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ORIGINAL ARTICLE

Gene introgression from common wheat into *Aegilops* L.

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Abstract Group of experiments were carried out to verify possibility of gene introgression from common wheat into *Aegilops*. The artificial indoor crossbreed was conducted using 7 genotypes from 4 wheat relative species as female, and common wheat as male. The experiment result shows that different species has variable cross ability. Among the 4 Aegilops species, the highest cross rate is from the combination of *Aegilops tauschii* × *Triticum aestivum* (46.49% for genotype Ae42, 22.58% for Y92), the second is from *Aegilops ovata* × *T. aestivum* (14.76% for Y100, 12.11% for Ae23), the third is from *Aegilops cylindrica* × *T. aestivum* (2.23% for Ae7, 8.50% for Y145), and the lowest is from *Aegilops speltoides* × *T. aestivum* (0.19%). Hybrid embryos from different combinations have different ability of callus initiation and germination. The hybrid embryos from *A. ovata*/*T. aestivum* and *Ae. tauschii*/*T. aestivum* have a higher level of callus initiation and germination. *Ae. cylindrica*/*T. aestivum* has a middle level, while the *Ae. speltoides* has a lower level. The interspecific hybrids between *Aegilops* and common wheat have so low fertility. In back-crosses, the seed-set rate of hybrids of *Ae. ovata*/*T. aestivum* is 3.71% and 4.36% respectively back-crossed with male and female parents, while for hybrids of *Ae. cylindrica*/*T. aestivum*, they were 0 and 0.33% respectively, and for *Ae. tauschii*/*T. aestivum*, 0.33% and 0 respectively. On selfing of the hybrids, the seed-set rate is 0 (no seed set from 9750 florets) for the combination of *Ae. cylindrica*/*T. aestivum*, 0.044% (3 selfed seeds out of 6870 florets) for *Ae. ovata*/*T. aestivum* and 0 (no seed set from 7253 florets) for *Ae. tauschii*/*T. aestivum*. The research suggests that the probability of gene introgression from *T. aestivum* into *Aegilops* species is very low in nature.

1. Introduction

It is a main problem demand consideration that possibility of objective gene introgression from common wheat into relative species when transgenic wheat field release. According to the existing research on wheat origin, *Aegilops* L. is an important source of common wheat gene and has a close phylogenetic relationship with wheat. For so many favorable gene resources

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as disease-resistant, insect-resistance, stress-resistance, and high protein content, more research were put on mining of gene base, introgression of beneficial gene into wheat genetic background (Pshenichnikova et al., 2005, 2010; Kozub et al., 2004; Cenci et al., 1999; Schoenenberger et al., 2005). Along with the appearance of transgenic wheat, introgression from common wheat into Aegilops L plants and transferring stability of the gene in crossbreed offspring, both are important evaluation indexes of transgenic wheat environmental safety. Gene introgression from common wheat into Aegilops L has been found in Spain and Switzerland (Saboe et al., 2008; Guadagnuolo et al., 2001), but no related report in China.

For the difference in parent genotype and growing development environment, cross rate has differences either. More factors should be considered in intergeneric cross, such as whether encountered in parent florescence, flowering characteristic, temperature and humidity. In our research, we use 4 Aegilops L. species containing *Aegilops speltoides* (2n = 2X = BB = 14), *Aegilops tauschii* (2n = 2X = DD = 14), *Aegilops cylindrica* (2n = 4X = CCDD = 28), *Aegilops ovata* ($2n = 2X = C^uC^uM^oM^o = 28$) as female parents to cross with common wheat, back-cross and self-cross. The research focused on the largest possibility of gene introgression into Aegilops L. and provided theory basis for transgenic wheat environmental safety.

2. Methodology

In this research, the material utilized as female parents, were *A. speltoides* Ae4, 2 variation type of *Ae. tauschii*, Ae42 and Y9, 2 variation type of *Ae. cylindrica*, Ae7 and Y14, 2 variation type of *Ae. ovata*, Ae23 and Y10. All the materials above were provided by Crops science institute of Chinese academy of agricultural science. Three common wheats were used, S4185, S9306 and J1316 as male parents, they were provided by Shijiazhuang academy of agricultural science.

Female and male parent materials were planted in a greenhouse by stages to ensure flower synchronization. Emasculating before flowering of wild relatives of wheat, pollinated them dust mixed by 3 common wheats in 1–2 d, and repeated in the next day. 2 or 3 days after pollination, than dripped 10 ml 100 mg/ml 2, 4-D solution on stigma one time every day. After 12–16 d pollination, hence got crossbreed spike and count seed-set rate.

