Science in China Series C: Life Sciences

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Inducement of chromosome translocation with small alien segments by irradiating mature female gametes of the whole arm translocation line

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***Haynaldia villosa* Schur. (syn. *Dasypyrum villosum* Candargy, 2*n*=14, VV) has been proved to be an important genetic resource for wheat improvement. The development of translocation with small alien chromosome segments, especially interstitial translocation, will be helpful for better utilization of its useful genes. Up to now, most of the reported *Triticum aestivum* – *H. villosa* translocation lines are involved in a whole arm or large alien fragments. In this paper, we report a highly efficient approach for the creation of small chromosome segment translocation lines. Before flowering, the female gametes of wheat-*H. villosa* 6VS/6AL translocation line were irradiated by $^{60}C_O$-γ ray at 160 Rad/M dosage rate and three dosages (1600, 1920, 2240 Rad). Anthers were removed from the irradiated florets on the same day and the florets were pollinated with normal fresh pollens of *T. aestivum* cv. Chinese Spring after 2–3 days. Genomic *in situ* hybridization (GISH) at mitosis metaphase of root-tip cell of M_1 plants was used to detect the chromosome structural changes involving 6VS of *H. villosa*. Among the 534 M_1 plants screened, 97 plants contained small segment chromosome structural changes of 6VS, including 80 interstitial translocation chromosomes, 57 terminal translocation chromosomes and 55 deletion chromosomes. For the 2240 Rad dosage treatment, the inducement frequencies of interstitial translocation, terminal translocation and deletion were 21.02%, 14.01%, and 14.65%, respectively, which were much higher than those previously reported. The M_2 seeds were obtained by backcrossing of 74 M_1 plants involving 146 chromosomes structural changes of 6VS, and it was found that the structural aberrations in the M_1 plants could be transmitted to their progenies. Irradiating mature female gametes of whole arm translocation is a new and highly efficient approach for creation of small segment chromosome structural changes, especially for interstitial translocations.**

irradiation, mature female gametes, small fragment translocation chromosome, wheat, *Haynaldia villosa*, genomic *in situ* hybridization

Haynaldia villosa Schur. (syn. *Dasypyrum villosum* Candargy, 2*n*=14, VV), a related species of wheat, has been identified to be resistant to powdery mildew, rusts, take all and eyespot diseases, and tolerant to drought and cold stresses[1–5]. Because of its wide genetic relationship, the chromosomes of *H. villosa* in the wheat background rarely pair and crossover with those from wheat[6,7]. Therefore, the manipulation of transferring useful genes from *H. villosa* into common wheat by recombination could be extremely difficult. However, this can be overcome by the development and use of alien translocation lines[8,9].

For the inducement of chromosome translocation between wheat and their relatives, several methods have been established and successfully used, such as ionizing

Received October 1, 2007; accepted November 19, 2007
doi: 10.1007/s11427-008-0048-2
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Supported by the National Natural Science Foundation of China (Grant No. 30270827) and Project of Higher Education for Innovation and Introduction of Resourcefullness (111 Project) (Grant No. B08025)

irradiation, tissue culture, gameticide chromosome effect and univalent chromosome mis-division of the amphiploid, alien addition lines and substitution lines[8]. Univalent chromosome mis-division can only cause whole arm translocation[10−12]. Most of the induced translocations through irradiation, tissue culture and gameticide chromosome effect using amphiploids, alien addition lines and substitution lines as initial materials were also whole arm translocation[13−15]. Because of the genetic linkage drag, many redundant genes on the chromosome arm were transferred into wheat accompanied with the interested genes in the whole arm translocations. The ideal gene transfer is to create introgression lines, which have the shortest alien chromosome segments with the target genes. These lines are cytologically more stable. Although Ph-system is a good strategy for inducing compensative translocation, it is lowly efficient and not suitable for wheat and *H. villosa* because of its wide genetic relationship[6,8]. Therefore, it is very important to explore a new approach not only to induce translocation with high frequency, but also to induce small fragment translocations, especially interstitial translocations, with high frequency.

Irradiation of dry seeds or plants with whole chromosomes rarely causes double breakage- reunion events in a single chromosome, which is necessary for creating interstitial translocations[8]. In the present study, we will explore a new, highly efficient strategy for the inducement and identification of small chromosome segment translocation using irradiation and GISH analysis. The new developed wheat-alien translocations will provide useful germplasms for wheat improvement. The results will be helpful for understanding the mechanism of chromosomal rearrangements.

