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Directional transduction of male sterile gene *rfv₁* of NIAN type in wheat

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Abstract A new method for producing a NIAN type wheat maintenance line with the male sterile gene *rfv₁* was described. That is the variety Xinong Fp1, a 1BL/1RS translocation line, as the acceptor and *Triticum macha* var. *subletschchumicum*, a non-1BL/1RS translocation line, as the donor, a directional substitution backcross was made and confirmed by chromosome of root tip preparations and SDS-PAGE analysis. The male sterile gene *rfv₁* of *Triticum macha* var. *subletschchumicum* was transferred to the genome of Xinong Fp1. A new NIAN type wheat maintenance line with the male sterile gene *rfv₁* was bred. The method described was successful in breeding a new male sterile type for hybrid wheat production.

Keywords wheat, male sterile lines of NIAN type, 1BL/1RS translocation chromosome, gene *rfv₁*, directional transduction

1 Introduction

Tremendous economic benefit has been achieved worldwide by using male sterility for controlling pollination and producing hybrid F₁ seeds for the utilization of heterosis in many crops, such as maize, rice, and sorghum (Zhang et al., 1996). Wheat, as one of the most important crops in the world, has obvious heterosis. Efficient utilization of hybrid varieties in wheat (*Triticum aestivum*) also offers an effective way of overcoming food shortages due to their yield heterosis and the improved proteins (Singh et al., 2004).

At present, four major systems have been utilized in studying heterosis and producing commercial hybrid wheat. They are cytoplasm-nucleus male sterility (CMS),

chemical hybridizing agents (CHA), genomic male sterility (GMS) and photoperiod–temperature sensitive male sterility (PMS) (Zhang et al., 1996, 2002). In addition, there exist other methods that combine two of these four systems to produce the hybrid seeds; for example, the combinations of GMS with CHA, or PMS with CHA. According to recent researches, each method has been proven successful only after a long period of study, but there are still many problems to be studied and solved in production (Zhang et al., 1996).

CMS is divided into two groups based on the cytoplasm, namely T-type and Nian-type. The T-type is the male sterile line for those with the cytoplasm of *Triticum timopheevi*. The prominent characteristics of this type include easy maintenance and stable sterility. The deficiencies include limited restoration resources, thin seeds, pre-harvest sprouting and lower rate of germination (Wilson and Ross, 1962). T-type CMS is still dissatisfactory in practice although some of the deficiencies have been overcome.

Nian-type is the male sterile line for those with the cytoplasms of *Aegilops kotschyi*, *Ae. variabilis*, *Ae. ventricosa*, and *Ae. bicornis* (Niu et al., 2003). The features of this type of CMS cytoplasm are easy maintenance, easy restoration and seed plumpness. Some common cultivars with good agronomic performance use this CMS as for male sterile lines or restoration lines. Hence, Nian-type is considered as the most effective male sterile cytoplasm and most widely used commercially. Nian-type CMS lines exist with either 1BL/1RS or non-1BL/1RS chromosomes. 1BL/1RS male sterile lines with some cytoplasms may produce haploid lines which have unstable sterility, a broad span of fertility restoration, and poor growth potential (Tsunewaki et al., 1978; Mukai, 1983; Tsunewaki, 1988; Zhang, 1993). Recently, non-1BL/1RS male sterile lines of the Nian-type bred by the Northwest Agriculture & Forestry University have overcome the flaws of 1BL/1RS male sterile lines of Nian-type CMS (Zhang, 1993; Zhang et al., 1994). So far, only a few lines with this combination of sterility and restoration are available.

Translated from *Journal of Triticeae Crops*, 2008, 28(1): 21–24 [译自: 麦类作物学报]

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CHA is superior to CMS in random breeding hybrid combinations (Le Gouis et al., 2002; Kindred and Gooding, 2005) as CHA can induce male sterility rapidly and flexibly. It doesn't require maintenance, and male sterile lines and restoration lines do not need to be bred. In addition, a major advantage of CHA is that almost any inbred line may be used as a female parent (Adugna et al., 2004; Kofoid, 1991; Ikeguchi et al., 1999). When a female parent is sprayed with the CHA at the correct stage of growth, it represents the male sterile line and hybrid seeds can be obtained from an outcross. Hence, it requires not only the effective and nontoxic chemical hybridizing agents, but also the techniques of utilizing the chemicals to produce hybrid seeds (Mahajan et al., 2000). Recently, many elite hybrids have been successfully bred by using CHA, such as "Xiza series" was the ones marketed. However, the CHA system has a higher seed production cost (Murai et al., 2008) and some chemical hybridizing agents, which when used improperly, may decrease the vigour of stigmas and the rate of seed-setting (Zhang et al., 2002).

