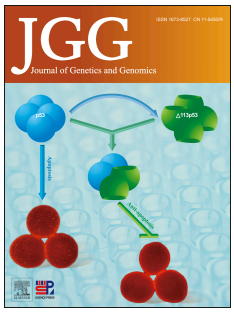
Journal Pre-proof



A heavy metal transporter gene *ZmHMA3a* promises safe agricultural production on cadmium-polluted arable land

Yuanyuan Chen, Zhen-Fei Chao, Min Jin, Ya-Ling Wang, Yaoyao Li, Jia-Chen Wu, Yingjie Xiao, Yong Peng, Qiao-Yan Lv, Songtao Gui, Xiaqing Wang, Mei-Ling Han, Alisdair R. Fernie, Dai-Yin Chao, Jianbing Yan

PII: S1673-8527(22)00215-6

DOI: https://doi.org/10.1016/j.jgg.2022.08.003

Reference: JGG 1103

To appear in: *Journal of Genetics and Genomics*

Received Date: 13 August 2022

Revised Date: 18 August 2022

Accepted Date: 21 August 2022

Please cite this article as: Chen, Y., Chao, Z.-F., Jin, M., Wang, Y.-L., Li, Y., Wu, J.-C., Xiao, Y., Peng, Y., Lv, Q.-Y., Gui, S., Wang, X., Han, M.-L., Fernie, A.R., Chao, D.-Y., Yan, J., A heavy metal transporter gene *ZmHMA3a* promises safe agricultural production on cadmium-polluted arable land, *Journal of Genetics and Genomics*, https://doi.org/10.1016/j.jgg.2022.08.003.

A heavy metal transporter gene *ZmHMA3a* promises safe agricultural production on cadmium-polluted arable land

In China, 19% of agricultural soils contain harmful heavy metal pollutants at levels exceeding environmentally recommended standards, whilst around 3 million hectares of arable land are too polluted to grow crops on (Zhao et al., 2015; Hu et al., 2016). Among the deleterious heavy metals, cadmium (Cd) is the most bioavailable toxic metallic pollutant and is rapidly transferable through the food chain (Wang et al., 2019). Concerning the current dilemma of the enhanced food demands of a rising population and decreasing availability of arable land, it is promising to cultivate field crops that produce enough safe foods for human consumption and simultaneously remove the pollutants from contaminated arable lands.

Many genes underlying Cd root uptake, vacuolar sequestration, root-to-shoot translocation, and accumulation have been demonstrated in rice (Zhao and Wang, 2020). Although knockout or overexpression of these key genes can efficiently reduce Cd accumulation in the grains, the field performance and even grain yield are unavoidably impaired when this strategy is followed (Sasaki et al., 2012; Sasaki et al., 2014; Lu et al., 2019; Chang et al., 2020). Owing to the massive biomass and high tolerance to Cd, maize (*Zea mays* L.) is a promising species for remediating Cd contaminations in agricultural soils (Wuana and Okieimen, 2010), as long as it can be engineered to be capable of producing safe kernels. It was reported that Cd content in maize varied widely among different genetic lines (Zhao et al., 2018; Baseggio et al., 2021), but only one gene regulating Cd accumulation in kernels has been functionally characterized in maize (Tang et al., 2021).

We initially observed that the leaf Cd content varied widely in two maize populations (Yang et al., 2011; Liu et al., 2020) (Fig. S1A and S1D). Then, a major peak (p = 7.05e–19) on chromosome 2 was identified in CUBIC, which explained 18.42% of the phenotypic variance (Figs. 1A and S1B). According to the parental IBD status of the peak bin (LRT = 69.84), JI53 group presents a significant difference from the other 15 groups (Non-JI53) and even greatly exceed the China guidance level of Cd contamination in cereals (0.1 mg/kg, hygienic standard for grains) (MHPRC, 2012), implying that the JI53-IBD is a high-Cd allele and Non-JI53 IBD is a low-Cd allele (Fig. S1C). By comparing the phenotypic difference against the genetic divergence among the progenies carrying JI53 bins

