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DEVELOPMENT AND STUDY ON NEW MAINTENANCE LINE SOURCES OF 5 MALE STERILE TYPES WITH EASILY RESTORING PERFORMANCE IN COMMON WHEAT*

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Abstract The alloplasmic wheat varieties Chris with 10 different *Aegilops* cytoplasms were used as the cytoplasm donors. A whole series of 1B/1R translocation lines, such as 77(2) and others, and both progeny plants or lines of reciprocal with same sterile nucleus gene but defferent cytoplasms served as nucleus donors. Under 5 sterile cytoplasm backgrounds the new maintenance lines with much easily restoring performance were bred by crossing, repeated substitution backcrossing and sterility coverting. The results from test cross and fertility analysis indicated that: 1) the restoring degree in F_1 between the new sterile lines and some varieties or lines is all over 90%. 2) The male sterile line with *Ae. uniaristata* cytoplasm is mainly conditioned by one major recessive gene, and the sterility with *Ae. ventricosa*, *Ae. kotschyi*, *Ae. variabilis* and *Ae. bicornis* sterile cytoplasms, namely their 1B/1R sterile lines, is the sterile induced by interaction between 1RS fragment and the 4 cytoplasms because of fertility gene deficiency. 3) No a haploid plant to be found in the sterile line and F_1 with *Ae. uniaristata* cytoplasm. The haploidy induced by 1B/1R sterile lines with *Ae. ventricosa*, *Ae. kotschyi*, *Ae. variabilis* and *Ae. bicornis* cytoplasms is side effects caused by the sterile induced by special interaction of 1B/1R traslocation chromosome and the 4 cytoplasms above. But their sterile lines without haploids bred by new maintenance lines showed that the haploid plants may disappear with different nucleus genetic background of sterile line. In ad-dition, the 5 sterile lines above not only possess widly easy restorer performance but also have a certain interaction ralationships. It supplied favourable condition for simplify procedure producing hybridseed.

Key words *Aegilops*; Alloplasmic wheat; 1B/1R translocation line; Male sterile line; new maintenance line sources

With the difficulties of the limited restoration sources, the wrinkled seeds and the low germination ability encountered in sterile cytoplasm of *Triticum timopheevi*, and the problems of a certain rate of haploid and a greater variation in the restoring degree appeared in the male sterile lines of 1B/1R wheat with *Ae. kotschyi*, *Ae. variabilis* cytoplasms. They have not been widely utilized to production so far[1,2,3,4,5]. Therefore, it is very necessary to make further efforts to find new sources of male sterile cytoplasm. The present investigation is a part of our research work on new maintainer lines of *Ae. kotschyi* and *Ae. variabilis* cytoplasms and new sterile cytoplasmic resources.

1 MATERIALS AND METHODS

The 10 fertile alloplasmic wheat varieties introduced from North Dakota University, USA[5]

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used as the female parents (Table1), and the 1B/1R translocation line 77(2)[5] as the male parent. Firstly, the 10 crosses and the subsequently repeated substitution backcrossed were made. Then on the basis of fertility the fertile combinations were eliminated and the sterile ones, besides continuous backcrosses with original recurrent male parent, were widely test crossed with a great number of wheat varieties or lines, including a series of 1B/1R translocation lines, so as to identify their maintenance, restoration performance and haploid frequency. Finally, the particular combination exhibited high-sterile but difficultly maintained in the repeated substitution backcrosses was again crossed and continu-ously backcrossed with both progeny plants or lines of reciprocal cross with different cytoplasms.

The fertility was identified by seed set of selfed main stem spike after maturity, and the pollen grain was observed with acetic carmine staining to partial materials. The haploid was identified by the chromosome number of root tip; the morpha of plants and the percentage of seed set in open pollination.

2 RESULTS AND DISCUSSION

2.1 Fertility Performance of F_1

The average percentages of seed set of 3cytoplasm donors from *Ae. ventricosa*, *Ae. variablis*, *Ae. kotschyi*, *Ae. bicornis* and *Ae. uniaristata* were obviously lower than thoes of the rest. The percentages of seed set in F_1 were 6.3, 16.7, 20.4, 24.8 and 38.2% in proper order. The fertility ranged from highly to half sterile. But the average percentages of seed set of the rest 5 combinations, namely cytoplasm donors from *Ae. cylindrica*, *Ae. crassa*, *Ae. longissima*, *Ae. squarrasa* and *Ae. juvenalis*, ranged from half to low sterile. They were 47.2, 60.5, 63.5, 66.0 and 72.8% respectively (Table 1).