In view of the advantages and disadvantages of CMS and CHA, an investigation of a system was conducted using *Triticum macha* var. *subletschchumicum*, a non-1BL/1RS translocation line as the donor, and the variety Xinong Fp1, a 1BL/1RS translocation line, as the acceptor. These lines were employed to study the directional transduction of the sterile gene *rfv₁* for establishing a new system of directional sterile gene transduction in male sterile lines, developing a new method of using heterosis in wheat, and laying a substantial foundation, both theoretically and technically, for the early and wide use of Nian male sterile lines.

2 Materials and methods

2.1 Materials

Xinong Fp1, the female parent of hybrid Xiza1 bred in the Key Laboratory of Crop Heterosis, Northwest Agriculture & Forestry University, China, and 1BL/1RS translocation line was used as the acceptor. *Triticum macha* var. *subletschchumicum*, a non-1BL/1RS translocation line and carries male sterile gene *rfv₁*, was used as the donor.

2.2 Methods

2.2.1 The experimental principle and design

Tsunewaki (1988) located the male sterile gene *rfv₁* on the short arm of 1B chromosome with *Triticum macha* var. *subletschchumicum*, which provides an excellent marker to directionally transfer the male sterile gene. Generally speaking, there were two pairs of satellites on the short arms of 1B and 6B chromosomes and four satellites could

be identified clearly under the microscope in every somatic cell of common wheat. The 1BL/1RS translocation lines had two satellites on 6B chromosomes because the short arms of 1B were substituted with the short arms of 1RS of rye (Li and Zhang, 1996; Qiao et al., 2001).

Xinong Fp1 1BL/1RS recurrent parent was crossed and backcrossed to *T. macha*, till the background of Xinong Fp1 contained the non-1BL/1BS chromosome of *T. macha* which carries the sterile gene *rfv₁*. This line will be a new male sterile maintainer line. We used the root satellites and the High-molecular-weight glutenin subunit (HWM-GS) to compare them with the standard protein subunit to select the target chromosomes. Based on the result of cytological identification, the seeds with three satellites (one from 1B, and two from 6B) were selected for continuous backcrossing. After 4 to 5 generations, we inbred the plant with three satellites. The plants with homozygosis *rfv₁ rfv₁* and agronomic traits of the recurrent parent Xinong Fp1 were selected.

The experimental design is shown in Figure 1.

2.2.2 Root tips sliding of wheat mitosis

Half remaining fungicide treated seeds with the embryo of the backcross progeny were germinated in a drainable Petri dish covered with sterilized filter paper in a 25°C incubator until the roots were 1–2 cm long. Root tips (1 cm long) were collected in glass vials with some water, pre-treated for 22–24 h by putting these glass vials in cold water at 4°C. Root tips were fixed in Canoy's Fluid containing ethanol/acetic acid (3:1) for 2–3 d at room temperature, stained in 1.5% fuchsin, and squashed in 45% acetic acid. At the same time, the seeds from which the root tips were collected were planted in the pots (Li and Zhang 1996; Qiao et al., 2001; Wang and Zhang, 2006).

2.2.3 SDS-PAGE analysis

Half of the seeds from backcross progeny without the embryo were used to extract the glutenin. The separation of the glutenin was analyzed using 12% SDS-PAGE, and the gels were stained overnight with 12% (w/v) trichloroacetic acid solution containing 0.05% Coomassie Brilliant Blue R250 in the absolute ethanol (5% w/v) and detained in 10% ethanol. (Gupta, 1991; Li and Gao, 2000; Duan and Zhao, 2004; Li et al., 2003).

