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Effect of trans-*Bacillus thuringiensis* gene on gibberellic acid and zeatin contents and boll development in cotton

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Abstract

Two experiments were conducted to investigate the effect of the *Bacillus thuringiensis* (Bt) transgene on gibberellic acid and zeatin contents and boll development in cotton using two types of Bt-transformed cultivars. In the 2003 study, boll size and weight, gibberellic acid 3 (GA_3) and zeatin (ZR) contents were investigated from 3 to 45 days after flowering (DAF). In 2004, the flowers were sprayed with GA_3, 6-benzyl adenine (6-BA) or a combination of both, and responses in boll size and weight, and endogenous GA_3 and ZR contents were determined. In comparison to the common parent, Simian 4, overall boll size and weight were lower for the conventional Bt cultivar, Sikang 1, but higher for the hybrid Bt cultivar, Sikang 3. Similarly, the boll GA_3 and ZR contents of Sikang 1 were lower than those of Simian 4, while those of Sikang 3 were higher than Simian 4. The largest difference between Sikang 1 and Simian 4 for boll GA_3 and ZR contents were 18.5 and 25.5%, respectively, observed at 17 DAF. The largest difference between Sikang 3 and Simian 4 for boll GA_3 and ZR contents were 25.5 and 85.7% at 31 DAF respectively. Application of GA_3, 6-BA or a combination significantly increased boll size and weight for the conventional Bt cultivar and Simian 4, but did not have a significant effect on these characteristics of the hybrid cultivar Sikang 3. GA_3 and ZR contents of the conventional Bt cultivar Sikang 1 were also significantly increased by application of these treatments. The combined application of GA_3 and 6-BA tended to have a larger effect than the application of either of them separately, but the differences were statistically not significant. These results suggested that the lower boll GA_3 and ZR contents, which could reduce boll nitrogen metabolism intensity, were responsible for the reduced boll development of the conventional Bt cultivar Sikang 1.

Keywords: Bt cotton; Boll development; Endogenous hormone; Gibberellic acid; Zeatin

1. Introduction

Bacillus thuringiensis (Bt) transgenic cotton was first commercialised in the mid-1990s (Jenkins et al., 1995; Pray et al., 2002). Due to its increased insect resistance Bt cotton was rapidly adopted in China. It is estimated that current annual plantings of Bt cotton are over 30 million acres in China, (Guo et al., 1999; Li and Wang, 1999; Zhao et al., 2000; Xing et al., 2001). The introduction of commercial cotton varieties producing CryIA insecticidal proteins increased farmer income and reduced chemical use, which in turn reduced environmental pollution from synthetic insecticides, increased worker safety, and improved grower profitability (Gould, 1988; Gasser and Fraley, 1989; Zipf and Rajasekaran, 2003). However, undesired changes in boll characteristics were frequently observed in some Bt cotton cultivars grown in different regions (Wilson, 1994; Tian, 1999). These included smaller bolls (Tian et al., 2000; Zhang et al., 2002), reduced lint percentage (Kerby et al., 1995; Fu et al., 2001) for conventional cultivars; and bigger bolls, and increased micronair (Hua et al., 1999; Cui et al., 2002; Zhang and Liu, 2001) for hybrid cultivars. However, the causes of these observed changes remained unclear. The introduction of the Bt gene and its expression of insecticidal proteins may cause some alterations of essential physiological processes including the metabolism of hormones, which affect the development of reproductive organs (Tian et al., 2000; Chen et al., 2000). Our recent work showed that the endogenous hormone content of the boll closely correlated with boll size (Chen et al., 2002). Therefore, the changed boll development characteristics might be related to altered boll hormone physiology of Bt cotton.

The primary objectives of the research reported here were to (a) characterize the change of boll GA_3 and ZA contents, (b) to test the responses of boll size and weight to external application

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of GA_3 and 6-BA in different types of Bt cotton cultivars (conventional and hybrid) and (c) to explain observed patterns in boll response in terms of hormonal effects.

