EFFECTS OF HETEROCHROMATIC KNOBS IN MAIZE SISTER INBRED LINES

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ABSTRACT

Maize heterochromatic knobs are chromosomal structures composed of highly repetitive DNA repeats. They are variable among races. A well-known effect of knobs is meiotic drive, causing their preferential segregation to egg cells, that occurs when knobs are converted into neocentromeres in the presence of the abnormal chromosome 10. It was demonstrated that distinct kinesin motors drive two types of maize neocentromeres composed of the K180 and TR1 repeats. We selected tropical sister inbred lines, homozygous for different knob compositions, to investigate knob effects. A group of these lines showed embryogenic response in tissue culture. Low frequency of mitotic instability was observed in the cultures and breaks occurred in chromosome possessing large knobs. Chromosome breakage was followed by breakage-fusion-bridge (BFB) cycles giving origin to aberrant chromosomes that suffered addition of telomeres. Fertile plants regenerated from short-term cultures had normal chromosomes. Correlations between genome size, knob content and flowering time has been reported. These relationships were not found in the inbred lines differing in the presence of four knobs and early flowering time was detected in lines and hybrids possesing the knob K9S.

Keywords: Maize, heterochromatic knobs, meiotic drive, callus cultures, BFB cycles, flowering time, genome size, unequal crossing-over.

RESUMO

Knobs heterocromáticos são estruturas presentes nos cromossomos do milho, compostas de sequências de DNA altamente repetitivo. Um efeito bem conhecido dos knobs é o direcionamento meiótico ("meiotic drive"), que causa sua segregação preferencial para a oosfera, e que ocorre quando os knobs são transformados em neocentrômeros na presença do cromossomo 10 anormal. Foi demonstrado que diferentes cinesinas atuam como motores que direcionam dois tipos de neocentrômeros, constituídos das sequências repetitivas K180 e TR1. Nós selecionamos linhagens tropicais irmãs, homozigóticas para diferentes constituições de knobs para investigar efeitos dos knobs. Um grupo dessas linhagens apresentou resposta altamente embriogênica em cultura de tecido. Foi observada baixa frequência de instabilidade mitótica nas culturas, bem como quebra em cromossomos possuidores de knobs de tamanho grande. Após a ocorrência de quebras cromossômicas, ciclos de quebra-fusão-ponte (BFB em inglês) ocorreram, dando origem a cromossomos aberrantes, aos quais novos telômeros eram adicionados. Plantas férteis, regeneradas a partir de culturas de curta duração, possuíam cromossomos normais. Tem sido relatada na literatura, correlações entre tamanho do genoma, conteúdo de knobs e tempo de florescimento. Estas relações não foram detectadas nas linhagens que diferiam na presença de quatro knobs, porém foi constatado florescimento mais precoce em linhagens e híbridos portadores do knob K9S.

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HETEROCHROMATIC KNOBS

Heterochromatic knobs are chromosomal structures localized in subterminal or terminal regions of maize chromosomes. They were firstly detected and mapped in the pachytene stage of meiosis in pollen mother cells (LONGLEY, 1938; RHOADES, 1950; McCLINTOCK et al., 1981). The first detection of knobs in mitotic metaphase was realized by the C-banding method (AGUIAR-PERECIN, 1985; AGUIAR-PERECIN & VOSA, 1985; WARD, 1980;). Knobs are variable among races according to their geographical localization and can be homozygous or heterozygous (McCLINTOCK et al. 1981). They are primarily composed of highly repeated DNA sequences (satellite DNAs) with 180-bp or 350-bp TR-1 element, or a mixture of both (PEACOCK et al., 1981; ANANIEV et al., 1998). Satellite arrays often contain complex internal structures called higher-order repeats (HORs), which may have functional significance. Recently, it was demonstrated that large knobs contain megabase-scale similarity blocks with repeated HOR patterns. These repeat units likely promote unequal crossing over, enabling rapid knob expansion, and may harbor motifs recognized by trans-acting factors involved in meiotic drive (PIRI et al., 2026).

