Bot. Bull. Acad. Sin. (2001) **42:** 109-114

Comparative analyses of disease resistant and nonresistant lines from maize × *Zea diploperennis* by GISH

Wen-Hui Wei[1], Rui Qin[1], Yun-Chun Song[1,*], Le-Qun Guo[2], and Ming-Guang Gu[2]

[1]*The Key Laboratory of MOE for Plant Developmental Biology, College of Life Sciences, Wuhan University, Wuhan Hubei 430072, The People's Republic of China*

[2]*Institute of Genetics, Chinese Academy of Sciences, Beijing 100101, The People's Republic of China*

(Received April 27, 2000; Accepted October 9, 2000)

Abstract. After a cross between maize inbred line Lu9 and *Zea diploperennis* (DP) and a backcross between their F_1 and Lu9, the BC_1 was reproduced with parthenogenesis induced by chemicals. In this study a_2-(1) and a_2-(6), the two tested, stable alloplasmic sister lines, were obtained through selecting and selfing of the parthenogenetical progenies for over ten generations. The line a_2-(1) showed DP characters, such as resistance to *Helminthosporium turcium* Pass, *H. maydis* Nisik, and *H. carbonum* ULLstrup while a_2-(6) exhibited no such resistance. The introgressed DP DNA segments were successfully detected, and their physical location on chromosomes were compared in these two lines by genomic in situ hybridization (GISH). In line a_2-(1), the hybridization signals were located on the long arms of chromosomes 1, 2 and 8, and on the short arm of chromosome 6; in line a_2-(6), they were located only on the long arms of chromosomes 1, 2 and 8, with no signal on chromosome 6 at all. The distribution of introgressed DP segments on chromosomes, reasons for differences of disease resistance between these two lines, and the existence of resistance genes in the introgressed segments are discussed.

Keywords: Alien introgressed segments; Disease resistance genes; Genomic in situ hybridization (GISH); Maize; *Zea diploperennis*.

Introduction

Many fine crop varieties showing high yield, quality, and disease resistance have been obtained by distant hybridization, including maize (Guo et al., 1997; 1998), rice (Mohan et al., 1994), barley (Pickering et al., 1997), wheat (Schwarzacher et al., 1992), onion (Keller et al., 1996), and their wild relatives. *Zea diploperennis* (DP) found by Iltis et al. (1979) is the immune source for several maize viruses, and it is also resistant to maize *Helminthosporium turcium* Pass, *H. maydis* Nisik, *H. carbonum* ULLstrup, foliar and root pathogens as well as insect pests such as corn earworms, stalk borers, and rootworms. In addition, DP has stress tolerance to drought, excess water, and low temperature (Nault et al., 1981). Guo et al. (1997; 1998) crossed maize inbred lines Lu9 and 330 with *Zea diploperennis* (DP) and obtained several maize lines showing DP characters, such as disease resistance, stress resistance, high yield, and quality. Whether the DP DNA segments were steadily introgressed into the genome of these lines or not—and if so, where such segments are located—is still unknown.

Plant breeders are interested in detecting the alien DNA segments in distant hybrids. The technique of genomic in situ hybridization (GISH) has been widely used in the analyses of genomic construction and the detection of alien chromatin (Durnam et al., 1985; Pinkel et al., 1986; Schwarzacher et al., 1989; Barre et al., 1998; Humphreys et al., 1998; Khrustaleva and Kik, 1998; Kamstra et al., 1999; Liu et al., 2000; Zhang et al., 2000). In this paper, we describe the detection and location of the introgressed alien segments in two alloplasmic pure lines from inbred Lu9 × DP, one disease resistant and the other susceptible.

*Corresponding author. E-mail: ycsong@whu.edu.cn

Materials and Methods

Plant Materials

The materials were obtained by Guo et al. (1997; 1998). Maize inbred line Lu9 was crossed with *Zea diploperennis* (DP) in 1985. Subsequently the F_1 was backcrossed with its maize parent and produced BC_1 in 1986. BC_1 was reproduced parthenogenetically by treatment with chemicals in 1987. Guo et al. further selected and bred maize lines of high yield and quality with some DP characters, such as disease and stress resistance, by selfing for over ten generations, eventually obtaining several stable alloplasmic lines. Two stable, pure lines a_2-(1) and a_2-(6) and their parents, maize inbred lines Lu9 and DP, were chosen as the tested materials. According to inoculated tests in field and greenhouse (Guo et al., 1997), line a_2-(1) was resistant to maize *Helminthosporium turcium* Pass, *H.*

maydis Nisik and *H. carbonum* ULLstrup, while a_2-(6) was sensitive. They were sister lines from the same ear in 1992 and showed no prominent phenotypic differences except for the disease resistance. In the present study, the tested seeds were produced in 1998.

