fluorescence in-situ hybridization

TT

Tertiary trisome

HO

Hypoploid

Abstract

The B-chromosome of maize contains an A-chromosome centromere-specific satellite CentC repeat in its centromere region (CENB) and at multiple locations in its distal heterochromatic regions (BDHs). Because CentC is highly repetitive, it is a challenge to study CentC sequences within individual centromeres or chromosome regions. The combined structure of CentC and a BDH-specific CL-repeat has allowed us to isolate CentC sequences from BDHs. In the study described herein, we have used a PCR method to amplify 13 CL-CentC variant products that were specifically mapped to A-centromeres (CENAs), the CENB, and BDHs via the tertiary trisomes and hypoploids of five B-10L translocations. Cloning and sequence analyses of these CL-CentC products have revealed a local CentC homogenization within the three CentC-containing regions. Phylogenetic analysis has indicated that the CentC sequences of BDHs are more closely related to those of CENAs in comparison to that of the CENB. Furthermore, the CentC monomers that are within the CENB are more diverse than those within BDHs and CENAs. These results shed light on the evolution of CentC repeats on the B-chromosome and provide a better understanding of B-chromosome evolution.

Introduction

Extra, supernumerary, or accessory chromosomes, which are called B-chromosomes, have been identified in a wide variety of plant and animal species. Their universal features include a failure of synapsis with any of the normal chromosomes (A-chromosomes) during meiosis, unusual cytological mechanisms that cause their accumulation in cells, and genetic inertness for plant development (reviewed in Jones et al. 2008). The B-chromosome was identified in maize nearly a century ago (Kuwada 1915); however, its detailed molecular organization and evolution remain largely unknown. The major reason for this gap in understanding is that its DNA defies isolation by conventional protocols, due to the repetitive nature of its sequences and its high homology to A-chromosomes (Chilton and McCarthy 1973; Stark et al. 1996; Cheng and Lin 2003, 2004; Lamb et al. 2005; Peng et al. 2005; Lo et al. 2009).

Several sequences of the maize B-chromosome have been identified in the past decade. A 1.4-kb repeat, ZmBs, was isolated from a B-chromosome-containing genomic library and was mapped to the B-centromere (CENB) (Alfentio and Birchler 1993). This sequence was subsequently found to be homologous with the centromere of chromosome 4, suggesting an evolutionary relationship between the two centromeres (Page et al. 2001). Using a B-specific random amplified polymorphic DNA (RAPD) marker, Stark et al. (1996) obtained the second B sequence, pBGBM18.2, which is located in the third and fourth distal heterochromatic (DH) regions of the B-chromosome (BDHs) (Lamb et al. 2007). Peng et al. (2005) have succeeded in cloning 14 B-specific amplified fragment length polymorphic (AFLP) fragments, which were mapped to 13 different B-regions defined by 12 B-10L translocations. More recently, Cheng and Lin (2003) and Lo et al. (2009) have isolated 78 B sequences from the microdissection libraries of the B-chromosome. One of these sequences is B-exclusive, and it has been revealed to be part of a tandem-arranged satellite DNA, the CL-repeat, which is specifically located in the first three BDHs (Cheng and Lin 2004; Cheng 2010).

To extend the analysis of the composition of the maize B-chromosome, Lamb et al. (2005) have used a collection of repetitive elements as probes for the fluorescence in-situ hybridization (FISH) analysis of chromosome spreads that contain B-chromosomes. They found that the various elements that are associated with A-centromeres (CENAs), including a centromere-specific tandem repeat (CentC), a retrotransposon-like repeated element (CentA), and a centromere-specific retrotransposon (CRM), are present not only in the CENB but also throughout BDHs. Using antibodies against centromeric histone H3 (CENH3), Lamb et al. (2005) observed that CENH3 is only associated with CentC in the CENB but not in BDHs, suggesting that there is no holocentromeric activity on the B long arm. The presence of CentC in BDHs had

previously been observed in sequences of a BDH-specific clone, pB51-15, which contains a CL-repeat array in conjunction with approximately seven copies of CentC (Cheng and Lin 2004).

Despite the clear observation of CentC in BDHs and the CENB, the evolution of CentC in the B-chromosome is unclear, because only a few CentC sequences are available from the two B-regions. In this study, we have used a primer pair that was designed using the CL-repeat and CentC to isolate a substantial number of CentC sequences from the CENB, BDHs, and CENAs of plants that were tertiary trisomes (TTs) or hypoploids (HOs), respectively, for maize B-10L translocations. Sequence and phylogenetic analyses of the resulting CentC monomers revealed evolutionary relationships among copies of CentC on the B- and A-chromosomes.

