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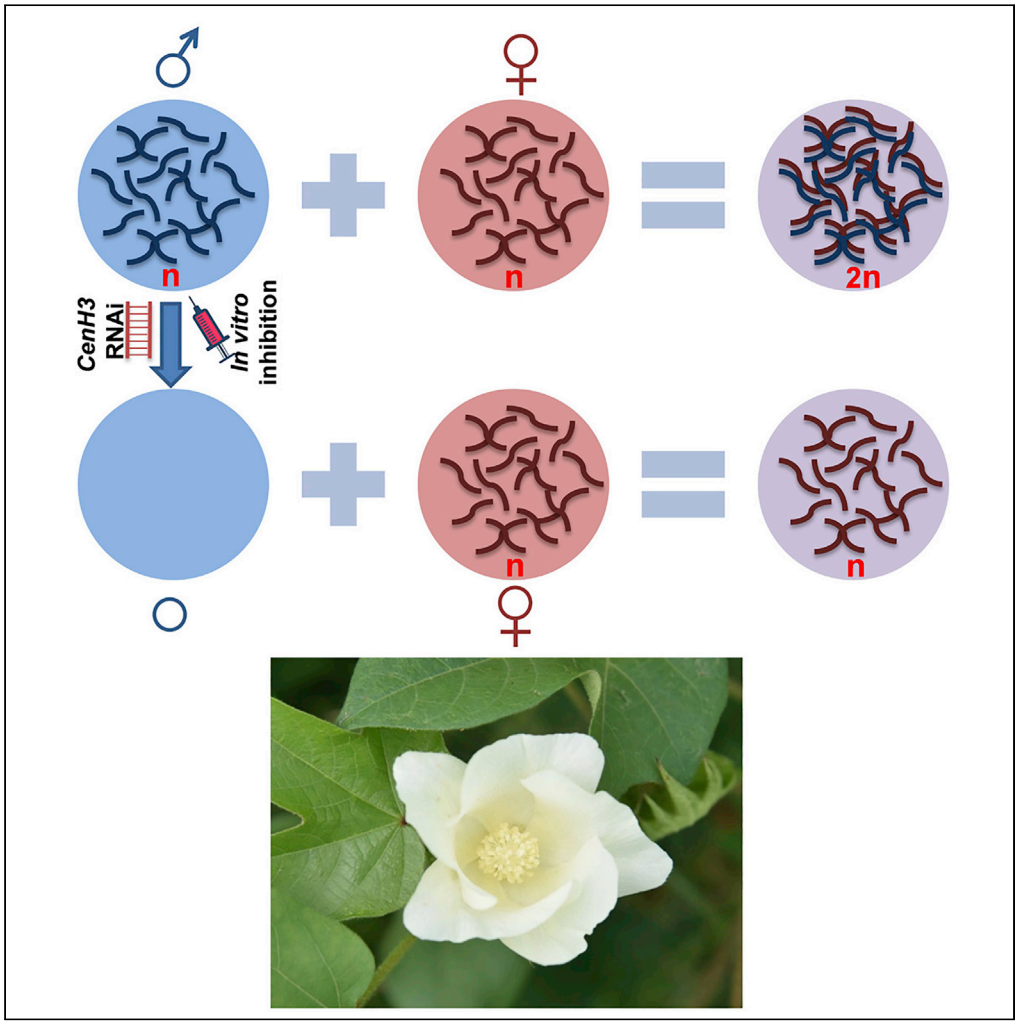

Xinpeng Gao, Huihui Guo, Jianfei Wu, ..., Tongtong Li, Cuixia Chen, Fanchang Zeng

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HIGHLIGHTS

CenH3 RNAi and *in vitro* inhibition can similarly induce haploids

We provide the cytological basis underlying parthenogenesis induction and haploids production

We report pseudo male gamete-mediated mock sexual reproduction during haploid parthenogenesis

We provide insights for the haploid parthenogenesis induction process

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Haploid Bio-Induction in Plant through Mock Sexual Reproduction

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SUMMARY

Haploidization is invaluable for basic genetic research and crop breeding. The haploid bio-induction principle is an important topic that remains largely unexplored. In this study, both CenH3 RNAi and *in vitro* inhibition were used to simulate and induce haploids in allopolyploid crop. Notably, *in vitro* CenH3 inhibition showed that the results were much the same to that of RNAi in phenotype, chromosome behavior, microspore production, and haploid induction. Cytological analyses of RNAi and inhibitor-treated progenies revealed elimination of chromosomes, defective microspores with empty nuclei, thereby giving rise to pseudo male gametes, and haploid parthenogenesis induction. We found distinct defective empty microspores that were positively correlated with the decrease of *CenH3* during RNAi manipulation. Investigation through both *in vivo* and *in vitro* studies revealed that haploidization was induced through the pseudo male gamete-mediated mock sexual reproduction. The present results provide insights for the haploid parthenogenesis induction process.

INTRODUCTION

Utilization of haploids in breeding procedures could significantly shorten the selection time or fix heterosis. Parthenogenesis and haploidy production are unique and crucial reproductive processes that naturally exist in plants. In the 1960s, Indian scholars Guha and Maheshwari (1964) cultured *Daturametel L.* anthers *in vitro* and successfully induced haploid plants. Mejza et al. (1993) successfully obtained the haploid plants of anther cultured wheat. Later, numerous optimization studies were performed to increase the haploid generation efficiency (Zhao et al., 2015). And the maize line Stock6 and derivatives can induce haploids when crossed with other strains (Coe, 1959; Lashermes and Beckert, 1988; Khakwani et al., 2015). Kasha and Kao (1970) reported high-frequency barley haploids in progenies of the cross *Hordeum vulgare* and *H. bulbosum*, which are caused by *H. bulbosum* centromeric loss of CENH3 protein and uniparental genome elimination, called the Bulbosum Technique (BT) (Sanei et al., 2011). Additionally, selective elimination of maize chromosomes has been reported in wheat × maize (Laurie and Bennett, 1988) and oat × maize (Marcínska et al., 2013) crosses and has been used in breeding cultivate varieties of these crops.