1 Materials and methods

1.1 Materials

The wheat-*H. villosa* 6VS/6AL translocation line (92R137) was developed by and preserved in Cytogenetics Institute, Nanjing Agricultural University[2].

1.2 Methods

1.2.1 Irradiation of female gametes and analysis of seed-set rates of M_1 aberrant plants. The mature female gametes of the translocation line 92R137 were irradiated by $^{60}C_O$-γ ray at the dosages of 1600, 1920, 2240 Rad and the dosage rate of 160 Rad/min 2−3 d before flowering. A randomized block design with three repeats and eight plants per repeat was employed. At the suitable stage, anthers of 2−3 spikes from each plant were removed. These spikes were covered with paper bags immediately after emasculation. After 2−3 d, the emasculated florets were pollinated with normal fresh matured pollens of common wheat variety "Chinese Spring", and the produced hybrids were named M_1. The (non-irradiated 92R137×"Chinese Spring") F_1 hybrids were used as control. The structurally aberrant chromosomes involving the short arm of 6V of *H.villosa* were detected by genomic *in situ* hybridization (GISH). The frequencies of the plants with small terminal translocation, interstitial translocation and chromosome deletion of 6VS were calculated respectively with the following formula:

The inducement frequency of plants with interstitial translocation chromosome (%)=(No. of plants with interstitial translocation chromosome in M_1)/(No. of plants observed in M_1)×100;

The inducement frequency of plants with terminal translocation chromosome (%)=(No. of plants with terminal translocation chromosome in M1)/(No. of plants observed in M_1)×100;

The inducement frequency of plants with deletion chromosome of 6VS (%)=(No. of plants with deletion chromosome involving 6VS in M_1)/(No. of plants observed in M_1)×100;

The mean inducement frequency of plants with structural aberration involving in 6VS (%)=(No. of plants with structure aberration involved in 6VS)/(No. of plants observed)×100.

One-three spikes on each plant were emasculated and pollinated with fresh pollens of "Chinese Spring", while the other spikes were covered with paper bags for self-pollination. The seed-set rates of self-crossing and back-crossing were calculated using the following formula:

Self-pollinating seed-set rate (%)=(No. of the seeds in two basal florets of the developed spikelets)/(No. of the two basal florets of the developed spikelets)×100;

Backcrossing seed-set rate(%)=(No. of the backcrossed seeds)/(No. of the backcrossed florets)×100

1.2.2 Cytogenetic analysis. Chromosome preparation followed Gill's method[16]. *In situ* hybridization using the genomic DNA of *H. villosa* labeled with Fluorescein-12-dUTP as probe followed Zhang's method[17].

The signals were visualized under an Olympus fluorescent microscope, and photographed with a CCD camera.

2 Results and analysis

2.1 Identification of small segment chromosome structural changes of 6VS in the M_1 plants

The mature female gametes of 6VS/6AL translocation lines were irradiated with $^{60}C_O$-γ ray before flowering, the anther-removed florets were pollinated with normal fresh pollens of "Chinese Spring", and M_1 were obtained. GISH analysis indicated that 97 of the 534 M_1 plants had chromosome structural changes of 6VS, and the frequency was 18.16%. Two plants had only interstitial translocation chromosome and deletion chromosome, respectively. The other 95 plants had two kinds of structurally changed chromosomes of 6VS. Among them, 38 had an interstitial translocation chromosome and a deletion chromosome (Figure 1), 41 had an interstitial and a terminal translocation chromosome (Figure 2), and 16 had a terminal translocation chromosome and a deletion chromosome (Figure 3), respectively. Totally, there were 192 structurally changed 6VS chromosomes in these lines, and the numbers for interstitial translocation, ter minal translocation and deletion chromosomes were 80, 57 and 55 respectively.

2.2 Effect of different irradiation dosages on the inducement frequency

In order to optimize the condition for efficient inducement of small alien fragment chromosome structural changes, three dosages were used in a randomized block design for irradiating mature female gametes of 6VS/6AL translocation line. The mean inducement frequencies of the M_1 plants were 24.84%, 21.30% and 10.58% in the treatments of 2240, 1920 and 1600 Rad respectively, whereas that of the control was 0.00% (Table 1). All the three dosages could induce chromosome structural changes and the 2240 Rad dosage was mostly efficient. The inducement frequency of M_1 aberrant plants (all three kinds of the chromosome structural changes, including interstitial translocation, terminal translocation and chromosome deletion) was signifi-