Chromosome Preparation

Chromosome preparation methods were developed by using the protoplast technique described by Song and Gustafson (1995) with some modifications. Root tips were treated in α-bromonaphthalene for 2 h at room temperature. The fixed root tips were immediately digested in 1% cellulase (Shanghai Institute of Biochemistry, Chinese Academy of Sciences) and 1% pectinase (SERVA) at 28°C for 2.5-3 h. The root tips were subjected to a hypotonic treatment in distilled water before being squashed.

DNA Extraction

The extraction of DP and inbred line Lu9 genomic DNA was performed using the procedure described by Ding et al. (1999). Lu9 genomic DNA, used as blocking DNA, was autoclaved for 5 min to produce 300-500 bp sequences.

Biotin Labeling of DNA and In Situ Hybridization

The DP genomic DNA was biotin-labeled with standardized procedures described by Sino-American Biotechnology Company, China. 10 µl dNTP(equal dATP, dGTP and dCTP), 5 µl 10×Buffer, 5 µl DP genomic DNA (0.2 µg/µl), 4 µl Bio-11-dUTP(0.45 µg/µl), 21 µl ddH_2O, 5 µl enzyme (equal polymerase I and DNase I) were components in a 50 µl labeling cocktail. After 1.5-2.5 h at 14-15°C, the labeling was stopped by adding 5 µl 0.2 mol/L EDTA (pH 8.0). The labeled probe was then separated through a Sepharose column and the labeling result was evaluated by means of dot blots.

In situ hybridization was performed with the procedure described by Gustafson and Dillé (1992), with some modifications. Chromosome preparations were pretreated with RNase A (100 µg/ml) in 2×SSC (0.3 mol/L sodium chloride plus 0.03 mol/L sodium citrate) at 37°C for 1 h, rinsed briefly in 2×SSC, and post-fixed in freshly depolymerised 4% paraformaldehyde for 10 min. Chromosomal DNA was then denatured by immersing the slides in 70% deionized formamide in 2×SSC at 70°C for 3.5 min, dehydrated in an ice-cold ethanol series (70%, 95% and 100%), and air-dried. The hybridization mixture contained 50% deionized formamide, 10% (w/v) sodium dextran sulphate, 2×SSC (1×SSC : 0.15 mol/L NaCl plus 0.015 mol/L sodium citrate), 0.25% (w/v) SDS, 4 µg/µl sonicated herring sperm DNA, 5 ng/µl labeled probe DP genomic DNA, 75 ng/µl blocking Lu9 genomic DNA. The blocking ratio was 1 probe DNA: 15 Lu9 DNA. The mixture was denatured in boiling water for 10 min and then placed on ice for at least 5 min; 40 µl of the hybridization mixture was applied per slide. The slides were denatured at 90°C for 10 min and hybridization was performed overnight at 37°C in a humid chamber.

Detection

The detection of fluorescent signals followed the procedure published by Dong and Quick (1995), with minor modifications. Slides were washed respectively for 10 min in 20% formamide, 2×SSC and 0.1×SSC at 42°C, in 0.1% TritonX-100 (diluted in 1×PBS) for 4 min and then in 1×PBS (0.13 mol/L NaCl, 0.007 mol/L $Na_2HPO_4 \cdot 12H_2O$ plus 0.003 mol/L $NaH_2PO_4 \cdot 2H_2O$) at room temperature for 5 min before detection.