The study by Ravi and Chan (2010) showed that *Arabidopsis thaliana* haploid plants can be easily generated through seeds by manipulating a single centromere protein, the centromere-specific histone H3 (CenH3) protein, which provided the zygotic mitosis model underlying selective elimination of chromosomes and has opened up new opportunities for constructing haploid inducer (HI) systems through genetic engineering. These HI lines can produce haploids without *in vitro* culture, and CenH3 can be found in all eukaryotes. Therefore, this type of HI can be applied to breeding in almost all plants. The *CenH3*-mediated haploid induction technique has so far been demonstrated and tested in plants (Ravi and Chan, 2010; Ravi et al., 2010; Lermontova et al., 2011; Kuppu et al., 2015; Karimi-Ashtiyani et al., 2015; Kelliher et al., 2016).

Seed haploid generation by manipulating the conserved CenH3 has been demonstrated and elaborated in *Arabidopsis*. However, many crop species are recalcitrant to generate haploids, and the haploid bio-induction complex process and principle remain largely unexplored. The cytological basis of chromosome elimination and the haploid induction process in cotton were investigated in this study. Haploid inducers mediated by the histone *GhCenH3* genes were obtained and identified through an *in vitro* inhibitor treatment strategy and an *in vivo* RNAi technique. Notably, our study revealed the cytological basis of haploid bio-induction through pseudo male gamete-mediated mock sexual reproduction, which provides a practical

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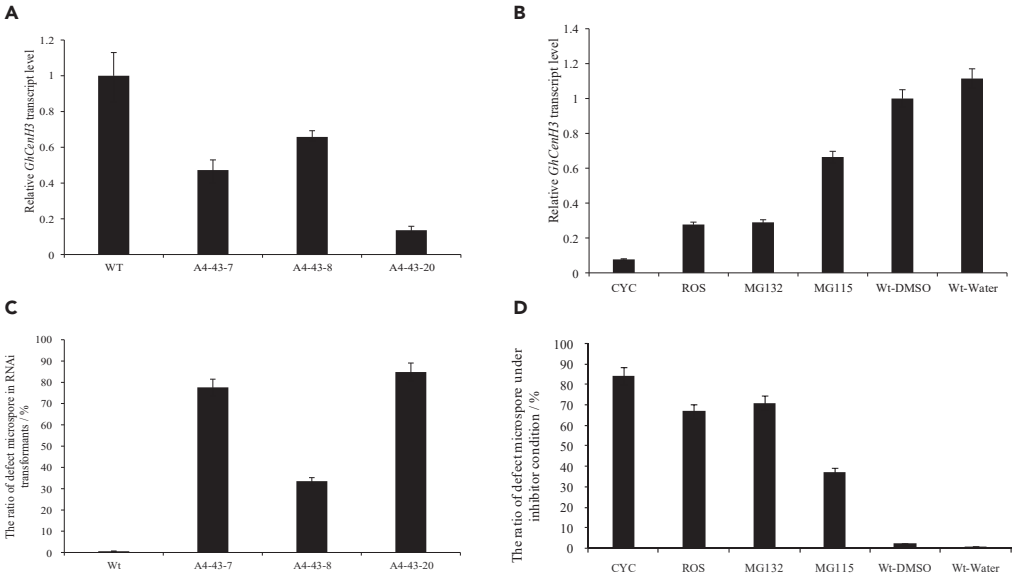

Figure 1. Suppression of Native *GhCenH3* Expression and Defective Microspore Production in Transgenic RNAi Lines and the Inhibitors Condition

(A) Suppression of native *CenH3* genes of flower anthers by RNAi transgenics.

(B) The expression of native *CenH3* genes of flower anthers under inhibitor treatment.

(C) Defective microspore production of RNAi transformants.

(D) Defective microspore production of CenH3 inhibitor-treated plants.

See also Figures S1 and S2. Data are represented as mean ± SD of three measurements.

biotechnology guide for crop haploid breeding. The findings in this study highlight the importance of mock sexual reproduction for understanding parthenogenesis bio-induction and haploid production in crops.

RESULTS AND DISCUSSION

Molecular Characteristics of Transgenic and Inhibitor-Treated Lines

In order to uncover the cytological basis and events underlying plant haploid bio-induction, RNAi and *in vitro* CenH3 inhibition were used to simulate and induce haploids in allopolyploid cotton. The selfing progenies of identified RNAi transformants will be investigated for further expression and functional studies. Among 23 different detected T2 positive transformant lines, the relative abundance of native *GhCenH3* transcript in the RNAi line A4-43-20 was reduced the most (over 80%) based on quantitative real-time RT-PCR (Figure 1A). The follow-up characterization and analysis will be carried out on the line.

Additionally, the previously reported four related inhibitors that reduce CenH3 expression and simulate the RNAi process were used in the study: CYC (Cycloheximide) (Lopes et al., 2011), ROS (Roscovitine) (Müller et al., 2014), MG115 (Z-leu-leu-norvalinal) (Lermontova et al., 2013), and MG132 (Z-Leu-Leu-Leu-CHO) (Moreno-Moreno et al., 2006). A microinjector was used to inject the inhibitor solution into cotton young flower buds during meiosis. The present study found that the four CenH3 inhibitors had efficient inhibitory effects on *GhCenH3* expression in cotton. CYC had the greatest inhibitory effect with over 90% decreased expression, followed by MG132, ROS, and MG115 (Figure 1B), compared with controls. Based on previous studies, complete silencing of conserved *CenH3* (Figure S1) is lethal. Therefore, severe but not complete suppressed status, such as that in the A4-43-20 line and CYC inhibition treatment plant (Figure S2), is ideal for further investigation.