Strip immature seed pollinated 12–16 d, put them into 0.1% corrosive sublimate to sterilize 8 min, and plant the rataria after washing by sterilized water. Some of the rataria were placed in callus induction MS culture medium added 2 mg/l 2, 4-D to induce callus and secondly, employed crossbreed callus to induce seedling in MS culture medium added no hormone, while put the others in MS_0 seedling germination culture medium to seed directly, or seed by directly method of in vivo-in vitro and plant embryo foster. Then transplanted the crossbreed seedlings into a greenhouse. While flowering, we employed some of the crossbreed to back-cross with female parents and male parents (carried out the pollination and hormone treatment method repeatedly). We counted the seed-set number in 12–16 d, and carried out an embryo salvation in back-cross crossbreed. And we employed the other crossbreed to self-cross naturally, and counted the seed-set number in a month.

$$\text{back-cross seed-set rates } (\%) = ((\text{seed-set numbers}/\text{numbers of little emasculation flower}) \times 100\%$$

$$\text{self-cross seed-set rates } (\%) = (\text{seed-set numbers}/\text{numbers of counting flower Result}) \times 100\%$$

3. Results

Genetic factors decide the cross ability of female parents and male parents. The cross ability of common wheat and Aegilops L. is different for different female parents. In general, the cross ability of *Ae. tauschii* and common wheat takes the highest place. The cross seed-set rates of 2 genotypes of Ae42 and Y92 run up to 46.49% and 22.58% respectively. The cross ability of *Ae. cylindrica* and common wheat is the second, and the cross seed-set rates of 2 genotype of Ae23 and Y100 is 12.11% and 14. 76% respectively. The cross ability of *Ae. ovata* and common wheat is the third and the cross seed-set rates of 2 genotype of Ae7 and Y145 is 2.23% and 8.50% respectively. The cross ability of *A. speltoides* L. and wheat takes the lowest place and the cross seed-set rates of the genotype of Ae48 and common wheat is just 0.19% (Table 1). The cross seed-set rates of common wheat and each species of Aegilops L. are relatively stable. it has not huge difference for relative decrease of emasculation spike numbers.

Callus rate and immediate seedling rate of rataria crossed by wheat and Aegilops L are different for female parents. Cross seed-set rate of Aegilops L/common wheat is very high, however, callus rate of cross embryo is so low, about 10%. The cross rataria callus rate of *Ae. ovata*/common wheat is the second (66.7–72.1%). The cross rataria callus rate of *Ae. cylindrica*/common wheat takes the highest place (80–100%) (Table 2). Immediate seedling rate of *Ae. ovata*/common wheat cross rataria takes the highest place(75–84.2%), Aegilops/common wheat is the second (28.6–33.3%) and *Ae. cylindrica*/common wheat takes the lowest place (15.4–25%). *Ae. speltoides*/common wheat gets only 3 cross rataria, callus rate and immediate seedling rate both are 0 (0/2 and 0/1).

Cross plant of Aegilops/common wheat grows vigorous and looks like female parents and male parents. Cross fertility is different for Aegilops L., but overall performance is bad. In the condition of the natural pollination, selfing seed-set rate of

Table 1 Cross between *T. aestivum* and *Aegilops* L.

Female parent	Enotype	No. of castrated spikes	No. of pollinated florets	No. of seed setting	Ratio of seed setting
Ae. speltoides	Ae48	100	1578	3	0.19
Ae. tauschii	Ae42	82	998	464	46.49
	Y92	103	1262	285	22.58
Ae. cylindrica	Ae7	98	628	14	2.23
	Y145	67	741	63	8.50
Ae. ovata	Ae23	47	809	98	12.11
	Y100	50	956	141	14.76

Table 2 Culture results of immature hybrid embryos.

Cross combination	No. of embryos cultured for calli	Ratio of embryos inducing calli	No. of embryos cultured for seedings	Ratio of seedlings produce directly
Ae. speltoides Ae48/*T. aestivum* Ae48	2	0	1	0
Ae. *tauschii* Ae42/*T. aestivum* Ae42	197	10.2	14	28.6
Ae. *tauschii* Y92/T. *aestivum* Y92	39	10.3	6	33.3
Ae. *cylindrica* Ae7/*T. aestivum* Ae7	10	100	4	25.0
Ae. *cylindrica* Y145/T. aestivum Y145	40	80.0	13	15.4
Ae. *ovata* Ae23/*T. aestivum* Ae23	46	72.1	14	75.0
Ae. *ovata* Y100/*T. aestivum* Y100	78	66.7	19	84.2

Table 3 Selfing of interspecific hybrids.

F_1 hybrid F_1	No. of spikes	No. of florets	No. of seed setting
Ae. tauschii/T. aestivum	225	7253	0
Ae. cylindrica/T. aestivum	468	9750	0
Ae. ovata/T. aestivum	539	6870	3 (0.044)

Table 4 Backcross of interspecific hybrids.