The signals were detected with 10 µg/ml sheep avidin-fluorescein isothiocyanate (Beijing Medical Academy), 5 µg/ml biotinylated-rabbit anti-sheep, and again 10 µg/ml sheep avidin-fluorescein isothiocyanate for 1 h at 37°C. The slides were washed in 1×PBS for 15 min between each of the above two steps at room temperature. The preparations were counterstained with 3 µg/ml PI (propidium iodide) and mounted in 10 µg/ml antifade (p-phenylenediamine dihydrochloride). Slides were examined with an Olympus BX-60 fluorescence microscope. Photographs were taken on Kodak 100 colour film.

A mean 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.

Results

The results of GISH with 1:15 blocking showed that all chromosomes were red and signal spots were yellow (Figure 1).

Line a_2-(1)

Sites of hybridization were located on the long arms of chromosomes 1, 2 and 8, and the short arm of chromosome 6. The mean percent distance from the centromere to the detection site was 84.87 ± 0.08, 52.95 ± 3.12, 67.56 ± 2.43 and 73.57 ± 0.38 on chromosomes 1, 2, 8, and 6 (Figure 1A-D, Table 1), respectively. The signals were detected on two members of each among chromosomes 1, 2, 8 and 6.

Line a_2-(6)

Hybridization signals were detected on the long arms of two members of each among chromosomes 1, 2 and 8. The mean percent distance of the hybridization signals on chromosomes 1, 2, and 8 was 90.63 ± 1.27, 59.26 ± 2.07, and 73.40 ± 1.87, respectively (Figure 1E and F, Table 1).

The physical locations of alien introgressed segments on chromosomes were similar between these two lines, except those on chromosome 6, where there was no hybridization signal at all in line a_2-(6).

As a control, the chromosome preparations of inbred line Lu9 were used for GISH with the same procedure mentioned above. No signal of hybridization was detected.

Table 1. The locations of hybridization signals in lines a_2-(1) and a_2-(6).

Lines	Chromosome and arm detected signals	Arm ratios	Average percent distance from the hybridized site to the centromere	Total number of the cells observed	Number of the cells detected signals	Detection rate
a_2-(1)	1L*	1.24±0.11***	84.87±0.08***	223	77	34.53
	2L	1.25±0.07	52.95±3.12	287	105	36.59
	8L	2.81±0.11	67.56±2.43	198	83	41.92
	6S**	2.27±0.15	73.57±0.38	210	54	25.71
a_2-(6)	1L	1.23±0.12	90.63±1.27	200	64	32.00
	2L	1.27±0.06	59.26±2.07	258	96	37.29
	8L	2.90±0.07	73.40±1.87	220	96	43.64

*Long arm; **Short arm; ***Standard deviation.