The results from F_1 indicate that there are certain fertility differentiations in cytoplasms tested because all F_1 are the same nuclear type and only the female cytoplasms are not quite the same.

Table 1 Fertility performances of F_1 hybrids between the alloplasmic Wheat varieties of 10 defferent Aegilops cytoplasms and 77(2)

表1 10种不同山羊草细胞质的异质小麦品种Chris与77(2)杂交F_1的育性表现

Combinations 组合	Total plants 总株数	Completely a) sterile (0) 全不育: No. of plants 株数	Completely sterile: % of total plants 占总株数	Highly sterile (1-20%) 高不育: No. of plants 株数	Highly sterile: % of total plants 占总株数	Half sterile (21-50%) 半不育: No. of plants 株数	Half sterile: % of total plants 占总株数	Low sterile (51-80%) 低不育: No. of plants 株数	Low sterile: % of total plants 占总株数	Completely fertile (81-100%) 全育: No. of plants 株数	Completely fertile: % of total plants 占总株数	Avg. % of seed set 平均结实率
(*Ae. ventricosa*)-Chris×77(2)	53	37	69.8	16	30.2							6.3
(*Ae. variabilis*)-Chris×77(2)	25			15	60.0	10	40.0					16.7
(*Ae. kotschyi*)-Chris×77(2)	27	2	7.4	14	51.9	11	40.7					20.4
(*Ae. bicornis*)-Chris×77(2)	59	29	49.2	12	20.3	18	30.5					24.8
(*Ae. uniaristata*)-Chris×77(2)	44	2	4.5	13	29.6	11	25.0	18	40.9			38.2
(*Ae. cylindrica*)-Chris×77(2)	49			12	24.5	15	30.6	15	30.6	7	14.3	47.2
(*Ae. crassa*)-Chris×77(2)	50			5	10.0	9	18.0	24	48.0	12	24.0	60.5
(*Ae. longissima*)-Chris×77(2)	49					14	28.6	25	51.0	10	20.4	63.5
(*Ae. squarrosa*)-Chris×77(2)	42					7	16.7	25	59.5	10	23.8	66.0
(*Ae. juvenalis*)-Chris×77(2)	34							23	67.6	11	32.4	72.8

Note: a) Fertility level is expressed as percentage of seed set in selfing:

$$\text{percentage of seed set in selfing} = \frac{\text{number of seeds set in basal two florets of fertile spikelets per spike}}{\text{number of fertile spikelets per spike} \times 2} \times 100$$

注：1)表头育性分类数字为自交结实百分率：

$$\text{自交结实率}(\%) = \frac{\text{每穗小穗基部两朵小花结实数}}{\text{每穗小穗数} \times 2} \times 100$$

2.2 Fertility Performance of Backcross Progenies

When the F_1 hybrids above were continuously backcrossed with the original male parent 77(2), the fertility was segregated. The fertility of the cytoplasms from *Ae. cylindrica*, *Ae. crassa*, *Ae. longissima*, *Ae. squarrosa* and *Ae. juvenalis* was normal in fertility. Such as in BC_2, the percentage of seed set was 63.7, 72.0, 70.7, 78.2, and 79.3% respectively. The fertility of the cytoplasms from *Ae. ventricosa*, *Ae. variabilis*, *Ae. kotschyi* and *Ae. bicornis* was completly sterile and only the initial generation exhibiting sterile was different(Table 2). Their anthers were all thin, dry and plumpless; although the filament extruded to glume outside, the anthers did not split; the pollen in anther was little and the few components in the pollen could only be seen using acetic carmine staining; most pollen generally kept in singlenucleus or twonuclei period; a little pollen could even develp to threenuclei period, but the male nuclei were abnormal and had no bearing ability.