Phenotype Analysis of Transgenic and Inhibitor-Treated Lines

The RNAi A4-43-20 plants were further inspected during their growth and development. The results exhibited that the growth and development of RNAi transformant were strongly inhibited (Figure S3). Additionally, stereoscopic microscope images of the flowers of *GhCenH3* RNAi transformant showed significant size reduction (Figures 2A–2D), with very short filaments (Figures 2B–2D), small anthers in the flower buds,

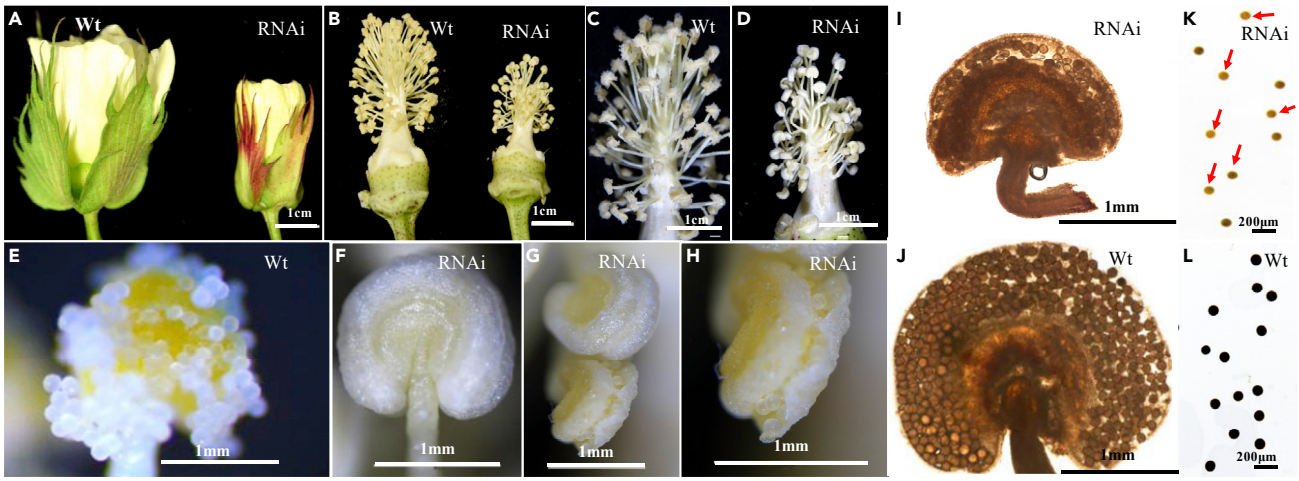

Figure 2. The Floral Phenotypic Traits of *GhCenH3* RNAi Transformant in Cotton

(A) The RNAi line A4-43-20 corolla compared with the wild-type.
(B) The RNAi line stamens and pistils compared with the wild-type.
(C) The wild-type stamens and pistils.
(D) The transformant stamens and pistils.
(E) The wild-type dehiscent anthers.
(F–H) The partially dehiscent anthers of the RNAi transformant. (F) longitudinal view; (G) Horizontal view; (H) enlarged view of G.
(I) The anther of the RNAi transformant stained by I_2-KI solution.
(J) The wild-type anther stained by I_2-KI solution.
(K) The RNAi transformant pollen stained by I_2-KI solution.
(L) The wild-type pollen stained by I_2-KI solution. The defective pollen marked by red arrows.
See also Figures S3–S5.

and abnormally dehiscent anthers (Figures 2E–2H), with less pollen in the anthers (Figures 2I and 2J) and partially defective pseudo pollen in the anthers (Figures 2K and 2L) compared with the wild-type plants. The CenH3 related inhibitor *in vitro* treatment lines showed similar phenotypes as the RNAi transformant (Figures S4 and S5).

Chromosome Elimination and Cytological Analysis during Male Meiosis

Interference of chromosome behavior was further investigated using both *in vivo* and *in vitro* strategies. Analysis of meiotic products during male meiosis of the *GhCenH3* RNAi transformant showed that *CenH3* reduction results in disturbed meiosis: an unequal amount of chromosomes in two plates of anaphase II (Figure 3B) and a high frequency of lagging chromosomes during anaphase II (Figure 3B and 3C), compared with wild-type (Figure 3A). The chromosome behaviors in the inhibitor-treated lines showed that inhibited *CenH3* expression caused defective meiosis with chromosome behaviors quite similar to that of the RNAi transformant (Figure 3D).

Interestingly, we detected abundant specific defective microspores with empty nuclei in both CenH3 inhibitor treatment and RNAi conditions (with a similar ratio above 80%, Figures 3E–3J). And the results showed a positive correlation between the defective microspore formation and *CenH3* decrease (Figure 1). The higher the reduction of CenH3, the higher number of defective microspores. Some microspores had shallower staining (Figures 3E–3J); we speculate that unequal meiosis leads to a decrease in chromosomes.

Combining the above findings, the RNAi transformant and inhibitor treatment line have similar characteristics. The *CenH3* RNAi and inhibitor treatment results indicate that *CenH3* reduction results in disturbed meiosis and produces defective microspores.

RNAi Haploid Progenies Induction and Identification

To determine the ploidy status and chromosome behavior of haploid progenies, mitotic preparations were made using root meristematic tissue of haploid progenies from selfing *CenH3* RNAi (Figure S6). Analysis of haploid progeny mitosis in selfing RNAi showed that *CenH3* reduction can also result in

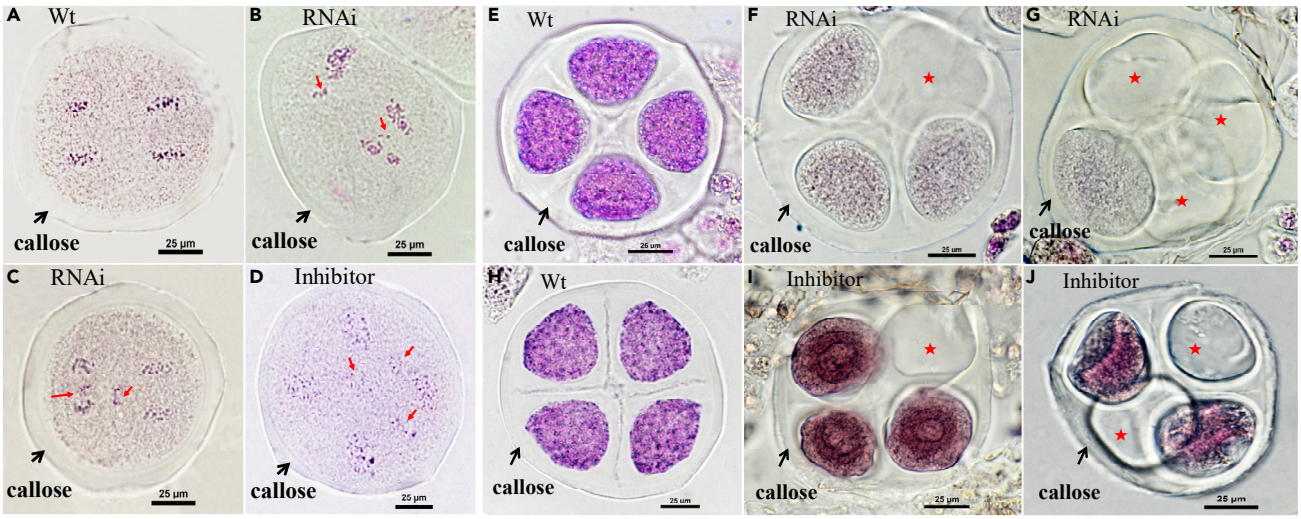

Figure 3. Chromosome Behaviors and Specific Defective Microspore Production after Disturbed Meiosis in CenH3 RNAi Transformant and Inhibitor Treatment Line in Cotton

(A) Anaphase II of the wild-type plant.

