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Letter

Development of High-HIR Maize Haploid Inducer Line CAU8

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INTRODUCTION

Maize yield improvement relies on the development of elite hybrids, which largely depends on the exploitation of heterosis between two inbred parental lines (Schnable and Springer, 2013). Doubled haploid (DH) technology has become a key approach to accelerate maize breeding, because completely homozygous inbred lines can be produced within two generations, haploid induction (HI) and chromosome doubling, rather than require 8-9 generations of selfing (Schnable and Springer, 2013). The DH technology mainly includes HI, haploid identification, and chromosome doubling, and induction efficiency directly determines the throughput of DH production (Ren et al., 2017).

Maize DH breeding was initiated from the discovery of the first haploid inducer line, Stock6 (Coe, 1959). However, Stock6 has several limitations, including a low haploid induction rate (HIR) and inferior agronomic performance, which restricts its direct use in large-scale production. Over the past 60 years, a series of modern inducer lines have been developed, such as WS14, RWS, RWS-GFP, UH400, CAU2-6, and CHOI4 (Prigge et al., 2012; Yu and Birchler, 2016; Liu et al., 2022). As a result, HIR has been increased from ~2% to ~15%. Nevertheless, this level of induction efficiency remains insufficient for large-scale DH breeding.

Two genes controlling HI, *ZmPLA1* (Gilles et al., 2017; Kelliher et al., 2017; Liu et al., 2017) and *ZmDMP* (Zhong et al., 2019), have been cloned. However, these genes alone cannot fully explain the ~15% HIR observed in modern inducer lines, suggesting that additional minor-QTL contribute to HI capacity. An important unresolved question is whether different haploid inducer lines harbor distinct QTL and whether these QTL can be combined through crossing and phenotypic selection to further enhance HI efficiency.

To test this possibility, we crossed two widely used haploid inducer lines, CAU6 and CHOI4, which both exhibit HIR values of approximately 14-15%. Through 8 generations of phenotypic selection, we developed CAU8, a new haploid inducer line with substantially improved HIR, while maintaining stable seed set and favorable agronomic traits.

RESULTS

Response to selection of HIR during the development of CAU8

Across successive selection cycles, the population mean HIR increased from ~14% in the early generations to ~21% (a 50% increase) and then stabilized in the later generations (Fig. 1-A), while the number of haploids per ear (NHPE) increased from ~34 to ~50 (Fig. 1-B). Importantly, the candidate inducer line maintained a stable HIR of ~22% over three consecutive generations in the ZD958 background, indicating that the enhanced HI capacity is stable.