2. Materials and methods

2.1. Plant materials and field design

Sikang 1, a conventional Bt cultivar, Sikang 3, a hybrid Bt cultivar, and Simian 4, the common parent of these two Bt cultivars were used in this study. These cultivars were grown in the experimental fields at Yangzhou University farm, Jiangsu Province, China (32°30′N, 119° 25′E), during two growing seasons (April-November) in 2003 and 2004. Seed was sown on 4 April in a warm room covered by plastic film. Seedlings were transplanted to the field on 17 May at a spacing of 0.83 by 0.32 m. The plot dimensions were in 15.0 by 5.4 m. A completely randomized block design with three replications was used to arrange the experiment in the field. The soil had a sandy loam texture (Typical fluvaquents, Entisols (U.S. taxonomy)], which contains 24.5 g kg^{-1} organic matter and available N-P-K at 108, 40.5 and 82.0.0 mg kg^{-1}, respectively. N (60 kg ha^{-1} as urea), P (300 kg ha^{-1} as single superphosphate), and K (120 kg ha^{-1} as KCl) were applied before transplanting. N (54 kg ha^{-1} as urea), P (300 kg ha^{-1} as single superphosphate), and K (120 kg ha^{-1} as KCl) were top-dressed at early flowering. Nitrogen as urea was again top-dressed at early boll developing stage (126 kg ha^{-1} as urea) and at peak boll stage (30 kg ha^{-1}). The chemical plant growth retardant DPC (1,1-dimethyl piperidinium chloride, $C_7H_{16}ClN$) was applied at peak square (15 g ha^{-1}), early flower (30 g ha^{-1}), peak flower (45 g ha^{-1}) and peak boll period (60 g ha^{-1}), to fine-tune vegetative and reproductive development. The two cultivars and their parents flowered on July 5 to 6, with bolls opening after August 25.

2.2. Preparation of plant material

For the 2003 experiment, 15 bolls were collected from the first position of the fruiting branches in the middle part of the plants at 3, 10, 17, 24, 31 and 45 days after flowering (DAF). Five bolls were frozen with liquid nitrogen, stored in a freezer, and used for the measurements of gibberellic acid 3 (GA_3) and zeatin (ZR) contents. The remaining 10 bolls were used for measuring boll volume and weight.

In 2004, labeled flowers from the first position of the fruiting branches at the middle part of the plants were sprayed with GA_3 at 20 μg g^{-1}, or 6-benzyl adenine (6-BA) at 10 μg g^{-1}, or the combination of both. Fifteen bolls were sampled at 3 and 31 DAF. Five bolls were used for GA_3 and ZR assays, and 10 bolls for measuring boll volume and weight.

2.3. Physiological measurements

2.3.1. Boll volume and boll weight

Boll size (volume) was determined by soaking the boll into water in a marked cylinder immediately after harvesting, and determining the resulting water displacement. The seed cotton from the boll was oven-dried at 70 °C until constant weight, and then weighed to obtain boll weight.

2.3.2. GA_3 and ZR concentration assay

The GA_3 and ZR concentrations in the boll extracts were determined by immunological analysis using ELISA (He, 1993). The boll tissue extracts were prepared by grinding the frozen boll tissue in 10 ml extraction buffer (Na_2CO_3, 1.33 g; DTT0, 192 g; NaCL, 1.461 g; Vc0.5 g dissolved in 250 ml distilled water) with a homogeniser fitted with a 10 mm grinding head; the content was moved to a 20 ml centrifuged tube; the grinding head was washed with 3 ml of buffer and this was added to the centrifuged tube. The contents of this tube were shaken by hand, and stored at 4 °C for 4 h. The extract was collected after centrifugation at 10000× at 4 °C for 20 min; the extracts (5 ml) was passed through a C18 Sep-Pak Cartridge (Waters, Milford, MA), and dried in liquid N_2. The residues were dissolved in PBS (0.01 Mol/L, PH 7.4) in order to determine the level of GA_3 and ZR. Microtitration plates were coated with the standard synthetic GA_3/ZR-ovalbumin conjugate in $NaHCO_3$ buffer (50 mMol/L, pH 9.6) respectively, incubated at 37 °C for 4 h; the ovalbumin solution (10 mg/ml) was added to each well in order to block non-specific binding. After incubation at 37 °C for 30 min, the standard GA_3/ZR samples and the antibodies were added to each well and incubated for a further 30 min at 37 °C. The antibodies against GA_3/ZR were obtained as described by Weiler et al. (1981). Then horseradish peroxidase-labelled goat antirabbit immunoglubolin was added to each well and incubated for 30 min at 37 °C. Finally, the buffered enzyme substrate (orthophenylenediamine) was added, the enzyme reaction allowed to proceed in the dark at 37 °C for 15 min, and then terminated using 3 M H_2SO_4 L^{-1}. The absorbency was recorded at 490 nm. Calculation of the ELISA data was performed as described by Weiler et al. (1981).

2.4. Statistics analysis

Analysis of variance was conducted for both of the experiments for all the characteristics measured using Proc ANOVA in SAS (SAS Institute, 1989). The differences between treatments (cultivars) were tested for significance using LSD. The assumption of homogeneity of variance is satisfied. Normality test detected moderate derivation for some of the measurements. However, various data transformation failed to provide better results. Therefore, results presented were from analysis conducted using original observations.

3. Results

3.1. Boll size and boll weight

The trend of boll size development over the growth period of 3–45 DAF was similar for all the studied cultivars (Table 1). Boll size increased rapidly from 3 to 17 DAF, slowly from 17 to 31 DAF, and stopped growth after 31 DAF. The differences in