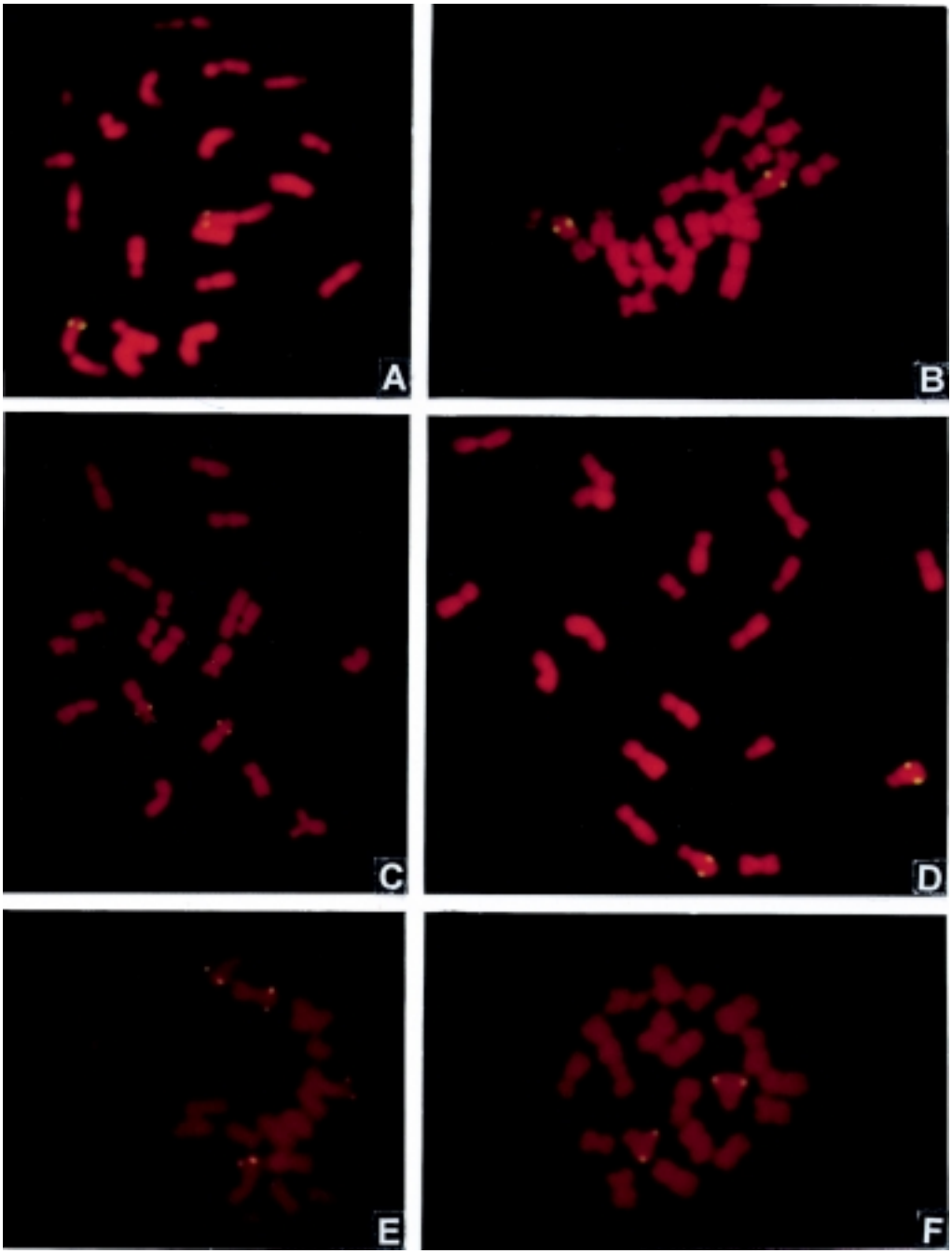

Figure 1. A-D were the detected results of alien introgressed segments in line a_2-(1); E-F were the detected results of alien introgressed segments in line a_2-(6). Double signals were detected on the homologous chromosomes. A, Hybridization signals were on the subterminal region of the long arm of chromosome 1; B, Hybridization signals were on the median part of the long arm of chromosome 2; C, Hybridization signals were on the median part of the short arm of chromosome 6; D, Hybridization signals were on the median part of the long arm of chromosome 8; E, Hybridization signals were on the subterminal region of the long arm of chromosome 1 and on the median part of the long arm of chromosome 2; F, Hybridization signals were on the median part of the long arm of chromosome 8.

Discussion

In our laboratory, several important disease resistance genes have been mapped by in situ hybridization with linked RFLP markers in maize. They include *Helminthosporium turcicum* Pass, *H. maydis* Nisik, and *H. carbonum* ULLstrup resistance genes *ht*, *htn*, *rhm* and *hm*. The gene *ht1* was located on the long arm of chromosome 2 (Li et al., 1998a), *htn1* on the long arm of chromosome 8 (Li et al., 1998b), *hm1* on the long arm of chromosome 1 (Li et al., 1998c), and *rhm* on the short arm of chromosome 6 (unpublished data). The physical locations on the chromosome arms corresponded to their positions in the genetic map (Coe, 1995). In this study, the results showed that the hybridization sites on the detected chromosome arms completely corresponded to those where *H. turcicum* Pass, *H. maydis* Nisik, and *H. carbonum* ULLstrup resistance genes *ht, htn, rhm* and *hm* were located. All the alien introgressions were located between the median and terminal regions, as were the disease resistant genes. DP was interfertile with maize (Iltis et al., 1979), meaning that during meiosis the pairing of homologous chromosomes could be performed in their hybrids. Therefore, the DP chromatin might be integrated into the maize genome by homologous or homoeologous recombination during meiosis. It can be deducted that the introgressed segments integrated in chromosomes 1, 2, 8 and 6 probably contain the genes *hm, ht, htn* and *rhm*, respectively. Chromosome microdissection developed recently is an efficient technique for the construction of specific library and the isolation of genes (Albani et al., 1993; Tian et al., 1999). We think that the introgressed segments can be isolated by microdissection based on their physical location and applied to the construction of a specific library. Then the related genes may be screened and characterized with the library if they are really positioned in these segments.

