RELEASE OF TWO NEW TRANSGENIC HIGH YIELDING HYBRID COTTON CULTIVARS, CCRI63 AND CCRI66, IN THE YANGTZE RIVER VALLEY, CHINA

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Abstract

Two new transgenic hybrid cotton cultivars, CCRI63 (Zhong001) and CCRI66 (ZhongCJ03B), both possessing exogenous genes Bt+CpTI, were jointly developed by Cotton Research Institute, Anyang, and Biotechnology Research Institute, Beijing, Chinese Academy of Agricultural Sciences(CAAS). CCRI63 and CCRI66 were approved for release for commercial production in the Yangtze River Valley by the Ministry of Agriculture, China, in 2007 and 2008, respectively. CCRI63 and CCRI66 were evaluated by China National Cotton Cultivar Regional Tests in a total of 72 locations in the Yangtze River cotton-growing region during 2004-2005 and 2005-2006, respectively. Statistical analyses showed that both hybrids had the top average lint yields in that CCRI63 (1479 kg ha^{-1}) had 10.0% higher yield than the standard Xiangzamian 2, while that CCRI66 (1566 kg ha^{-1}) increased yield by 15.1% over the average of two standards Xiangzamian 2 and Xiangzamian 8. Both hybrids exhibited some good to excellent characteristics such as yield stability, early maturity, resistance to lepidopterous pests and *Fusarium* wilt and *Verticillium* wilt diseases, and good fiber quality.

Abbreviations: Bt, *Bacillus thuringiensis* ; CpTI, Cowpea trypsin inhibitor; CCRI,China Cotton Research Institute.

Introduction

As a large cotton-growing country, China was comprised of five cotton-growing regions (Feng Z.F.). The Yangtze River cotton-growing region has about 1.4 million ha of cotton area, representing over one-third of the total cotton area of the country. In recent years, most of the cotton cultivars in the Yangtze River cotton-growing region are transgenic hybrids for lepidopterous pests resistance. In accordance with the seed laws and regulations of People's Republic of China, every cotton cultivar is not approved for release for commercial production until it is tested and evaluated two or three years by the National or Provincial Cotton Cultivar Regional Tests and at least one year's Yield Potential Trial. The China National Cotton Cultivar Regional Tests in the Yangtze River cotton-growing region have been administered by the Ministry of Agriculture, China, since 1956(Yang F.X. et al).

Materials and Methods

1. Breeding of CCRI63 and CCRI66

CCRI63 was a F_1 hybrid of 9053 x CCRI41(sGK9708), and CCRI66 was a F_1 hybrid of Zhong9018 x CCRI45. Both 9053 and Zhong9018 were conventional upland cotton lines which revealed some good to excellent characteristics in the Yangtze River Cotton-growing region such as yield stability, resistance to *Fusarium* wilt and *Verticillium* wilt diseases, and good fiber quality. CCRI41 and CCRI45 posses exogenous genes Bt+CpTI. And they have been approved for release for commercial production in the Yellow River Valley by the Ministry of Agriculture, China, since 2002 and 2003, respectively.

2. China National Cotton Cultivar Regional Tests in the Yangtze River Cotton Region during 2004-2006

The Regional Tests conducted in 18 annual test sites which were located in Sichuan(2 sites), Hunan(3 sites), Hubei(5 sites), Jiangxi(1 site), Zhejiang(1 site), and the southern parts of Henan(1 site), Anhui(2 sites) and Jiangsu(3 sites) during 2004-2006. Experimental procedures were very similar for all test sites. Nine to eleven entries including CCRI63 and CCRI66, along with control cultivar - Xiangzamian 2 in 2004 and 2005 and Xiangzamian 8 in 2006, were evaluated for agronomic and fiber quality traits in replicated, multi-location field trials. Seeds for experiments were requested from the breeders. All transgenic varieties received the same recommended cultural

practices as the non-transgenic varieties. Plots were arranged in a randomized block design with three replications. Every plot was 20 square metres, three to six rows wide, and the length was depending on location. The plant density per hectare equals to about $34.5*10^3$. There was a border which was at least four rows of cotton plants round the test field at each location. Yield and pre-frost yield were determined before November 20 and October 31 respectively. Grab samples were collected by hands at the harvest time before October 20 from 3 replications at each location to determine boll weight, lint percentage, seed index(weight in grams of 100 fuzzy seeds) and fiber quality. HVI fiber property determinations were made by Supervision, Inspection and Test Center of Cotton Quality, Ministry of Agriculture, China. Cotton pink bollworm (*Pectinophora gossypiella*), *Fusarium* wilt and *Verticillium* wilt diseases resistance were evaluated by Huazhong Agricultural University, China. Cotton bollworm [*Helicoverpa armigera* (Hübner)] resistance was evaluated by Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, China. The safety assessments were carried out by Institute of Plant Protection, Hubei Academy of Agricultural Sciences, China.

