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Forum

Chinese cabbage: an emerging model for functional genomics in leafy vegetable crops

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Abstract

Leafy vegetable crops (LVCs) are consumed worldwide and offer essential nutrients for humans. Unlike model plant species, systematic characterization of gene function is lacking although whole-genome sequences are available for various LVCs. Several recent studies in Chinese cabbage report high-density mutant populations linking genotype-to-phenotype, providing blueprints for functional LVC genomics, and beyond.

Among hundreds of plant species with edible leaves, LVCs are rich in nutrients and beneficial to human health. *Brassica* crops produce nutritious leaves, leafy heads, stems, roots, and tubers, used as food and vegetables, and oilseeds, used for production of edible oil. Six globally cultivated *Brassica* species include diploid *Brassica rapa* (AA), *B. nigra* (BB) and *B. oleracea* (CC), and amphidiploid *B. juncea* (AABB), *B. napus* (AACC) and *B. carinata* (BBCC). The first LVC whole-genome sequence (WGS) was determined on *B. rapa ssp. pekinensis* (Chinese cabbage) [1]. Genomes of numerous morphotype-different *B. rapa* and *B. oleracea* accessions and *B. juncea* were further sequenced [2-4]. High-quality WGS for *B. rapa* Z1 (yellow sarson), *B. oleracea* HDEM (broccoli), and *B. rapa ssp. chinensis* (pak choi, a non-heading leafy vegetable) were the latest being assembled [5,6]. Such copious WGS provide valuable resources not only for investigation into plant evolution, diversification and domestication, but also functional genomics and targeted crop improvement. Indeed, bioinformatic mining of *Brassica* genomes has uncovered thousands of genes predicted to have similar functions in diverse physiologies as found in model species [1-4,6], and several genes such as *Br-dyp1* conferring dark yellow petal color trait, *BrKAO2* for leafy head formation, and *BrMS1* encoding a PHD-finger transcription factor for induction of male sterility have been verified to act in Chinese cabbage growth and development [7-9]. These studies have shifted research trends from generating static WGS to defining dynamic *Brassica* gene

functions. However, systems approaches of functional genetics and/or genomics and insightful investigations into molecular mechanisms underpinning gene functionality vs traits are sporadic in *Brassica*. But these shortcomings are about to be overcome with several recent seminal studies laying the groundwork establishing Chinese cabbage as a model for LVC functional genomics [6,10-12].

High-density Chinese Cabbage Genetic Mutants Populations (designated CCGMP hereafter) have been created in Chinese cabbage inbred line *B. rapa ssp. pekinensis* cv. A03 [10]. Plants developed mutant phenotypes in leaf, flower, and plant size, such as leaf color mutant *yellow-leaf* resulted from the *yl1* mutation, leaf morphology mutant *leaf curling* caused by the *cl1* mutation, and leafy head mutant *inward curling leafy head top* due to the *ic3* mutation (Figure 1). WGS of 300 stable mutants provide 1.93 trillion-bp datasets, covering approx. 16-fold genome-depths for each mutant line. 750,629 SNPs and 46,272 InDels have been found to scatter in genes, intergenic regions and (retro)transposon elements (TEs) across the entire A03 genome. Of SNPs, 216,054 present in ORFs of 41,322 genes, and 105,430 SNPs result in 35,748 amino-acid (aa)-mutated proteins. Moreover, 13,271 of 14,459, 9243 of 10,144 and 7815 of 8572 genes in three subgenomes contain SNPs that lead to amino-acid mutations. The tremendous >10-year endeavours have generated a high-density CCGMP and an intensive genetic-to-phenotypic database (www.bioinformatics-lab.cn/EMSmutation/home) in Chinese cabbage. It represents the first forward-genetic resource that links diverse phenotypes to genotypes among any LVCs, and shows potentials of Chinese cabbage as an emerging model for LVC functional genomics.