It has been demonstrated that active genes are mainly located on the distal chromosome regions (Pedersen et al., 1997). It is evident that the introgressed segments detected in this study all are located on these active gene regions, indicating that the introgressed segments may be functional genetically. We never found any signals on the centromere regions, and this can be explained by the following two reasons: (*i*) The synapses near the centromeres are hindered by some unknown factors (Khrustaleva and Kik, 1998). (*ii*) Most recombination events occur in gene-rich regions (Künzel et al., 2000).

Like a_2-(1), the line a_2-(6) also displayed the hybridization sites on chromosomes 1, 2 and 8. It did not display on chromosome 6, but it was sensitive to the diseases mentioned above. Probably resistance or sensitivity to disease depends on more than the existence or inexistence of the introgressed segments on chromosome 6 because, as we discuss above, the DP chromosome segments integrated in chromosomes 1, 2 and 8 should contain DP resistance genes. We think the line a_2-(6) might lose some regular sequences activating the resistance gene expression and that these sequences were probably contained in the introgressed segments on chromosome 6 or somewhere else. Whether this is true or not remains to be proved in further study.

The signals on each detected chromosome are all small spots instead of large blocks, a phenomenon that has also been reported by other researchers (Kamstra et al., 1997; Karlov et al., 1999; Poggio et al., 1999). We think it is to be expected that when tested stable alloplasmic maize lines are obtained by selection over many generations, the wild characters of DP, except for those associated with disease resistance, must be gradually thrown away during that process. The materials that carried large DP segments might be those which showed more wild characters and were even unstable.

As a rule, the DP DNA sequences homologous with maize should be blocked by maize genomic DNA, and most of the DP genes should be homologous with maize genes. How could the DP segments on chromosomes 1, 2, 8 and 6 not be blocked by maize genomic DNA and still show the DP DNA signals? The reason is that the unexpressed species-specific repetitive sequences probably are nonhomologous between DP and maize, and the introgressed *Zea diploperennis*-specific repetitive sequences might not be blocked by maize genomic DNA. Therefore, the signals observed should represent the unexpressed DP-specific repetitive sequences rather than the expressed ones. Studies on identifying alien chromatins and genomic construction with FISH (fluorescence in situ hybridization) of species-specific repetitive DNA sequences have been reported by some researchers (Mukai and Nakahara, 1993; Pedersen and Langridge, 1997; Kamstra et al., 1999), and they also detected the hybridization signals just like those we observed in this study. Because differences in the repetitive sequences between DP and maize were not absolute, the integrated DP chromatins were still blocked to a certain degree by maize genomic DNA with a high blocking ratio.

Acknowledgements. This work was supported by the National Natural Science Foundation of China (39870423) and the Doctorate Vesting Point Foundation of the Educational Ministry of China.

Literature Cited

Albani, D., M.J. Cote, and K.C. Armstrong. 1993. PCR amplification of microdissected wheat chromosome arms in a single “single tube” reaction. Plant J. **4:** 899-903.

Barre, P., M. Layssac, A.D. Hont, J. Louarn, A. Charrier, S. Hamon, and M. Noirot. 1998. Relationship between parental chromosomic contribution and nuclear DNA content in the coffee interspecific hybrid C. *pseudozanguebariae* × C. *liberica* var. “dewevrei”. Theor. Appl. Genet. **96:** 301-305.

Coe, E.H. 1995. Gene list and working maps. Maize Genet. Coop. Newsl. **69:** 247-258.

Ding, Y., X.P. Chen, and L. Yan. 1999. Botanical status of wild relatives to barley from the Qing-Zang plateau of China. Hereditas **130:** 111-116.

Dong, H. and J.S. Quick. 1995. Detection of 2.6 kb single/low copy DNA sequence on chromosomes of wheat (*Triticum aestivum*) and rye (*Secale cereale*) by fluorescence in situ hybridization. Genome **38:** 240-249.