Table 2　Fertility performances of backcross progenies between 4 alloplasmic wheat varieties and 77(2)

表2　4种异质小麦品种与77(2)回交的育性表现

Combinations 组合	Back-cross generation 回交世代	Total plants 总株数	Sterility 不育状况: No. of sterile plants 不育株数	Sterility 不育状况: % of[a] sterile plants 不育株率(%)	Sterility 不育状况: Sterile[b] degree 不育度(%)	Fertility 可育状况: No. of fertile plants 可育株数	Fertility 可育状况: % of fertile plants 可育株率(%)	Fertility 可育状况: Fertile[c] degree 可育度(%)	Fertility 可育状况: Fertility range 可育幅度(%)	Cenetic ratio 可育株与不育株比率	P(X^2) 机率
(*Ae. ventricosa*)-Chris×77(2)	BC_1	117	90	76.9	100.0	27	23.1	9.5	2.8-18.4		
	BC_2	39	32	82.1	100.0	7	17.9	4.2	2.8—5.6		
	BC_3	66	66	100.0	100.0	0	0	0	—		
	BC_4	115	115	100.0	100.0	0	0	0	—		
(*Ae. variabilis*)-Chris×77(2)	BC_1	45	18	40.00	100.0	27	60.0	21.1	2.5-39.5	1:1	0.1-0.25
	BC_2	65	65	100.0	100.0	0	0	0	—		
	BC_3	94	94	100.0	100.0	0	0	0	—		
(*Ae. kotschyi*)-Chris×77(2)	BC_1	33	14	42.4	100.0	19	57.6	17.4	2.6-38.5	1:1	0.25-0.50
	BC_2	40	40	100.0	100.0	0	0	0	—		
	BC_3	69	69	100.0	100.0	0	0	0	—		
(*Ae. bicornis*)-Chris×77(2)	BC_1	20	20	100.0	100.0	0	0	0	—		
	BC_2	32	27	84.4	100.0	5	15.6	5.7	2.6-8.8		
	BC_3	84	62	73.8	100.0	22	26.2	31.5	2.8—69.4		
	BC_4	115	115	100.0	100.0	0	0	0	—		
	BC_5	60	60	100.0	100.0	0	0	0	—		
(*Ae. uniaristata*)-Chris×77(2)	BC_1	34	6	17.6	100.0	28	82.4	27.6	3.3-42.9		
	BC_2	37	7	18.9	100.0	30	81.1	15.4	2.3-52.6		
	BC_3	26	0	0	—	26	100.0	30.9	18.4-57.9		
	BC_4	29	4	13.8	100.0	25	86.2	18.7	2.7-45.2		

Note: a) The rate of sterile plants is the percentage of completely sterile plants covering total ones examined.

b) $\text{Sterile degree} = \frac{\text{number of sterile florets in basal two florets of fertile spikelets per spike}}{\text{number of setting spikelets per spike} \times 2} \times 100.$

c) The fertile degree is the percentage of seed set in selfing.

注：1)不育株率(%)=全不育株数占调查总株数的百分数.

2) $\text{不育度}(\%) = \frac{\text{每穗小穗基部两朵小花的不育小花数}}{\text{每穗小穗数} \times 2} \times 100.$

3)可育度为自交结实率.

Notably, the fertility of backcross progenies in (*Ae. uniaristata*)-Chris × 77(2) has been segregated and all the percentages of the sterile plants were lower than the ones of the fertile. But the fertile degree of all fertile plants was very low, and it was stably between the highly to half sterile. Thus both progeny plants of lines of reciprocal cross with different cytoplasms were selected as male parents again. The male parent 1 was the high-sterile plant of F_1 between ms (*T. aestivum*)-Aiganzao and (*Ae. squarrosa*)-84-241, of which the average percentage of seed set in selfing was 16.3%. The male parent 2 was the progeny plant or line derived from (*Ae. squarrosa*)-84-241 ×[*ms* (*T. aestivum*) -Aiganzao × (*Ae. squarrosa*)84-241] F_1[6]. First, (*Ae. uniaristata*)-Chris was crossed with the male parent 1, and the F_1 was strictly selfed. Then the completely stierile plants selected from F_2 were successively backcrossed with the male parent 2 in pairing. Following this way, when the female parent was crossed with the màle parent 1, their F_1 were fertile, but the fertile degree was much lower (only 26.3%). When the F_1 was selfed the F_2 fertility was segregated. 42 out of 53 plants obtained were fertile and the other 11 plants were completely sterile. The rate between the fertile and sterile plants was nearly 3 : 1. The completely sterile plants segregated from F_2 were again backcrossed with the male parent 2 and the fertility from BC_1 to BC_4 was segregated, but the rate of the sterile plants in progenies was greatly increased and all plants were completely sterile in BC_5 and BC_6. The morpha of the aberrant anthers was similar to the sterility of 4 sterile cytoplasms above, but the pollen was gener-ally kept in single-nucleus or two-nuclei period.

