Summary Report (Peter Kamau)

Project Title: Map based cloning of *GIC* (*GIANT CHLOROPLAST*) gene (Rough mapping)

Summary

Project purpose

This project was established to identify linkage in nipponbare rice variety of a mutant gene responsible for a phenotypic change of the chloroplast size. Mutation was induced in the nipponbare rice variety using ethyl methyl sulfonide (EMS) to bring about this mutation. The nipponbare was then crossed with kasalath rice variety to develop an F2 population (Fig. 1). A backcross FI population was also developed using the nipponbare rice variety (Fig. 2). Both the F2 and F1 seeds were planted in the green house at ambient temperature conditions and the 10 day old plants were screened under a fluorescent microscope to observe for the plants with the giant chloroplast (*GIC*). DNA was isolated from plants observed to have the giant chloroplast and screened with molecular markers to identify the chromosome where the responsible mutant gene for *GIC* was located. This study aims at resolving the key question of chloroplast control in monocotyledons.

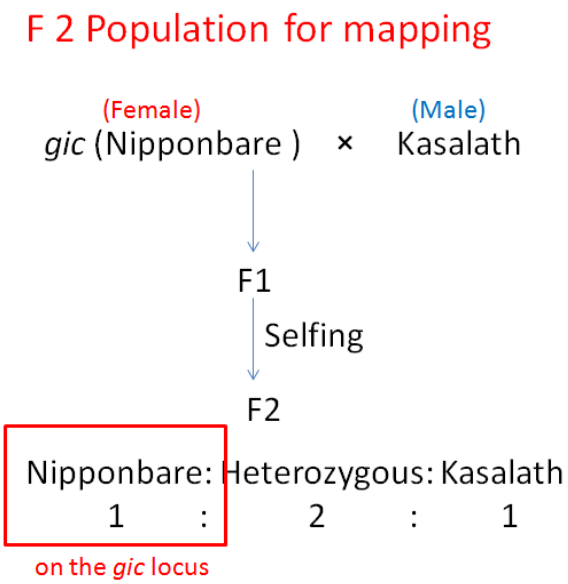

Fig 1. Schematic diagram for the development of the F2 population seeds

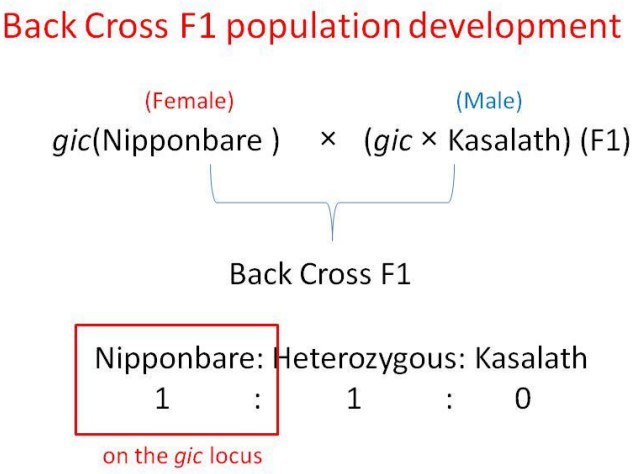

Fig 2. Schematic diagram for the development of the F1 population seeds

Objectives of the study

1. Identify the giant chloroplast in plants from the F2 and F1 population through screening using a fluorescent microscope
2. Establish linkage by identifying the chromosome where the mutant gene responsible for the giant chloroplast is located.

Outcome

Objective I. Identify the giant chloroplast in plants from the F2 and F1 population through screening using a fluorescent microscope

F2 and F1 seeds were incubated in at 30^0C for 48 hours and followed by transferring the germinating seedlings in the greenhouse. After 10 days the young seedlings were screened to observe the plants with the phenotypic change of the chloroplast. This involved taking a small section of the inner back of the stem using fine forceps and observing under a fluorescent microscope at 100x magnification (Fig. 3). The giant chloroplasts were observed to be significantly larger than the normal chloroplast in size (Fig. 4).