Agronomic traits of CAU8

Agronomic performance is essential for the large-

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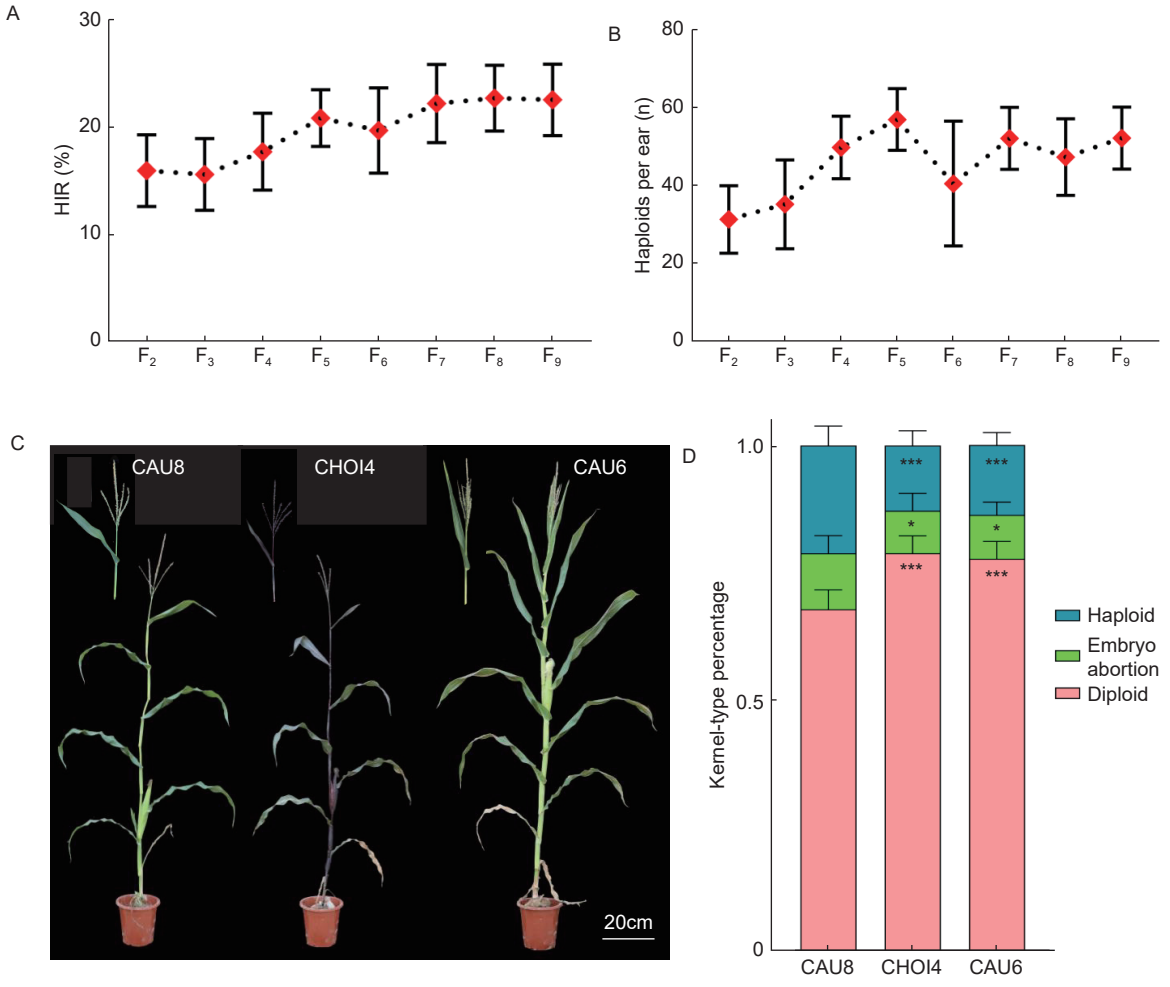

E

Testers	Inducer	Haploids per ear	HIR(%)	EmAR(%)	Kernels per ear	Kernels in total
ZD958	CHOI4	24.75±5.18***	11.11±2.26***	10.44±2.90	224.63±32.60	1797
	CAU6	31.00±6.26***	13.33±2.53***	6.19±1.75***	233.38±29.12	1867
	CAU8	52.10±7.92	22.42±3.34	11.55±3.07	234.70±34.12	2347
ZND678	CHOI4	27.40±10.74	12.38±4.59**	8.59±6.32	220.60±21.71	1103
	CAU6	27.50±14.50	15.44±1.89*	10.08±2.53	192.33±77.44	1154
	CAU8	37.40±7.75	19.14±3.06	9.83±3.52	195.90±29.87	1959
JK968	CHOI4	34.78±3.83***	14.03±1.74***	7.92±1.18*	250.00±30.39	2250
	CAU6	41.75±1.26*	15.26±2.68*	9.03±1.41	278.50±37.14	1114
	CAU8	56.00±13.70	22.75±5.17	10.27±2.31	248.50±42.14	2485
XY335	CHOI4	33.50±7.06**	14.04±2.49***	6.16±3.03**	238.50±25.62	1431
	CAU6	26.50±3.89***	12.06±1.87***	10.14±2.16	222.17±34.56	1333
	CAU8	46.38±6.91	20.58±2.74	12.80±4.48	225.38±16.83	1803

Fig. 1. Development and HI performance of the maize haploid inducer line CAU8.