Materials and methods

Maize lines

The inbred maize L289 lines with and without B-chromosomes, respectively, were used to prepare mitotic and pachytene chromosome spreads for the FISH analyses. The genomic DNA of L289+2B and L289+0B was used as a template for polymerase chain reaction (PCR) amplification. Five B-10L translocations (TB-10L7, TB-10L18, TB-10L20, TB-10L26, and TB-10L38) in the W23 background (Lin 1972) were used to generate translocation heterozygous, tertiary trisomic, and hypoploid plants. The procedure for producing the three plants of each B-10L translocation has been previously described (Cheng 2010). Each translocation has a distinct breakpoint along the length of both arms of the B-chromosome and a second breakpoint in the long arm of chromosome 10 (Lin 1974, 1978; Cheng and Lin 2003). These breakpoints result in two translocation chromosomes, 10-B and B-10. The 10-B chromosome carries the centromere of chromosome 10, which is attached to the distal portion of the B long arm, whereas the B-10 chromosome contains the B centromere, which is connected to the terminal portion of the long arm of chromosome 10. A translocation heterozygote contains 10, 10-B, and B-10; a tertiary trisome has 10, 10, and B-10; and a hypoploid has 10 and 10-B. As shown in Fig. 1, the breakpoint of TB-10L18 is located in the B short arm (BS); in TB-10L7, the breakpoint is in the proximal euchromatic (PE) region; in TB-10L38, the breakpoint is between DH1 and DH2; in TB-10L26, the breakpoint is close to the junction of DH2 and DH3; and in TB-10L20, the breakpoint is near the junction between DH3 and DH4.

The amplification and cloning of CL-CentC variants

The following primers were designed from the B-clone sequences, pB51-15 (AY426743; Cheng and Lin 2004), and were used to amplify the portion between the CL-repeat and the CentC arrays: CL-1, ggtaacatgactaaacaag and BCentC-1, tgtttggagtggtttcacgc (Supplementary Figure 1). The PCR was carried out using DNA from L289+2B, L289+0B, and the B-10L translocation derivatives as templates (30 cycles of 94°C, 30 s; 50°C, 30 s; and 72°C, 90 s). The products were ligated into the pGEM-T easy vector (Promega) and transformed into competent cells of *Escherichia coli* (DH5α).

Chromosome preparation and FISH analysis

Metaphase chromosome spreads and pachytene chromosomes were prepared according to published protocols (Cheng and Lin 2003, 2004). Probe preparation and FISH procedures were performed as previously described (Cheng 2010). FISH images were captured using an Olympus DP72 CCD camera on an Olympus BX51 fluorescence microscope and processed using Photoshop (Adobe, San Jose, CA).

Sequence analysis

The sequences that were obtained in this study were deposited into GenBank with the accession numbers of HN174141 to HN174150. The CentC monomers in each CL-CentC clone were identified using Tandem Repeats Finder (version 3.21) (Benson 1999). The sequence logo analysis was created using the online WebLogo application (Crooks et al. 2004). CentC sequence alignments were performed using Alignment Explorer/ClustalW, and the phylogenetic tree was constructed via the neighbor-joining method using Mega4 (Tamura et al. 2007) with 100 bootstrap replicates.

Results

Chromosome localization of CL-CentC PCR products

It was previously demonstrated that the maize B-chromosome contains centromere-specific satellite repeat CentC (Ananiev et al. 1998) in the CENB and BDHs (Lamb et al. 2005). In addition, a CentC tract was observed to adjoin the BDH-specific CL-repeat in the B-clone, pB51-15 (Cheng and Lin 2004). To isolate CentC from BDHs, we designed a primer pair (CL-1 and BCentC-1; Supplementary Figure 1) to amplify CL-CentC region-specific sequences. The chromosomal locations of the resulting CL-CentC products were determined by FISH analyses of root-tip spreads and pachytene B-chromosomes from L289+2B, using labeled CL-CentC products as probes. As shown in Fig. 2a, FISH signals were concentrated on the mitotic CENB and CENAs, indicating that the products contain sequences located in those centromere regions. In addition, there were dispersed signals on the long arm of B-chromosome. In the pachytene B-chromosomes (Fig. 2b–2e), FISH signals were observed on the CENB and all four BDHs, which is consistent with the distributions of CentC that have been observed by Lamb et al. (2005).

The mapping of CL-CentC variant products in B-10L translocations

Using the CL-1 and BCentC-1 primers, 11 CL-CentC variant products (variants c to m) and smeared backgrounds were observed in the L289+2B preparations (Fig. 3). Only two major products (variants a and b), with several minor products, were observed in the L289+0B. These CL-CentC variant products were physically mapped via the amplification of each variant from the TTs and HOs of five TB-10L translocations (TB-10L7, TB-10L18, TB-10L20, TB-10L26, and TB-10L38; Lin 1978; Cheng and Lin 2003). As shown in Fig. 1, TB-10L18 breaks in the BS and the remaining four translocations in the long arm of B-chromosome, as follows: TB-10L7 at the PE; TB-10L38 between DH1 and DH2; TB-10L26 close to the junction of DH2 and DH3; and TB-10L20 near the junction of DH3 and DH4. The rationale of the mapping is as follows: a PCR product that could be amplified from a TT of a B-10L translocation would come from the B portion that is carried on

the B-10 chromosome, and a product that could be amplified from an HO of a B-10L translocation would come from the B part on the 10-B chromosome.

