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Combining Ability and Heterotic Performance of Bt Transgenic Lines in Cotton (*Gossypium hirsutum* L.)

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Abstract: Five transgenic lines of cotton (*Gossypium hirsutum* L.) carrying the cry1Ac (*Bt*, *Bacillus thuringiensis*) gene were developed from a cultivar JH321 by independent transformation events. Fifteen F_1 hybrids were obtained from diallel crosses among the five transgenic lines and JH321. When compared with the transformation receptor cultivar JH321, significant variations in agronomic traits including improved yield or yield components were found among these transgenic lines. General combining abilities (GCA) among these transgenic lines varied greatly, and two of these lines with significant positive GCA effects were identified. Heterosis in F_1 hybrids also exhibited significant variations. It was found that the average heterosis of crosses with two transgenic lines was lower than that with only one transgenic line as parent in yield performance. The economic trait improvement for transgenic lines and the heterosis produced between transgenic line and receptor cultivar might be raised from different integration sites of *Bt* gene.

Key words: upland cotton; combining ability; transgenic lines; *Bt* genes

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1 Introduction

Use of transgenic Bt cottons can reduce the environmental pollution from synthetic pesticides, increase worker safety, and improve profitability of the farmers, so they have been widely used recently in China, reaching over two million ha in 2002[1].

Cotton is a leading crop for fiber production, but the average cotton yield in most countries have been stagnated since the early 1990s[2]. Hybrid cotton contributing an increasing part to total yields of cotton in many countries. In India, at least 40% of cotton production is derived from hybrids varieties[3]. The conventional wisdom in cotton hybrid breeding has been to cross between genetically distant and unrelated cultivars, but there are no generally accepted criteria on how to select these parents. In recent years, cotton breeding practice in China showed that the hybrids with a *Bt* transgenic variety as parent(s) had significant yield heterosis through increasing the boll number. More than 10 hybrids were released. Hybrid cotton comprised nearly 15% of the total hectarage in China in 2002[4]. However, the genetic basis of these observations was not clear. The objective of this study was to evaluate the genetic variation among a set of transgenic *Bt* cotton lines as

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compared with their parental cultivars. We also analyzed their differences in Combining abilities and the heterosis in the hybrids derived from these transgenic lines.

2 Materials and Methods

2.1 Plant material

Five transgenic cotton lines, T908, T912, T915, T921, and T923, were selected from a transgenic population originated from *Agrobactertium*-mediated genetic transformation of the "JH321" with the *Cry1Ac* gene[5]. Each line was derived from independent transformation events of the embryogenic calli and has been propagated to T4 generation showing stable inheritance of agronomical traits. Genetically, the five transgenic lines and the receptor cultivar, JH321, should have the same background.

2.2 Verification of transgenic lines

Genomic DNA was extracted from leaf tissue of JH321 and each of the five selected transgenic lines[6]. For Southern analysis, 20 μg genomic DNA from each sample was digested with 150 units of *Hind* III restriction enzyme. The digested DNA samples were separated in a 1.2% agarose gel, blotted onto a nylon membrane and hybridized with an α-^{32}P-dCTP labeled *Cry1Ac* gene sequence as the probe, following the procedure of Sambrook et al[7].

2.3 Field experiments

The experiments, enclosed 21 entries (15 F_1 hybrids and six parental lines), were arranged in a random complete block design (RCB) with three replications. One entry was planted in two rows (7.0 m length and 1.0 m apart) and the distance of neighbor plants was about 0.33 m. The experiments were conducted at the Plant Experiment Station of Henan Academy of Agricultural Sciences, Henan Province, China. Standard agronomic practices were used throughout the growing season. Particularly, when necessary, pesticides were applied on all test materials to avoid possible effects of pest damage on yield or yield components.

Yield data were obtained by actual harvest of each plot. Data for boll number, boll weight and lint percent were also collected. Fiber quality parameters were determined by High-Volume Instrument (HVI900) at the Cotton Fiber Quality Testing Center, Ministry of Agriculture, Anyang, Henan, China.

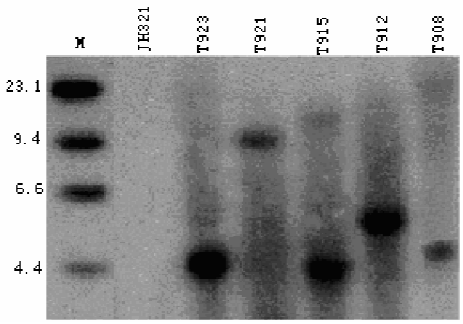
2.4 Statistical analysis

Statistical analysis of diallel data used Model I, Method IV to partition the sum of squares for hybrids and combining ability were calculated[8].