The promise

CCGMP is undoubtedly a timing resource and holds remarkable promises for linking specific phenotypes to underpinning genes and delineating related functional modes in Chinese cabbage. CCGMP was immediately exploited, uncovering *BraYL-2* and *BraY-4* responsible for yellow leaf phenotype and five genes for regulating glucosinolate biosynthesis [10]. Morphological categorization of CCGMP further revealed a leaf inward curling mutant (*ic1*). The intronless 1980-bp *BrOPS*, a homolog of *Arabidopsis thaliana* *OCTOPUS*, mapped to the *ic1* locus, encodes a 659-aa cell-membrane protein [11]. However, the *ic1* allele *BrmOPS* bears a premature stop codon resulted from a G809A SNP and can only produce a non-

functional N-terminal 269-aa polypeptide. *BrOPS* involves brassinosteroid signal transduction pathway to control early leafy head development and *BrOPS* transcription responds to BR treatment. *BrOPS* interacts with Brassinosteroid Insensitive-2 (*BrBIN2*), a GSK3 kinase known as a negative regulator in brassinosteroid signaling. The *BrOPS*-*BrBIN2* complex anchors *BrBIN2* to cell membrane. This prevents *BrBIN2* from accessing and phosphorylating transcriptional factor *BRINSENSITIVE1* (*BRI1*)-*EMS-SUPPRESSOR1* (*BrBES1*). Unphosphorylated *BrBES1* accumulates in nucleus where it binds the promoter of axial polarity gene *BrAS1* and suppresses its transcription. However, *BrmOPS* fails to interplay with and cannot restrict *BrBIN2* to plasma membrane. Free *BrBIN2* then targets and phosphorylates *BrBES1*. Phosphorylated *BrBES1*^P cannot repress *BrAS1* transcription in *ic1*. Thus, a functional *BR/BrOPS-BrBIN2/BrBES1/BrAS1* module modulates leaf axial patterning and growth to warrant normal development of leaf curvature and leafy head in Chinese cabbage. Such regulatory module, once disrupted by loss-of-function *BrmOPS*, causes abnormal leaf inward curling (Figure 2, Left panel).

Moreover, mining CCGMP unravelled that 'gain-of-function' *ferrochelatase-2* (*dBrFC2*, 3143-bp) is responsible for dark green leaf in *dg* mutant [12]. Overexpression of *dBrFC2*, not wildtype *BrFC2*, increases accumulation of chlorophyll and heme contents. *dBrFC2*-transgenic lines develop dark green leaves. *BrFC2* consists of 10 exons and 9 introns plus 5'- and 3'-UTRs. The 3'-end exon contains a C-T SNP causing a sense-mutation in *dBrFC2*. *dBrFC2* encodes a 495-aa protein with K464 substitution of R464 in *BrFC2* within the conserved chlorophyll a/b-binding motif. *dBrFC2* and *BrFC2* localize inside leaf chloroplasts, form homodimer and interact with protochlorophyllide oxidoreductase B1 and B2 (*BrPORB1* and *BrPORB2*). However, R464K enhances *dBrFC2* to form more homodimers and higher ferrochelatase activity to catalyse more heme biosynthesis. R464K also increases affinities of *dBrFC2*-*BrPORB1* and *dBrFC2*-*BrPORB2* complexes to bind protochlorophyllide (Pchl_{id}), and enzymatic activities of *BrPORB1* and *BrPORB2* for higher bioproduction of chlorophyllide (Chl_{id}) and chlorophyll in *dg* chloroplasts (Figure 2, Right panel).

The challenge

Usefulness of CCGMP is evident not only in functional characterization of loss/gain-of-function genes, but also in elucidation of structural bases, molecular mechanisms, and biochemical processes that underlie growth, development and metabolism in Chinese cabbage [10-12]. However, the challenge to make full use of CCGMP lies ahead. A particular confinement is lack of an effective and efficient system to transform LVCs and many *Brassica* species [12]. However, transgene-free gene editing may offer a gleeful opportunity to create knockout or gene-repairing lines to confirm functions of genes identified from CCGMP. Transformation-free plant virus-based technologies such as virus-induced gene complementation [13], virus-induced gene editing [14], and virus-induced gene silencing (VIGS) including Sir (siRNA-mediated), Mr (microRNA-mediated), and Pr (pseudo-recombinant virus-mediated) VIGS [15], can also be explored to complement genes in CCGMP or knockout/down genes in wildtype plants. Thus, development of novel functional genetic/genomic toolboxes will be necessary to fully realize potentials of CCGMP and *Brassica* WGS to bridge gaps between gene-to-function vs genotype-to-phenotype in Chinese cabbage and other LVCs.