As shown in Fig. 3 and Table 1, in terms of the product distribution, the TTs of TB-10L26 (referred to as 26TT) and TB-10L20 (referred to as 20TT) were indistinguishable, as were the HOs of TB-10L7 (referred to as 7HO) and TB-10L38 (referred to as 38HO). Variants d and f were present in all TTs but absent in all HOs, suggesting that their locations were in the CK (including the CENB) that was not involved in any of the HOs. The variant e, appearing in the TT of TB-10L38 (referred to as 38TT) and 38HO but not in the HO of TB-10L 26 (referred to as 26HO), was located in DH1 and DH2. The map position of variant l could be assigned to DH2 and DH3, because it was visible in 26HO and 26TT but not in 38TT and the HO of TB-10L20 (referred to as 20HO). Variant j was present in 38TT and 26HO but absent in 7TT and 20HO, suggesting that its position was in DH1 and DH3. Finally, the presence of variants c, g, h, i, k, and m in 38HO and 26TT but not in 38T and 26HO indicates that the position of these products was specific to DH2.

The cloning and sequence analysis of CL-CentC variants

To understand the sequence structure of the CL-CentC variants in the CENB, BDHs, and CENAs, we cloned and sequenced 10 variants (accession numbers are reported at the end of this paragraph) from the PCR products of 7TT, 7HO, and L289+0B. As shown in Fig. 4, two clones (CC7TT-1 and CC7TT-2) were obtained from 7TT, five (CC7HO-1–CC7HO-5) from 7HO, and three (CC0B-1–CC0B-3) from L289+0B. Both CC7TT-1 and CC7TT-2 contained sequences with high identity levels to CentC and the CENB-specific repeat ZmBs, but low identity levels to the CL-repeat, suggesting that the two sequences were amplified from the CENB. All of the five 7HO clones carried the CL-repeat and three carried CentC, supporting their locations in BDHs. Because L289+0B contains no B-chromosome, the CentC that was obtained from the three CC0B clones should be amplified from CENAs.

Using the Tandem Repeats Finder software, 30 complete CentC monomers (~157 bp; Fig. 4, red boxes) and eight partial CentC monomers (Fig. 4, green boxes) were identified. The complete CentC monomers had an overall A+T content of 56.2% in the CC7TT clones, 54.7% in the CC7HO clones, and 53.3% in the CC0B clones. In addition, the pairwise similarity of CentC monomers ranged from 71.1 to 99.0% within the CC7TT clones, from 86.8 to 96.0% within the CC7HO clones, and from 86.0 to 98.0% within the CC0B clones. Sequence logo analysis of these complete CentC monomers

showed that the majority of the CentC monomers from the CC7TT, CC7HO, and CC0B clones could be distinguished by nucleotide number 42, 49, 64, 65, 77, 79, 83, 95, 96, 99, 126, 127, 129, 137, 141, and 150 (Supplementary Figure 2). These results suggest a greater level of homogeneity within a localized B-chromosome region than between different regions.

(Accession numbers of the CL-CentC clones: CC7TT-1, HN174141; CC7TT-2, HN174142; CC7HO-1, HN174143; CC7HO-2, HN174144; CC7HO-3, HN174145; CC7HO-4, HN174146; CC7HO-5, N174147; CC0B-1, HN174148; CC0B-2, HN174149; and CC0B-3, HN174150)

The evolutionary relationships of CentC on B- and A-chromosomes

To investigate the evolutionary relationships between CentC copies that are located in the CENB, BDHs, and CENAs, 30 complete CentC monomers from CC7TT, CC7HO, and CC0B clones (Fig. 4, red boxes), in addition to six CentC monomers from the pB51-15 (Cheng and Lin 2004) and five CentC monomers from the centromeric BAC clone ZM16H10 (Nagaki et al. 2003), were used to construct a phylogenetic tree using the neighbor-joining method (Fig. 5). These CentC monomers were sorted into the following three distinct clades: the first clade contained 11 CentC monomers (seven from CC7TT-1 and four from CC7TT-2), the second clade had 13 CentC monomers (four from CC7HO-1, two from CC7HO-2, one from CC7HO-5, and six from pB51-15), and the third clade consisted of 17 CentC monomers (five from CC0B-1, four from CC0B-2, three from CC0B-3, and five from ZM16H10). Each of the three clades could be assigned to a specific region, as follows: the first clade to the CENB, the second clade to BDHs, and the third clade to CENAs (Fig. 5). According to the phylogenetic tree, CentC monomers from BDHs were more closely related to those from CENAs in comparison to those from the CENB. Furthermore, CentC monomers from the CENB showed greater sequence heterogeneity than those from CENAs and BDHs.

Discussion

Using a primer pair that is specific to the sequences of the CL-repeat and CentC, 13 CL-CentC variant products were identified and mapped to the CENB, BDHs, and CENAs in tertiary trisomic and hypoploid plants that contained one of five B-10L translocations, respectively. Subsequent cloning and sequence analysis of complete CentC monomers, which were embedded in these products, indicated that a local homogenization process occurred in the CentC sequences of the B-chromosome. Phylogenetic analyses of 41 CentC monomers from the CENB, BDHs, and CENAs revealed that CentC repeats from CENAs were more closely related to those that were derived from BDHs than to those from the CENB. Furthermore, CentC monomers in the CENB showed more sequence heterogeneity in comparison to those in BDHs and CENAs.