GENETIC EFFECTS OF KNOBS

The knowledge on the genetic effects of knobs has been a challenge for geneticists. It is well known that there are cases in which they are preferentially transmitted to the egg cell. This meiotic drive happens in the presence of an uncommon form of chromosome 10, the abnormal chromosome 10 (Ab 10) In its presence, the knobs of other chromosomes are converted into neocentromeres, that drive the knobbed chromatid to the viable megaspore during the female meiosis (RHOADES & DEMPSEY, 1966; DAWE & HIATT, 2004). It was demonstrated that distinct kinesin motors drive two types of maize neocentromeres composed of the repeats K180 or TR1: the **Kindr** motor for knob180 repeats (DAWE et al., 2018) and the **Trkin** motor which powers minor TR-1 repeat neocentromeres (SWENTOWSKY et al., 2020). Both kinesins are encoded by genes localized on the abnormal chromosome 10.

Other characteristic of knobs is that they are located in gene-dense areas and suppress local recombination, when they are in heterozygous condition (GHAFFARI et al., 2013).

Chromosome alterations in knobbed chromosomes were detected in plants regenerated from tissue culture, as commented bellow.

DEVELOPMENT OF INBRED LINES HOMOZYGOUS FOR VARIABLE KNOB CONTENTS

We developed sister inbred lines to investigate knob effects. To select lines homozygous for different knob contents, C-banded mitotic metaphases from S_3 to S_9 progenies were analyzed. These progenies were derived from one plant of a S_2 progeny derived from the tropical variety Jac Duro. Four families of lines were selected and designed JD 1-3, JD 2-1, JD 4-1 and JD 4-4 (see FLUMINHAN & AGUIAR-PERECIN, 1998; CARVALHO et al., 2022).). All the lines were obtained in the experimental field of the Department of Genetics, ESALQ, USP, Piracicaba, SP, Brazil. During the analysis of the progenies with different levels of selfing, until S_9, plants heterozygous for some knobs, were detected, except for lines of the family JD 2-1, homozygous since S_3. The lines belonging to the four families were homozygous for the knobs visualized in the pachytene phase of meiosis: K6L2, K6L3, K7L, K8L1 and K8L2 and variable for K3L, K5L, K7S and K9S. According to the literature, K refers to knob, number to

chromosome, S to short arm and L to long arm of the chromosome (McCLINTOCK et al., 1981).

Lines homozygous for variable knob contents were obtained and were useful for some investigations. Their karyotypes were characterized using fluorescent *in situ* hybridization (FISH) with probes of repetitive sequences of DNA to identify unequivocally the somatic chromosomes (MONDIN et al., 2014). The probes used were the 180-bp knob repeat, centromeric satellite (CentC), centromeric satellite 4 (Cent4), specific for chromosome 4, subtelomeric clone 4-12-1, characteristic of some chromosomes, 5S ribosomal DNA (5S rDNA), located on chromosome 2, and nucleolus organizing region (NOR) DNA specific for chromosome 6. Previously, the somatic karyotypes of temperate commonly used inbred lines were characterized with the mapping of nine probes for repetitive DNA sequences (Kato et al., 2004).

Embriogenic response and mitotic instability in callus cultures derived from maize inbred lines differing in heterochromatic knob content

Twenty-two lines belonging to the four JD families were analyzed to investigate their embryogenic response, mitotic instability and chromosome stability in callus cultures. Only lines of the family JD 1-3 developed highly embryogenic calli type II (FLUMINHAN & AGUIAR-PERECIN, 1998). Mitotic abnormalities were investigated in conventional stained and C-banding preparations of callus cells. Anaphase bridges resulting from delayed chromatid separation at knob levels, typical bridges and fragments were observed. Delayed chromatids were shown to be held together at heterochromatic knob sites, while typical bridges would be formed by dicentric chromatids arising from breakage-fusion-bridge (BFB) cycles initiated by chromosome arms broken during the primary event (FLUMINHAN et al., 1996). The frequency of mitotic abnormalities varied from 2.01 to 12.50% in lines of the four families (FLUMINHAN & AGUIAR-PERECIN, 1998). Previous meiotic studies of maize plants regenerated from tissue culture showed chromosome aberrations and that most breakpoints were located between the centromere and the heterochromatic knobs or the nucleolus organizer (BENZION & PHILLIPS, 1988; LEE & PHILLIPS, 1987). A hypothesis proposed to explain the role of knobs in inducing chromosome breakage is that normally late-replicating heterochromatic regions may replicate even later in the culture environment, leading to the formation of chromosome bridges, due to delayed separation of siter chromatids at heterochromatic regions (LEE & PHILLIPS, 1987, 1988). Our results supported this hypothesis (FLUMINHAN et al., 1996)

