Creation of the technique of interspecific hybridization for breeding in cotton

LIANG Zhenglan (梁正兰)[1], JIANG Ruqin (姜茹琴)[1],
ZHONG Wennan (钟文南)[1], HE Jianxing (何鉴星)[1], SUN Chuanwei (孙传渭)[1],
QOU Zhongjin (邱仲锦)[1], LIU Diliang (刘棣良)[1], ZHANG Xinxue (张欣雪)[1],
ZHAO Guozhong (赵国忠)[2], NIU Yongzhang (牛永章)[3],
WANG Jiadian (王家典)[4], WANG Yuan (王　远)[5], LIANG Limin (梁理民)[5]
& WANG Liumin (王留民)[6]

1. Institute of Genetics, Chinese Academy of Sciences, Beijing 100101, China;
2. Shijiazhuang Academy of Agricultural Science, Shijiazhuang 050041, China;
3. Shanxi Academy of Agricultural Science, Taiyuan 030031, China;
4. Henan Academy of Agricultural Science, Zhengzhou 450002, China;
5. Shaanxi Academy of Agricultural Science, Yangling 713800, China;
6. Shandong Academy of Agricultural Science, Jinan 250100, China
Correspondence should be addressed to Liang Zhenglan (email: jxhe@genetics.ac.cn)

Received November 14, 2001

Abstract The new technique of interspecific hybridization was created in *Gossypium*, which could remarkably overcome abortion of interspecific hybridization and hybrid sterility of F_1. A large number of germplasm resources were obtained from seventy cross combinations among the cultivated species and between the cultivated and the 14 wild species, respectively. 8 varieties have been developed, of which 4 were from the cross combination of *G. hirsutum*×*G. arboretum* and the other are the first breed from the hybrids between *G. hirsutum* and 4 wild species, respectively. Of them Shiyuan 321 (jimian 24) is a new variety which had the highest increase in the national Yellow River Valley Regional test, with planting area added up to 933333 ha in the recent three years. The breeding system of interspecific hybridization was established.

Keywords: *Gossypium*, interspecific hybridization, breeding system.

Intervarietal crossing and pedigree method have been mainly used in cotton breeding for a long time. The closer relationship, narrow genetic base and poor germplasm result in the slightly increased yields of varieties and the poor fiber strength and adversity resistance. Therefore, cotton breeders consider that the technique of introgression of useful characteristics from wild species into cultivars should be an important breeding method[1,2].

1 Materials and methods

1.1 Experimental materials

14 wild species and 4 cultivated species (including 52 varieties) were used.

1.2 Experiment design and methods

Overall design included three steps: (i) The isolation mechanisms of interspecies were approached and the effective method for overcoming interspecific incompatibility was established. (ii) The advanced hybrid populations were obtained among cross combinations, and the peculiar genetic law of breeding of interspecific hybridization was discovered. (iii) The new varieties and new germplasms with excellent characteristics were developed.

2 Results

Three research goals in the above designs have been achieved through continuous experiments in the past 27 years, and a new system of breeding of interspecific hybridization has been established in *Gossypium*. The system consists of the following results.

2.1 Establishment of a new point of view on genetic isolation and a new technique system of interspecific hybridization in *Gossypium*

On the basis of genetics, reproductive physiology and experiments for many years, a new concept that the incompatibility of interspecific hybridization was, in essence, due to the disturbance of phytohormones metabolism in generative organ of the maternal plant, was advanced. Owing to disturbance of hormones, the high concentration of phytohormones and high activities of enzymes could not be formed in the processes of fertilization. Therefore, the transport channel of nutrients from maternal tissues (nucleus, integument ovary) to hybrid embryo was blocked. Consequently, the endosperm degenerated and the embryo died at the early stage. By spraying or dropping with phytohormones the metabolic function of hybrid endosperm and embryo could be improved remarkably, which has been confirmed by a series of tests. The results showed that compared with non-spraying group, the number of seeds with good developmental endosperm increased 45-fold, and the number of large embryos increased 42-fold[3—5].