Maize is an important model system for research concerning plant centromeres. Its CENAs contain CentC and a centromere-specific retrotransposon, CRM (Ananiev et al. 1998), both of which are located within the functional centromere domain that is marked by CENH3 (Zhong et al. 2002). To understand the large-scale DNA organization of CENAs, Jin et al. (2004) have used a DNA fiber-based FISH approach to analyze individual maize CENAs from oat (*Avena sativa*)-maize chromosome addition lines and have found that the cores of maize CENAs primarily contain CentC arrays that are intermingled with CRM clusters. Following the same approach, a similar CentC/CRM organization was observed in the CENB, wherein the functional domain consists of several CentC/CRM regions that are embedded within multimegabase arrays of ZmBs; however, only a small number of the ZmBs associate with CENH3 (Jin et al. 2005). Meanwhile, Theuri et al. (2005) have extracted 23 retrotransposons from BAC clones that are specific to the CENB and CENAs. A comparison of these retrotransposons indicates that 11 retroelements are common to the CENB and CENAs, five are specific to CENAs, and seven are unique to the CENB. These results reveal that CENAs and the CENB are similar in organization but differ in composition; however, the sequence divergence between the two centromeres is still poorly understood.

The local homogenization of centromere satellite repeats has been well demonstrated in several plant species. Lee et al. (2006) have analyzed CentO monomers from the centromeres of rice chromosomes 1 and 8 via dot plot and phylogenetic analyses, and they have found that CentO monomers from the same centromere are more similar to one another than to those from different centromeres. Similarly, Hall et al. (2005) examined centromere satellite monomers from the BAC clones of *Arabidopsis arenosa*, *Capsella rubella*, and *Olimarabidopsis pumila* and observed that satellite monomers from each BAC are formed a distinct clade, which indicates the occurrence of local homogenization. Similarly, our observations

that the CentC monomers from CENAs and the CENB fall into different clades and exhibit a specific number of nucleotides (Fig. 5 and Supplementary Figure 2) support the model of local CentC homogenization in the centromeres of maize B- and A-chromosomes. Furthermore, due to the homogeneity of CentC monomers within BDHs, the homogenization mechanism seems not only to affect centromere satellite repeats within the functional centromeres but also to influence CentC repeats on the long arm of maize B-chromosome.

The evolution of centromere satellite repeats may be constrained by centromere function. Interactions with centromere-binding proteins could be expected to provide a selective pressure that restricts changes in centromere satellite repeats (Malik and Henikoff 2002). In maize B-chromosome, both the CENB and BDHs contain centromere-specific CentC, whereas only the CENB exhibits interactions with CENH3 (Lamb et al. 2005). This observation suggests that the evolution of CENB CentC may be under greater selective pressure than the CentC of BDHs; hence, the level of CentC homogenization in the CENB would be expected to be higher than that in BDHs. Notably, our result conflicts with this expectation. The divergence of CentC monomers within the CENB is higher than that observed within BDHs (Fig. 5). In general, neither all CentC sequences of CENAs are associated with CENH3 (Jin et al. 2004), nor are all of those of the CENB (Jin et al. 2005). Therefore, it is possible that the CentC repeats of the CENB that were isolated in this study are not located in the CENH3-associated domain of the CENB. There is also a potential for sampling bias that might skew the results with only a few CentC monomers from each CentC-containing region. However, considering the unusual accumulation mechanism of B-chromosome, including nondisjunction at the second pollen mitosis (Longley 1927; Roman 1947) and preferential fertilization (Roman 1948; Carlson 1969), an alternative possibility is that the evolution of CentC repeats in the B-chromosome might be caused by a mechanism that is different from that responsible for the evolution of CentC repeats in A-chromosomes.

Although substantial sequences are available from the maize B-chromosome (see "Introduction" for details), its origin and evolution are still unclear. The observation of CENA-specific CentC on the CENB and BDHs supports the idea of an intraspecific origin of the B-chromosome from a dicentric chromosome (Lamb et al. 2005), from which one centromere was inactivated by a series of deletions, insertions, mutations, amplifications, and rearrangements to generate the current BDHs during the formation of the B-chromosome (Cheng 2010). This idea leads to the question of whether the two centromeres of the dicentric chromosome were duplicated from a single centromere or derived from two different centromeres. In the former case, CentC repeats in the CENB and BDHs on the current B-chromosome should be similar if there was no selective pressure from centromere-binding proteins. On the contrary, if the centromere of the B-chromosome arose from two different centromeres, the divergence of CentC repeats of the CENB and BDHs should be observed. Thus, the

clustering of CentC monomers from the CENB and BDHs into two distinct clades (Fig. 5) indicates that the centromeres of the hypothetical dicentric chromosome would have been derived from different chromosomes.

