

## Transgene Stacking in Cotton Improvement

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To date, more and more transgenic varieties of upland cotton (*Gossypium hirsutum* L.) generated with transgenes, which derived from various of alien species, are playing important role in agricultural production. Stacking of multi-transgenes has a potential for combining all the merits of distinct transgenic lines in a cultivar and possibly makes a significant contribution to cultivar improvement. We selected four transgenic lines, which had the same genetic background as the receptor variety (Huakangmian No. 1) in the *Agrobacterium*-mediated transformation to investigate the effects of the transgene stacking. The lines were divided into three groups: the first group covers two lines with big boll, among which one is transposon-induced mutant (*En*) (average boll weight 8.2 g), another one is the *rolB* transformant (average boll weight 7.6 g). The second is the line transformed with the dimer of *Bt*-insect resistant gene and mannosyltransferase gene (*mnt*), showing heavy boll setting. The third is the line transformed with monooxygenase gene (*mnx*). It has fine fiber quality (fiber length 34 mm, micronaire 4.7, fiber strength 30.5 cN · tex<sup>-1</sup>). Both *mnt* and *mnx* genes were derived from *Arabidopsis thaliana*. We made five hybrid combinations in 2004 from these four lines to see if the variations of the parental traits occurred in F<sub>1</sub> populations. And then, successive selections were carried out in the F<sub>2</sub> aimed to recover the original characteristics of the parental lines. Parallel PCR analyses of selected plants were done to check the stability of stacked transgenes in cotton genome, which used the coding sequences of the individual transgenes as primers. Compared with the control variety Huakangmian No. 1, four of the five F<sub>1</sub> populations had a significant increase in cotton yield and improved fiber quality. In the case of boll sizes, two combinations of big-boll lines crossed with the heavy boll setting line, with a paternal average boll weight 5.1 g, performed more boll-setting, with enlarged boll size (average boll weight 7.4 g and 6.8 g, respectively). The boll weight of the hybrid of two big-boll lines reached 8.5 g, surpassing those of both parents, but the boll number per plant reduced, which resulted in a cotton yield reduction. As all the parental lines of the F<sub>1</sub> combinations had the same genetic background as the receptor variety in transformation, the performances of F<sub>1</sub> plants was apparently attributed to the collective effects of the transgenes rather than heterosis. Individuals selected from F<sub>2</sub> populations maintained the major target characteristics of their ancestors, in 2007, for instance, one line combined big bolls together with insect resistance and long fiber. Further verification of this line by PCR showed that the plants had all the positive signals of the transgenes, including *Bt*, *mnt*, and *En* genes or marker. We also have more than twenty mutants induced by transposon tagging systems, such as the mutants of blight resistance (*Xanthomonas campestris* pv *malvacearum*), spherical boll, male sterility, deep green blade, which need to be further investigated in molecular biology and cotton genomics, and evaluated for their utilities in cotton breeding.