and Non-JI53 bins, we refined this QTL to a 6 Mb interval (158–164 Mb, Fig. 1B), which was comparable to *qLCd2* (153.75–167.58 Mb on chromosome 2) for maize leaf Cd accumulation (Zhao et al., 2018) and *qCd1* for maize kernel Cd variation (Tang et al., 2021). The same QTL *qCd2* (162.8 Mb–164.4 Mb, B73RefGen_v4.32) was also detected in AMP, which accounted for 17.98% of the phenotypic variance (Fig. 1C and 1D). Based on the B73 gene model annotations at *qCd2*, *Zm00001d005189* and *Zm00001d005190* were annotated as heavy metal ATPases (Table S1). In the published studies (Cao et al., 2019; Tang et al., 2021), these two genes were directly named *ZmHMA4* (*GRMZM2G455491*) and *ZmHMA3* (*GRMZM2G175576*). However, phylogenic analysis in our study shows that both Zm00001d005189 and Zm00001d005190 are the closest homologous to OsHMA3 (Ueno et al., 2010; Miyadate et al., 2011) (Fig. S2). Hereafter, it is more reasonable to designate *Zm00001d005190* as *ZmHMA3a* and *Zm00001d005189* as *ZmHMA3b* and nominate them as the candidate genes (Fig. 1D). Consistent with the B73 transcriptome profile (Walley et al., 2016), RNA profiling of 24 parental lines of CUBIC revealed that *ZmHMA3a* was mainly expressed in roots while *ZmHMA3b* was essentially not expressed in any tissues (Figs. S3 and S4). Knockout and overexpression of *ZmHMA3b* did not alter the leaf Cd content (Fig. S5), which demonstrated that *ZmHMA3b* was not the causal gene for natural variation in the leaf Cd.

According to the IBD status of *ZmHMA3a*, the CUBIC progenies were classified into JI53 and Non-JI53 groups. Compared to Non-JI53 allelic individuals, JI53-allelic progenies showed significantly higher Cd levels in the leaf and kernel (Fig. S6A). Additionally, 12 significantly associated variations (P < 8.9e-08) on *ZmHMA3a* were identified in AMP, which included the top SNP (chr2.s_163039506, P = 7.0e-15) identified in AMP is on the 5th exon of *ZmHMA3a* (Fig. 1E). Based on the 3 nonsynonymous SNPs (SNP27, SNP144, and SNP2945) and 2 structure variations (INS381 and INS1384) in *ZmHMA3a*, 409 accessions of AMP were classified into 6 distinct haplotypes (Fig. S6B). Comparatively, the leaf Cd content of Hap 6 was significantly higher than the other five groups, while no significant difference was observed among the other five groups (Fig. S6C). Moreover, the leaf Cd content of maize accessions carrying INS381 was significantly higher than that of maize lines not carrying INS381 in AMP (Fig. S6D).

Subsequently, we resequenced *ZmHMA3a* in high-Cd accessions (JI53, Mo17, and KN5585) and low-Cd accession (B73 and HZS) and verified this insertion (INS381) (Fig. 1F and 1G), which is annotated as an unspecified class LTR-retrotransposon (ID = RLC00217Zm00014a00006)

(Anderson et al., 2019). In addition, JI53-type progenies harboring this intronic insertion accumulated more Cd in the leaf and kernel than Non-JI53 lines without this insertion (Fig. 1H), but the expression level of *ZmHMA3a* in roots was not statistically different among these individuals (Fig. 1I). Furthermore, the full CDS sequences information revealed that all *ZmHMA3a* in the high-Cd accessions are mis-transcribed, which generates a premature stop-codon, while the transcripts from B73 and HZS are normal (Figs. 1J and S7). The above evidence implied the LTR-retrotransposon is the causal variant, which did not alter the gene expression level but induced the dysfunctional protein. Therefore, we speculated *ZmHMA3a* might be the causal locus of leaf Cd variations in maize. In support of this, knockout of *ZmHMA3a* in KN5585 background (KO#5 and KO#6; Fig. S8A) did not result in any change in Cd concentration either in leaves or kernels (Fig. 1K and 1L); two EMS mutants *hma3a.1* and *hma3a.2* (Fig. S8B) accumulated more Cd in leaves and kernels than the wild accession B73 (Fig. 1M and 1N).

