Wuhan University Journal of Natural Sciences Vol. 3 No. 4 1998,495~498

Physical Location of *Helminthosporium Carbonum* Susceptibility Gene hm1 by FISH of a RFLP Marker umc119 in Maize

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Abstract **A fluorescence *in situ* hybridization (FISH) procedure was adopted to physically map a RFLP marker, umc119 near the centromere of the long arm of linkage group1 in maize. The hm1 gene (*Helminthosporium carbonum* susceptibility gene) was linked closely with the marker umc119. RFLP markers are very good landmarks for mapping genes. Therefore, we also determined the position of the gene hm1 on the chromosome based on the physical location of umc119. The disease induced by infection of *Helminthosporium carbonum* is one of the serious maize diseases and it distributes in many countries including China. Hybridization sites were showed on 1 L (long arm of chromosome1) and 5 L. The percentage distance from centromere to the hybridization site was 22. 86 on 1 L and 58. 23 on 5 L The detection rate was about 12% for mitotic cells. In interphase nuclei five hybridized sites were detected. It demonstrated that umc119 was multiplicated sequences. FISH has more advantages over *in situ* hybridization (ISH) detected by DAB for increasing the detection ratio and contrast between chromosomes and hybridization signals. The ability to detect the hybridization signal of a small low copy DNA sequence is a very important key towards wide application of FISH for plant genome mapping.**

Key words **FISH, maize, RFLP marker, *Helminthosporium carbonum* susceptibility gene hm1**

In situ hybridization (ISH) technique is a powerful tool to physically locate specific DNA sequences or genes directly on chromosomes. The sensitivity of ISH techniques for humans is now down to 0. 25 kb[1]. Many single copy genes have been located onto human chromosomes. However, the ISH of small single or low copy DNA sequences in plants was more difficult than in humans. Recent development and numerous refinements of methods including chromosome preparations and detection procedures have made it possible to detect signals of small single or low copy DNA sequences in plants[2-5]. In these studies, the signals were detected by DAB and the detection rates were only about 6% in rice and around 10% in maize. Recently, fluorescence *in situ* hybridization (FISH) has been developed rapidly. Some researches[6,7] about it have been reported in plants. Dong and Quick mapped a 2. 6 kb in size genomic DNA sequence on metaphase chromosomes of wheat and rye by FISH[8]. It was demonstrated that FISH had many advantages over ISH with enzymatic detection methods, for example, higher sensitivity and higher contrast between signals and chromosomes[7,8]. The FISH of DNA segments around 1kb in size has never been reported so far in plants. The disease induced by infection of *Helminthosporium carbonum* is a epidemic and distributed in many countries including China. To locate the hm1 gene is very important for maize breeding enhancing its resistance to the disease. RFLP markers are very good landmarks for mapping genes. Physical location of the

* Supported by the National Natural Science Foundation and Doctorate Vesting Point Foundation of the Education Committee of China
Received Mar. 10, 1998. Li Lijia; born in 1967. Ph. D
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gene could be deducted by the physical position of the RFLP markers linked closely with it with In this study, physical location of *Helminthosporium carbonum* susceptibility gene hm1 with FISH of a RFLP marker umc119 is reported. The marker umc 119 is 1.01 kb in size.

1 Materials and methods

1.1 Materials

Maize inbred line Huang Zao 4 , which derived from a native cultivar in China, was used as the tested plant material; seeds were provided by professor Song Jiancheng, Shandong Agricultural University, Shandong province, China. The tested probe RFLP marker umc119 is close to hm1 gene dictating *Helminthosporium carbonum* susceptibility 1 near the centromere of the long arm in linkage group 1 of the genetic maps[9]. It is a 1.01 kb DNA fragment cloned in pUC19 and was kindly provided by the RFLP laboratory and probe bank of the US Department of Agriculture; Agriculture Research Service, and Department of Agronomy, University of Missouri, Columbia, USA.