3 Results

3.1 Genetic variation of transgenic lines

Southern hybridization of genomic DNA from the five transgenic lines with the *Bt* gene *Cry1Ac* sequence as the probe (Fig. 1) confirmed that all these lines carry this *Bt* gene. Since not all the hybridization patterns in these lines were the same, this might suggest that different lines may come from independent transformation events and the *Bt* gene may have different insertion locations.



Note: Lane M, DNA size marker (λ-*Hind* III); Numbers to the left of marker lanes are band size in kilobase pairs (kbp).

Fig. 1 Southern hybridization profile of five Bt transgenic cotton lines and the receptor cultivar, JH321

Variance analysis revealed significant differences in yield, yield components and fiber quality parameters among the five transgenic lines and "JH321" (data not shown). The performance of these traits showed either increase or decrease compared with the receptor "JH321" (Table 1). The boll numbers per plant, boll weight and lint percentages all contributed to the yield

increase in T923. Yield decrease in T908 was due to lower boll number and boll weight. From Table 1, it seems that there were negative correlations in yield improvement and fiber quality parameters. For example, while the yield of T923 was significantly higher than that of "JH321", the micronaire of its fiber was coarser (higher micronarie) than that of "JH321". It is evident that the agronomic traits of transgenic lines varied greatly even though they were originated from the same receptor cultivar (JH321).

3.2 The combining abilities of transgenic lines and "JH321"

There were significant differences in combining abilities among the five transgenic lines (Table 2). T915 and T923 had positive general combining ability (GCA) effects. T915 exceeded "JH321" significantly in yield and yield components, but had negative effects on fiber qualities. The two lines also had big variations of special combining components, but had negative effects on fiber qualities. The two lines also had big variations of special combining ability (SCA) effects, indicating high yield potentials in hybrids from them. It seems that a larger population of gene transformation is needed to select transgenic lines with higher combining abilities. Significant positive correlation was observed between the yield performance and the GCA effect as well as the variance of SCA of the transgenic lines (data not shown). Obviously, good agronomic performance of transgenic lines would also benefit selection of parents in hybrid breeding.

Table 1 Yield, yield components and fiber quality parameters of transgenic lines and JH321

lines	yield /(kg • hm^{-2})	yield components			fibre quality parameters		
		boll number	boll weight /g	lint percentage /%	fiber length /mm	fiber strength /(cN • tex^{-1})	micronaire
JH321	846.27	15.40	4.93	39.17	28.90	30.10	4.60
T908	681.00**	13.10*	3.96**	39.46	29.80	31.70*	4.30
T912	824.74	15.63	4.64	38.42	29.40	30.10	4.70
T915	920.63	15.83	4.86	39.64	27.77	27.67**	5.27**
T921	535.03**	11.77**	4.85	38.17	30.50	30.37	4.50
T923	954.79*	16.87	5.15	40.23	27.67	29.27	5.07**

Note: Value followed by one asterisk (*), or two asterisks (**) indicated significantly different from that of JH321 at $P=0.05$ and $P=0.01$, respectively.

3.3 The heterosis of yield

The five transgenic lines were supposed to have the same genetic background as the acceptor cultivar "JH321". Heterosis over "JH321" was evident in F_1 hybrids from crosses between transgenic lines, and between the transgenic lines and "JH321" (Table 3). The average heterosis of yield across all 15 hybrids was 8.82%, ranging from -26.20% to 37.14%. In yield components, the boll number contributed to the yield most. The heterosis for the boll number was 8.87%, ranging from -10.40% to 36.05%, which was similar to the yield. The boll weight was second. There seems to have negative heterosis in lint percentage and no heterosis in fiber quality traits (Table 3).

Interestingly, heterosis in the hybrids from crosses involving only one transgenic parent was much higher than that in crosses in which both parents were transgenic lines for the yield (13.15 versus 8.82%). This indicated that the combination of two transgenic lines did not show cumulative effect on heterosis, but rather reduced the effect in yield performance of F_1 to some extent.

4 Discussion

There are many reports in the literature on the variation of morphological and agronomical traits in transgenic plants[9-10]. Most of these changes showed undesirable effects on agronomic traits. However, in this study, the five trans-

genic lines should have the same genetic background as JH321, but two lines have significantly higher yield over JH321 (Table 1). One reason may be the gene insertion effects of alien genes and its interactions with native genes[11]. Another one may be the somaclonal variations.