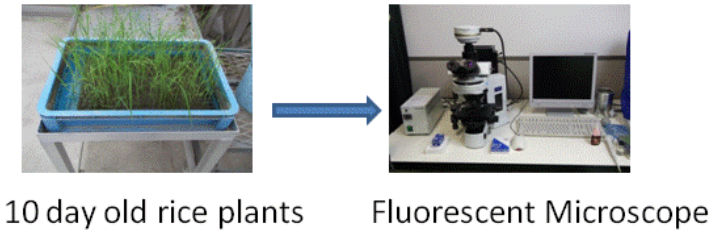

Fig 3. Ten days old F2 and F1 population were screened under a fluorescent microscope to identify plants the mutation causing the giant chloroplast phenotypic change

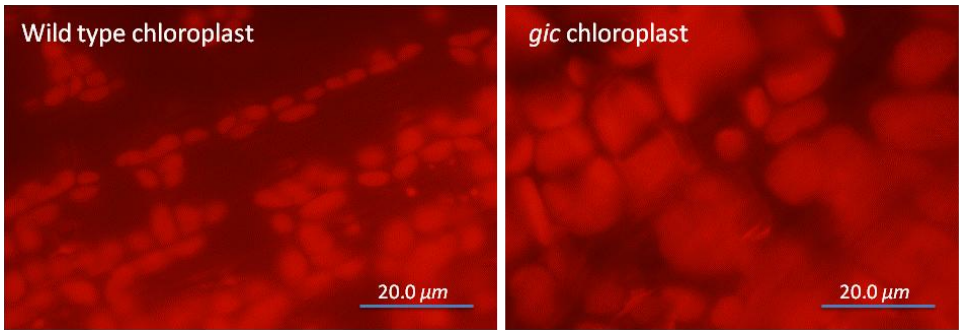

Fig 4. The giant chloroplasts were observed to be significantly larger than the normal chloroplast in the F2 and F1 population.

Objective 2. Establish linkage by identifying the chromosome where the mutant gene responsible for the giant chloroplast is located.

DNA Isolation

A portion of the leaf was taken from the F2 and F1 population observed to have the giant chloroplasts. The leaf was dried in an incubator at 50^0C for 24 hours. The dried leaf was put in an eppendorf tube and crushed in a shaker. 300ul of Isolation buffer was added and the mixture incubated at room temperature for 30 minutes. The mixture was then centrifuged at 13,000xg for ten minutes. The supernatant was then transferred to a new eppendorf tube and 1ml of absolute ethanol was added. After vortexing the mixture was centrifuged at 13,000xg for ten minutes. The supernatant was discarded and 1 ml of absolute ethanol was added again and centrifuged to clean the pellet. The supernatant was decanted out and the pellet dried at room temperature for 2 hours. 100ul of Tris EDTA (T.E.) buffer was added to the pellet to resuspend the DNA and stored at 4^0C

PCR

Rice markers (Fig 5a and 5b) were used to amplify a 100 bp region. PCR was carried out in 10 µl reactions containing, a final concentration 300 µM of each of the dNTPs, 0.5 pM of each primer, ≈ 10 ng of the template, 2 units of quick *Taq* DNA polymerase and nuclease free water. The amplification regime consisted of an initial denaturation of 94^o C 30 seconds, 30 cycles of 1 minute seconds denaturing at 94^o C, 30 seconds annealing at 55^o C and 40 seconds extension at 68^o C.