To validate *ZmHMA3a* functions in limiting Cd translocation from root to shoot, we generated two independent overexpression lines (OE8 and OE32) and the respective non-transgenic lines (CK8 and CK32) of *ZmHMA3a* (Fig. 1O). We quantified the Cd levels in the roots and the shoots of CKs and OEs seedlings grown in hydroponic solution with/without 80 μM $CdCl_2$ contamination and found that the OE plants accumulated significantly more Cd in roots and greatly limited Cd translocation from root to shoot (Fig. S9A and S9B). Moreover, the fluorescence signals of the Cd-indicator dye from the OE8 root protoplasts were observed to be stronger in the vacuole than in the cytoplasm (Fig. S9C) whereas the fluorescence signals from the CK8 root protoplast were weaker in the vacuole than in the cytoplasm (Fig. S9D). Furtherly, *ZmHMA3a*^{B73}::GFP fusion protein was identified to be localized at the tonoplast in *Arabidopsis* mesophyll protoplasts when the plasma membrane and the tonoplast were separated by the chloroplasts (Fig. 1P) and the functional allele of *ZmHMA3a* was able to complement the function of Cd transportation in Cd-hypersensitive yeast mutant *ycf1* (Fig. 1Q). These data confirmed that *ZmHMA3a* is a core valve located at the tonoplast which mediates Cd compartmentalization in the root vacuoles.

Given that *ZmHMA3a* can sequester Cd into root vacuoles, overexpression of *ZmHMA3a* would be valuable for Cd-tolerance improvement. We therefore carried out the field trials (soil pH = 6.4) with *ZmHMA3a*^{B73} overexpression lines (OE8 and OE32) together with the non-transgenic lines (CK8 and CK32) under three Cd levels: background (0.1 ppm), slightly-polluted (1 ppm), and

heavily-polluted (3 ppm). Across the three Cd-level trials, the ear development of CK plants was normal in the field trials with 0.1 ppm and 1 ppm Cd but was remarkably impeded in the 3 ppm field trial (Fig. 1R). The kernel weight per ear (KWPE) of OE plants and the CK plants were not significantly different from that in the background (0.1 ppm) and lightly-polluted (1 ppm) soil. KWPE of CK plants was tremendously decreased by 47.6% (P = 7.4e–07) in the heavily polluted soil (3 ppm Cd) compared with that of CK lines grown in the background field trial (Fig. 1S). These data indicate that heavily polluted soil significantly retards the growth development and yield of maize. By contrast, *ZmHMA3a* overexpression plants produced normal ears in the three field trials, and the yields (KWPE) were not significantly affected in the heavily polluted field compared with that in the 0.1 ppm Cd field (Fig. 1R and 1S), exhibiting the power of *ZmHMA3a* overexpression in improving maize tolerance to Cd. In heavily-polluted field trial, Cd accumulation was decreased up to 95.5% in the OE kernels (OE32 vs. CK32 at 3 ppm, P = 3.2e–05) (Fig 1T), while other essential micronutrients, such as zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn), were unaffected or slightly decreased in any given circumstance (Fig. S10). Remarkably, the Cd concentrations were only 0.005–0.013 mg/kg in the OE kernels harvested from 1 ppm field and 0.006–0.033 mg/kg in the OE kernels from 3 ppm field (Fig. 1T), which were markedly lower than the hygienic standard for grains in China (MHPRC, 2012).

To assess the potential of *ZmHMA3a* overexpression for resolving agricultural Cd pollution, we grew these OE lines together with the CK lines and the transgenic receptor KN5585 in pots containing field soil (pH = 5.6) supplemented with 18 ppm Cd and the same batch soil without Cd (0.1 ppm Cd). KN5585 and CK plants performed well in Cd-free soil pots, but they were severely impaired either in growth or yield under extremely Cd-polluted environment (18 ppm Cd) (Fig. 1U). In such conditions, the Cd contents in KN5585 and CK kernels were averagely 11-fold higher than 0.1mg/kg (Fig. 1V). By contrast, OE plants can normally grow without any observable defects and the yield was mildly impacted (Fig. 1U). The Cd levels of OE kernels were far below 0.1mg/kg when grown on 18 ppm Cd-supplemented soil (Fig. 1V). Notably, OE plants could accumulate up to 3 mg Cd per kilogram of dry leaves under 18 ppm Cd condition (Fig. S11), suggesting that *ZmHMA3a* overexpression lines have a great potential to efficiently extract Cd from the contaminated field. Considering that maize is a widely grown crop with massive biomass, the non-edible tissues can be processed into ash for Cd recycle to achieve eco-friendly and sustainable

development. These results showed that *ZmHMA3a* overexpression lines can be perfectly used for safe agricultural production without yield penalty in Cd polluted areas.