The prospect

Despite challenges ahead, **prospects** of CCGMP and WGS of leafy heading and non-heading Chinese cabbages plus other LVCs and *Brassica* sp. for their applications in functional genomics **look** bright. CCGMP covers mutants predominantly in leaf growth and development (Figure 1). Leaf is the main consumable **organ** and primary target for genetic improvement in LVCs. Novel genes involved in control of leaf growth and development, and biosynthesis of nutritious metabolites will be almost certainly identified from CCGMP, and then used for targeted Chinese cabbage breeding. Moreover, common evolutionary routes among *Brassica* sp. and other LVCs suggest that any functional genetic/genomic information gained from Chinese cabbage is translatable and can serve as a framework for improving production of leaves, leafy heads, stems, roots, tubers and seeds in these crops. However, the present CCGMP encompasses mutants largely related to leaf development. Other types of mutants defective in disease resistance, abiotic stress tolerance or nutritional traits may be less represented, but could and should be expanded to produce even denser CCGMP using latest phenomics technologies. CCGMP also contains mutants resulted from mutations in TEs that are

often associated with plant epigenetic regulation. Therefore, CCGMP may offer an opportunity to define functional epigenetics in Chinese cabbage. Such work may lead to establish epigenetic mutant populations as an extra resource for epi-breeding of Chinese cabbage, LVCs and other vegetable crops.

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Declaration of interests

No interests are declared.

References

1. Brassica rapa Genome Sequencing Project Consortium. (2011) The genome of the mesopolyploid crop species *Brassica rapa*. Nat. Genet. 43, 1035-1039.
2. Cheng, F. et al. (2016) Subgenome parallel selection is associated with morphotype diversification and convergent crop domestication in *Brassica rapa* and *Brassica oleracea*. Nat. Genet. 48, 1218-1224.
3. Su, T. et al. (2018). A genomic variation map provides insights into the genetic basis of spring Chinese cabbage (*Brassica rapa ssp. pekinensis*) selection. Mol. Plant. 11, 1360-1376.
4. Yang, J. et al. (2016) The genome sequence of allopolyploid *Brassica juncea* and analysis of differential homoeolog gene expression influencing selection. Nat. Genet. 48: 1225-1232. Erratum in: Nat. Genet. 50, 1616 (2018).
5. Belser, C. et al. (2018) Chromosome-scale assemblies of plant genomes using nanopore long reads and optical maps. Nat. Plants. 4, 879-887.
6. Li, P. et al. (2021) Assembly of the non-heading pak choi genome and comparison with the genomes of heading Chinese cabbage and the oilseed yellow sarson. Plant Biotechnol. J. 19, 966-976.

7. Yang, S. et al. (2022) Map-Based cloning and characterization of *Br-dyp1*, a gene conferring dark yellow petal color trait in Chinese cabbage (*Brassica rapa* L. *ssp. pekinensis*). *Front. Plant Sci.* 13, 841328.
8. Huang, S. et al. (2022) BrKAO2 mutations disrupt leafy head formation in Chinese cabbage (*Brassica rapa* L. *ssp. pekinensis*). *Theo. App. Genet.* 135, 2453-2468.
9. Dong, S. et al. (2022) Defect in BrMS1, a PHD-finger transcription factor, induces male sterility in ethyl methane sulfonate-mutagenized Chinese cabbage (*Brassica rapa* L. *ssp. pekinensis*). *Front. Plant Sci.* 13, 992391.
10. Sun, X. et al. (2022) Construction of a high-density mutant population of Chinese cabbage facilitates the genetic dissection of agronomic traits. *Mol. Plant.* 15, 913–924.
11. Zhang, X. et al. (2022) OCTOPUS regulates BIN2 to control leaf curvature in Chinese cabbage. *Proc. Natl. Acad. Sci. U.S.A.* 119, e2208978119.
12. Liu, M. et al. (2022) Mutation in a chlorophyll binding motif of Brassica ferrochelatase enhances both heme and chlorophyll biosynthesis. *Cell Rep.* 41, 111758.
13. Zhou, T. et al. (2012) Virus-induced gene complementation reveals a transcription factor network in modulation of tomato fruit ripening. *Sci. Rep.* 2, 836.
14. Zhang, X. et al. (2020) An RNAi suppressor activates in planta virus-mediated gene editing. *Funct. Integr. Genomics.* 20, 471-477.
15. Li, H. et al. (2021) Efficient and high-throughput pseudorecombinant-chimeric Cucumber mosaic virus-based VIGS in maize. *Plant Physiol.* 187, 2865-2876.

