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Haploid embryos: Being like Mommy or like Daddy?

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Spotlight

Haploid Embryos: Being Like Mommy or Like Daddy?

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ABSTRACT:

In planta haploid embryo induction is a powerful plant breeding tool, but is limited to few crops. Two studies reported seed-based haploid systems producing paternal haploid embryos by engineering CENTROMERIC-HISTONE3. Along with recent translation of maize maternal haploid induction ability in wheat and rice, it extends haploid inducer lines collection.

Background

Haploid embryos contain only one set of parental chromosomes (n), instead of the classical situation with two sets of chromosomes ($2n$), one from the mother and one from the father. Nevertheless, haploid embryos and their subsequent haploid plantlets represent the basis of the Doubled Haploid (DH) technology which is a significant plant breeding tool [1,2]. DH technology could be simply summarized by: (i) the production of haploid embryos, and (ii) duplication (copy-paste) of the haploid genome to recover normal ploidy state. DH technology allows efficient plant breeding cycles, mainly by shortening the time for creating fixed genetic material (inbred line), since only two generations are needed to obtain homozygous plants, instead of six or more generations when using conventional crosses [1,2]. Thus, DH pipelines can offer rapid evaluation of phenotypic traits in

30 homogenous progeny, and the production of haploid embryos is central to this process. Although *in*
31 *vitro* methods are most widely used to produce haploid embryos starting from the maternal or paternal
32 germline (gametes or their precursors), they are costly and genotype-dependent [1]. The use of haploid
33 inducer lines represents an attractive way to induce haploid embryos entirely *in planta*. These *in planta*
34 methods produce haploid embryos within seeds, circumventing a major bottleneck of both *in vitro* and
35 wide-hybridization methods, namely embryo rescue. A simple intraspecific cross between a plant of
36 interest and a haploid inducer line allows the production of viable seeds, among which a certain
37 proportion have haploid embryos (**Figure 1**). The uniparental haploid embryos lack the haploid inducer
38 genome, making either paternal- or maternal haploid (**Figure 1**). Two main types of *in planta* haploid
39 inducer lines were reported: (i) the CENH3-based induction system based on engineering of
40 Centromeric Histone 3 (CENH3) (**Figure 1A**), and (ii) the maternal maize haploid induction ability which
41 is based on mutation of the *MATRILINEAL (MATL) / NOT LIKE DAD (NLD) / PHOSPHOLIPASE-A (PLA)*
42 gene (**Figure 1B**) [1–5]. Few additional haploid inducer lines were reported in sorghum and potato for
43 example, but the molecular bases remain unknown [1].
44

45 **A long-awaited discovery: efficient CENH3-based haploid induction in crops**

46 The CENH3-based haploid induction system was born in 2010 thanks to a discovery using the
47 model plant *Arabidopsis thaliana* [6]. Although this pioneering work has held great potential for plant
48 breeders, efforts to translate this technology to crops have been hindered by the low haploid induction
49 rate [1]. However, by engineering CENH3 genes in both maize and wheat crops, two recent studies
50 reported paternal haploid induction with a rather high haploid induction rate (~5-8%) (**Figure 1A**) [7,8].
51 Wang *et al.* [7] initially failed to translate *Arabidopsis* CENH3-based haploid induction system to maize,
52 but then surprisingly identified a new and simple way to induce haploid embryos. Crossing a female
53 maize line heterozygous for the *cenh3* null mutation (*cenh3/WT*) with wild-type pollen led to ~5% of
54 seeds with haploid embryos [7]. In wheat the situation is more complex since there are two *CENH3*
55 genes (α and β), and because bread wheat is a hexaploid crop with three sub-genomes (named A, B
56 and D), making a total of 12 *CENH3* alleles. Thanks to huge efforts in wheat transformation (more than
57 1150 events screened!) and rigorous analyses of gene editing events, Lv *et al.* were able to isolate a
58 specific mutagenic scenario that led to haploid embryo induction [8]. They found that a specific
59 mutation type on *CENH3 α -A*, called restored frameshift (RFS), coupled with knockout alleles for both
60 *CENH3 α -B* and *CENH3 α -D* triggered haploid induction (the 6 *CENH3 β* alleles were left at wild-type
61 state). The RFS consists of a wild-type protein sequence with the replacement of few amino acids from
62 the N-terminal domain by unrelated ones [8]. They demonstrated the RFS on *CENH3 α -A* gene is critical
63 for haploid induction but, unexpectedly, the haploid inducer lines heterozygous for *CENH3 α -A* RFS
64 (RFS/WT) outperformed the homozygous ones (RFS/RFS) [8]. To sum-up, good paternal haploid
65 induction systems based on CENH3 engineering were obtained in both maize and wheat (**Figure 1A**).
66 Of note, maternal haploid embryos were reported at a low rate (0.5%) using the maize CENH3-based
67 induction system [7], although the maternal origin was not double-checked.
68