In summary, we identified a major QTL *qCd2* for maize leaf Cd variations, which has been consecutively detected by previous studies (Zhao et al., 2018; Baseggio et al., 2021; Tang et al., 2021), suggesting that some essential genes underlying this region controlling Cd accumulation in maize. A comprehensive analysis of 12 *ZmHMA* genes (*ZmHMA1–ZmHMA12*) in maize identified *ZmHMA3* were significantly associated with maize leaf Cd variations (Cao et al., 2019). Collectively, these publications only speculated *ZmHMA3* might be responsible for Cd variations in maize without convincing data. Recently, Tang et al. (2021) functionally characterized this essential gene for Cd accumulation in maize kernels. However, the adjacent homolog gene *ZmHMA4* was not excluded with any genetic evidence as a potential gene that coexisted in the QTL. In contrast, we firstly confirmed that *ZmHMA3b* was not the causal gene for natural variation in the leaf Cd via knockout and overexpression lines (Fig. S5). Then, we provided shreds of sound evidence to conclude that *ZmHMA3a* mediates Cd compartmentalization in the vacuoles of maize roots (Figs. 1P, 1Q, S9). Notably, the *ZmHMA3a* overexpression lines can produce Cd-free kernels without yield and quality penalties when grown under different Cd-contaminated levels (1 ppm, 3 ppm) and even extremely contaminated (18 ppm Cd) (Fig. 1R–1V), which provided genetic basis for breeding an ideal maize cultivar with high Cd accumulation in the non-edible tissues for phytoremediation and low Cd in the grains for safe food.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We thank Mr. Hao Liu from the National Key Laboratory of Crop Genetic Improvement (Huazhong Agricultural University) for the help in managing the high-throughput computing system. The yeast strain *ycf1* was kindly provided by Dr. Xinyuan Huang from Nanjing Agriculture university. This research was supported by National Key Research and Development Program of China (2020YFE0202300), National Natural Science Foundation of China (31961133002), Chinese Academy of Sciences (XDB27010000), and Sichuan Science and Technology Program (2018HH0160).

References

Anderson, S.N., Stitzer, M.C., Brohmmer, A.B., Zhou, P., Noshay, J.M., O'connor, C.H., Hirsch, C.D., et al., 2019. Transposable elements contribute to dynamic genome content in maize. Plant J. 100, 1052–1065.

Baseggio, M., Murray, M., Wu, D., Ziegler, G., Kaczmar, N., Chamness, J., et al., 2021. Genome-wide association study suggests an independent genetic basis of zinc and cadmium concentrations in fresh sweet corn kernels. G3 11, jkab186.

Cao, Y., Zhao, X., Liu, Y., Wang, Y., Wu, W., Jiang, Y., et al., 2019. Genome-wide identification of *ZmHMAs* and association of natural variation in *ZmHMA2* and *ZmHMA3* with leaf cadmium accumulation in maize. PeerJ 7, e7877.

Chang, J.D., Huang, S., Konishi, N., Wang, P., Chen, J., Huang, X.Y., Ma, J.F., et al., 2020. Overexpression of the manganese/cadmium transporter *OsNRAMP5* reduces cadmium accumulation in rice grain. J. Exp. Bot. 71, 5705–5715.

Hu, Y.N., Cheng, H.F., Tao, S., 2016. The challenges and solutions for cadmium-contaminated rice in China: a critical review. Environ. Int. 92–93, 515–532.

Liu, H.J., Wang, X.Q., Xiao, Y.J., Luo, J.Y., Qiao, F., Yang, W.Y., Zhang, R.Y., et al., 2020. CUBIC: an atlas of genetic architecture promises directed maize improvement. Genome Biol. 21, 1–17.

Lu, C.N., Zhang, L.X., Tang, Z., Huang, X.Y., Ma, J.F., Zhao, F.J., 2019. Producing cadmium-free Indica rice by overexpressing *OsHMA3*. Environ. Int. 126, 619–626.

MHPRC., 2012. China national food safety standard: maximum limit of contaminants in food (GB 2762–2012). MHPRC Beijing, China.

Miyadate, H., Adachi, S., Hiraizumi, A., Tezuka, K., Nakazawa, N., Kawamoto, T., Katou, K., et al., 2011. OsHMA3, a P1B-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles. New Phytol. 189, 190–199.

Sasaki, A., Yamaji, N., Ma, J.F., 2014. Overexpression of *OsHMA3* enhances Cd tolerance and expression of Zn transporter genes in rice. J. Exp. Bot. 65, 6013–6021.

Sasaki, A., Yamaji, N., Yokosho, K., Ma, J.F., 2012. Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. Plant Cell 24, 2155–2167.