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Table 1 The distribution of CL-CentC variant products by five B-10L translocations

Variant	Size (kb)	2B	0B	TB-10L										Position [a]
				18TT	18HO	7TT	7HO	38TT	38HO	26TT	26HO	20TT	20HO	
a	3.8	-	+	-	+	+	-	+	-	-	+	-	+	CENAs
b	2.8	-	+	-	+	+	-	+	-	-	+	-	+	CENAs
c	1.3	+	-	+	-	-	+	-	+	+	-	+	-	DH2
d	1.2	+	-	+	-	+	-	+	-	+	-	+	-	CK
e	1.1	+	-	+	-	-	+	+	+	+	-	+	-	DH1, 2
f	0.85	+	-	+	-	+	-	+	-	+	-	+	-	CK
g	0.78	+	-	+	-	-	+	-	+	+	-	+	-	DH2,
h	0.72	+	-	+	-	-	+	-	+	+	-	+	-	DH2
i	0.62	+	-	+	-	-	+	-	+	+	-	+	-	DH2
j	0.52	+	-	+	-	-	+	+	+	-	+	-	-	DH1, 3
k	0.48	+	-	+	-	-	+	-	+	+	-	+	-	DH2
l	0.3	+	-	+	-	-	+	-	+	+	+	+	-	DH2, 3
m	0.15	+	-	+	-	-	+	-	+	+	-	+	-	DH2

2B L289+2B, *0B* L289+0B, *TT* tertiary trisome, *HO* hypoploid, *CENAs* A centromeres, *CK* centromeric knob, *DH* distal heterochromatic region, + presence of CL-CentC variant, - absence of CL-CentC variant

[a] The position where the variant is located from analyses of tertiary trisomes and hypoploids of the five B-10L translocations

Figure legends

Fig. 1

Break positions of five B-10L translocations. The maize B-chromosome in pachytene stage consists of the short arm (*BS)*, centromeric knob (*CK*), proximal euchromatic region (*PE*), distal heterochromatic regions (*DH1–DH4*), and distal euchromatic tip (*DE*). *Arrows* indicate breakpoints of the five B-10L translocations on the B-chromosome

Fig. 2

FISH analysis of CL-CentC PCR products. The CL-CentC PCR products from L289+2B (*green*) and the *CENB*- specific repeat, B1.1a (*red*) were used as FISH probes to hybridize the mitotic chromosomes (**a**) and the pachytene B-chromosome (**b–c**) of L289+2B. The inverted DAPI image of the pachytene B-chromosome is provided in **e**, and the short arm (*BS*), centromeric knob (*CK*), proximal euchromatic region (*PE*), distal heterochromatic regions (*DH1–DH4*), and distal euchromatic tip (*DE)* are indicated. *Bars* represent 10 μm

Fig. 3

Isolation and mapping of the CL-CentC variant products by five B-10L translocations. The CL-CentC products were amplified from the genomic DNA of L289+2B (*2B*), L289+0B (*0B*), translocation heterozygous (*H*), tertiary trisomic (*TT*), and hypoploid (*HO*) plants of the five B-10L translocations (TB-10L18, TB-10L7, TB-10L38, TB-10L26, and TB-10L20). -, no template DNA, *a–m* CL-CentC variants. The molecular weights are marked on *left*

Fig. 4

Schematic sequence organization of CL-CentC clones. The locations of identified elements are indicated with colored boxes. *Black boxes* ZmBs, *gray boxes* the CL-repeat, *green boxes* partial CentC monomer, *red boxes* complete CentC monomer, and *white box* unidentified element. The binding sites of primer CL-1 and BCentC-1 are indicated by *black* and *red arrows*, respectively

Fig. 5

Phylogenetic analysis of CentC monomers from the CENB, BDHs, and CENAs. The neighbor-joining method (ClustalW) was used to construct the phylogenetic tree of 41 CentC monomers extracted from CC7TT, CC7HO, and CC0B clones as well as the pB51-15 (Cheng and Lin 2004) and the centromeric BAC clone ZM16H10 (Nagaki et al. 2003). *Bar length* represents estimated substitutions per site

Supplementary Figure 1

Diagram of the pB51-15 sequences showing the PCR primers landing sites. The CL-repeat primer (*CL-1*) and CentC primer (*BCentC-1*) were designed from sequences of the B-clone (pB51-15, AY426743; Cheng and Lin 2004). *Gray box* CL-repeat monomer, *red box* CentC monomer, *arrow* primers binding site

Supplementary Figure 2

Sequence logo analysis of CentC monomers. Logos display the consensus sequences of complete CentC monomers from CC7TT, CC7HO, and CC0B clones (*upper panel*), and the selected nucleotides significantly different among CentC sequences of the three origins (*lower panel*)

Figure 1

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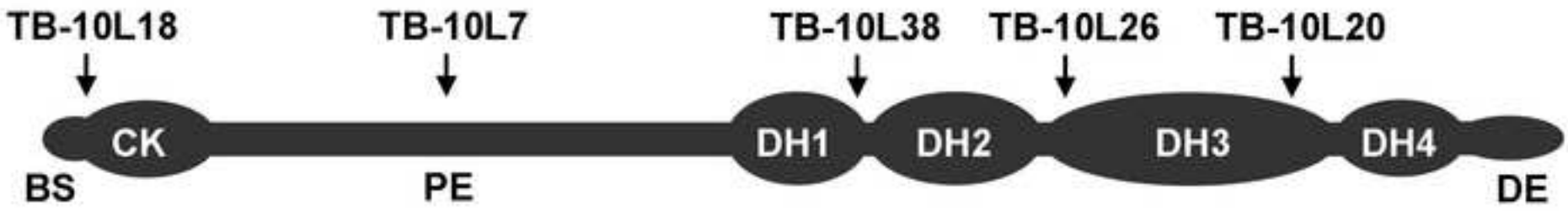