The fertility of backcross progenies further verified the differentiation of 10 *Aegilops* cytoplasms in table 1 in fertility. Based on this differentiation, new male sterile cytoplasm can be found under special interaction between nucleus and cytoplasm.

2.3　Identification of Maintenance and Restoration Performance of 5 Male Sterile Types

The 5 male sterile types bred above were test crossed with 153 wheat varieties or lines as correspondingly as possible. When the male parents were the 1B/1R traslocation lines, such as Yanshi 9, 80(6) and so on, the plants of F_1 were all completely sterile under the sterile cytoplasm backgrounds of *Ae. ventricosa*, *Ae. variabilis*, *Ae. kotschyi* and *Ae. bicornis*. When the non-1B/1R lines were used as male parents, such as Xiaoyan 6, Xiaoyan 168 and so on, all the plants of F_1, except the haploid plants, were fertile, but the fertile degree was different in different cytoplasms and different combinations of the same cytoplasm. The average percentages of seed set of all F_1 ranged from 5.7 to 97.7%. In addition, it is a few F_1 hybrids segragated in fertility. In the sterile line with *Ae. uniaristata* cytoplasm the F_1 fertility of 4 test cross parents (89-10-139, 89-7-97, 90-7-7 and 90-7-88) out of 90 combinations were completely sterile. They only accounted for 4.4% of all test cross combinamions. The other 86 ones were all fertile, up to 95.6%. Notably, there is also obvious difference in the restoring degree of different combinations. The percentages of seed set were between 2.4 and 94.7%. Their direct reason remains to be further studied. According to the results from this study it may be considered to be related to the components of restorer gene in nucleus of every male parent of test crosses. In the test cross F_1, the fertility is conditioned by 4 different forms: there are both the major restorer gene and the modifying gene enhancing the fertility in nucleus; there only exists the major restorer gene; besides the major restorer gene, there also exists the inhibiting gene; and there only exists the modifying gene enhancing the fertility. The F_1 percentages of seed set of the 4 different nucleus genotypes are 81%～100%, 51%～80%, 21%～50% and 1%～20% in proper order. Of course, fertility may be influenced by the new genetic background of F_1 and the environment.

2.4　Haploid Frequency of Backcross Progenies and F_1 from Test Crosses

The haploid frequency of ms (*Ae. ventricosa*)-77(2), ms (*Ae. variabilis*)-77(2), ms (*Ae. kotschyi*)-77(2) and ms (*Ae. bicornis*)-77(2) were investigated and they were 13.3, 16.7, 19.7 and 3.7% respectively. The haploid plants were also found in F_1 of test crosses, but under different cytoplasm backgrounds and different combinations of the same cytoplasm the haploid frequency of F_1 was obviously different. The order of *Ae. ventricosa*, *Ae. variabilis*, *Ae. kotschyi* and *Ae. bicornis* cytoplasms was 0-50.8%, 0-55.6%. 0-77.8% and 0-25.0% respectively. It shows that there are inhibiting and enhancing effects, as for the male parent, for haploid. Notably, when the sterile lines with *Ae. ventricosa*, *Ae. variabilis* and *Ae. kotschyi* cytoplasms were successively backcrossed using 90-110 no haploid plant was found from BC_2 to the subsequent backcross generations, and when the sterile cytoplasm of *Ae. bicornis* was repeatedly backcrossed with 89-78 no haploid plant was found from BC_2 to high generations either and their progenies were all compltely sterile. Especially, both 90-110 and 89-78 are semidwarfing cultivars, and the growth vigor is normal, and the seeds are plump under sterile cytoplasms, and easily restoring performance is much highly. They can be used directly to combine the fine combinations in bybrid wheat. In the sterile line with *Ae. uniaristata* cytoplasm no haploid plant is found.

