

Centromere Inactivity Facilitates Haploidy in Crop Breeding

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Abstract: *The current article seeks to buttress the vast growing importance of double haploidy in crop breeding. Double haploidy in a large number of plant species occurs as a result of chromosome elimination in the associated parent crops. In the paper reviewed, the authors investigated the specific processes involved in selective elimination of parental chromosomes during early embryonic development of two barley species; *Hordeum vulgare* and *Hordeum bulbosum*. The method used by the authors involved making unstable crosses with gametes of *Hordeum vulgare* and *Hordeum bulbosum* above 18°C and stable crosses of the same plants below 18°C. The results revealed that crosses above 18°C led to complete elimination of the genome from *Hordeum bulbosum* and formation of haploid *Hordeum vulgare* embryos. The authors made the following conclusions. First, chromosome elimination was triggered by the inactivity of a centromere-specific histone H3 variant (CENH3) in *Hordeum bulbosum*. Secondly, centromere inactivity is caused by centromeric loss of CENH3 protein, as opposed to silencing of CENH3 genes in *Hordeum bulbosum*. Thirdly, diploid barley species encode two types of CENH3 proteins which are sandwiched within in the centromeres during mitosis and meiosis. And lastly, when multiple types of CENH3 are present in fertilizing parental species, not all CENH3 proteins are incorporated in the centromeres. Arising from the authors's conclusions, it can be said that double haploidy could serve as a basis for many crop breeders to select beneficial traits for their crops such as drought tolerance, improved fruit flavour and pest resistance, among others.*

Keywords: *Centromere inactivity, Double haploidy, CENH3, Barley, Mitosis, Meiosis*

1. INTRODUCTION

Haploid plants are commonly produced by rare interspecific crosses which result in complete chromosome elimination of one parent after fertilization. These hybrids are currently gaining advantages in crop breeding because doubled haploid homozygous cell lines can easily be generated from them, thus simplifying plant breeding by skipping several generations of inbreeding. In the past decade, a good number of hypotheses were put forward to explain the mechanisms underlying uniparental chromosome elimination; however the actual process still remained vague. The authors of the reviewed article recently reported that centromere inactivity arising from loss of a centromere specific histone (CENH3) plays a major role in uniparental chromosome elimination in hybrid barley embryos [1]. This approach provides clearer insights into the mechanism involved in generating haploid progenies in plants, thus improving crop breeding.

Upon fertilization of an egg with the sperm of a different species, a fairly common phenomenon that occurs is the elimination of chromosomes from one parental genome [1], which produces haploid cell lines. During mitotic cell division, centromeres serve as anchor points for spindle microtubules and ensure fidelity in the distribution of chromosomes to the daughter cell. Furthermore, in this process, CENH3 is also closely linked with the centromere and the newly formed daughter progeny. It has been demonstrated in mammals [2] and fruit-flies [3] that loss of CENH3 protein from the centromeres causes failure in chromosome segregation and centromere formation during cell division. The authors analyse the mechanism of centromere inactivity involved in selective elimination of the parental chromosomes during early development of barley hybrids; *Hordeum vulgare* × *Hordeum bulbosum* [1]. Specifically the authors demonstrate that centromeric loss of CENH3 protein leads to centromere inactivity, which precedes uniparental chromosome elimination in the generation of haploid progenies.

2. METHODS

The authors made unstable crosses (test experiment) with gametes of *Hordeum vulgare* (*Hv*) × *Hordeum bulbosum* (*Hb*) above 18°C and stable crosses (control experiment) below 18°C [1]. Crosses above 18°C led to complete elimination of the genome from *Hb* and formation of haploid *Hv* embryos (figure 1). To determine centromeric and transcriptional activity of micronucleated *Hb* chromatin during interphase, the presence of CENH3 and RNA Polymerase II were assayed by Fluorescence in situ hybridization (FISH).

3. RESULTS AND DISCUSSION

As expected from the unstable crosses, the *Hb* genome was not eliminated in the control experiment. The selective parental chromosome elimination was shown by the lagging or abnormal segregation of the *Hb* chromosome during mitotic anaphase. At this stage, the centromere activity was also examined by immunostaining dividing cells with anti-grass CENH3. The results showed that active centromeres (CENH3 positive) were found in segregating chromatids, but lagging chromosomes of *Hb* were devoid of functional centromeres (CENH3 negative).

