4 - 34 Progress of Ion Beam Breeding Research at IMP in 2016

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1. The mutation breeding of plants

In 2016, the mutation mechanism of various plant mutants including *Arabidopsis thaliana*, Geranium, *Tradescantia fluminensis*, *Taraxacum koksaghyz Rodin* and algae obtained by heavy ion beam mutation breeding techniques were investigated. The genome-wide profiling of an Arabidopsis mutant named *civar* (Carbon ion beams Induced Variegated) was revealed by using re-sequencing and the rough map-based cloning techniques. A phenotype screening of *Arabidopsis thaliana* irradiated by carbon ion beams based on high-throughput imaging technique combining the principal component analysis and scatter matrix clustering analysis was built and optimized which can screen the variation of plant mutant populations. The pigment components and quantities and expression profiles of key genes involved in anthocyanin biosynthesis of flower-color mutant of Geranium and leaf-color mutant of *Tradescantia fluminensis* were investigated. Mutation breeding of *Taraxacum koksaghyz Rodin* induced by carbon ion beam irradiations which is notable for its production of high quality rubber and inulin was started. In addition, the photosynthetic response of the mutants of *Scenedesmus quadricauda* with altered pigment features were analyzed.

2. The mutation breeding of microorganisms

The mutation breeding research of microbes including *Aspergillus niger*, *Saccharomyces cerevisiae*, and *Acidithiobacillus ferrooxidans* induced by heavy ion beam irradiations were investigated this year. Concerning *Aspergillus niger*, the cellulase production affected by aeration rate and the radioprotective effect of melanin from the spores were analyzed. In addition, the combination fermentation of *Aspergillus niger* mixed with *Trichoderma reesei* was carried out. Effects of carbon sources on the biomass accumulation of a high-yield strain of *Saccharomyces cerevisiae*, 100G-9, was studied.

3. Sweet sorghum industry chain of circular economy

Up to 2016, more than 1.12 million Chinese mu cultivation of sweet sorghum has been promoted in Gansu, Qinghai, Ningxia provinces and Xinjiang Uygur Autonomous Region in northwest of China. A perfect industrial chain of sweet sorghum which can produce various bio-products including yeast, ethanol, β-glucan, silage, *etc.*, was constructed. More important, the whole industry demonstration project of sweet sorghum benefits the development of local agriculture and pasturage aquaculture extremely.

4 - 35 Identification of Mutant Gene Induced by Carbon Ion Beams in *Arabidopsis* by Whole Genome Re-sequencing and Rough Map-based Cloning

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In recent years, heavy ion beams have been recognized as an effective and efficient physical mutagen due to their capacity to induce mutations with high frequency and broad spectrum. Nowadays, it is not so difficult to produce various mutants in plants. However, to mine the responsible mutated genes has been an important challenge. For mutation isolation, map-based cloning is one of the major traditional ways to isolate the mutant genes that control traits of interest in forward genetics studies. However, the process of map-based cloning is usually complicated and time-consuming. As the urgent demands of genome-wide studies, the High Throughput Sequencing (HTS) techniques have been rapidly developed and enabled rapid sequencing and mutation detection for genomic mutation identification, species evolution, genetic diseases, *etc.* But as everything has two sides, researchers do not know how to start when facing the huge data which produced by HTS. Since rough mapping is able to narrow down the screen ranges, while re-sequencing can reveal the genome-wide mutation profiling of carbon ion beams irradiation on *Arabidopsis*, association analysis of this two techniques will accelerate the identification of the mutant genes that are responsible for the phenotypes.

In the present study, an *Arabidopsis* mutant named *civar* (carbon ion beams Induced Variegated) displaying variegated stem, rosette and cauline leaves, sepals, and siliques, was induced by carbon ion beams accelerated by the Heavy Ion Research Facility in Lanzhou (HIRFL). Based on the IIlumina HiSeq2500 platform, whole genome re-sequencing of M_3 *civar* was performed in order to characterize mutations induced by heavy ion beams at DNA level comprehensively. To mine the candidate genes that are responsible for the mutant phenotypes of *civar*, the rough map-based cloning was associated to narrow down the genetic ranges.