(B and C) Anaphase II of the CenH3 RNAi line A4-43-20. (B) Field of typical cell one; (C) Field of typical cell two.

(D) Anaphase II of inhibitor-treated plants. Lagging chromosomes were marked by red arrows.

(E) Normal microspores in wild-type.

(F and G) Defective microspores with empty nuclei in RNAi, marked by red stars. (F) Field of typical cell one.

(G) Field of typical cell two.(H) Normal microspores in wild-type mock treatment. (I and J) Defective microspores produced by inhibitor treatment. (I) Field of typical cell one; (J) Field of typical cell two. All cells have a complete callose protective layer.

disturbed mitosis: a high frequency of chromosomes were stranded on the equatorial plate, which then became lagging chromosomes during mitosis anaphase (Figure 4A), compared with the normal haploid progenies generated from maternal wild-type plants induced by RNAi lines (Figure 4B). This result suggests that there is a steady chromosome status in bio-induced haploid in wild-type plants but a chromosome elimination effect due to reduced *CenH3* in selfing RNAi progenies. And the haploid status of bio-induced haploid progenies from wild-type plants by chromosome counting was shown (2n = 26 chromosomes) in Figure 4C.

Lermontova et al. (2011) found that *CenH3* RNAi in *Arabidopsis thaliana* resulted in dwarf plants, decreased fertility, and changed ploidy levels in somatic cells. Kelliher et al. (2016) also found that the *CenH3* RNAi transgenic plants of maize were seriously affected in growth and development and they also produced haploids after selfing. The *CenH3* RNAi interference research of *Brassica juncea* showed slow plant growth, dwarf plants, seed sterility phenomena, and genetic offspring can induce haploids and aneuploid plants by selfing (Watts et al., 2016). In addition, the aneuploids were detected in the *A. thaliana CenH3* RNAi transformants (Lermontova et al., 2011). Considering the aneuploids, our results are consistent with those of Ravi and Chan (2010) and Watts et al. (2016) but differ from those of Kelliher et al. (2016) who found no aneuploids in *CenH3*-mediated HI lines of maize.

The application of haploid reproductive technology has the potential to revolutionize crop breeding. Haploids have been conventionally induced mainly through the generation of plants from cultivated gametophic cells and tissues by *in vitro* culture technologies or through the selective loss of a parental chromosome set by inter- or intraspecific hybridization *in vivo* (Kalinowska et al., 2019). However, owing to the application limits of current haploid technologies, scientists and plant breeders are highly interested in underlying principles as well as methodological improvements of haploidization. Recently, seed haploid induction technologies like *CenH3* manipulation and haploid parthenogenesis induction have been developed. The haploid progenies could be obtained from the maternal wild-types, induced by RNAi (♀WT×RNAi♂). In this study, the *CenH3* transgenic RNAi line was generated to suppress native *CenH3* to simulate haploid inducer (HI) lines with relative high productivity of haploid progenies ~8%. However, to this date, the principle controlling haploidization and parthenogenesis remains almost completely

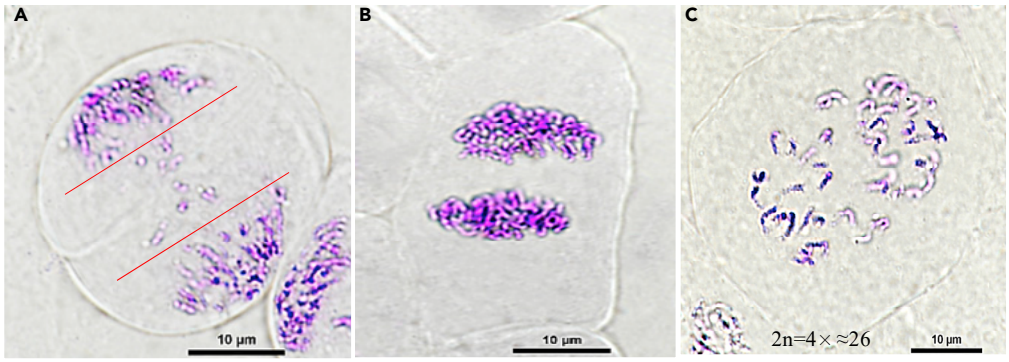

Figure 4. Cytology of Haploid Progeny Plants Derived from the *CenH3* RNAi Transformants

(A) The behavior of chromosomes during mitosis anaphase in haploid progenies from the RNAi line A4-43-20 selfing.

(B) The behavior of chromosomes during mitosis anaphase in haploid progenies from maternal wild-type haploid bio-induction.

(C) The chromosome counts of the haploid progenies produced from maternal wild-type haploid bio-induction.

See also Figure S6.

unknown. Very little progress has yet been made so far on elucidating the mechanism of haploid formation and parthenogenesis.