Tang, B., Luo, M., Zhang, Y., Guo, H., Li, J., Song, W., et al., 2021. Natural variations in the P-type ATPase heavy metal transporter gene *ZmHMA3* control cadmium accumulation in maize grains.

J. Exp. Bot. 72, 6230–6246.

Ueno, D., Yamaji, N., Kono, I., Huang, C.F., Ando, T., Yano, M., Ma, J.F., 2010. Gene limiting cadmium accumulation in rice. Proc. Natl. Acad. Sci. USA 107, 16500–16505.

Walley, J.W., Sartor, R.C., Shen, Z.X., Schmitz, R.J., Wu, K.J., Urich, M.A., Nery, J.R., et al., 2016. Integration of omic networks in a developmental atlas of maize. Science 353, 814–818.

Wang, P., Chen, H.P., Kopittke, P.M., Zhao, F.J., 2019. Cadmium contamination in agricultural soils of China and the impact on food safety. Environ. Pollut. 249, 1038–1048.

Wuana, R.A., Okieimen, F.E., 2010. Phytoremediation potential of maize (*Zea may*s L.). A review. African Journal of General Agriculture 6, 275–287.

Yang, X.H., Gao, S.B, Xu, S.T., Zhang, Z.X., Prasanna, B.M., Li, L., Li, J.S., et al., 2011. Characterization of a global germplasm collection and its potential utilization for analysis of complex quantitative traits in maize. Mol.Breeding 28, 511–526.

Zhao, F.J., Ma, Y.B., Zhu, Y.G., Tang, Z., Mcgrath, S.P., 2015. Soil contamination in China: current status and mitigation strategies. Environ. Sci. Technol. 49, 750–759.

Zhao, F.J., Wang, P., 2020. Arsenic and cadmium accumulation in rice and mitigation strategies. Plant and Soil 446, 1–21.

Zhao, X.W., Luo, L.X., Cao, Y.H., Liu, Y.J., Li, Y.H., Wu, W.M., Lan, Y.Z., et al., 2018. Genome-wide association analysis and QTL mapping reveal the genetic control of cadmium accumulation in maize leaf. BMC Genomics 19, 91.

Figure legend

Fig. 1. *ZmHMA3a* is the causal locus of natural variation in leaf and kernel Cd of maize plants and overexpression *ZmHMA3a* in maize holds a great potential for resolving the agricultural Cd pollution. **A**: Manhattan plot of Cd content in leaf via sGWAS in CUBIC. The black dashed line depicts the cutoff -$\log_{10}$(P = 9.9e–08) = 7.0. **B**: Fine-mapping of the major QTL controlling leaf Cd content on chromosome 2 using CUBIC offspring. Values are mean ± S.E. and different letters denote significant differences (P < 0.05) from a Tukey's HSD test. n is the number of CUBIC offspring belonging to the IBD-status groups. **C**: Manhattan plot for GWAS on leaf Cd variations in AMP. chr2.s_163039506 is the top SNP (P = 7.0e–15). **D**: Locuszoom of *qCd2* with 22 putative genes marked by rectangles. Arrows on the horizontal black lines show the direction of transcription. **E**: Natural variations in *ZmHMA3a* were significantly associated with leaf Cd content in AMP. The top SNP chr2.s_163039506 is highlighted by the black arrow and the other genetic variants are colored according to their LD (R^2) with the top SNP. The triangles denote structure variants and the dots represent SNPs. The horizontal dashed line represents the significance threshold (P = 8.9e–08) of genome-wide association study in AMP. **F**: The gene structure of *ZmHMA3a* in low-Cd line (B73 and Non-JI53) and high-Cd line (Mo17 and JI53) with white boxes representing exons, gray box representing 5′ UTR and blue triangle representing the LTR-retrotransposon inserted in 1st intron. Green bar represents CopZ domain and yellow bars represent ATPase-IB2-Cd domain. **G**: Agarose gel electrophoresis (AGE) image of the PCR product of LTR retrotransposon insertion in low-Cd line (B73) and high-Cd line (Mo17, KN5585, and JI53). **H** and **I**: Comparison of Cd content in the kernel and leaf (**H**) as well as the expression level of *ZmHMA3a* (**I**) in root between the JI53-haplotype offspring and the Non-JI53 type offspring in CUBIC. **J**: Transcript isoforms of *ZmHMA3a* in low-Cd line (B73 and HZS) and high-Cd line (Mo17, KN5585, and JI53). **K** and **L**: Comparison of Cd content in the leaf (**K**) and kernel (**L**) between the knockout mutants (KO#5 and KO#6, Cas9-free T_3 generation) and the transgenic receptor KN5585. **M** and **N**: Comparison of Cd content in the leaf (**M**) and kernel (**N**) between the EMS mutants *hma3a.1* and *hma3a.2* and the wild accession B73. **O**: The relative expression level of *ZmHMA3a* in transgenic overexpressing lines (OE8 and OE32) and the segregated non-transgenic lines (CK8 and CK32). Gene-expression level is analyzed using quantitative PCR with two biological replicates, each with three technical replicates. The maize gene (*Zm00001d044172*) is used as an internal control. **P**: Subcellular localization of *ZmHMA3a*. *ZmHMA3a*::GFP from B73 (Lower panel) and only GFP (Upper panel) were transiently expressed in *Arabidopsis* mesophyll protoplasts. From left to right: images of the GFP signal and chlorophyll autofluorescence, bright-field images, and merged images. White arrow indicates the area where the tonoplast is separated from the plasma membrane. Scale bars, 8 μm. **Q**: Heterologous expression of *ZmHMA3* in Cd-sensitive yeast strain *ycf1*. *ycf1* (Cd-sensitive) expressing ZmHMA3, AtHMA3(as positive control) and empty vector (pYES2) after three days growth at 30°C on SD-Ura media with and without 50 μM Cd, and galactose was used for induction of HMA3. Wide type