F_1 hybrid F_1	Recurrent parent	No. of castrated spikes	No. of castrated. florets	No. of seed setting
Ae. tauschii/T. aestivum	*T. aestivum*	30	916	3 (0.33)
	Ae. tauschii	86	2432	0
Ae. cylindrica/T. aestivum	*T. aestivum*	70	1358	0
	Ae. cylindrica	70	1204	4 (0.33)
Ae. ovata/T. aestivum	*T. aestivum*	154	1886	70 (3.71)
	Ae. ovata	65	871	38 (4.36)

Ae. tauschii/common wheat and *Ae. ovata*/common wheat both are 0 and that of *Ae. ovata*/common wheat is only 0.044% (Table 3).

In backcross of repeat pollination and hormone treatment, the backcross of *Ae. tauschii*/common wheat and *Ae. tauschii* dose not set seed, the backcross seed-set rate of *Ae. tauschii*/common wheat and common wheat is 0.33%. The backcross seed-set rate of *Ae. cylindrica*/common wheat and *Ae. cylindrica* dose seed, is 0.33%. The backcross of *Ae. cylindrica*/common wheat and common wheat have no offspring. The backcross seed-set rate of *Ae. ovata*/common wheat and common wheat is higher, the backcross seed-set rate, using *Ae. ovata* as male parents is 4.36%, while using common wheat is 3.71% (Table 4).

4. Discussion

Aegilops L. genus has 5 fundamental genomes, which are C, D, M, S and U (D and S be called Balso). The genomes play an important role in the origin and evolution course of common wheat (Davoyan et al., 2012; Li et al., 2011; Timonova et al., 2013). In our research, the material involves 4 species of Aegilops L genus, containing 5 chromosome groups. Aegilops L. genus belongs to self-pollination and often cross-pollinated plants have ability of nature outcross. Cross of common wheat and *A. speltoides*, *Ae. Tauschii*, *Ae. cylindrica*, *Ae. ovata* genus all have been reported (Kozub et al., 2008; Loureiro et al., 2006a,b; Gill, 1981; Sharma and Gill, 1986), but most of the research focuses on distant related cross applying at genetic breeding. In the research, we used the 4 species of Aegilops L to cross with common wheat. In condition of embryo rescue, the easiest cross is *Ae. tauschii*/common, getting 46.49% genus cross superlatively, the second is *Ae. ovata*/common, getting 14.76% genus cross superlatively, the third is *Ae. cylindrica*/common, getting 12.11% genus cross superlatively and *A. speltoides* gets the lowest cross seed-set rate in our research. The cross of common wheat and different Aegilops L. species or different gene of the same Aegilops L species all perform different cross ability, it means that they have different potential cross ability with common wheat. Guadagnuolo et al. (2001) used common wheat and *Ae. cylindrica* to carry out pollination experiment in field, and got 1% and 7% genus cross seed, Loureiro et al. (2006a,b) got 0.39% *Ae. ovata*-common wheat genus crossbreed in experiment of field condition. The experiment result shows that the seed-set rate of *Ae. cylindrica*/common wheat exceeds 10%. In the experiment, implementing embryo rescue enhances seed-set rate, overcomes the uncertainty conducted by nature factor in field and reveals cross seed-set ability of Aegilops L. and common wheat (Sharma and Gill, 1986).

Backcross and selfing seed-set situation decide whether common wheat gene could introgress in Aegilops L. stably. In our research, we used the Aegilops L. as female parents to get crossbreed, then carried out backcross by two parents respectively. *Ae. ovata* backcross her crossbreed crossed with common wheat, the high seed-set rate is 4.36%. *Ae. cylindrica* backcross her crossbreed crossed with wheat can seed, the seed-set rate is 0.33%. The selfing of *Ae. ovata*/wheat crossbreed also can seed, the seed-set rate is 0.044%. Loureiro et al. (2006a,b) also confirmed the fact, that the backcross of *Ae. ovata*/common wheat can produce back-cross offspring (seed-set rate is 8%). Genus crossbreed of *Ae. ovata*/common wheat is better than that of *Ae. cylindrica*/common wheat for fertility. Genus crossbreed of *Ae. ovata*/common wheat and *Ae. cylindrica*/common wheat both have low fertility. Whether wheat gene can introgress into Aegilops L. genus relates to many factors, such as whether can encounter in flower, whether crossbreed pollen and female parents can back-cross or seed and whether the backcross course can continue in offspring for a long time. Each factor can affect the introgression of genus gene.

5. Conclusions

1. Though implementing repeat pollination and hormone treatment, cross and back-cross seed-set rate of Aegilops L./common wheat is low, while the nature cross and back-cross seed-set rate is lower.
2. In nature condition, it is very difficult that common wheat gene introgress into Aegilops L. and transfer in the latter population.
3. We can't overlook the possibility of some special species that become gene introgression object, such as *Ae. cylindrica* and *Ae. ovata*.

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