In vivo maternal haploid induction based on genome editing of DMP in Brassica oleracea

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Brassica oleracea is an important plant species that includes many globally cultivated vegetable crops (cole crops), such as cabbage, broccoli, cauliflower, kale and Brussels sprouts. These plants provide human beings with not only plentiful nutrients such as carotenoids, minerals, vitamins A and C, dietary fibre but also unique health-promoting compounds like glucosinolates (Xu et al., 2014).

Heterosis utilization in crops, including cole vegetables, requires the development of homozygous lines usually generated by multiple rounds of selfing or backcrossing (Zhong et al., 2019). Doubled haploid (DH) technology enables the generation of complete homozygous lines within two generations, dramatically accelerating the breeding progress (Zhong et al., 2020). However, traditional haploid induction (HI) in Brassica oleracea depends on an in vitro anther/microspore cultivation approach, which is not only complicated but also highly limited by plant genotype. In recent years, MTL/NLD/ZmPLA1, ZmDMP and ZmPOD65 were found to be responsible for inducing in vivo maternal haploid embryos in maize (Jiang et al., 2022 and references therein). Although orthologues of MTL/NLD/ZmPLA1 have not been found in dicots, ZmDMP-like genes are present in dicots and have been demonstrated to trigger in vivo maternal HI in Arabidopsis, Medicago truncatula, tomato, rapeseed and tobacco (Li et al., 2022; Wang et al., 2022; Zhong et al., 2020, 2022a,b). However, it is still not known whether this approach can be applied to cole crops.

We found fifteen putative DMP-like proteins in the Brassica oleracea genome. Among the proteins identified above, BoC04.DMP9 and BoC03.DMP9 were highly similar to ZmDMP, with 61% and 60% sequence identity, respectively, and they were assigned to a subclade together with AtDMP9 and AtDMP8 (Figure 1a). qRT–PCR analysis indicated that both BoC03.DMP9 and BoC04.DMP9 are highly expressed in pollen and flower buds, with BoC03.DMP9 being more highly expressed (Figure 1b). We cloned BoC03.DMP9 and BoC04.DMP9 from multiple cabbage inbred lines. Intriguingly, BoC04.DMP9 was lost in these cabbage lines due to a 1-bp deletion in exon. We further investigated DMPs in the Brassica genus, which showed that DMP8 was completely lost, and DMP9 experienced duplication and then lost. Both A and B genomes retained two normal DMP9 genes, whereas in the C genome, differential DMP9 orthologues were lost, including BoC04.DMP9 in B. oleracea and BnaC03g03890D in B. napus, indicating that the loss of DMP9 is a recent event after the formation of B. napus.

We employed the CRISPR/Cas9 approach to knock out BoC03.DMP9 in the cabbage ‘MW’ background. A CRISPR/Cas9 construct with a specific guide RNA sequence targeting the exon of BoC03.DMP9 was generated and introduced into cabbage by Agrobacterium-mediated transformation (Figure 1c). We obtained 8 lines with mutations in the target region, among which two homozygous or biallelic boc03.dmp9 mutants with deletions/insertions that led to frameshift and premature termination were selected for further studies (Figure 1d,e). Upon selfing or serving as pollen donors for crossing, the boc03.dmp9 mutants showed significantly reduced seed sets (Figure 1f,g).
To test whether boc03.dmp9 mutants could induce the production of haploids, we crossed boc03.dmp9 mutants (as male parents) with the tester line ms4, a curly kale male-sterile line. ms4 is an ideal HI testing material owing to its two characteristics: (i) completely green, a natural phenotype resulting from the abolishment of anthocyanin accumulation (Figure 1h), and (ii) male sterility, which prevents the occurrence of selfing. We found that six out of 255 progenies exhibited a completely green phenotype (Figure 1i). Flow cytometry analysis revealed that all six non-purple plants were true haploids, which corresponded to a haploid induction rate (HIR) of 2.35% (Figure 1j,k).

We further carried out a set of tests using boc03.dmp9 mutants to cross cabbage materials, including two inbred lines (19Z2053 and 2039) and one hybrid (2085). Molecular markers showing InDel polymorphism between boc03.dmp9 and the female parents were developed to screen all the progenies. Haploid would show genotype identical to the corresponding female (Figure 1l). Potential haploids identified by molecular markers were further confirmed by cytometry analysis and plant phenotyping. The HIRs ranged from 0.41% to 1.60% (Figure 1k).

In summary, we demonstrated that boc03.dmp9 mutants could induce in vivo maternal haploids in cole crops. The reported DMP-based in vivo HI system offers a novel, simple and cost-effective DH technology without genotype recalcitrance. Importantly, this system is applicable to one-step creation of homozygous male-sterile lines for hybrid seed production. In summary, this HI system could accelerate cultivar improvement and genetic studies of these important vegetable crops and provides reference information for extending this system to other dicotyledonous crop species.

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Conflicts of interest

The authors declare no conflict of interest.

Author contributions

F.H. and H.L. conceived and designed the work. X.Z., K.Y., Y.L. and N.Z. performed the experiments. F.H. and X.Z. wrote and revised the manuscript. L.Y., Y.Z., Y.W., J.J. and Z.F. analysed the data and revised the manuscript. All authors have read and approved the final manuscript.

References

Jiang, C., Sun, J., Li, R., Yan, S., Chen, W., Guo, L., Qin, G. et al. (2022) A reactive oxygen species burst causes haploid induction in maize. Mol. Plant, 15, 943–955.
Li, Y., Li, D., Xiao, Q., Wang, H., Wen, J., Tu, J., Shen, J. et al. (2022) An in planta haploid induction system in Brassica napus. J. Integr. Plant Biol. 64, 1140–1144.
Wang, N., Xia, X., Jiang, T., Li, L., Zhang, P., Niu, L., Cheng, H. et al. (2022) In planta haploid induction by genome editing of DMP in the model legume Medicago truncatula. Plant Biotech. J. 20, 22–24.
Xu, F., Zheng, Y., Yang, Z., Cao, S., Shao, X. and Wang, H. (2014) Domestic cooking methods affect the nutritional quality of red cabbage. Food Chem. 161, 162–167.
Zhong, Y., Liu, C., Qi, X., Jiao, Y., Wang, D., Wang, Y., Liu, Z. et al. (2019) Mutation of ZmDMP enhances haploid induction in maize. Nat. Plants, 5, 575–580.
Zhong, Y., Chen, B., Li, M., Wang, D., Jiao, Y., Qi, X., Wang, M. et al. (2020) A DMP-triggered in vivo maternal haploid induction system in the dicotyledonous Arabidopsis. Nat. Plants, 6, 466–472.
Zhong, Y., Chen, B., Wang, D., Zhu, X., Li, M., Zhang, J., Chen, M. et al. (2022a) In vivo maternal haploid induction in tomato. Plant Biotech. J. 20, 250–252.
Zhong, Y., Wang, Y., Chen, B., Liu, J., Wang, D., Li, M., Qi, X. et al. (2022b) Establishment of a dmp based maternal haploid induction system for polyploid Brassica napus and Nicotiana tabacum. J. Integr. Plant Biol. 64, 1281–1294.