3. Yield Potential Trial in 2006

The Trial conducted in 10 annual trial sites which were located in Sichuan(1 site), Hunan(1 site), Hubei(2 sites), Jiangxi(1 site), Zhejiang(1 site), and the southern parts of Henan(1 site), Anhui(1 sites) and Jiangsu(2 sites) in the Yangtze River cotton-growing region in 2006. Five entries including CCRI63 and CCRI66, along with one control cultivar which was Xiangzamian 8 in 2006, were evaluated for agronomic and fiber quality traits in replicated, multi-location field trials. Experimental procedures were very similar for all trial sites. Plots were arranged in a randomized block design with two replications. Every plot was 150 square metres. The plant density and cultural practices were very similar to the cotton plants growing on the farms.

Results and Discussion

1. CCRI63

Statistical analyses of China National Cotton Cultivar Regional Tests in the Yangtze River Cotton-growing Region showed that new transgenic hybrid cotton cultivar CCRI63(Zhong 001) had the top average lint yield with 1479 (1517-1441) kg per hectare, and had a significant increase of 10.0%(4.1%～15.9%) over the control Xiangzamian 2 during 2004-2005. On average, its growth period was 125(122～128) days, approximately a day earlier than the control. The plant height was 115.3(109.5～121.0) cm. The number of fruiting branch was 17.62(17.00～18.23). The boll number per plant at September 15^{th} was 25.57 (24.93～26.21), and it was average 1.85(1.31～2.32) more than the control. Boll weight was 5.71(5.68～5.74) g. Lint percentage was 41.52% (41.48%～41.56%). Seed index was 9.81(9.73～9.89) g. The fiber properties appeared to be very satisfactory. The upper-half mean length was 30.0(30.5～29.4) mm，the fiber strength was 29.1(29.0～29.2) $cN \cdot tex^{-1}$， the micronaire was 4.8(4.6～4.9)，the fiber elongation was 7.0%(7.1%～6.9%)， and the fiber uniformity was 84.2%(84.3%～84.1%). It was highly resistant to cotton bollworm [*Helicoverpa armigera* (Hübner)] and pink bollworm (*Pectinophora gossypiella*). *Fusarium* wilt severity index was 12.39(14.07～10.70). *Verticillium* wilt severity index was 18.82(15.63～22.00). It had shown very good *Fusarium* wilt tolerance and good *Verticillium* wilt resistance (see table 1).

Table 1. Average lint yield, seed cotton yield, and fiber quality of CCRI63 orthogonally contrasted to the standard Xiangzamian 2 based on 36 test sites of China National Cotton Cultivar Regional Tests in the Yangtze River Cotton-growing Region during 2004-2005.

Variety	Year	Average lint yield			Average seed cotton yield			Fiber quality			
		Kg/ ha	Contrasted to CK (±%)	Order	Kg/ ha	Contrasted to CK (±%)	Order	Pre-frost yield percentage (%)	Lenth (mm)	Strength (cN/tex)	Micronaire
CCRI63	2004	1517	+4.1**	1	3666	+5.4**	1	83.1	30.5	29.0	4.6
	2005	1441	+15.9**	2	3470	+14.8**	2	94.0	29.4	29.2	4.9
	Mean	1479	+10.0**	1	3567	+10.1**	1	88.6	30.0	29.1	4.8
Xiang-zamian 2 (CK)	2004	1457	0	4	3476	0	4	84.1	29.8	30.3	4.9
	2005	1243	0	10	3021	0	10	92.9	28.6	28.7	5.2
	Mean	1331	0	7	3205	0	7	88.5	29.2	29.5	5.1

Note: ** indicates highly significant difference at the 0.01 probability level.

Statistical analyses showed that its general adaptation was significantly superior to the control cultivar, and it was determined to be very adaptable throughout most of the Yangtze River cotton growing region.