Figure Legends

Figure 1. Generation of genetic mutation populations and summary of mutant phenotypes in Chinese cabbage. Workflow from EMS mutagenesis to whole genome sequencing (WGSeq) to functional genomics assay in the Chinese cabbage inbred line *B. rapa ssp. pekinensis* cv. A03 is shown. EMS mutagenesis was performed on high-quality wild-type A03 seeds. These potentially mutated seeds (designated M1 seeds) were sown and M1 plants were obtained. After self-pollination, thousands of M2 seeds were collected from M1 plants and randomly used to produce M2 plants. Among them, 2,744 developed mutated leaves, while 41 and 18 produced atypical flowers or irregular plant sizes, respectively. Detailed changes in the 3 groups of leaf, flower and plant size for M2 mutants were further phenotyped against wild-type A03 Chinese cabbage that produces a cylindrical leafy head with outward-curving leaves at various vegetative-to-reproductive growth and development stages. Self-progenies of the EMS mutants were carried out to the sixth

generation (M6) plants, and heritable phenotypes were confirmed in M4-M6 mutant plants. Subsequently, whole genomes of stable EMS-mutated Chinese cabbage mutants were sequenced to ratify the entablment of the Chinese cabbage genetic mutant populations (CCGMP). In summary, plants in CCGMP develop three main types of mutant phenotypes in leaf, flower, and overall size of Chinese cabbage. Leaf mutants are predominant. Subclassifications of these mutants as well as numbers (in bracket) of each type or subtype of mutants are shown. Original data for this figure were adapted from Table 1 [10].

Figure 2. Molecular mechanisms underlying the genetic links between genotype and phenotypes in loss-of-function *ic1* and gain-of-function *dg* mutants. **Left,** BrOPS involves brassinosteroid (BR) signaling to control leafy head development. BrOPS is a receptor-like cell membrane protein. It interacts with and recruits kinase BrBIN2 to plasma membrane. This fails BrBIN2 to phosphorylate transcriptional repressor BrBES1 which then binds *BrAS1* promoter and blocks the transcriptional complex to initiate *BrAS1* transcription in nucleus. However, loss-of-function BrmOPS cannot interact with BrBIN2 which freely targets BrBES1 for phosphorylation (P). Phosphorylated BrBES1^P cannot bind *BrAS1* promoter and *BrAS1* is transcribed, resulting in inward curling leaf in *ic1* mutant. Thus, a functional BR-BrOPS signal transduction from plasma membrane to nucleus is required to guarantee normal leafy head development in Chinese cabbage [11]. **Right,** gain-of-function dBrFC2 enhances biosynthesis of heme (red dot) and chlorophyll (green dot) in *dg* mutant. Compared to wildtype BrFC2, dBrFC2 possesses (i) increased capacity to form protein homodimer and enhanced enzymatic activity to catalyse heme biosynthesis, and (ii) improved functionality to interact with BrPORB1 and BrPORB2, leading to higher affinities of the dBrFC2-BrPORB1 and dBrFC2-BrPORB2 complexes to bind substrate pchl_{ide} to produce more chlorophyll inside the chloroplasts of the *dg* leaf tissues [12]. This figure was created using BioRender (<https://biorender.com/>).