At the end of mitosis, segregated chromosomes normally form nuclear membranes, however lagging chromosomes create micronuclei which finally degrade to form a haploid *Hv* embryo. From the determination of centromeric and transcriptional activity of micronucleated *Hb* chromatin during interphase, the authors discovered that RNA polymerase II was scanty within the micronuclei. This observation is consistent with the fact that further segregation of the *Hb* chromosomes is impossible after micronuclei formation and the transcriptional activity of the micronuclei is drastically reduced. Furthermore, FISH technique demonstrated that at interphase, the *Hb* centromeres also carry less CENH3 proteins. This buttresses the observation that centromere inactivity in *Hb* due to loss of CENH3 causes mitosis-dependent uniparental chromosome elimination in unstable crosses between *Hv* and *Hb*. Other roles of CENH3 in the mechanism of uniparental chromosome elimination were summarised by the authors as follows:

- Two CENH3 variants (α CENH3 and β CENH3) are encoded in diploid barley species. However not all variants get incorporated into centromeres if multiple CENH3 are present in interspecies combination.
- Uniparental chromosome elimination is dependent on environmental conditions (for example, temperature above 18°C promotes chromosome elimination) and genotype.
- Centromere inactivity results from centromeric loss of CENH3 protein, rather than gene silencing.

4. CONCLUSION

The aim of virtually all plant breeders is to produce crops that are homozygous. Breeding of these crops enables beneficial traits (e.g. pest resistance, drought tolerance and improved fruit flavour) to be passed on to their offsprings. The authors' of the reviewed paper have shed more light on the mechanism of selective chromosome elimination and crucial roles of CENH3 in the production of haploid plants. This also provides an excellent basis for gene mapping which can be used for mutation breeding in plants. In this regard, extensive mutant libraries of crop genes carrying the desired CENH3 mutation necessary for loss of CENH3 and centromere inactivity can be created in order to facilitate the generation of homozygous haploids and largely improve crop breeding. The authors' research also uses CENH3 variants to cast light on the evolution of plant species including barley, rice, maize and *Arabidopsis*. Invariably, this again proves that CENH3 is the fastest evolving sequence in the genome of several plant species.

(A). After fertilization between the *Hordeum vulgare* (*Hv*) and *Hordeum bulbosum* (*Hb*) gametes, a diploid zygote containing chromosomes from both parents, is formed. (B). During mitotic division, *Hv* centromeres become inactive and lead to loss of *Hb* CENH3. *Hv* centromere and *Hv* CENH3 remain intact and active. (C). Loss of *Hb* CENH3 consequently leads to complete elimination of only *Hb* chromosome (which appear as disintegrated micronucleated membranes) and formation of a haploid daughter embryo. (D). The haploid embryo can be selfed to form a doubled haploid homozygous embryos containing chromosomes from only *Hv* species.

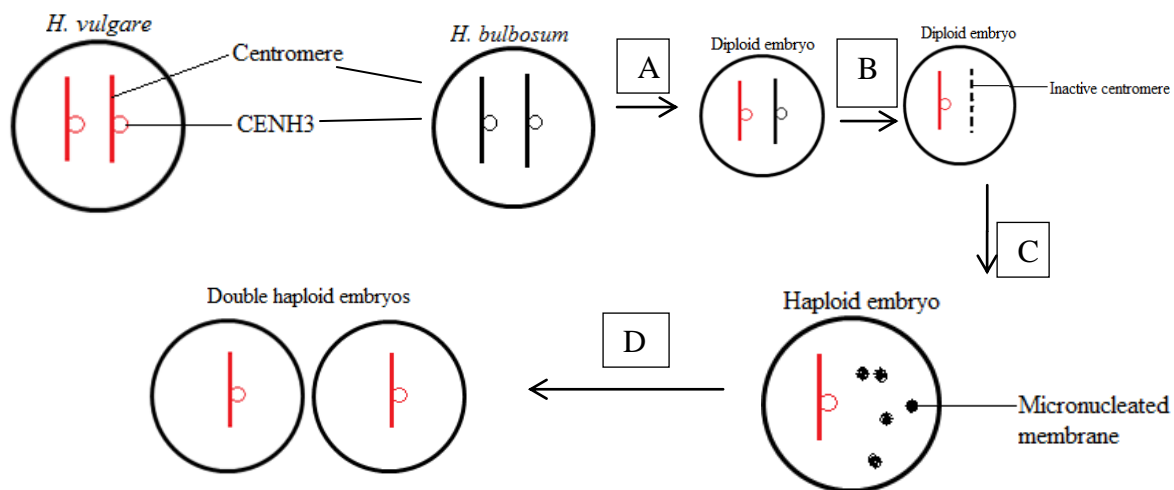


Figure1. Mechanism involving centromere inactivity and loss of CENH3-dependent uniparental chromosome elimination in plants.

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