Three lines and respective hybrids belonging to the JD 1-3 family were investigated for their frequency of embryogenic calli, that was high (83-99%), on 45 days after the beginning of the cultures. Fertile plants with chromosomal stability were derived from these cultures. The results showed that the genotypes analyzed would be useful in programs of Biotechnology involving genetic transformation (SANTOS-SEREJO & AGUIAR-PERECIN, 2000). Furthermore, the results showed that short-term cultures should be used to regenerate plants without chromosome aberrations. Long-term cultures may accumulate chromosome aberrations, for alterations in chromosomes 7 and 9 were accumulated by the occurrence of BFB cycles and de novo telomere formation in 18 to 46-month-old samples of tissue cultures (SANTOS-SEREJO & AGUIAR-PERECIN, 2016). Also, amplification of the knob on the long arm of chromosome 7 was observed to occur by unequal crossing-over events in tissue cultures (SANTO-SEREJO et al., 2018).

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The relationship between maize heterochromatic knobs, genome size (GS) and flowering time (FT) is a long debate

Quantification of DNA content through flow cytometry has documented significant variability in the maize genome size between landraces and inbred lines (LAURIE & BENNETT, 1985; REALINI et al., 2015). The influence of this variation has been demonstrated in some studies involving correlations between genome size, knob content and flowering time in maize (GONZÁLEZ et al., 2026; JIAN et al., 2017; RAYBURN et al., 1985; 1994). Recently, González et al (2026) considered that variation in the abundance of transposable elements is thought to play a major role in DNA content variation, since long terminal repeats (LTR) retrotransposons, which represent more than 70% of the nuclear genome, differ in both copy number and chromosomal distribution in maize (SAN MIGUEL & BENNETZEN 1998; (SCHNABLE et al., 2009). It is important to emphasize that the genetic architecture of flowering time in maize has been widely studied, given that this trait reflects the plant adaptation to the environment (see review in CARVALHO et al., 2022). The surveys on the relationship between knob content and flowering time have considered just the knob numbers or estimated the knob abundances by low coverage sequencing (BILINSKI et al., 2018; G0NZÁLEZ et al, 2026; JIAN et al., 2017; REALINI et al., 2015). However few studies have evaluated the knob effect depending on its homozygous or heterozygous condition (CHUGHTAI & STEFFENSEN, 1987). In a studied carried out by Carvalho et al. (2022), an analysis of genome size, knob composition and flowering time in lines of the families JD 1-3, JD 2-1 and JD 4-4 (reported in the present review) and hybrids homozygous or heterozygous for knobs did not detect correlations between knobs, genome size and flowering time. However, an association between the large knob on chromosome 9 (K9S) and earliest flowering time was found. As knob inhibits crossing-over in their proximity (GHAFFARI et al., 2013), the interpretation was that a gene affecting plant development was linked to K9S. Also, as the variability in the number and size of the knobs were small between the inbred lines studied, differences in influence on the flowering time would not occur.

CONCLUDING REMARKS

The C-banding method was used in studies aiming the selection of lines with different knob contents and chromosomal abnormalities in callus cultures, because the knob composition of the population examined was well known and previously confirmed by the analysis of pachytene chromosomes. Now, the unequivocal identification of mitotic chromosomes is performed using the mapping of repetitive DNA sequences by FISH. As for the selection of lines showing embryogenic response in tissue cultures, the results indicate that lines with low content of knobs are recommended, to avoid chromosome breakage in callus cultures. As for the correlation between knobs and flowering time, future studies should involve the analysis of the knob content in a significant number of lines with great differences in their flowering time. Also, the variability of the genome size must be influenced by the amount of retrotransposons, that are abundant in maize genome.

Author contribution:

MLRAP wrote this review and coordinated research on the mapping of knobs on maize somatic cells, selection of inbred lines homozygous for different knob compositions and selection of lines with embryogenic response in tissue culture and analysis of their chromosomal stability, and co-supervised studies on genome size and flowering time of

inbred lines and respective hybrids.

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