Durnam, D.M., R. Gelinas, and D. Myerson. 1985. Detection of species specific chromosomes in somatic cell hybrids. Somatic Cell Mol. Genet. **11:** 571-577.

Guo, L.Q., M.G. Gu, and T.X. Yang. 1997. Studies on alloplasmic pure line derived from chemically-induced parthenogenic maize distant hybrid and its breeding. Chinese Acta Genet. Sin. **24(6):** 537-543.

Guo, L.Q., M.G. Gu, T.X. Yang, S.J. He, and Z. Zhang. 1998. Production of alloplasmic pure line through chemically-induced parthenogenesis of female gametophyte of maize distant hybrid. Develop. Reprod. Biol. **7(1):** 55-62.

Gustafson, J.P. and J.E. Dillé. 1992. Chromosome location of *Oryza sativa* recombination linkage groups. Proc. Natl. Acad. Sci. USA **89:** 8646-8650.

Humphreys, M.W., A.G. Zare, I. Pasakinskiene, and H. Thomas. 1998. Interspecific genomic rearrangements in androgenic plants derived from a *Lolium multiflorum* × *festuca arundinacea* (2n=5x=35) hybrid. Heredity **80:** 78-82 .

Iltis, H.H., J.F. Doebley, R.M. Guzman, and R. Pazy. 1979. *Zea diploperennis* (Cramineae): a new teosinte from Mexico. Science **203:** 186-188.

Kamstra, S.A., A.G. Kuipers, M.J. De Jeu, M.S. Ramanna, and E. Jacobsen. 1997. Physical localisation of repetitive DNA sequences in *Alstroemeria*: karyotyping of two species with species-specific and ribosomal DNA. Genome **40:** 652-658.

Kamstra, S.A., A.G. Kuipers, M.J. De Jeu, and M.S. Ramanna. 1999. The extent and position of homoeologous recombination in assessment of first generation backcross progenies. Chromosoma **108(1):** 52-63.

Karlov, G.I., L.I. Khrustaleva, K.B. Lim, and J.M. van Tuyl. 1999. Homoeologous recombination in *2n*-gamete producing Interspecific hybrids of *Lilium* (Liliaceae) studies by genomic in situ hybridization (GISH). Genome **42(4):** 681-686.

Keller, E.R.J., I. Schubert, J. Fuchs, and A. Meister. 1996. Interspecific crosses of onion with distant Allium species and characterization of the presumed hybrids by means of flow cytometry, karyotype analysis and genomic in situ hybridization. Theor. Appl. Genet. **92:** 417-424.

Khrustaleva, L.I. and C. Kik. 1998. Cytogenetical studies in the bridge cross *Allium cepa* × (*A. fistulosum* × *A. rylei*). Theor. Appl. Genet. **96:** 8-14.

Künzel, G., L. Korzun, and A. Meister. 2000. Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. Genetics **154:** 397-412.

Li, L.J., Y.C. Song, H.M. Yan, L. Wang, and L.H. Liu. 1998a. The physical location of the gene *ht1* (*Helminthosporium turcium* resistance 1) in maize (*Zea mays* L.). Hereditas **129:** 101-106.

Li, L.J., Y.C. Song, and H.M. Yan. 1998b. Chromosome location of two RFLP markers umc84 and umc30 tightly linked to the htn1 gene in maize. Chinese Acta Phytopathol. Sin. **28(2):** 117-121.

Li, L.J., Y.C. Song, H.M. Yan, L. Wang, and L.H. Liu. 1998c. Physical location of *Helminthosporium carbonum* susceptibility gene hm1 by FISH of a RFLP marker umc119 in maize. Wuhan Univ. J. Natural Sci. **3(4):** 495-498.

Liu, W.X., P.D. Chen, and D.J. Liu. 2000. Studies of the development of *Triticum aestivum-Leymus racemosus* translocation lines by pollen irradiation. Chinese Acta Genet. Sin. **27(1):** 44-49.

Mohan, M., S. Nair, and J.S. Bentur. 1994. RFLP and RAPD mapping of the rice *Gm*2 gene that confers resistance to biotype 1 of gall midge (*Orseolia oryzae*). Theor. Appl. Genet. **87:** 782-788.