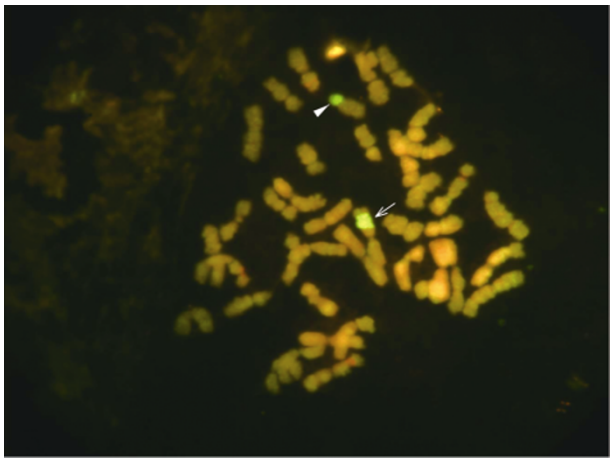

Figure 1 After genome *in situ* hybridization (GISH), the chromosome fragments of *H. villosa* at the mitosis metaphase in root-tip cells of M_1 plants turned yellow and green. The arrow shows an interstitial translocation with small fragments, and the arrow-head shows a deletion of 6VS.

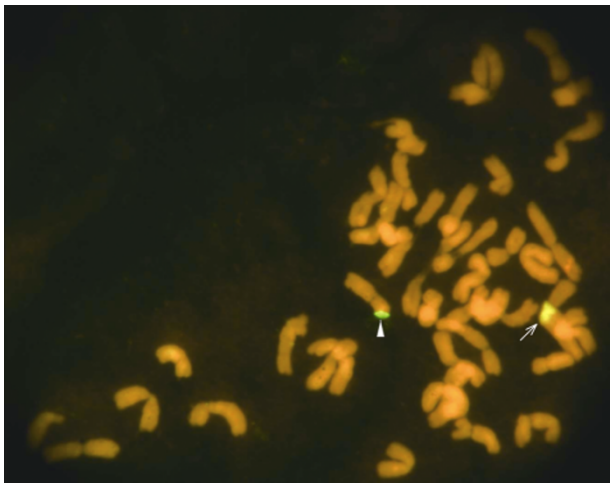

Figure 2 After genome *in situ* hybridization (GISH), the chromosome fragments of *H. villosa* at the mitosis metaphase in root-tip cells of M_1 plants turned yellow and green. The arrow shows an interstitial translocation with small fragments, and the arrow-head shows a small fragment terminal translocation of 6VS.

Figure 3 After genome *in situ* hybridization (GISH), the chromosome fragments of *H. villosa* at the mitosis metaphase in root-tip cells of M_1 plants turned yellow and green. The arrow shows a terminal translocation chromosome, and the arrow-head shows a deletion of 6VS.

cantly increased by 10.72% when the dosage increased from 1600 to 1920 Rad, but by only 3.54% from 1920 to 2240 Rad, whereas the frequency of the induced deletion was significantly increased from 1920 to 2240 Rad. For both 1920 and 2240 Rad dosages, it was observed that interstitial translocation had the precedence over the other two types. These results indicated that irradiation of mature female gametes of wheat-*H. villosa* 6VS/6AL translocation line at 2240 Rad and 160 Rad/min was the best choice to induce interstitial translocation, terminal translocation and chromosome deletion of 6VS simultaneously, especially for the interstitial translocation with a small alien fragment inserted.

2.3 Seed-set rate of the M_1 aberrant plants

A total of 534 M_1 plants were analyzed by GISH and 97 plants were found to have chromosome structural changes of 6VS. Twenty-three of the 97 were dead before heading. Self-pollinated and backcrossed seeds with Chinese Spring were obtained from 62 and 74 plants respectively. The seed-set rates of the M_1 plants with chromosome structural changes of 6VS derived from 3 irradiation dosages were calculated, and the data are shown in Table 2. Compared with those of the control, both the self-pollinated and backcrossed seed-set rates of the three irradiation dosages were significantly reduced. There was a negative correlation between the seed-set rate and the dosage applied. The seed-set rate of backcrossing was higher than that of self-pollinating for all the three dosages. The results indicated that the vigor of both male and female gametes was infected by the irradiation, with the male gametes being more sensitive. Backcrossing of M_1 plants with normal pollens of Chinese Spring contributed to the transmission of the chromosome structural changes to their progenies by escaping the use of the sterile male gametes, and this should be a critical step to improve the efficiency of saving small segment chromosome structural changes.