69 **What can we learn from maize and wheat CENH3-based haploid inducer lines?**

70 The novelty brought by these two studies is the importance of heterozygosity to induce
71 haploid embryos, indicating that one copy of the CENH3 wild-type allele is required to obtain the
72 highest haploid induction rates [7,8]. However, the wheat situation differs from what has been
73 reported in maize, since the *CENH3 α -A* allele in a “knock-out/wild-type” configuration does not induce
74 haploid embryos, but needs to be heterozygous in a “RFS/wild-type” state [8]. This contradictory
75 situation could lie in the fact that the “RFS/wild-type” wheat haploid inducer line still has three copies
76 of *CENH3 β* gene in a wild-type state, knowing that wheat has a complex centromere organization with
77 the two versions of CENH3 (α and β) localizing in complementary centromeric domains.

78 Interestingly, Wang *et al.* [8] took advantage of the relative large size of maize kernels to
79 genotype endosperm from seeds with haploid embryos. They were able to deduce that haploid

80 induction occurred when the embryo sacs (and thus egg cells) carried the *cenh3* null mutation [8]. The
81 proposed working model is that these *cenh3* null embryo sacs have a diluted quantity of the wild-type
82 CENH3 protein leading to defective centromeres. Indeed, just before meiosis, the diploid megaspore
83 mother cell is heterozygous in a *cenh3* null/WT state. After meiosis, half of the resulting megaspores
84 have the *cenh3* null allele in their haploid genome, but should have nevertheless inherited wild-type
85 CENH3 protein in their centrosomes. This initial CENH3 protein pool is then diluted by the three post-
86 meiotic cell divisions, creating egg cells with defective centromeres. Thus, when these “CENH3 diluted”
87 egg cells are fertilized by wild-type sperm cells, it could lead to postzygotic maternal genome
88 incompatibility that generates paternal haploid embryos [7]. A similar scenario could be envisioned in
89 the wheat CENH3 engineered system, but with RFS allele instead of *cenh3* null allele. Nevertheless, the
90 mode of action of wheat RFS alleles remains to be investigated. Since the RFS alters putative histone
91 post-translational modification sites, it can be speculated that these CENH3 modifications have an
92 important role in centromere stability. Experimental testing of RFS mutation on diploid plants with a
93 single-copy *CENH3* gene should also help to clarify the situation.

94

95 **Maize and wheat now have many ways to induce haploid embryos and multiple breeding** 96 **applications**

97 The discovery of efficient CENH3-based paternal haploid inducer lines places maize and wheat
98 in a unique situation because different methods are now available to induce both maternal and
99 paternal haploid embryos. The two historical wheat haploidization methods are: interspecific crosses,
100 and *in vitro* culture of male haploid cells, the latter method being also reported for maize [1].
101 Furthermore, wheat, together with rice, have recently benefited from successful translation of maize
102 *in planta* haploid induction system [9,10] (**Figure 1B**). Whereas all these methods could be used to
103 speed-up the production of fixed material (DH lines), the CENH3-based haploid induction system offers
104 the possibility to obtain an unusual cellular arrangement in which the paternal genome is surrounded
105 by maternal cytoplasm. This allows cytoplasm swapping in a single-step, simplifying hybrid crop
106 management [1].