1.2 Methods

Chromosome preparation methods were developed using the protoplast technique as described by Song *et al.* (1994)[4] with some modifications. Root tips were collected from germinating seeds and treated in saturated α-bromonaphthalene solution for 2 h at room temperature, then washed in deionized water for 5 min. The root tips were fixed in freshly prepared methanol : acetic acid (3 : 1) fixative overnight at 4℃, washed in deionized water for 5 min. Then root tips were digested in 1% cellulase (Shanghai institute of Biochemistry, Chinese Academy of sciences) and 1% pectinase (SERVA) at 28℃ for 2.5 h. After enzymatic treatment, cells were subjected to a hypotonic treatment in water, then 2-3 root tips were squashed with fixative on cold slide and dried in air.

The probe was biotin-labeled with the procedure of the kit supplied by Sino-American Biotechnology Company China.

In situ hybridization was performed using the procedure described by Gustafson and Dille (1992)[6].

Fluorescent signals were detected based on the procedure published by Griffor *et al.* (1991)[2], Gustafson and Dille (1992)[6] and Dong and Quick (1994)[8] with some modifications. The procedure includes following steps:

1) after hybridization, the coverslip was removed by dipping the slides in a 2×SSC solution and the slides were washed in 20 % formamide (in 2×SSC) at 42 ℃ for 15 min, 2×SSC at 42 ℃ for 4 min, and PBS at room temperature for 5 min, then the slides were drained but not dried;

2) each slide was added 20 μL goat anti-Biotin antibody-FITC at a final concentration of 1 μg/mL (Sigma), covered with a coverslip, incubated in a humid chamber at 37 ℃ for 30 min and the slides were washed 3 times, each for 5 min in PBS at room temperature;

3) the slides were added 20 μL of biotinylated rabbit anti-goat antibody (Gibcol) for 30 min at 37 ℃ in a humid chamber, then were washed as above;

4) repeat step 2;

5) after washing in 2×SSC at room temperature for 5 min , PBS at room temperature for 5 min, the slides were added 10 μL (1 μg/mL) propidium iodide (PI) in an anti-fade solution (10 μg/mL p-thenylenediamine dihydrochloride), covered with a coverslip, and observed and photographed under a Zeiss fluorescence microscope.

An average of the hybridization site measurements was taken by calculating the distance from the centromere to the detection site and using that as a percentage of the arm on which the site was located. The arm ratio of the chromosome showing a detection site was also measured in order to determine on which chromosome the site was located.

2 Results and discussion

The chromosomes stained with PI appeared red and the probe signals detected with FITC showed yellow. The tested probe umc 119 was hybridized onto the long arm near the centromere of chromosome 1 and its percentage distance from hybridization site to the centromere was 22.86 (Fig. 1b, d).

The signal was also detected on the long arm of chromosome 5 and the percentage distance was