A deeper knowledge of the haploid parthenogenesis process in plants is required. This knowledge is a significant prerequisite to understand the formation and control of the apomictic development process from sexual reproduction. The *in vitro* CenH3 inhibition pollinator used to simulate the RNAi HI can generate the haploid progenies. We note that the inhibitors have a transient effect, different from the continued chromosome elimination effect in transgenic RNAi approach during early zygotic embryogenesis. Therefore, haploid progenies from ♀WT×inhibition (inhibitor-treated pollinator)♂ could be generated only from female gametes stimulated with defective pseudo male gametes. It suggested that haploid progenies could be similarly produced from mock sexual reproduction using RNAi HI line as pollinator of wild-type parents. *CenH3*-meditated haploid induction has been conventionally considered through chromosome elimination that typically takes place during early zygotic embryogenesis. Besides this basic principle, our results revealed a notable parthenogenesis engineering process by mock sexual reproduction for haploid bio-induction, which is distinct from the model of genome elimination during zygotic mitosis (Ravi and Chan, 2010; Maheshwari et al., 2015). It can be inferred that those two processes could occur alternatively or simultaneously during *CenH3*-meditated haploid induction. This study updates the haploid bio-induction principle and provides a practical biotechnology guide for haploid production in crops. It is of great fundamental and practical importance in crop science, holding significant promise for its advancement in engineering of apomixis in crop breeding. Future research is necessary to explain how mock sexual reproduction is established and to elucidate the molecular mechanisms regulating haploid parthenogenesis. Except the present strategy in this study, selectively modifying and editing key amino acids in the CenH3 will be complementary to build HAI system as reported in *A. thaliana* (Karimi-Ashtiyani et al., 2015; Kuppu et al., 2015). Furthermore, the improvement and implementation of haploidization approaches in crop breeding remains a mission to be fulfilled.

Limitations of the Study

- The difference between natural and bio-induced parthenogenesis/apomixis has not been investigated extensively so far.
- Further research is needed to elucidate the molecular mechanisms regulating haploid parthenogenesis mediated by mock sexual reproduction.
- More efficient haploid induction approaches for crop breeding need to be investigated in the future based on this study.

Resource Availability

Lead Contact

Further information should be directed and will be fulfilled by the Lead Contact, Fanchang Zeng (fczeng@sdau.edu.cn).

Materials Availability

Available from the Lead Contact under the public/local regulation and Material Transfer Agreements (MTAs) for education and research purpose if they are used not for commercial.

Data and Code Availability

The data can be found and is available within the manuscript and Supplemental Information.

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101279.

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AUTHOR CONTRIBUTIONS

F.Z. and X.G. conceived and designed the research project. X.G., H.G., and Y.F. performed gene cloning, vector construction, and genetic transformation. H.G., J.W., X.L., and Z.G. performed all morphological and other molecular experiments. X.G. and Y.F. performed the cytological studies. X.G., J.W., and L.Z. performed tissue inhibitor treatment. H.G., C.Z., Y.F., and T.L performed tissue and cell sampling. X.G., H.G., C.C., and F.Z. analyzed the data and wrote the article.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental Information

Haploid Bio-Induction in Plant through Mock Sexual Reproduction

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Supplementary information:

Transparent Methods

1. Plant material

Upland cotton varieties TM-1 and Luyanmian21 were grown in the experimental field at Shandong Agricultural University in Tai'an, Shandong province, PR China. TM-1 is the basic genetic germplasm material for various studies as the standard strain of upland cotton (*G. hirsutum.* L). While Luyanmian21 is a popular cultivar germplasm of upland cotton (*G. hirsutum*. L) in China.

2. Sequence alignment and phylogenetic analysis

The CDS and protein sequence of *GhCenH3*-A (KP177475), *GhCenH3*-D (KP177466), *GbCenH3*-A (KP177468), *GbCenH3*-D (KP177472), *GaCenH3* (KP177465), *GrCenH3* (KP177464), *TcCenH3* (XP_007051593), *NtCenH3* (AB366153.1), *AtCenH3* (AF465800), *BnCenH3* (ACZ04984), *DcCenH3* (KJ201903), *GmCenH3* (FK014964), *ZmCenH3* (AF519807), and *OsCenH3* (AY438639) were downloaded from the public database Pub Med (http://www.ncbi.nlm.nih.gov/pubmed/) according to the Gene Bank ID. ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/) was used to align the *CenH3* gene protein sequences, using default parameter values. Then, the Mega6.06 software version was used to establish the Neighbor-Joining Tree (NJ) phylogenetic tree.

3. Cloning RT-PCR

GhCenH3-A (KP177475) and *GhCenH3*-D (KP177466) were aligned using ClustalW (http://www.ebi.ac.uk/ Tools/msa/clustalw2/) to determine the conserved region for RNAi fragment amplification. Total RNA was isolated from 50 mg plant tissue with TRI reagent according to the manufacturer's instructions (T9424, Sigma-Aldrich, USA). Then, 0.5 μg RNA was used for first-strand cDNA synthesis using the *EasyScript*® One-Step gDNA Removal and cDNA Synthesis SuperMix (TRAN, Beijing, CN). Next, cDNA was used as a template to amplify a fragment of the *CenH3* gene using the listed primers (forward 5'-TAAGAAACCAAGACGGAAGCCATCT-3'; reverse 5'-AAGAGGCAGCAGAGGAC-TATTTGAT-3'). RT-PCR was performed using an ABI 9902 (Applied Biosystems, Veriti® 96-Well Thermal Cycler 9902, USA), and the PCR program was as follows: 95℃ for 6 min,

followed by 35 cycles of 95℃ for 30 s, 60℃ for 30 s, 72℃ for 30 s, and finally 72℃ for 2 min. Then, the amplified fragments were cloned and sequenced for RNAi construction.

4. Vector construction and plant transformation

To make the RNAi constructs with double-stranded RNA (hairpin RNA), the conserved region of *GhCenH3* was amplified by RT-PCR with specific primers (forward 5'-CACCTGAAGCGGGAAACATAACTTGAAAT-3'; reverse 5'-CTGGAGTTGGTGTGGCC GG-3'), and the PCR products were inserted into the RNAi vector plasmid pB7GWIWG2(Ⅱ), using the LR reaction to generate the P_{35S}*:GhCenH3* RNAi construct (Helliwell *et al.*, 2002). The construct was transformed into *G. hirsutum* L. mediated by *Agrobacterium tumefaciens* (LBA4404) according to previous reports (Bibi *et al.*, 2013; Wang *et al.*, 2013).