Based on the above result, a new technique system was established, which combined the phytohormones (GA_3 and NAA) spraying trails with embryo culture *in vitro* and colchicine treatment (7.5—10 mg/L) in test tube into a complete program. Compared with the previous methods, this method had the following advantages: (i) The boll setting percentage was 30%— 100%, while it was 0%—5% by the previous methods. (ii) In general, the number of large embryos per 100 pollinated flowers was 119—1600, while 0—15 by the previous methods. Both increased more than 10—30-fold. (iii) The fertile F_1 plant was 25%—100%, which also increased significantly compared with that by the previous methods. In addition, the procedure of the new technique was single, and could be done in the same year when the cross was made, and the fertile F_1 plant could be obtained in the winter in a green house[6—8] (see tables 1 and 2).

2.2 Successful crosses were made between *G. hirsutum* and 14 wild species, and high generation hybrids (10—20 generations) were obtained.

In our serial experiments, we first made successful crosses in 70 combinations from the

Table 1 Comparison of percentage of boll setting and embryo development between various combinations of interspecific hybridization in *Gossypium*

Cross combination	No. of flower pollinated	No. of boll with large embryo	Setting boll (%)	No. of embryo arvested	Size of embryo (length × width, mm)	No. of large embryo per 100 flowers pollinated
Acalasj-1 $(AD)_1$×*G. raimondii* (D_5)	26	21	80.8	80	(7×2.5)—(9.5×4)	307.7
Acalasj-1 $(AD)_1$×*G. sturtianum* (C_1)	2	2	100.0	32	(7×2)—(7×3)	1600.0
Delfos184 $(AD)_1$×*G. sturtianum* (C_1)	1	1	100.0	14	(9×4)—(10×4)	1400.0
86-1 $(AD)_1$×*G. sturtianum* (C_1)	8	7	87.5	78	(6×2)—(8×3)	975.0
G. herbaceum (A_1)×*G. sturtianum* (C_1)	15	12	80.0	56	(2.5×1.5)—(4×3)	373.3
Keyi 181 $(AD)_1$×*G. anomalum* (B_1)	10	8	80.0	74	(5×1.5)—(10×5)	740.0
Shan 401-1 $(AD)_1$×*G. anomalum*	4	1	25.0	7	(5×2)—(9×3)	175.0
Keyi 2 $(AD)_1$×*G. anomalum*	7	5	71.4	29	(5×2)—(10×4.5)	328.6
86-1$(AD)_1$×*G. gossypioides* (D_6)	6	3	50.0	46	(5×1.5)—(7×2.5)	766.6
Delfos 429 $(AD)_1$×*G. longicalyx* (F_1)	49	3	6.1	4	3.5×2.5	8.2
86-1 $(AD)_1$×*G. stocksii* (E_1)	74	16	21.6	34	(3×1)—(7.9×3.1)	45.9
Keyi 181 $(AD)_1$×*G. bickii* (G_1)	54	8	14.8	11	(4.5×1)—(11×6)	20.4
Wantzu×*G. bickii* (G_1)	124	100	80.8	551	(2.5×1.5)—(5×3)	444.4
Keyi 2 $(AD)_1$×*G. davidsonii* ($D_{3\text{-}d}$)	24	20	83.0	53	(3×1)—(7×2.5)	220.8
Keyi 2 $(AD)_1$×*G. thurberi* (D_1)	28	9	32.1	19	(3×1)—(6×3.5)	68
Yunhai $(AD)_2$×*G. thurberi* (D_1)	39	13	33.3	31	(3×2)—(6×3)	79.5
Giza45 $(AD)_2$×*G. thurberi* (D_1)	23	14	60.9	27	(6.5×3.6)—(8×5)	119.4
Keyi 181 $(AD)_1$×*G. trilobum* (D_8)	80	4	5.0	24	10.1×5.5	30.0
Shan 1155 $(AD)_1$×*G. trilobum* (D_8)	45	3	6.5	5	7.8×4.2	11.1
86-1$(AD)_1$×*G. somalense*(E_2)	30	12	40.0	43	(5.5×2)—(9.6×2.1)	143.3
Keyi 2 $(AD)_1$×*G. arboreum* (A_2)	8	8	100.0	29	(3×1)—(2.5×7)	362.5