Figure 2

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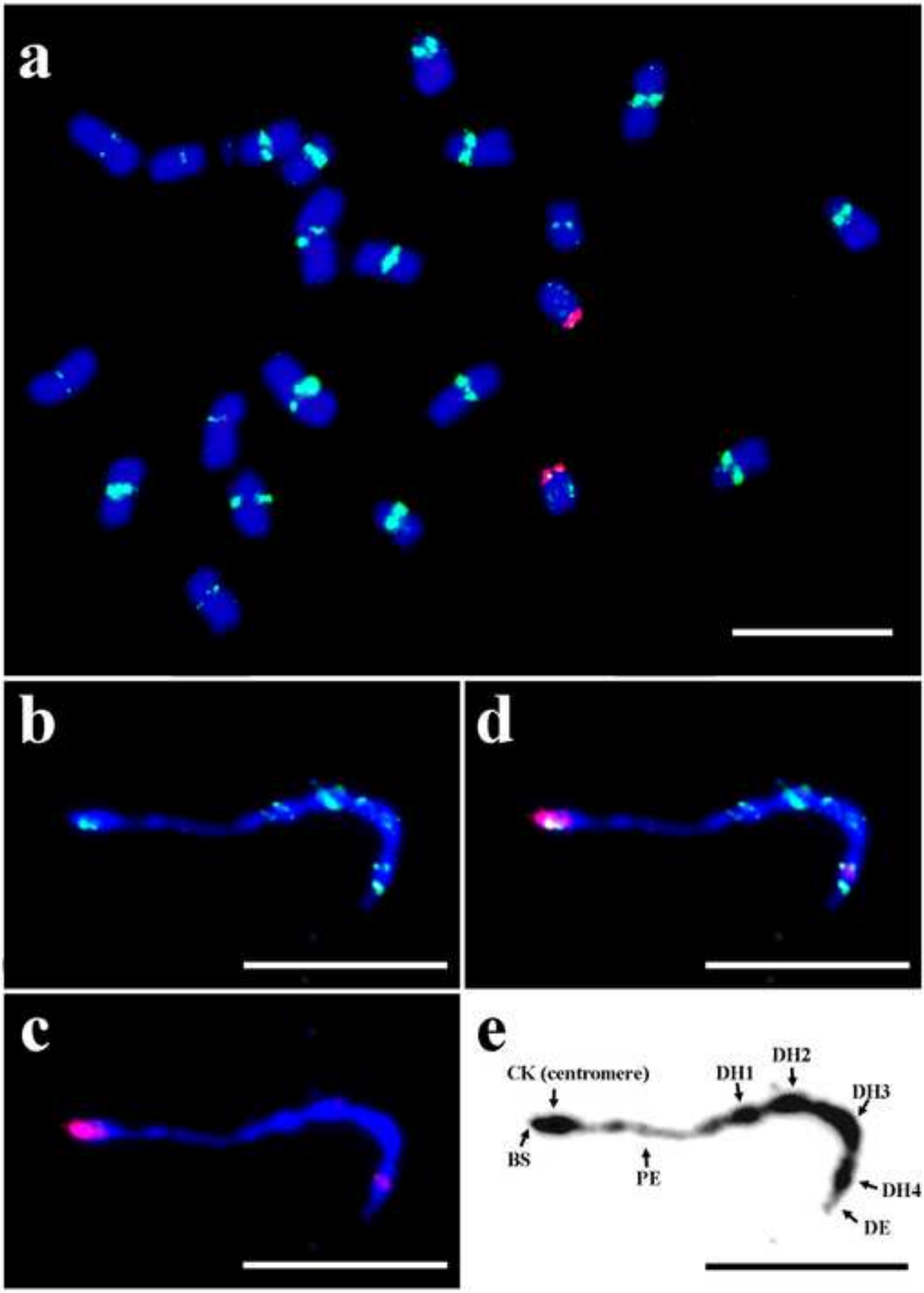