2.5 Maintenancerestoration Relationships Among 5 Sterile Cytoplasms

The results from test cross indicate that the most 1B/1R translocation lines [77(2), Shaan 7859, 78(2), 8222 and others] are all the maintainer lines of the male sterile lines with *Ae. ventricosa*, *Ae. variabilis*, *Ae. kotschyi* and *Ae. bicornis* cytoplasms, but they are all the direct restorer lines for sterile cytoplasm of *Ae. uniaristata*; The 89-7-97 is the restorer line of *Ae. kotschyi* and *Ae. variabilis* sterile cytoplasms, but completely sterile for *Ae. uniaristata* cytoplasm. The E32-1 is completely sterile for *Ae. ventricosa* cytoplasm, but normaly fertile for *Ae. variabilis*, *Ae. kotschyi*, *Ae. bicornis* and *Ae. uniaristata* cytoplasm. On the conrary, the 89-10-139 is the restorer line of *Ae. ventricosa* cytoplasm, but completely sterile for *Ae. uniaristata* cytoylasm. The 90-110 is sterile for *Ae. ventricosa*, *Ae. variabilis* and *Ae. kotschyi* cytoplasms, but completely fertile for *Ae. bicornis*. If the maintenancerestoration relationships among these sterile cytoplasms are further studied, the breeding procedure of hybrid wheat will be greatly clarylied and the single sterile cytoplasm existed in hybrid wheat utilization can also overcome so as to speed the application of bybrid wheat to production as early as possible.

REFERENCES

1 Wilson J A and Ross W M. Malesterility Interaction of *Triticum aestivum* nucleus and *Triticum t..opheevi* cytoplasm. *Wheat Inj. Serv* 1962. (14):29

2 Milohnic J. Effect of cytoplasmic male sterility on the quality of seed in wheat. *Poljopr.* 1976, 38(48):27

3 Tsunewaki K. In: *Genetic Diversity of the Cytoplasm in Triticum and Aegilops*, Japan soc. Prom. Sci., Tokyo, 1980, 237

4 张改生. 粘、易型 1B/1R 小麦雄性不育系产生单倍体的遗传机理及育性恢复性能的研究. 遗传学报, 1992, 19(3):266～277

5 张改生等, 偏型、粘型和易型小麦雄性不育系的初步研究. 作物学报, 1989, 15(1):1～10

6 张改生, 单型小麦雄性不育系的育成及其育性恢复性能的初步研究. 科学通报, 1992, 37(7):641～645

7 Zhang Gaisheng. Breeding of male sterile line of wheat with *Ae. uniaristata* cytoplasm and its performance of fertility restoration. *Chinese Science Bulletin.* 1993, 38(1):75～80

5种易恢复小麦雄性不育类型新保持系资源的建拓与研究

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摘要　以10种不同山羊草细胞质的异质小麦品种Chris为胞质供体，77(2)等一系列1B/1R易位系及两个核不育基因相同而胞质不同的正反杂交后代株(系)为核供体，采用杂交、置换回交和转育等方法，在单、偏、粘、易和二角型5种不育胞质源中，选育出易恢复性能极高的新保持系资源。测交和育性分析结果表明：(1)其不育系的F_1杂种恢复度均在90%以上。(2)单型不育性主要由一对主效隐性基因控制；偏、粘、易和二角型(1B/1R不育系)则是一种育性基因位点缺失，而仅由1RS片段与对应4种异质构成的互作不育。(3)单型不育系及F_1不产生单倍体；偏、粘、易和二角型1B/1R不育系产生单倍体是1B/1R易位染色体与4种异质专一互作后产生不育所带有的一种副效应；但从这4种异质中由新保持系资源培育出的无单倍体不育系可看出，单倍体性可随不育系核遗传背景不同而消失。此外，上述这5种异质不育系不但具有广泛的易恢复性，而且彼此间还存在着一定的互作关系，为简化制种程序提供了极方便的条件。

关键词　山羊草；异质小麦；1B/1R易位系；雄性不育系；新保持系资源

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