Table 2 The general combining ability (GCA) effect (values in upper row) and the variance of the special Combining ability (SCA) effect (values in lower row) of JH321 and five transgenic lines of cotton

traits	JH321	T923	T921	T915	T912	T908
lint yield	45.81	110.53	-146.71**	150.62**	-102.49**	-57.75**
	2671.46	4610.52	779.48	4630.00	2370.41	1659.89
boll number	0.49	1.81*	1.90**	0.98*	-1.75**	-0.62
	0.78	-0.26	-0.36	0.28	-0.08	0.32
boll weight	0.38	0.13*	0.17**	0.16*	-0.21**	-0.30**
	0.07	0.04	0.04	0.10	0.07	0.08
lint percent	-1.00	0.32**	0.54	0.73**	-0.07	0.56**
	0.66	0.18	-0.12	0.00	-0.32	0.32
fibre length	-0.30	-0.61**	0.83**	-0.43	0.13**	0.38**
	0.01	0.18	0.23	0.09	0.07	0.29
fibre strength	-1.18	-0.88	1.12**	0.03**	0.05**	0.92**
	0.81	0.33	0.49	0.31	0.33	0.69
micronarie	-0.16	0.24**	0.08	0.37**	-0.22	-0.15
	0.03	0.01	-0.01	0.01	0.00	0.02

Note: Value of each transgenic line followed by one asterisk (*) and two asterisks (**) is significantly different from the corresponding value of JH321 at $P=0.05$ and $P=0.01$ level, respectively.

Table 3 Heterosis of cotton yield, yield components and fiber quality parameters in F_1 hybrids over JH321

crosses	lint yield	boll number	boll weight	lint percentage	fibre length	fibrestrength	micronaire
cross with one transgenic line as parent							
JH321×T908	1.06	-0.15	11.37	-7.60	1.38	-4.82	-16.30
JH321×T912	-7.76	-4.86	8.47	-5.75	1.38	-5.32	-11.96
JH321×T915	37.14	36.05	7.39	-1.71	-1.21	-1.99	4.35
JH321×T921	4.95	-1.30	15.18	-8.10	4.15	3.82	-8.70
JH321×T923	30.36	28.35	11.62	-1.67	-2.08	-3.16	5.43
means 1[a]	13.15	11.62	10.80	-4.97	0.73	-2.29	-5.43
cross with two transgenic lines as parents							
T908×T912	-6.59	2.25	-8.39	-0.09	2.42	4.15	-8.70
T908×T915	17.45	17.17	6.04	0.46	2.42	6.64	6.52
T908×T921	-20.18	-10.40	-6.83	-1.30	3.29	10.80	-5.43
T908×T923	25.06	19.01	5.02	-0.36	3.46	-0.33	-2.17
T912×T915	23.33	6.55	11.42	-2.35	-0.69	2.33	0.00
T912×T921	-26.20	-9.64	-5.72	-4.65	6.40	2.82	-7.61
T912×T923	12.86	4.40	8.55	-2.51	0.00	0.83	-4.35
T915×T921	12.75	6.42	6.66	-1.43	4.33	1.66	0.00
T915×T923	24.64	29.38	4.31	-2.16	-3.11	-4.82	7.61
T921×T923	3.42	9.84	4.38	-4.67	1.04	0.00	1.09
means 2[b]	6.65	7.50	2.54	-1.91	1.96	2.41	-1.30
means 3[c]	8.82	8.87	5.29	-2.9	1.50	0.84	-2.70

Note: [a] means 1 is the average heterosis across five F_1 hybrids involving JH321 and each of the five transgenic lines;

[b] means 2 is the average heterosis across the 10 F_1 hybrids among five transgenic lines;

[c] means 3 is the total average heterosis of all 15 F_1 hybrids.

Heterosis was also observed in crosses between natural, physical, or chemical mutants derived from the same parent in a number of crops[12]. It seems that changes in a limited number of genes are directly responsible for yield heterosis in F_1 hybrids. However, in this study, yield heterosis in the hybrids from crosses in which both parents were transgenic lines was much lower than that in crosses involving only one transgenic parent(Table 3). It is possibly because that the level of heterotic effects of mutated genes may depend on their interactions with other mutated genes or with genes from the parental genotype.

It has been a general assumption that the genetic distance between two parents is positively correlated with heterosis of F_1 hybrids. The molecular technology made it possible to evaluate directly whether the genetic diversity between the parents in DNA level can be as a tool in predicting heterosis. There are many studies to address this problem in crops such as maize, wheat, rice, and soybean, but the results are still far from conclusive. In this study, transgenic lines and their parental variety are similar to near-isogenic lines, differing only in a limited number of loci. The high level of heterosis in F_1 hybrids from transgenic lines observed in this study was an obvious contradiction to the conventional wisdom on selection of parental lines for hybrid breeding. The heterosis in these transgenic line-derived hybrids appeared to be related with specific interactions of mutated genes rather than the overall genetic diversity between the parents. However, further investigation is needed to take on to understand the underlying genetic and molecular mechanisms of heterosis.

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