107 Finally, haploid inducer lines have recently been elegantly used to deliver genome edit
108 machinery to crop genotypes that are recalcitrant to genetic transformation [11,12]. This *trans* editing
109 system allows the production of genome edited elite cultivars that are both transgene-free plants
110 (because CRISPR-cassette is unstable like the haploid inducer genome) and homozygous (because they
111 are DH plants).

112 To sum-up, *in planta* haploid induction systems allow not only convenient production of
113 haploid embryos within viable seeds, but represent the entry point for increasing numbers of breeding
114 applications, not forgetting that haploid inducer lines also represent entry points to better characterize
115 plant reproduction.

116

117

118 **Figure 1. Two different kind of haploid inducer lines to trigger either paternal (A) or maternal (B)** 119 **haploid embryos through seeds in wheat.**

120 (A) Engineering of CENH3 allows creation of haploid inducer lines which are able to generate viable
121 seeds containing embryos with a paternal haploid genome. These CENH3-based haploid inducer lines
122 trigger paternal haploid embryos when used as a female parent in outcrossing with wild-type pollen.

123 (B) Translation of maize haploid induction system to wheat allows induction of viable seeds containing
124 embryos with a maternal haploid genome. In this situation, the haploid inducer line is used as a male
125 and outcrosses with wild-type female. In all cases, the haploid embryos lack the genome from the
126 haploid inducer line. The main breeding advantages of these *in planta* inducer lines are (i) shortening
127 the creation of inbred lines, (ii) shortening the creation cytoplasm swapping (for CENH3-based haploid
128 inducer lines only) and (iii) allowing *trans* editing of non-inducer genome. This figure was created using
129 BioRender (<https://biorender.com/>).

130

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136

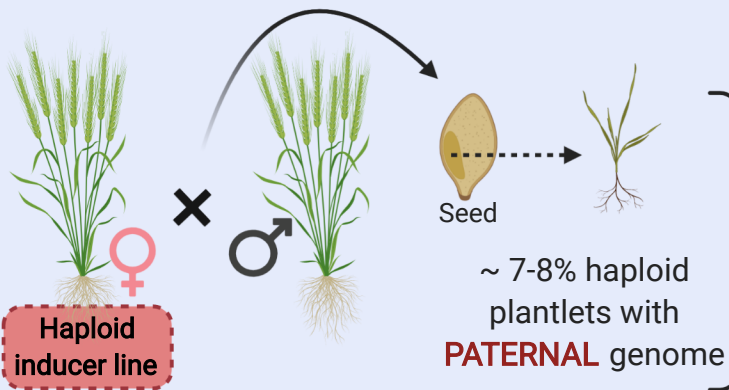
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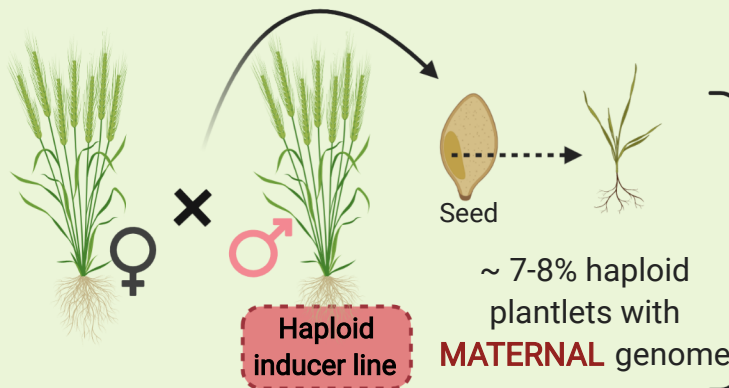
(A) Haploid embryo induction by CENH3 engineering



Breeding applications

- One-step cytoplasm swapping
- Shortening inbred line creation
- *trans* editing

(B) Haploid embryo induction by translation of maize MATL/NLD/PLA1 system



Breeding applications

- Shortening inbred line creation
- *trans* editing