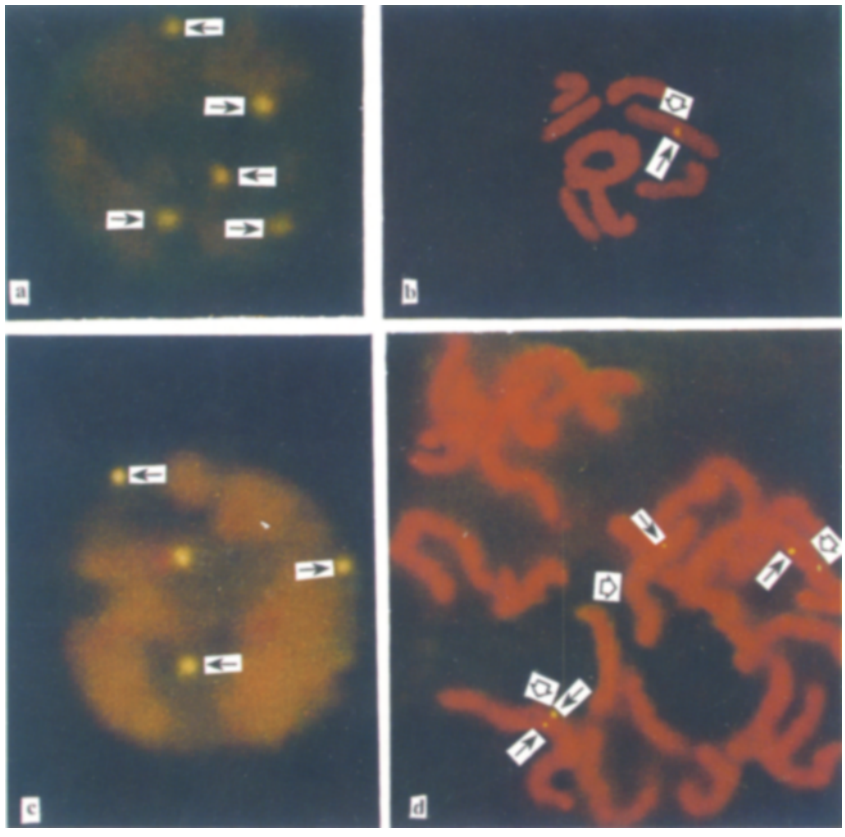

Fig. 1 a-d. The hybridized sites of the probes umc 119 on the chromosome in maize

In all figures, the solid arrows denote the hybridization signals, the open arrows denote the centromeres. a. The interphase cell has five hybridization signals of umc119. b. The hybridization signal of umc199 is in 1L. c. The interphase cell has four hybridization signals of umc119. d. The hybridization signal of umc119 is in 1L and 5L

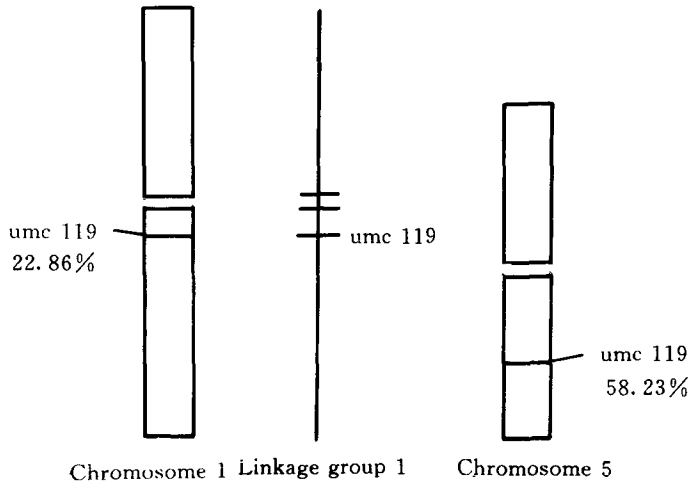

Fig. 2 The comparisons between physical map and genetic map of the RFLP marker umc119. The left and right are physical maps and the middle is the genetic map.

located in the same region (Song *et al.* 1997)[11]. It demonstrated that the maize physical map is basically consistent with the genetic map for RFLP markers umc119 and umc58. some sciemtists indicated that considerable variations existed between genetic and physical maps[2,3]. We think there appeared different relationship between genetic and physical maps in different regions. In some of them there were considerable variations and in the others this two kinds of maps might be cosistent with each other.

It was suggested that the concentration of PI should be well controlled and the probe concentration should be higher. In our experiment, PI concentration was 1.0 μg/mL and probe concentration was 500 μg/mL. The main obstacale in FISH mapping of single copy DNA in plants is chromosome preparation. In our study, the protoplast chromosome preparation method was adopted because this method developed by Gustafson and Dille (1992)[2] in ISH could get rid of the cell debris very effectively and improve the detection rate. With the improvement and development of FISH technique in mapping of single or low copy small sequence, more sequences or genes will be located on the chromosomes in plants.

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