Table 2 Advances of cotton breeding in cultivated species×wild species

Cross combinations	Hybrid generation (1997)	Results of breeding
G. hirsutum×*G. anomalum* [$(AD)_1$×B_1]	BC_2F_{14}, BC_3F_{13}	new variety Jin. 21 and fine quality lines
G. triphyllum×*G. hirsutum* [B_2×$(AD)_1$]	F_1	breeding material
G. hirsutum×*G. sturtianum* [$(AD)_1$×C_1]	BC_2F_{12}, BC_1F_{13}	new variety Qinyuan 4 and wilt resistance lines
G. herbaceum×*G. sturtianum*(A_1×C_1)	BC_1F_1	breeding material
G. herbaceum×*G. australe*(A_1×C_3)	BC_2F_2	breeding materials
G. herbaceum×*G. sturtianum*×*G. hirsutum* ([A_1×C_1×$(AD)_1$]	BC_2F_{12}	new lines of frost resistance
G. herbaceum×*G. australe*×*G. hirsutum*[A_1×C_3×$(AD)_1$]	BC_2F_6	good fibre and Aphis resistances
G. barbadense×*G. thurberi* [$(AD)_2$×D_1]	F_1BC_1	breeding material
. *barbadense*×*G. thurberi*×*G. hirsutum* [$(AD)_2$×D_1×$(AD)_1$]	BC_2F_{10}	new variety Shiyuan 321 and 6 types of new germplasms
G. barbadense×*G. klotzschianum*[$(AD)_2$×$D_{3\text{-}k}$]	F_3	breeding materials
G. barbadense×*G. raimondii*[$(AD)_2$×D_5]	BC_2F_{11}, BC_1F_{13}	long fiber and high lint percentage lines
G. barbadense×*G. gossypioides*[$(AD)_2$×D_6]	BC_1F_{11}	new lines
G. hirsutum×*G. trilobum*[$(AD)_1$×D_8]	BC_2F_7	high fiber strength lines
G. hirsutum×*G. stocksii*[$(AD)_1$×E_1]	BC_2F_7	high fiber strength and dry resistance lines
G. hirsutum×*G. somalense*[$(AD)_1$×E_2]	BC_2F_7, BC_1F_8	new strain Shiyuan 406 and specially long fiber lines
G. barbosanum×*G. hirsutum*[E_3×$(AD)_1$]	F_1	breeding materials
G. hirsutum×*G. bickii*[$(AD)_1$×G_1]	BC_3F_{13}, BC_4F_{12}	new variety Jin. 27 and new germplasms
G. herbaceum×*G. bickii*(A_1×G_1)	F_1	breeding materials
G. hirsutum×*G. hirsutum* ssp. *mexicanum*	BC_2F_{11}	new lines of dry resistance
G. hirsutum×*G. mustelinum*[$(AD)_1$×$(AD)_4$]	BC_1F_7	new lines of high fiber strength

crosses of *G. hirsutum* and *G. barbadense* with *G. arboreum* and *G. herbaceum*, and then a lot of hybrids were also obtained from 14 wild species crossed with the cultivated species. Then a great number of new germplasms, new strains and new varieties derived from the hybrids involving 13 wild species were selected (see tables 2 and 3).

Table 3 New varieties of interspecific hybrids in *Gossypium*

Varieties	Cross combinations	Date of approval	Superior to CK (%)[a)]		Cumulative planting area by 1999 (ha.)[b)]
			regional test	performance test	
'Shiyuan 321' (Ji 24)	*G. barbadense*×*G. thurberi*×*G. hirsutum*	1996.4	19.7	26.5	933.3
Yuan 91406 (Qinyuan 4)	*G. hirsutum*×*G. sturtianum*	1998.2	13.8	17.2	20.3
Yuan 820 (Jin 21)	(*G. hirsutum*×*G. anomalum*)×(*G. hirsutum*×*G. thurberi*)	1997.4	11.3	17.1	16.7
Yuan 2918 (Jin 27)	(*G. hirsutum*×*G. bickii*)×(*G. hirsutum*×*G. thurberi*)	1999.5	11.0	15.0	0.3
Yuan 394 (Yu 11)	*G. hirsutum*×*G. arboreum*	1994.4	10.2*	10.1	415.3
Yuan 2 (Qinli 514)	*G. hirsutum* × *G. arboreum*	1990.12	18.9	20	55.5
Yuan 3 (Qinli 534)	*G. hirsutum*×*G. arboreum*	1990.12	22.0	23	38.7
Shiyuan 345	*G. hirsutum*×*G. arboreum*				
Total					1479.8