5. Molecular analysis of transformants

Total genomic DNA was isolated from young leaves of transgenic and wild-type (Wt) control using the CTAB method (Murray and Thompson, 1980), which was used for transgenic PCR screening with specific primers (forward 5'- AAGATGGACCCCCACCCA-CGAG-3'; reverse 5'- CACTTTGATCTTTTGCGAGGCTTTG-3') that stretched across the pCaMV 35S sequence and *GhCenH3* sequence. Total RNA was isolated from plant flower anther tissue with TRI reagent according to the manufacturer's instructions (T9424, Sigma-Aldrich, USA), which was used for cDNA synthesis and then for qRT-PCR to assess the expression level of the native *GhCenH3* gene. qRT-PCR was performed with Ultra SYBR Mixture (Low ROX, TRAN, Beijing, CN) using an Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher, USA). The PCR program was as follows: 95℃ for 10 min, followed by 40 cycles of 95℃ for 15 s and 60℃ for 1 min. The specificity of the amplified PCR products was determined by melting curve analysis (95℃ for 15 s, 60℃ for 1 min, 95℃ for 15 s and 60℃ for 15 s). *GhUB7* (DQ116411) of *G. hirsutum* L. was used as internal control to standardize the results (Guo *et al*., 2016). For each gene, quantitative PCR assays were repeated with triplicate runs. Relative expression levels were measured using the $2^{-\triangle\triangle Ct}$ analysis method.

6. Morphological and cytological analysis

Whole plant architecture and floral organs of T2 generation in RNAi cotton transformant lines and wild-type plants were photographed using a Nikon D750 SLR camera (Nikon, JPN).

Microstructure of upland cotton tissue was photographed using a Nikon SMZ25 stereo microscope (Nikon, JPN).

The young buds and root meristem were collected. The samples were then fixed in 3:1 ethanol: acetic acid solution. Then, the samples were softened using 1 M HCl at 65-70℃ for a few minutes. Small bits of immature anther sample were placed on a clean glass slide along with a drop of modified phenol and safranine (Sigma-Aldrich, USA) staining solution, and a cover glass was placed over the sample. The slide was placed between bibulous paper, and the tissue was compressed with a pencil. To evenly spread the cells, the cover slip was pressed manually. The slides were observed using a Nikon ECLIPSE Ni biomicroscope (Nikon, JPN) with a bright field microscope at 400-1000X magnification. And flow cytometry analyses using the procedure described by Sun *et al*. (2004).

7. Treatment with inhibitors

Four CenH3 related inhibitors were used in this study, including CYC (Cycloheximide, Sigma-Aldrich, USA), ROS (Roscovitine, Sigma-Aldrich, USA), MG115 (Z-leu-leu-norvalinal, Sigma-Aldrich, USA) and MG132 (Z-Leu-Leu-Leu-CHO, Sigma-Aldrich, USA). The inhibitor solution was prepared according to the previously reported literature method (Lermontova *et al*., 2013). Two controls were designed in this detection, add only DMSO and water equal to CenH3 inhibitor concentrated solution. And a microinjector was used to carefully inject the inhibitor solution (approximately 5 μl) into the cavity of cotton young flower buds (approximately 3 mm) before anthesis, which were then marked with a plastic tag. The next day after treatment, part of the flower bud was processed to observe the meiosis behavior. The developmental phenotypes were recorded in primary generation and induced progenies.

8. Statistical analysis

Statistical analyses were performed with one-way ANOVA, with “treatment” as the factor, followed by Tukey’s Honestly Significant Differences (HSD) test with a cut-off significance at $P< 0.05$ or $P< 0.01$ to evaluate differences among treatments.