Mukai, Y. and Y. Nakahara. 1993. Simultaneous discrimination of the three genomes in hexaploid wheat by multicolor fluorescence in situ hybridization using total genomic and highly repeated DNA probes. Genome **36:** 489-494.

Nault, L.R. and W.R. Findley. 1981. Primitive relative offers new traits for corn improvement. Ohio-Rep. Res. Dev. **66 (6):** 90-92.

Pedersen, C., J. Zimny, and D. Becker. 1997. Location of introduced genes on the chromosomes of transgenic barley, wheat and triticale by fluorescence in situ hybridization. Theor. Appl. Genet. **94:** 749-757.

Pedersen, C. and P. Langridge. 1997. Identification of the entire chromosome complement of bread wheat by two-colour FISH. Genome **40:** 589-593.

Pickering, R.A., A.M. Hill, and R.G. Kynast. 1997. Characterization by RFLP analysis and genomic in situ hybridization of a recombinant and a monosomic substitution plant derived from *Hordeum vulgare* L. × *Hordeum bulbosum* L. Crosses. Genome **40:** 195-200.

Pinkel, D., T. Straume, and J.W. Gray. 1986. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Natl. Acad. Sci. USA **83:** 2934-2938.

Poggio, L., V. Confalonieri, C. Comas, A. Cuadrado, N. Jouve, and C.A. Naranjo. 1999. Genomic in situ hybridization (GISH) of Tripsacum dactyloides and *Zea mays* ssp. *Mays* with B chromosomes. Genome **42(4):** 687-691.

Schwarzacher, T., K. Anamthawat-Jonsson, G.E. Harrison, and A.K.M.R. Islam. 1992. Genomic in situ hybridization to identify alien chromosomes and chromosome segments in wheat. Theor. Appl. Genet. **84:** 778-786.

Schwarzacher, T., A.R. Leitch, and M.D. Bennett. 1989. In-situ localization of parental genomes in a wide hybrid. Ann. Bot. London **64:** 315-324.

Song, Y.C. and J. P. Gustafson. 1995. The physical location of fourteen RFLP markers in rice (*Oryza stiva* L.). Theor. Appl. Genet. **90:** 113-119.

Tian, J., Y.F. Lu, J.X. Deng, B. Li, X.Y. Zhang, and G.T. Liu. 1999. The microdissection of addition chromosomes in *Triticum aestivum-Th. intermedium* addition line TAI-27 and the selection of specific probes. Sci. in China (Series C) **29 (2):** 174-179.

Zhang G.F., J.W. Liu, Y.Z. Huang, M. Ding, S.X. Tang, and X. Jia. 2000. Isolation and verification of *Triticum aestivum-Eremopyrum orientale* addition lines and substitution lines by GISH. Chinese Acta Genet. Sin. **27(1):** 50-55.

玉米×二倍體多年生類玉米抗病與非抗株系的 GISH 分析

魏文輝[1] 覃 瑞[1] 宋運淳[1] 郭樂群[2] 穀明光[2]

[1]武漢大學植物發育生物學教育部重點實驗室
[2]中國科學院遺傳研究所

玉米自交系旅 9 與二倍體多年生類玉米（*Zea diploperennis,* DP）雜交，F_1 與旅 9 回交，回交一代（BC_1）通過化學藥物誘導進行孤雌生殖，本研究用於實驗的兩個穩定異質姐妹系 a_2-(1) 和 a_2-(6) 是對孤雌一代進行自交並選擇了十多代以後得到的。株系 a_2-(1) 顯示出 DP 一些性狀，如抗玉米大斑病、小斑病和圓斑病，a_2-(6) 則不抗病。利用基因組原位雜交（GISH）成功地檢測了這兩個株系中滲入的 DP DNA 片段，並比較了它們在染色體上的物理位置。在 a_2-(1) 中，雜交信號定位在 1，2，8 號染色體長臂及 6 號染色體短臂，在 a_2-(6) 中，雜交信號只定位在 1，2，8 號染色體長臂，6 號染色體短臂沒有檢出雜交信號。對染色體上 DP 滲入片段的分佈，兩株系間抗病性不同的原因，以及滲入片段中存在的抗病基因進行了討論。

關鍵詞：玉米；二倍體多年生類玉米；外源滲入片段；抗病基因；基因組原位雜交。