a) CK of Qinli 534, Qinyuan 4, Jin 21, Jin 27 are Liao 7, Zhongmiansuo 19, Jin 6, Jin 10, respectively, and the others are Zhongmiansuo 12. b) Data from the national cotton variety joint test.

2.3 New germplasms with various extra fine traits were obtained

Those new germplasms were examined by appraisal committee, and possessed 1—2 extra fine traits each.

They were (i) extra fiber strength (27.4—33.3 cN/tex); (ii) superior fiber length (36.5—40.6 mm); (iii) high lint percentage (47%—48.6%); (iv) fine fiber (micronaire reading 3.7—4.0); (v) big boll (7.6—9.95 g); (vi) aphis resistance (degree I); (vii) bollworm resistance (degree I); (viii) both *Fusarium* and *Verticillium* wilt resistance (disease index *Fusarium* wilt 0—8, *Verticillium* wilt 8—25); (ix) drought resistance (degree I, superior to ck BPA_{68}); (x) red flower purple spot *G. hirsutum* type new germplasm, of which those derived from the hybrid of *G. hirsutum*×*G. bickii* and *G. hirsutum*×*G. somalense* were selected for the first time[7—10].

2.4 Eight new varieties and eight new strains were bred

All of 8 new varieties possessed the characteristics of high yield, fine quality and multi-adversity resistance, of which 4 varieties were derived from the hybrids of *G. hirsutum*×*G. arboreum*, and the others were selected for the first time from the hybrids of *G. hirsutum*, *G. barbadense* with 4 wild species, respectively[9,11,12].

Among those 'Shiyuan 321' is the best, of which lint yield was 19.7% higher than that of the control 'Zhongmiansuo 12', ranking first in the regional cotton variety test of the Yellow River valley from 1993 to 1995, with cumulative planting area of 933333 ha, during 1997—1999. 'Shiyuan 321' (together with 'Qinyuan 4') set a new record of extremely high yield, 3790.5 kg/ha, in

0.35 ha demonstration field at Cele County, Xinjiang autonomous region, which is rare in the world cotton growing history.

Eight new strains had been approved by the cotton regional test in Henan, Hebei, Shaanxi and Shanxi provinces respectively. They were: Yuan 406 (*G. hirsutum*×*G. somalense*), Yuan 1505 (*G. hirsutum*×*G. bickii*), Yuan 159 (*G. hirsutum*×*G. arboreum*), Yuan 6926 (*G. hirsutum*×*G. barbadense*×*G. thurberi*), Yuan 93089 (*G. hirsutum*×*G. thurberi*×*G. arboreum*), Yuan 637 (*G. barbadense*×*G. thurberi*×*G. hissutum*), Yuan 1062 (*G. hissutum*×*G. thurberi*×*G. arboreum*), Yuan 809-3 (*G. hirsutum*×*G. hissutum* ssp. *maxianum*×*G. bickii*).

2.5 Discovery of new rules involved in interspecific hybridization in *Gossypium*

Through a series of tests for many years, we found a number of important rules involved in interspecific hybridization. They included the following: (i) the incompatibility of species cross was, in essence, due to the metabolic disturbance of phytohormones in the processes of fertilization; (ii) the crosses between the wild species and different varieties of the cultivated species presented evident selectivity of fertilization, in which the difference in a number of large embryos among different cross combinations reached even more than 10—20-fold; (iii) effect of the female parent on the improvement of hybrid characteristics in backcross; (iv) the segregation of posterity could be shortened by using the cultivar as female parent in back-crossing, which was especially important to some combinations which could hardly get hybridized, such as *G. hirsutum*×*G. somalense*, *G. hisrtum*×*G.bickii*.