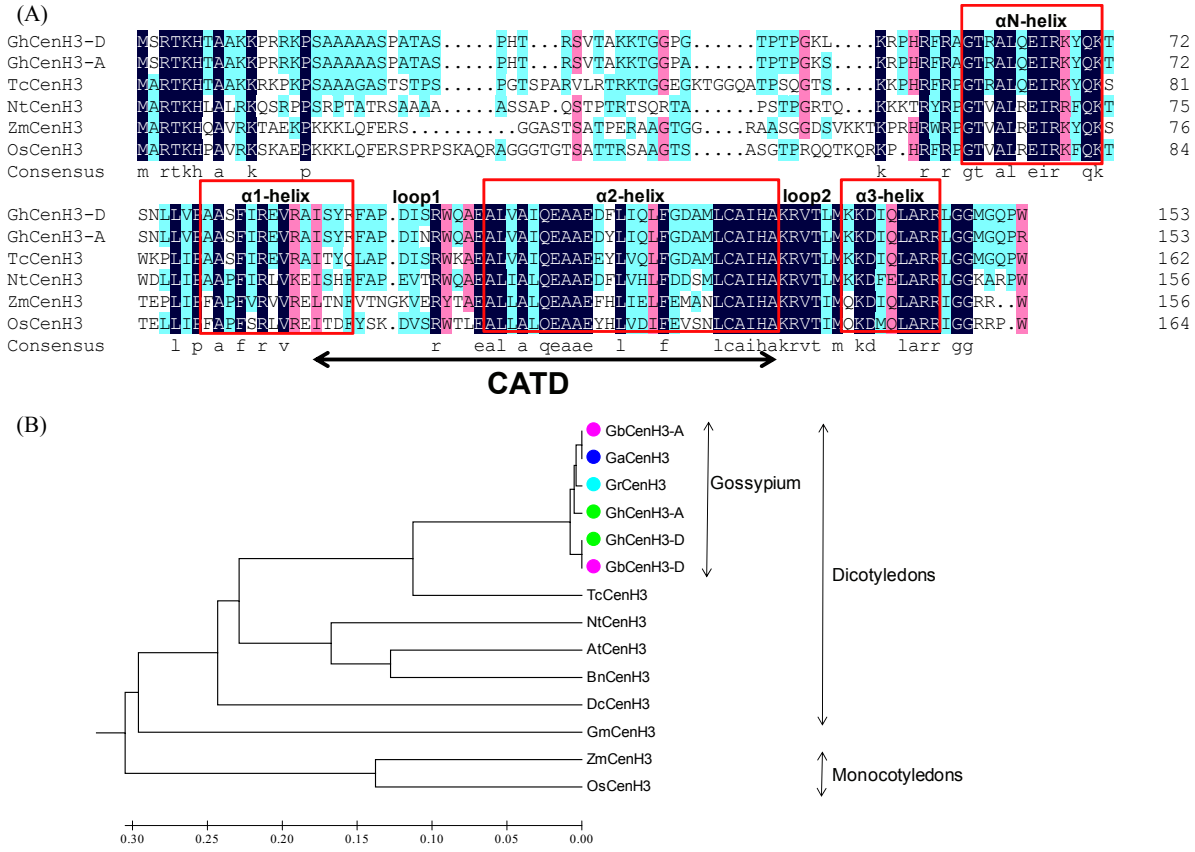

Figure S1. Conservation and phylogenetic analysis of GhCenH3-D and GhCenH3-A of cotton, related to Figure 1. (A) Alignment of deduced amino acid sequences. The CenH3-typical αN-helix, α1-helix, α2-helix, α3-helix, loop1 region, loop2 region and CTA-domain are indicated. (B) Phylogenetic analysis shows that *Gossypium CenH3* forms a subcluster with part dicotyledons and monocotyledons crops CenH3s. Gh, *G. hirsutum*; Gb, *Gossypium barbadense*; Ga, *Gossypium arboreum*; Gr, *Gossypium raimondii*; Tc, *Theobroma cacao*; Nt, *Nicotiana tabacum*; At, *Arabidopsis thaliana*; Bn, *Brassica napus*; Dc, *Daucus carota*; Gm, *Glycine max*; Zm, *Zea mays*; Os, *Oryza sativa*.

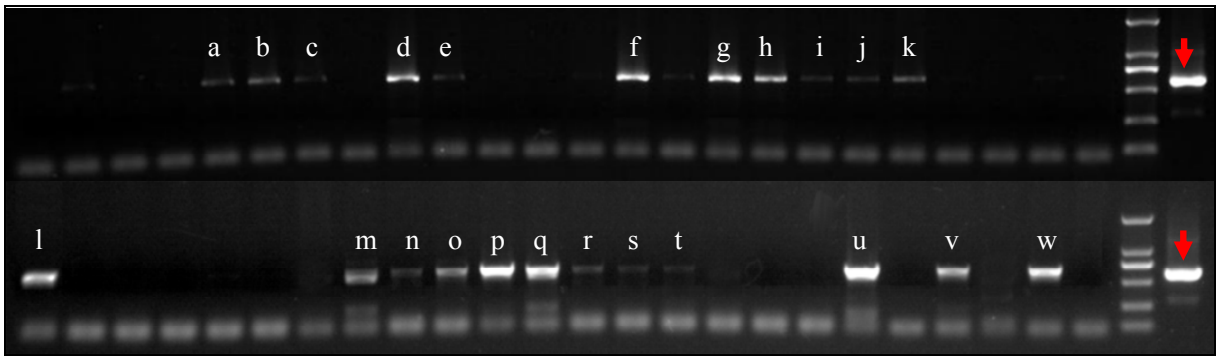

Figure S2. PCR identification of *Gossypium hirsutum* P_{35S}*:GhCenH3* RNAi construction transgenics, related to Figure 1. a-k, TM-1 cotton transgenics; l-w, Luyanmian21 (a variety in China) cotton transgenics. Red arrows indicate positive control.

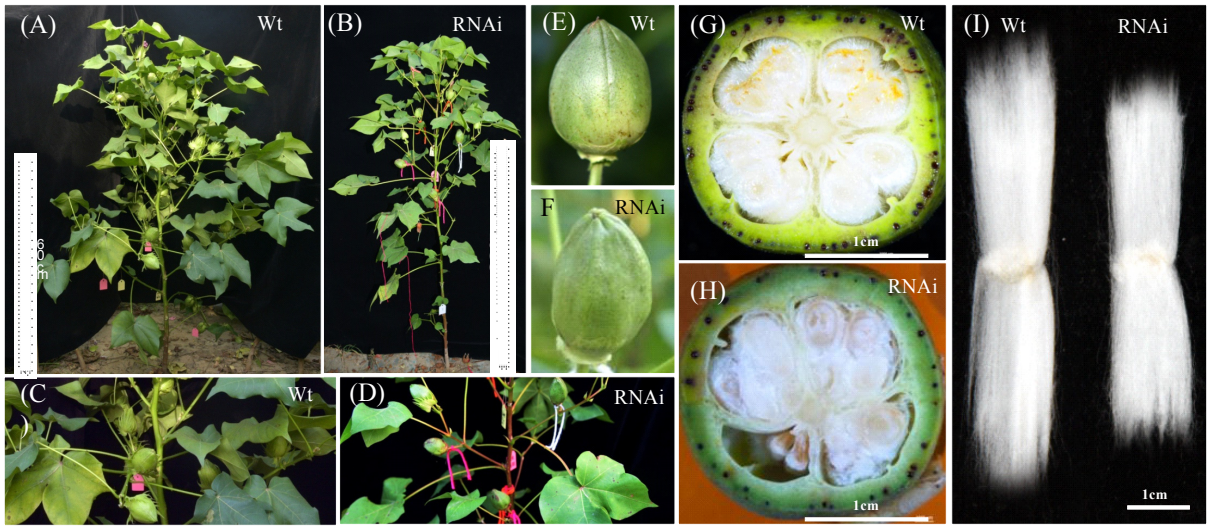

Figure S3. The general growth and development traits of *GhCenH3* RNAi transformant in cotton, related to Figure 2. (A) The wild-type plant. (B) The RNAi line A4-43-20. (C) Part of figure A. (D) Part of figure B. (E) The boll of the wild-type plant. (F) The boll of the RNAi transformant. (G) Transverse section of the wild-type boll. (H) Transverse section of RNAi cotton transformant. (I) The RNAi transformant fiber compared to the wild-type.