BY4741 expressing empty vector was also used as a positive control. **R**: Ear morphology of *ZmHMA3a* overexpression lines (OE8 and OE32) and the non-transgenic lines (CK8 and CK32) grown in three Cd levels field trials. ppm: parts per million. **S**: Comparison of kernel weight per ear of *ZmHMA3a* overexpression lines and the non-transgenic lines in three Cd levels field trials. Values are mean ± S.E.. **T**: Comparison of Cd content in kernels between *ZmHMA3a* overexpression lines and the non-transgenic lines grown in three Cd levels field trials. **U**: The growth performance of *ZmHMA3a* overexpression plants and the non-transgenic plants as well as the transgenic receptor KN5585 grown in the pots supplemented w/o 18 ppm Cd. **V**: Comparison of Cd content in kernels between *ZmHMA3a* overexpression lines and the non-transgenic lines as well as the transgenic receptor KN5585 grown in the pots supplemented w/o 18 ppm Cd. Values are mean ± S.D. and the statistical significance is estimated by two-sided Student's *t*-test. ***, $P < 0.001$. *n* is the number of maize accessions assigned to the corresponding group. NS, not significant. *n* is the sample size. DW, dry weight. S.E., standard error; S.D., standard deviation.

Yuanyuan Chen[1]

National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, Hubei 430070, China

Zhen-Fei Chao[1]

National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China

University of Chinese Academy of Sciences, Beijing 100049, China

Min Jin

National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, Hubei 430070, China

Ya-Ling Wang

National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China

Yaoyao Li

School of Life Sciences, State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, South China Agricultural University, Guangzhou, Guangdong 510642, China

Jia-Chen Wu

National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China

University of Chinese Academy of Sciences, Beijing 100049, China

Yingjie Xiao

National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, Hubei 430070, China

Hubei Hongshan Laboratory, Wuhan, Hubei 430070, China

Yong Peng

National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, Hubei 430070, China

Qiao-Yan Lv

National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China

University of Chinese Academy of Sciences, Beijing 100049, China

Songtao Gui

National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, Hubei 430070, China

Xiaqing Wang

Beijing Key Laboratory of Maize DNA Fingerprinting and Molecular Breeding, Maize Research Center, Beijing Academy of Agriculture & Forestry Sciences (BAAFS), Beijing 100097, China

Mei-Ling Han

National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China

Alisdair R. Fernie

Department of Molecular Physiology, Max-Planck-Institute of Molecular Plant Physiology, Am Mühlenberg 1, 14476 Potsdam-Golm, Germany

Dai-Yin Chao[*]

National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China

Jianbing Yan[*]

National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, Hubei 430070, China

Hubei Hongshan Laboratory, Wuhan, Hubei 430070, China

[1] *These authors contributed equally to this work.*

[*]*Correspondence authors.*

Email addresses: dychao@cemps.ac.cn (D.-Y. Chen), yjianbing@mail.hzau.edu.cn (J. Yan)