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.

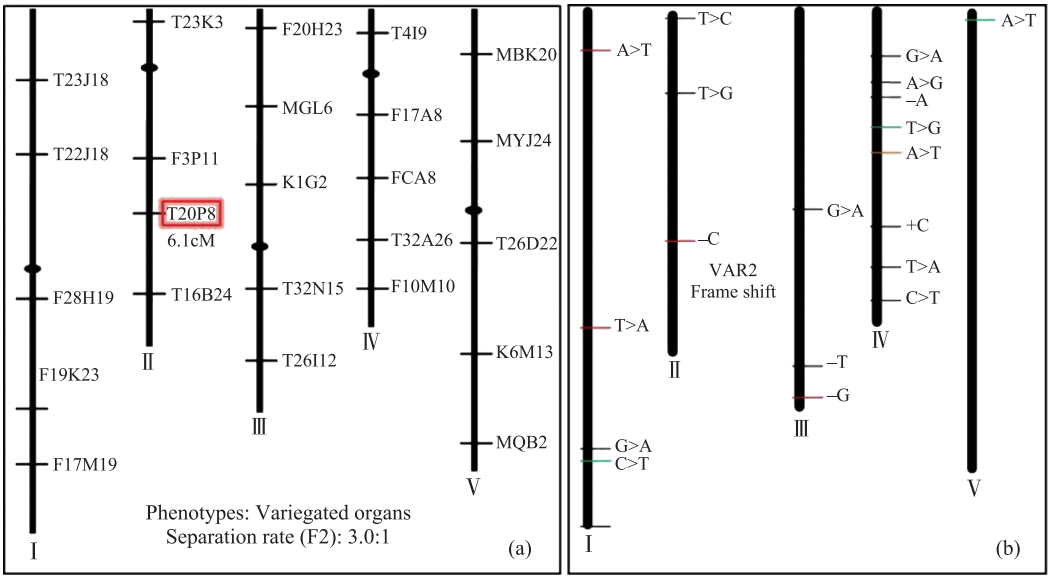

Fig. 1 Map-based cloning for responsible regions in chromosomes of *civar* (a) and mutations induced by carbon ions in *civar* genome(b).

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

Wang Jie, Li Xin, Li Wenjian, Du Yan, Wei Wei, Lu Dong, Ma Liang and Wang Jufang

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

expressed differently, and the photosynthetic characteristics of the two progenies changed markedly after carbon-ion irradiation. From mutagenic effects we found that the low dose of carbon ion irradiation caused hormetic effects on the photosynthetic efficiency, thermal dissipation ability and transcriptional regulation of the light harvesting complex II antenna proteins in *Scenedesmus quadricauda*.

4 - 37 DNA Damage in Bone Marrow Mononuclear Cells of Mice Detected by Two Dimensional Comet Assay

Wang Zhuanzi, Liu Fang, Li Wenjian, Wei Wei and Dang Bingrong

Heavy ion irradiation attract a large interest for two applications: radiotherapy and space radiation protection in manned space missions. Exposure to heavy ions radiation results in multiple effects through DNA damage induction. Single-cell gel electrophoresis or comet assay is known for its ability to detect DNA damage at the single cell level and has been used for years to assess DNA damage. It can detect low levels of DNA strand breaks in a short time, just using a few sample cells. DNA double strand breaks (DSBs) are measured at the neutral comet assay condition; under the alkaline comet assay condition both DNA single strand breaks (SSBs) and part DSBs are detected. The two dimensional comet assay is a modification of the two original comet assay, can simultaneously detect DNA SSBs and DSBs in the same human spermatozoa. If the two dimensional comet assay can be adapted to simultaneously assess different DNA break types in other cell types, DNA damage induced by heavy ion radiation can be assessed more accurately. The purpose of this paper is to validate the two-dimensional comet assay as a reliable method to simultaneously detect both DNA single and double strand breaks in the same bone marrow mononuclear cells (BMMNCs).

BMMNCs were incubated with different concentrations of H_2O_2 for 5 min to induce DNA SSBs or incubated with reaction buffer containing DNase I enzyme for different time to induce DNA DSBs, DNase I and H_2O_2 were used successively to induce SSBs and DSBs in the same cells. Then two dimensional comet assay was performed, the images were analyzed with CASPLab 1.2.2 Software.

The electrophoresis results were shown in Fig. 1. The TM and OTM of SSBs and DSBs were analyzed separately.

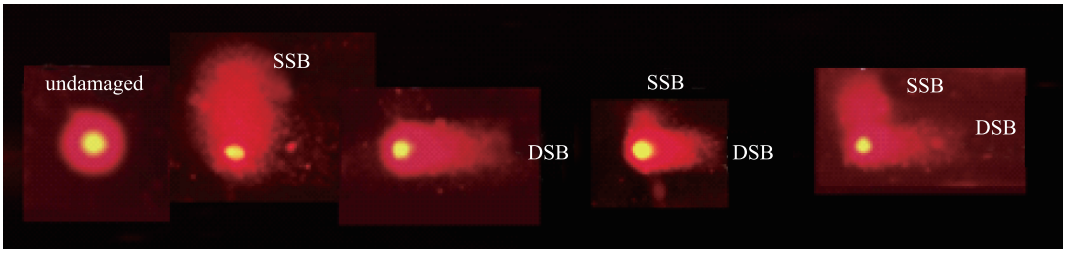

Fig. 1 Two dimensional comet assay detects different comet types.

In the BMMNCs treated with H_2O_2, the yield of SSBs increased significantly with the increase of H_2O_2 concentration, and there was no obvious DSBs (Fig. 2(a)). The results of DNase I treatment were shown in Fig. 2(b), the TM and OTM of DSBs increased significantly with the time of enzyme treatment, while SSBs remained at the control level. In the cells treated with H_2O_2 and DNase I successively, the TM and OTM of X-axis had the same trends as the results of independently DNase I treatment, and the results of Y-axis were similar to the H_2O_2induction, and no significant statistically differences were found (Fig. 2).

This study indicated that two dimensional comet assay is adapted to simultaneously detect BMMNCs SSBs and DSBs. It can be expected to more accurately assess heavy ions radiation induced BMMNCs DNA damage with this technique.