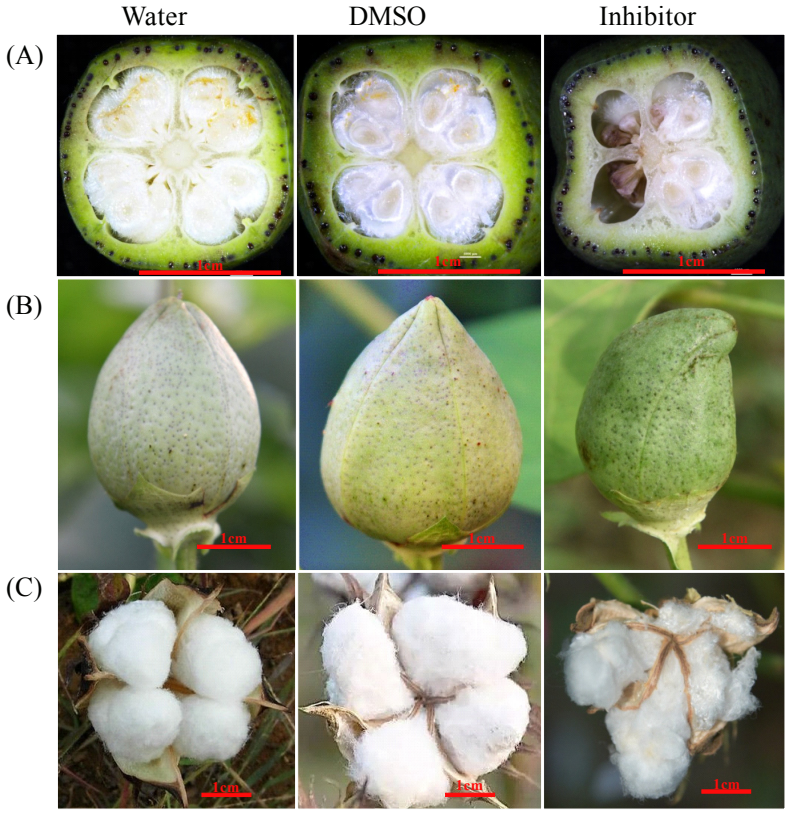

Figure S4. The boll traits of Wt, DMSO and inhibitor treatment in cotton, related to Figure 2. (A) 5 d bolls of plants. (B) 35 d bolls of plants. (C) The crack bolls of plants. Water, treated with water; DMSO, treated with DMSO; Inhibitor, treated with CenH3 related inhibitor.

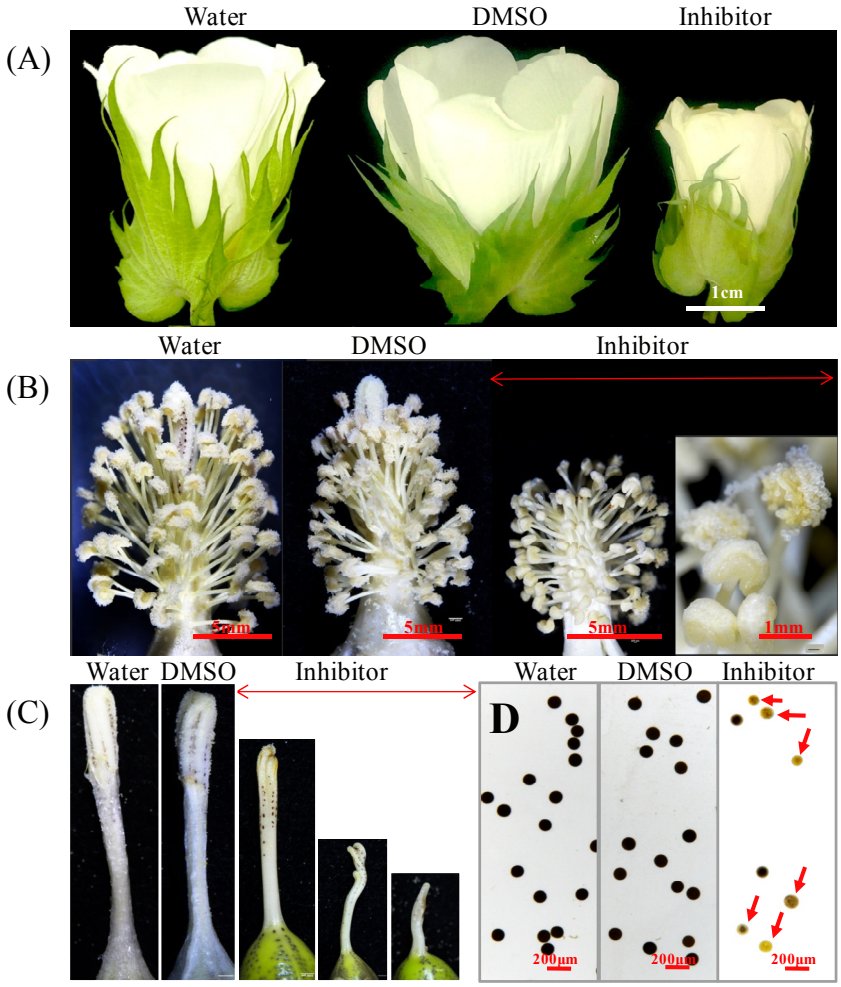

Figure S5. The floral phenotypic traits observed in inhibitor induced progenies plants, related to Figure 2. (A) The corolla of plants. (B) The stamens and pistils of plants. (C) The stigma of plants. (D) The plant pollen stained by I2-KI. The defective pollen was marked by red arrowheads. Water, treated with water; DMSO, treated with DMSO; Inhibitor, treated with CenH3 related inhibitor.

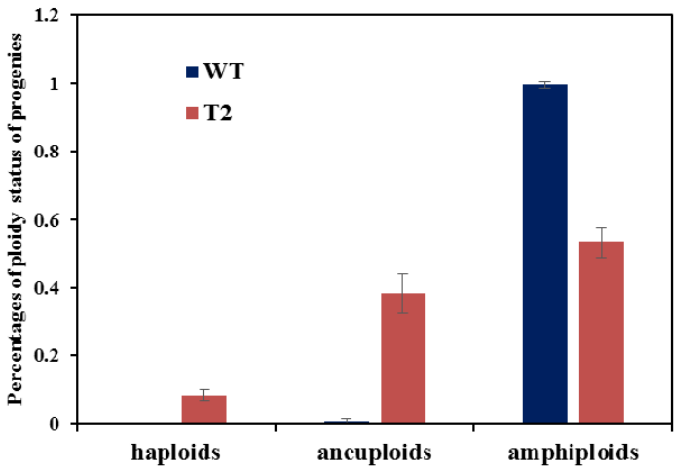

Figure S6. Statistics of the percentages of haploids and aneuploids in wild-type and T2 progenies, related to Figure 4. Data are represented as mean +/- SD of three measurements.

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