The M_3 progeny of *civar* was crossed with wild type ecotype *Landsberg erecta* (Ler), and then DNA was collected from 60 F_2 individuals that displayed the variegated phenotypes. The segregation of mutant gene in F_2 progeny corresponded to the Mendelian ratio of 3:1, which demonstrated that the phenotypes of the *civar* mutant were caused by a recessive single gene mutation. According to rough mapping, *civar* showed the lowest exchange rate at T20P8 on the chromosome 2 (about 6.1%)(Fig. 1(a)). On the other hand, genomic DNA of *civar* (M_3 progeny) was extracted by using CTAB protocol. The whole genome re-sequencing was performed based on the IIIumina HiSeq2500 system. After sequence alignment and rigorous filtering, 15 SNPs (Single Nucleotide Polymorphisms), 2 small InDels (insertion-deletion) were identified (Fig. 1(b)). Associated with rough mapping, there were only 1 variant site (chr2, 13175805) with deletion of a single cytosine, which led to frameshift_variant and synonymous_variant effects of *VAR2* gene. Precisely, *VAR2* showed a variegated phenotype. Therefore, it showed that association analysis of rough mapping and whole genome re-sequencing provided crucial guides for identifying the responsible genomic regions that may contribute to mutant phenotypes.

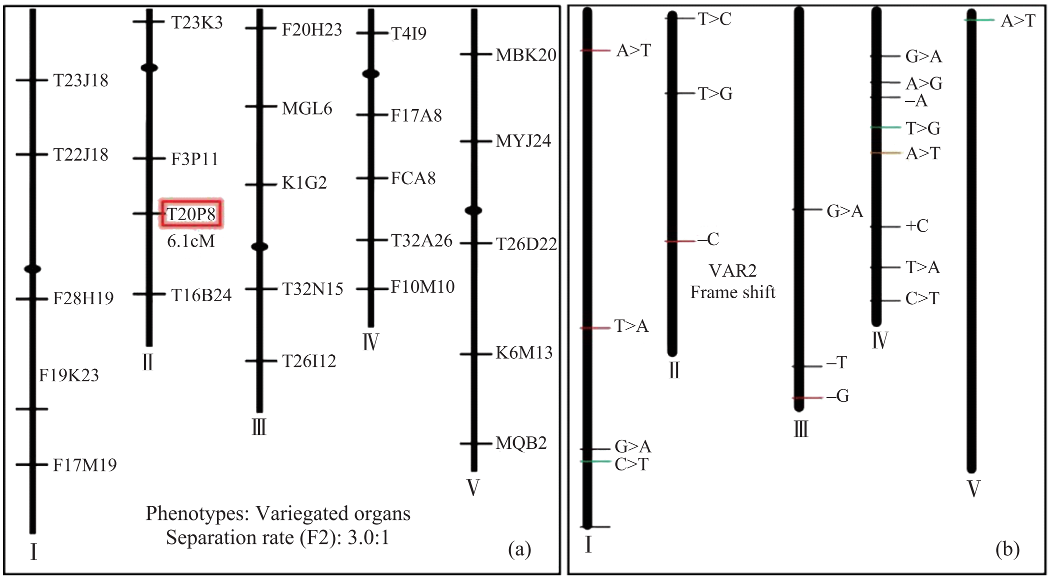

Fig. 1 Map-based cloning for responsible regions in chromosomes of *civar* (a) and mutations induced by carbon ions in *civar* genome(b).

4 - 36 Photosynthetic Response in *Scenedesmus quadricauda* after Carbon-ion Irradiation

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A large proportion of mutants with altered pigment features have been obtained via exposure to heavy-ion beams, a technique that is efficient for trait improvement in the breeding of plants and algae. However, little is known about the changes of the photosynthetic response of microalgae after exposure. In our group, six progenies of *Scenedesmus quadricauda* deficient in chlorophyll *a* were isolated after carbon-ion exposure that were provided by the heavy ion research facility in Lanzhou (HIRFL), China. Two progenies were picked up because their photosynthetic efficiency and the photoprotection ability were markedly different from the wild type. What is more, the proteomics studies of the two progenies were analyzed. The most differential proteins in the two progenies were from light harvesting complexes. In the other aspect, our group analyzed the chlorophyll fluorescent parameters (Fv/Fm, ϕPSII, and NPQ), the photoprotective pigment lutein, and the transcriptional expression of Lhcb1 and Lhcb2 in *Scenedesmus quadricauda* after exposure to$^{12}C^{6+}$ ions. Exposure to 20 Gy of carbon ions improved the photosynthetic efficiency of *Scenedesmus quadricauda* more quickly than exposure to 60 or 120 Gy during 48 h of culture after irradiation. The thermal dissipation by *Scenedesmus quadricauda* was initiated more quickly after exposure to 20 Gy than exposure to 60 or, 120 Gy. The transcriptional expression of Lhcb1 and Lhcb2 was up-regulated within 4 h of culture after exposure to 20 Gy of carbon ions. Thus, from the mutant strains we found that light harvesting proteins