Statistical results of Yield Potential Trial in the Yangtze River cotton-growing region in 2006 showed that CCRI63 had an average lint yield with 1586 kg per hectare, and had an increase of 2.3% over the new control Xiangzamian 8. The rest results were similar to the official regional variety tests during 2004-2005 as above.

2. CCRI66

Statistical analyses of China National Cotton Cultivar Regional Tests in the Yangtze River cotton-growing region showed that new transgenic hybrid cotton cultivar CCRI66 (Zhong CJ03B) had the top average seed cotton yield with 3495.0 kg per hectare, the top average lint yield with 1443.0 kg per hectare, and had a highly significant increase of 16.0% over the control Xiangzamian 2 in 2005. In addition, CCRI66 had the top average seed cotton yield with 4061 kg per hectare, the top average lint yield with 1689 kg per hectare increased yield by 14.2% over the control Xiangzamian 8 in 2006. On average, its growth period was 123(123～123) days, a day earlier than the controls. Its plant height was 112 (111～113) cm. The number of fruiting branch was 17.81(17.82～17.80). The boll number per plant was 26.8(26.0～27.6)， and it exceeded in number of 2.42(2.20～2.64) in comparison with the controls. Boll weight was 6.1(5.9～6.4) g. Lint percentage was 41.5% (41.3～41.8%). Seed index was 10.1 (10.0～10.2) g. The average fiber properties appeared to be good during 2005-2006. The upper-half mean length was 29.6(29.0～30.1) mm，the fiber strength was 28.6(29.2～28.1) cN•tex^{-1}，micronaire was 5.1(5.0～5.2)，fiber elongation was 6.6%(6.9%～6.4%)， fiber uniformity was 84.7%(83.9%～85.4%). It was highly resistant to cotton bollworm [*Helicoverpa armigera* (Hübner)] and pink bollworm (*Pectinophora gossypiella*). *Fusarium* wilt severity index was 7.68 (8.50～6.86). *Verticillium* wilt severity index was 24.40(28.40～20.39). It had shown very good resistance to *Fusarium* wilt, and good tolerance to *Verticillium* wilt (see table 2).

Table 2. Average lint yield, seed cotton yield, and fiber quality of CCRI66 orthogonally contrasted to the standards Xiangzamian 2 or Xiangzamian 8 based on 36 test sites of China National Cotton Cultivar Regional Tests in the Yangtze River Cotton-growing Region during 2005-2006.

Variety	Year	Average lint yield			Average seed cotton yield			Fiber quality			
		Kg/ ha	Contrasted to CK (±%)	Order	Kg/ ha	Contrasted to CK (±%)	Order	Pre-frost yield percentage (%)	Lenth (mm)	Strength (cN/tex)	Micronaire
	2005	1443	+16.0**	1	3495	+15.7**	1	93.3	29.0	29.2	5
CCRI66	2006	1689	+14.2**	1	4061	+7.4**	1	93.4	30.1	28.1	5.2
	mean	1566	+15.1**		3778	+11.5**	1	93.5	29.6	28.6	5.1
CK1	2005	1243	0	10	3021	0	10	92.9	28.6	28.7	5.2
CK2	2006	1479	0	10	3781	0	9	96.4	30.5	28.2	4.8
	mean	1361	0	10	3401	0	10	94.6	29.6	28.5	5.0

Note: 1) ** indicates significant difference at the 0.01 probability level.
2) CK1 represents control cultivar Xiangzamian 2; CK2 represents control cultivar Xiangzamian 8.

Based on the evaluations, its general adaptation was superior to Xiangzamian 2, and it was similar to Xiangzamian 8.

Statistical results of Yield Potential Trial in the Yangtze River cotton-growing region for CCRI66 in 2006 showed that the results were similar to the official regional variety tests in the same year as above.

Summary

As new high-yielding transgenic hybrid cotton cultivars, both CCRI63 and CCRI66 possess exogenous genes Bt+CpTI which were inherited by their male parents CCRI41 or CCRI45. Safety evaluation results for CCRI63 and CCRI66 showed that no damage was observed to the health of human beings, animals, and our environment when they were applied in the Yangtze River cotton-growing region in 2004 through 2008. Fiber quality appears to be very satisfactory. They will allow producers to have improved lepidopterous pests control and *Fusarium* wilt, *Verticillium* wilt diseases mitigation as well.

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