3 Results

3.1 Morphological characteristic of root tips chromosomes

Chromosomes of root tips of Xinong Fp1 and *T. macha* were observed under the microscope in order to identify

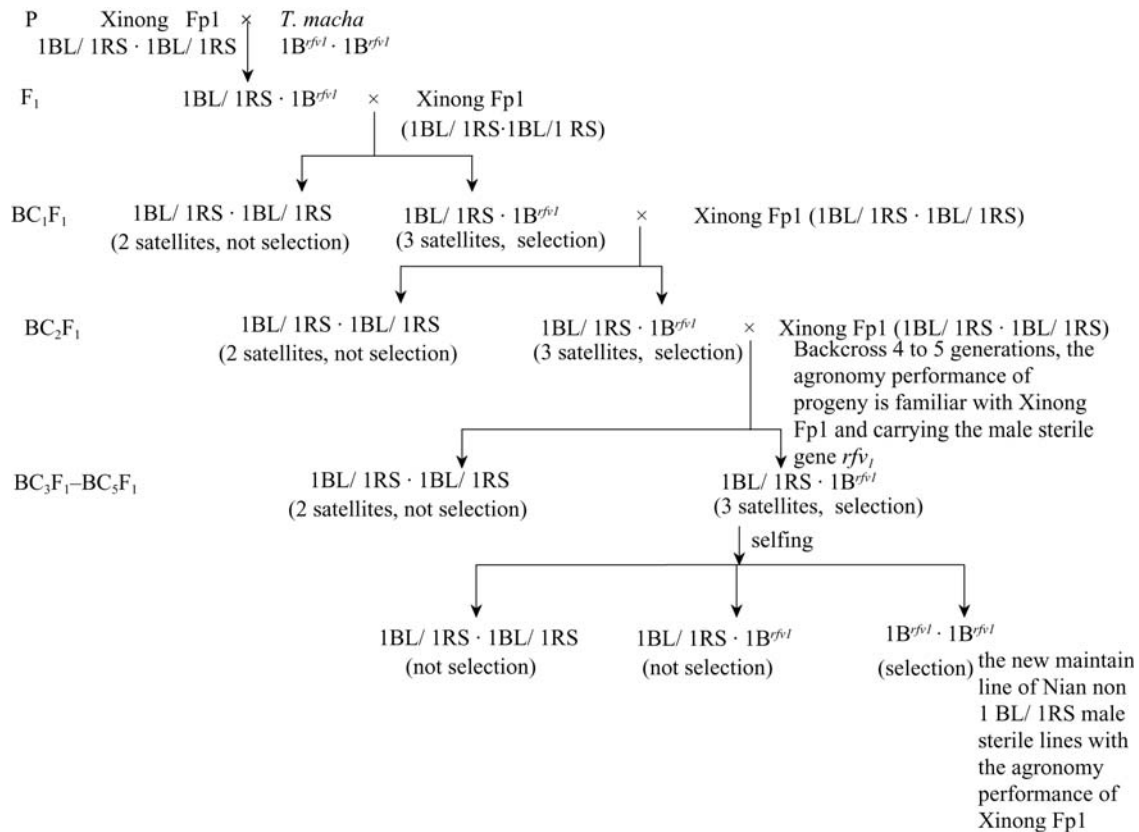


Fig. 1 The experimental design

the property of their 1B chromosome. Two satellites were observed clearly in Xinong Fp1 (Fig. 2), and four satellites in *T. macha* (Fig. 3). The result indicated that Xinong Fp1 was a 1BL/1RS translocation line, and *T. macha* was non-1BL/1RS with the male sterile gene *rfv₁*. Theoretically there are three satellites in all the F₁ cells and half of backcross progeny cells. By observing the roots tip under the inferoscope, we could see three satellites in F₁ and some of BC₁F₁ cells clearly. Cells with three satellites are 75% of the total in progeny cells observed, which is identical with the expected result (Fig. 4).

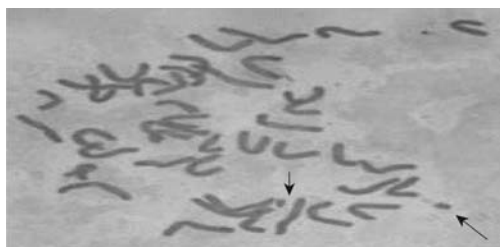


Fig. 2 Chromosomes of Xinong Fp1 root-tip
Note: The arrows show 2 satellites.

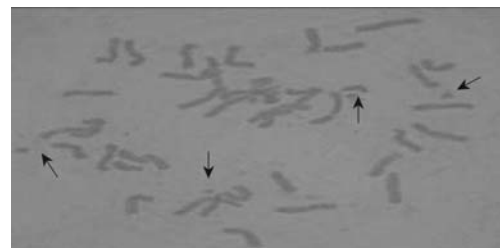


Fig. 3 Chromosomes of root-tip of *T. macha* root-tip
Note: The arrows show 4 satellites.