3 Discussion

Sears successfully created chromosome translocation between wheat and *Ae. umbellulata*, and proved that ionizing irradiation was an efficient method to induce chromosome translocation[18]. Since then, many translocations involving wheat and *H. villosa*, *S. cereal, L. racemosus*, etc., have been developed[2,14,15,19,20]. However, only one of them was interstitial translocation, in which an alien chromosome fragment was inserted into the chromosome of wheat[21]. Amphiploid, addition line and substitution line of wheat-alien species were usually used as initial materials to induce alien chromosome translocations. Ionizing irradiation more frequently induced single breakage-reunion events in the centromere or the adjacent region, and produced whole arm translocations or large alien fragment translocations between wheat and the alien chromosomes. Both the occurrence frequencies of double breakages in the same chromosome and interstitial translocations are very low[2,13,22−25].

In order to improve the efficiency for creation of interstitial translocations, instead of amphiploids, alien addition lines, or alien substitution lines, the whole arm

Table 1 Effect of different irradiation dosages on the inducement frequency of small fragment structural changes of 6VS

Treatments	Inducing frequencies of small fragment structural changes	Inducing frequencies of interstitial translocation	Inducing frequencies of terminal translocation	Inducing frequencies of chromosome deletion
2240 Rad	24.84 (39/157) a	21.02 (33/157) a	14.01 (22/157) a	14.65 (23/157) a
1920 Rad	21.30 (36/169) a	18.93 (32/169) a	12.43 (21/169) a	10.06 (17/169) b
1600 Rad	10.58 (22/208) b	7.21 (15/208) b	6.73 (14/208) b	7.21 (15/208) c
Control	0.00 (0/98) c	0.00 (0/98) c	0.00 (0/98) c	0.00 (0/98) d

The values followed by a, b, c or d within the same column are significantly different at P=0.05.

Table 2 Comparison of the seed-set rates (%) of the M_1 aberrant plants in different treatments

Treatments	No. of M_1 aberrant plants	Backcrossing seed-set rates (No. of the backcrossed seeds/No. of the backcrossed florets)	Self-pollinating seed-set rates (No. of the seeds in the two basal florets of developed spikelets/No. of two basal florets of developed spikelets)
Control	15	89.98±2.74(1096/1218) a	95.58±1.79(1190/1245) a
1600 Rad	17	82.52±2.63(1275/1545) b	76.77±8.50(1269/1653) b
1920 Rad	28	76.25±2.61(1930/2531) c	64.10±11.30(1732/2702) c
2240 Rad	29	70.18±5.58(1883/2683) d	47.82±12.93(1403/2934) d

The values followed by a, b, c or d within the same column are significantly different at P=0.05.

translocation was used in the present research for ironizing irradiation. In the whole chromosome arm translocation, one breakage in the alien chromosome will generate small segment interstitial or terminal translocations, or alien chromosome deletions. Our results confirmed this hypothesis, and we observed that not only small fragment terminal translocations and deletions but also intercalary translocations were observed at a high frequency (21.02%), which was much higher than the previously publicated records.

Seeds, mature pollens and adult plants at meiosis were usually used for irradiation. By $^{60}C_O$-γ ray treatment of mature pollens of the monosomic addition line and adult plants at meiosis of the disomic addition line, Liu et al.[22,23] obtained more than 10 intergeneric translocation chromosomes between wheat and *L. racemosus*, but none of them was interstitial translocation. By irradiation of mature pollens of *T. durum-H. villosa* amphiploid, Bie et al.[25] obtained 98 intergeneric translocatiom chromosomes, and only six of them were interstitial translocation. The average ratio of interstitial translocation for each *H. villosa* chromosome was lower than 1%, much lower than that of the present study (21.02%). To enhance the inducement frequency of translocation, especially interstitial translocation, mature female gametes of the whole arm translocation line were irradiated, and the satisfactory result was obtained. From our data, the use of mature female gametes had several advantages. First of all, female gametes were less lethal sensitive and could endure higher dosages and dosage rates. For the M_0 plants, the average seed-set rates of the three dosage treatments (1600, 1920 and 2240 Rad) were as high as 74.62 ± 7.66%, 67.43 ± 10.66% and 56.75 ± 12.29%, three dosages were all lower than the half-lethal dosages. The increase of dosage and dosage rate will significantly increase the frequency of breakage-reunion events, including double breakage-reunion events, and hence produce more chromosome structural changes, especially interstitial translocation. Secondly, female gametes were irradiated just before fertilization and they were pollinated with normal fresh pollens after irradiation. In this case, the structural aberrations had more chances to be involved in the fertilization process before restoration or elimination and transmitted to the next generation. Thirdly, these irradiated female gametes could be pollinated with mature and fresh pollens of normal wheat. This avoided the elimination of structurally aberrant chromosomes due to the fertilization competition mainly occurred in the male gametes, and high proportion of the chromosome aberrations could be saved in the M_1 plants. M_2 seeds were obtained also by backcrossing with normal fresh pollens of wheat. Various structural changes (including interstitial translocations) observed in the M_1 were recovered in the M_2 (Figures 4 and 5). Backcrossing could improve the transmission frequency and vitality of the progenies. The fertility is enhanced while the generation is increased, and hence the small fragment chromosome changes in the M_2 plants would be easily transferred to the next generation. In addition, GISH was applied in the M_1. This enabled us to detect more chromosome structural changes of alien segments in a small population.