Figure 3

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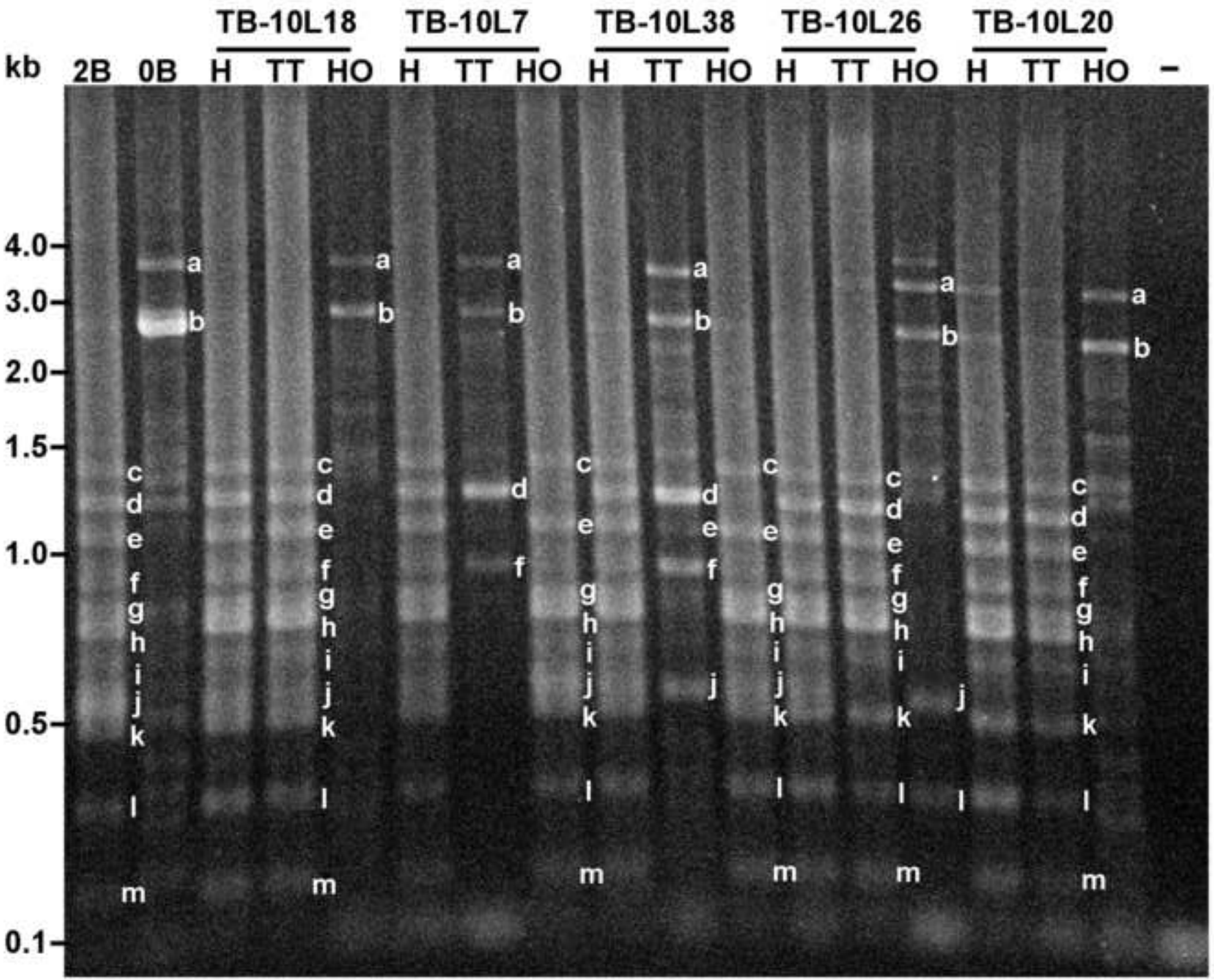

Figure 4

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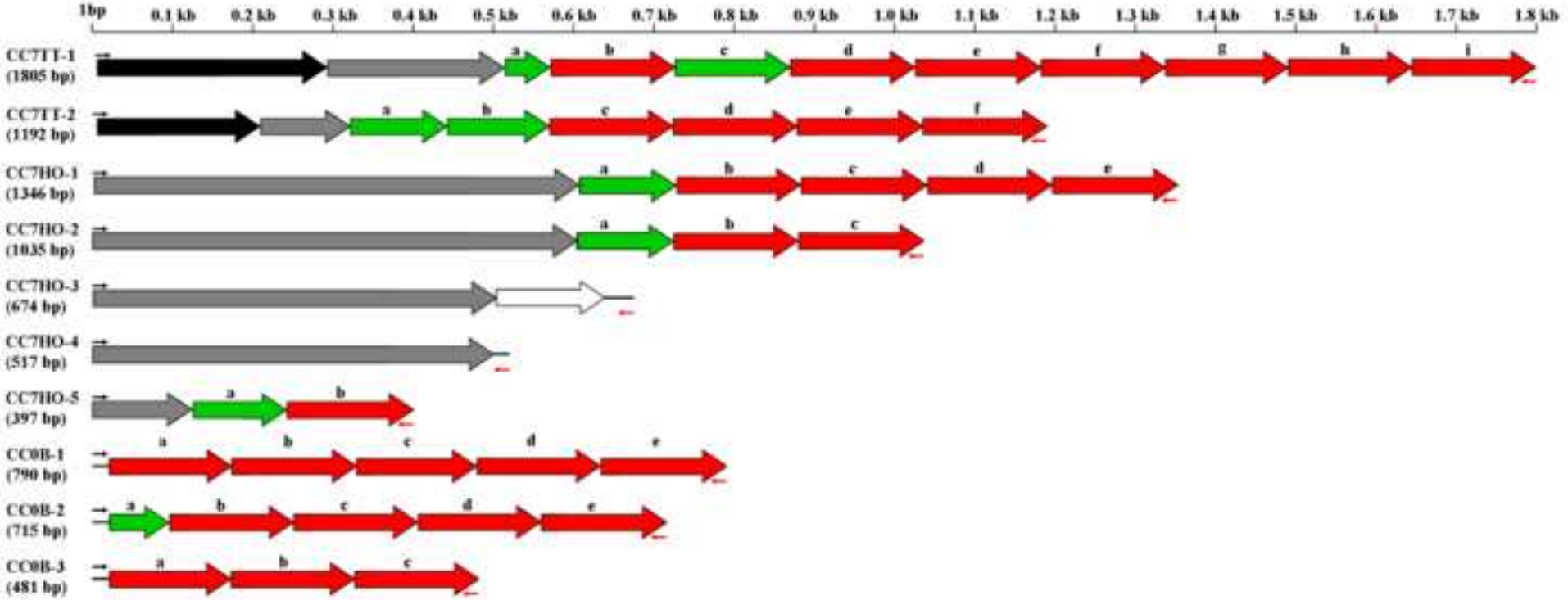