2.6 Establishment of the breeding procedure for interspecific hybridization in *Gossypium*

On the basis of the new discovery as mentioned above, the new breeding system was established. The essential procedures are:

(i) fertile hybrids are obtained fast by using the new technique of interspecific hybridization (spraying GA_3 and NAA on hybrid boll-embryo culture *in vitro*-colchicine treatment in test tube);

(ii) for the purpose of increasing the successful frequency of hybridization several cultivars as the female parent are crossed with the same wild species simultaneously;

(iii) the low generation hybrids are back-crossed with the mixed pollens of multiple cultivars to increase the frequency of recombinants with fine traits;

(iv) for shortening the period of segregation of posterity the cultivars are used as female parents in back-crossing at a good time;

(v) the experimental breeding lines are examined and identified in several places under different ecological conditions. Both high yield and fine quality as well as wide adaptability are selected simultaneously.

This new system has the following advantages: the operative procedure is simple; the breeding period is short; and it can be used in the numerous cross combinations between different species. It is a new creation of breeding system for interspecific hybridization in *Gossypium*.

References

1. Stewart, J. M., Hsu, C. L., Hybridization of diploid and tetraploid cotton through in ovulo embryo culture, J. Hered., 1978, 69: 404—408.
2. Ndunga, V. J., Maxech, R., The improvement of cotton (*Gussypium hirsutum*) using interspecific hybridization, Bull. Rech. Agrom. Gembloux, 1998, 23(3): 283—316.
3. Liang Zhenglan, Sun Chuanwei, The significant effect of endosperm development on the interspecific hybrid formation of cotton, Acta Genetics Sinica (in Chinese), 1982, 9(6): 447—454.
4. Qiu Zhongjin, Liang Zhenglan, Wu Lianying et al., The effects of phytohormone spray on the ultrastructural development of endosperm cell of interspecific hybrid on *Gossypium*, Acta Gossypii Sinica (in Chinese), 1994, 6(2): 74—77.
5. Liang, Z. L., Sun, C. W., Liu, T. L. et al., Studies on interspecific hybridization in cotton, Scientia Sinica, 1978, 11(4): 545.
6. Altman, D. W., Exogenous hormone application at pollination for *in vitro* and *in vivo* production of cotton interspecific hybrids, Plant Cell Reports, 1998, 7: 257—261.
7. Zhao Guozhong, Liang Zhenglan, Feng Hengwen et al., Improvement of the cotton by interspecific hybridization using eight wild species and two diploid cultivated species, Acta Agriculture Boreali-Sinica (in Chinese), 1994, 9(4): 44—48.
8. Niu Yongzhang, Liang Zhenglan, Li Binglin et al., Breeding for new germplasm lines by interspecific hybridization in *Gossypium*, China Cotton (in Chinese), 1998, 25(1): 16—17.
9. Liang Zhenglan, Jiang Ruqin, Zhao Guozhong et al., Studies on the trispecific hybrid (*Gossypium barbadense-G. thurberi-G. hirsutum*) and new variety breeding, Acta Agronomica Sinica (in Chinese), 1996, 22(6): 673—680.
10. He Jianxing, Jiang Ruqin, Zhang Xinxue et al., The cytogenetics and fiber properties of an allohexaploid between *Gossypium hirsutum* and *G. somalense*, Chinese Science Bulletin, 2000, 45(16): 1742—1747.
11. Liang Zhenglan, Jiang Ruqin, Liang Limin et al., Study on the interspecific hybridization of *Gossypium hirsutumum* × *G. sturtianum* and selection of a new variety Qinyuan 4, Scientia Agricultura Sinica (in Chinese), 1999, 32(4): 14—19.
12. Liang Zhenglan, Genetics and Breeding of Interspecific Hybridization in *Gossypium* (in Chinese), Beijing: Science Press, 1999, 193—201, 236—242.