Figure 4 The structural aberration involved in the short arm of 6V chromosome of *H. villosa* detected by GISH from backcross M_2 plants. The arrow shows interstitial translocation with small fragments, and the arrow-head shows a deletion of 6VS.

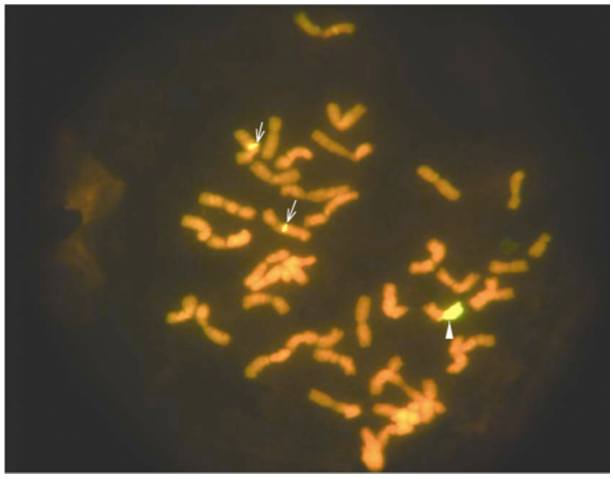

Figure 5 The structural aberration involved in the short arm of 6V chromosome of *H. villosa* detected by GISH from self M_2 plants. The arrow shows an interstitial translocation of small fragments, and the arrow-head shows a deletion of 6VS.

Using the established strategy, various chromosome changes with different breakage points and fragment sizes of chromosome 6VS have been obtained (Figure 6). They are useful germplasm not only for wheat improvement, but also for fine mapping the interest geneslocated on 6VS.

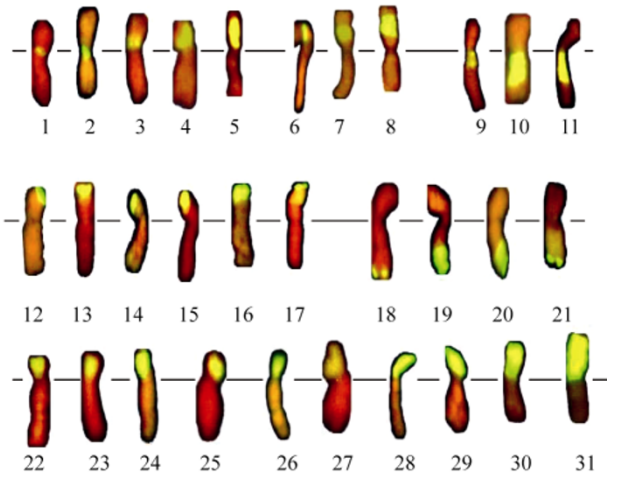

Figure 6 Some structurally changed chromosomes involving the short arm of 6V chromosome of *H. villosa* detected by GISH in M_1 plants. 1—11: Interstitial translocation chromosomes with small fragments; 12—21: terminal translocations chromosomes; 22—31: deletion chromosomes of 6VS.

4 Conclusion

Irradiation of mature female gametes of whole arm translocation line by $^{60}C_O$-γ ray at the 160 R/M dosage rate and 2240 Rad dosage, followed by pollination with fresh and mature pollens of normal common wheat could significantly increase the inducement frequency of translocation chromosome with small alien fragments and obtain more M_1 seeds. The GISH technique could also improve the accuracy and efficacy for the rapid identification of alien chromosome translocation. Backcrossing the aberrant plant (♀) with common wheat (♂) could increase the transmission frequency of the chromosome structural aberration. This should be a new, efficient technique system for the creation of small fragment chromosome structural aberrations, especially interstitially translocation, between wheat and alien species.

We are grateful to Institute for Application of Atom Energy, Jiangsu Academy of Agricultural Sciences, for their kind help with irradiation.

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