3.2 Characteristic of SDS-PAGE

The composition of HMW glutenin subunits of Xinong Fp1 and *T. macha* were determined by SDS-PAGE, using wheat lines Chinese Spring (N, 7+8, 2+12) as control. The results indicated that the composition of HMW glutenin subunits was different in Glu-B1 between Xinong Fp1 and *T. macha*. Xinong Fp1 contained HMW glutenin subunit 7+9 of Glu-B1, while *T. macha* contained HMW glutenin subunit 7+8 of Glu-B1. So the backcross progeny can be classified for chromosome constitution. If the HMW glutenin subunits 7+8 and 7+9 appear at the same lane on the gel, the genome of the seed will contain both the 1BL/1RS and 1B chromosomes with the male sterile gene *rfv₁*.

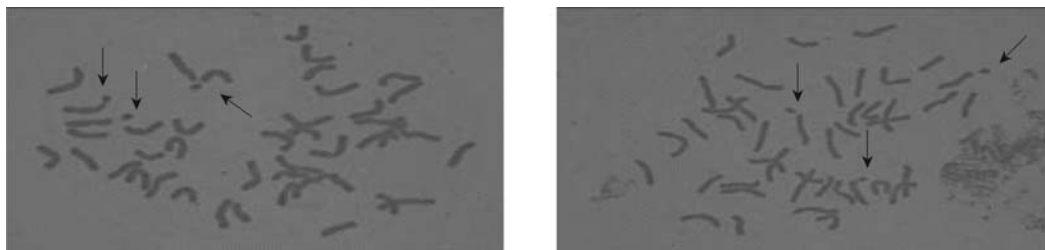


Fig. 4 Chromosomes of backcross generation's root-tip
Note: The arrows show 3 satellites contain one chromosome with 1B^{rfv₁}.

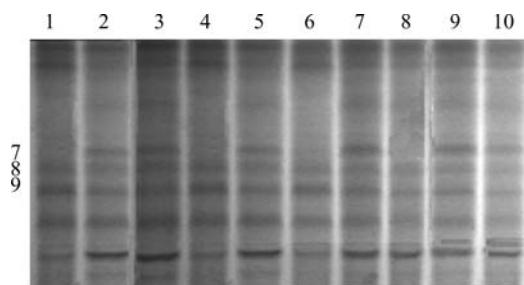


Fig. 5 Electrophoresis lanes of partial generation

In our experiment, half of the seeds without the embryo of BC₁F₁ were analyzed for HMW glutenin subunit by SDS-PAGE (Fig. 5). The results indicated that HMW glutenin subunits 7+8 and 7+9 appeared in lanes 2, 3, 5, 7, 9 and 10. So the seeds contain both 1BL/1RS chromosome and 1B chromosome. The result of SDS-PAGE coincided with the root tip identification. In this way, all other backcross progenies were classified, and it was confirmed that 1B chromosome with the male sterile gene *rfv₁* was introduced into Xinong Fp1 from *T. macha*.

4 Discussion

In order to utilize CMS cytoplasm of the Nian type to overcome some disadvantages of the CHA system used to produce the new hybrid 'XiZa 1' cultivar, the 1B chromosome with the male sterile gene *rfv₁* of *T. macha* was transferred into the female parent, and Xinong Fp1 replaced the 1BL/1BR chromosome. This provides a new maintenance line. An innovative screening method to effectively induce this transfer was described. The satellite number of the root tips was different between materials carrying the 1BL/1RS and 1B chromosomes. The 1B lines carrying the male sterile gene *rfv₁* were linked with the satellites, which supplies a method for effectively screening the target gene *rfv₁*. Furthermore, the transference of Male Sterile Gene *rfv₁* could be confirmed by SDS-PAGE. The consistency between the satellite classification and SDS-PAGE analysis demonstrated there was none or almost no recombination between the 1BL/1RS chromosome in Xinong Fp1 and 1B chromosome in *T. macha*. The above mentioned study lays a theoretical and

technical foundation for an exact and simple method in introducing the male sterile gene *rfv₁* on 1B to a 1BL/1RS line.

Acknowledgements This research was financially supported by the National Natural Sciences Foundation of China (Grant No. 301705760), the National High Technology Research and Development Program of China (No. 2002AA207004), the Agricultural Biotechnological Breeding Foundation of Yangling, Shaanxi Province, China (No. 99-1A), and Funding Project for Academic Tip-top Talent Support in Northwest Agriculture & Forestry University, China. In addition, we are grateful to Dr. I. H. DeLacy from the University of Queensland, Australia for reviewing the manuscript.

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