A. HIR performance across generations (F_2-F_9). **B**. Haploids per ear across generations (F_2-F_9). **C**. Representative plant phenotypes of the inducer lines (left to right: CAU8, CHOI4, and CAU6; scale bar = 20 cm). **D**. Proportional distribution of kernel categories from tester ears crossed with CAU8 and its parental lines, including haploid kernels, embryo-abortion kernels, and diploid kernels. **E**. HI performance of CAU8, CHOI4, and CAU6 across four hybrid tester backgrounds (ZD958, ZND678, JK968, and XY335), including NHPE (number of haploids per ear), HIR, EmAR, kernels per ear, and total kernels. Data are presented as mean ± SD. Statistical significance was determined by one-way ANOVA. ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

scale deployment of haploid inducer lines. Under winter nursery conditions in Hainan Island, CAU8 showed a plant height of 170.1 cm and an ear height of 35.2 cm. The tassel had an average of 4.2 branches with a main tassel length of 29.1 cm (Fig. 1-C). The mean selfed seed set was 102.0 kernels per ear. Overall, compared with both parental lines, CAU8 exhibited a relatively reduced plant stature and a prolonged pollen-shedding duration. These traits are important for practical application because they improve the operability and reliability in breeding programs.

Performance of CAU8 in haploid induction across different backgrounds

To assess HI efficiency in different genetic backgrounds, we crossed CAU8 to four different hybrid testers (Fig. 1-E). CAU8 achieved a mean HIR of 22.42%, 19.14%, 22.75%, and 20.58% in ZD958, ZND678, JK968, and XY335, respectively. In all four tester backgrounds, the HIR of CAU8 was significantly higher than that of CHOI4 and CAU6. Specifically, CAU8 increased the HIR by 46.6% to 101.8% compared with CHOI4, and by 24.0% to 70.6% compared with CAU6.

In addition to HIR, CAU8 showed a significant increase in NHPE. Across the four testers, CAU8 produced 52.10, 37.40, 56.00, and 46.38 NHPE in ZD958, ZND678, JK968, and XY335, respectively (Fig. 1-E). Importantly, the total number of kernels per ear did not differ significantly between CAU8 and either of its parents across the four hybrid tester backgrounds (Fig. 1-E). These results demonstrate that the HI capacity of CAU8 is significantly enhanced without any obvious reduction in seed set, indicating its potential value for improving DH breeding efficiency.

DISCUSSION

Compared with CHOI4 and CAU6, CAU8 produced significantly higher numbers of haploid kernels and embryo-aborted kernels, but fewer diploid kernels. Although the underlying molecular mechanism remains unknown, it is reasonable to speculate that CAU8 may enrich minor QTL that contribute to HI from both parents. More importantly, unlike the functions of *ZmPLA1* and *ZmDMP*, CAU8, with improved HI efficiency, did not show decrease in seed set, this phenomenon is not only highly valuable for DH breeding, but also very interesting for scientists to explore the genetic mechanism underlying. The successful development of CAU8 demonstrates that HI efficiency can be further improved through phenotypic selection, which provides both a practical strategy and genetic resources for developing high-HIR haploid inducer lines.

METHODS

Plant materials

To develop a haploid inducer line with improved haploid induction rate (HIR) and number of haploids per ear (NHPE), two maize haploid inducer lines, CAU6 and CHOI4, were used as parental lines. Both CAU6 and CHOI4 were developed by China Agricultural University, with HIRs of approximately 15% and 14%, respectively. During the breeding process, phenotypic selection was carried out with an emphasis on two core indices, HIR and NHPE. In the early generations, the hybrid ZD958 was used as the tester female parent for evaluation. At a later stage (F_9), we performed a combined evaluation using four hybrid testers: ZD958, ZND678, JK968, and XY335, to confirm induction stability and background adaptability.

Phenotyping of individuals from F_2 to F_9

Both CAU6 and CHOI4 carry the *R1-nj* color marker. Hybrid kernels produced from crosses between inducer lines and tester lines can be classified into three categories based on the *R1-nj* marker phenotype: heterozygous diploid kernels (purple aleurone and purple embryo), putative haploid kernels (purple aleurone with colorless embryo), and embryo-aborted kernels (without embryo).

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YAN Tongzheng, GUO Shuwei, LIU Jinchu, LU Zhixiao, LI Yuqing, GENG Zhao, CHEN Shaojiang, LIU Chenxu
(*Frontiers Science Center for Molecular Design Breeding (MOE) / National Maize Improvement Center of China / College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China*)

Corresponding authors:

Chen Shaojiang (chen368@126.com);
Liu Chenxu (liucx@cau.edu.cn)