Figure 5

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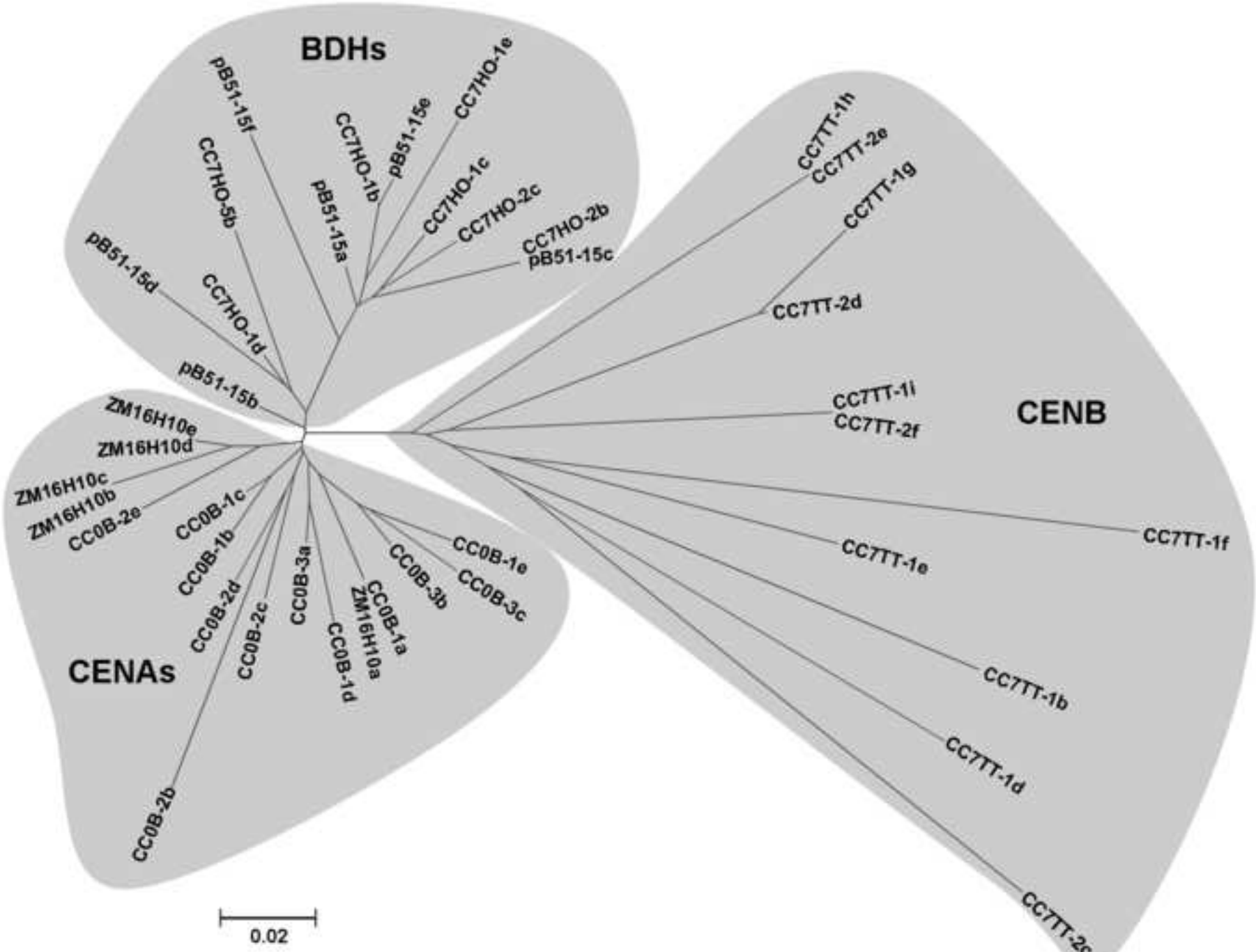

Supplementary Figure 1

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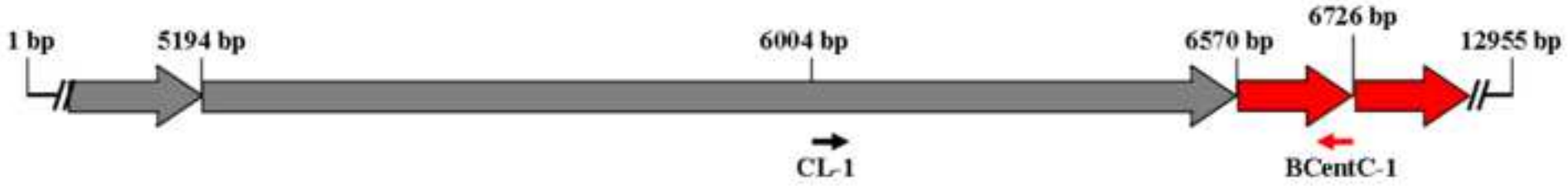

Supplementary Figure 2

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