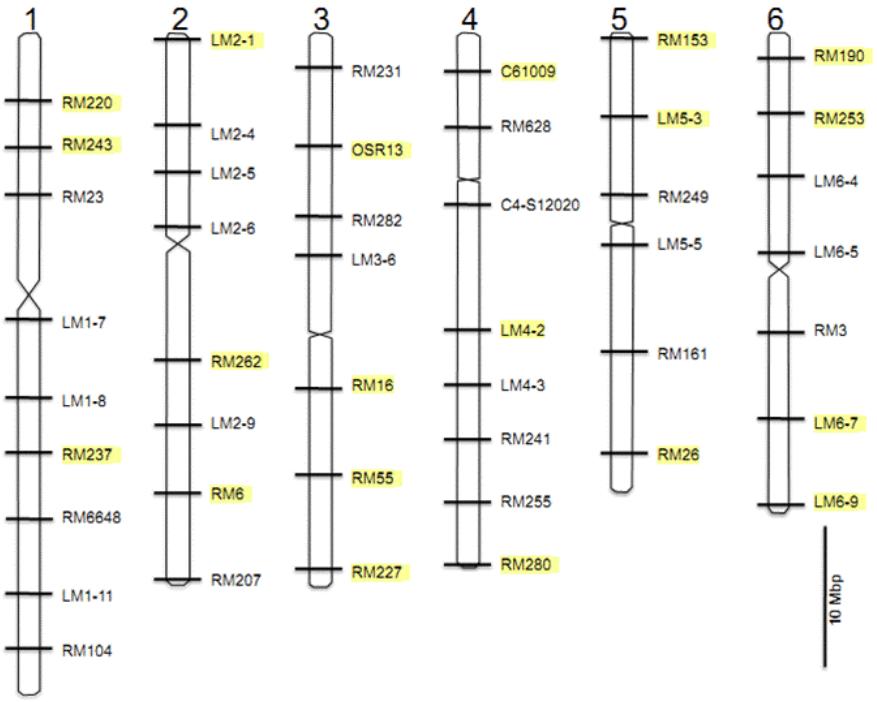

Fig 5a. Rice markers in chromosome 1-6 that were used to screen the F2 and F1 population to establish linkage. The yellow highlighted markers are the ones that were done

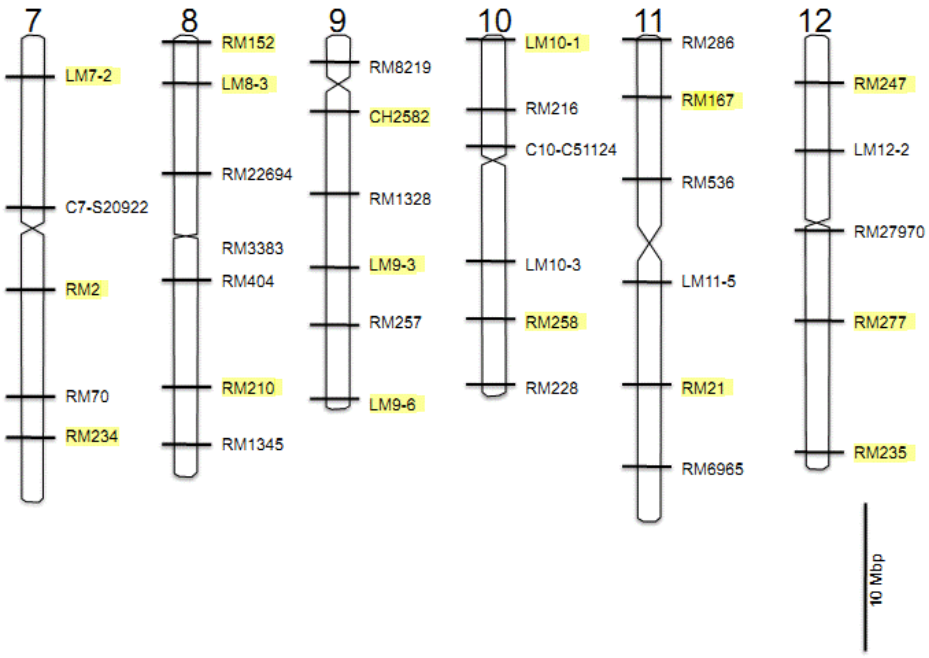

Fig 5b. Rice markers in chromosome 7-12 that were used to screen the F2 and F1 population to establish linkage. The yellow highlighted markers are the ones that were done

Linkage

Linkage was established by identifying chromosome 4 (Fig. 5a) to be the location of the mutant gene responsible for the giant chloroplast. This was achieved using the molecular marker RM280 in chromosome 4. Amplification of the F1 population had 18 out of the 19 amplicons corresponding to the nipponbare positive control (Fig. 6)

Fig 6. F1 population amplicons corresponded with the nipponbare positive control thus establishing chromosome 4 as the location of the mutant gene responsible for the giant chloroplast. K is the kasalath positive control whereas N is the nipponbare positive control.

Stay in Japan and at the Institute of Plant Science and Research (IPSR)

My stay in Japan and working at IPSR was very pleasant. Everyone at the institute and especially in Professor Sakamoto's laboratory was very friendly and helpful. They made it possible to make our two months stay in Japan very comfortable and successful in our work. I also realized Japanese people are generally polite and very hard working. Their work ethics I believe is what has made Japan such a developed country. My adviser Professor Sakamoto was kind enough to spare some time from his busy schedule to take us round in parts of Japan to enjoy the beautiful scenery such as the country side, mountains and the marvelous technology in Japan such as the suspension bridge. I also got to enjoy the local cuisine which is not found in my country such as sushi, noodles among others. I would like to thank Professor Sakamoto and everyone in his lab for making my stay in Japan memorable. Below are some of photos that captured some of these memorable moments.