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RESEARCH ARTICLE

Development of glyphosate-tolerant transgenic cotton plants harboring the *G2-aroA* gene

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Abstract
Given that glyphosate weed control is an effective strategy to reduce costs and improve economic outcomes of agricultural production in China, the development of glyphosate-resistant cotton holds great promise. Using an *Agrobacterium*-mediated transformation method, a new *G2-aroA* gene that encodes 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) was transformed into cotton cultivar K312. The transgenic cotton plants were regenerated from a callus tissue culture *via* kanamycin selection. Ten regenerated cotton plants were obtained and allowed to flower normally to produce fruit. The results from polymerase chain reaction (PCR) and Southern and Western blot analyses indicated that the target gene was integrated into the cotton chromosome and was expressed effectively at the protein level. The glyphosate tolerance analysis showed that the transgenic cotton had a high resistance to glyphosate. Further, even cotton treated with 45.0 mmol L^{-1} of glyphosate was able to slowly grow, bloom and seed. The transgenic cotton may be used for cotton breeding research of glyphosate-tolerant cotton.

Keywords: cotton (*Gossypium hirsutum* L.), *Agrobacterium*-mediated method, glyphosate, *G2-aroA*, genetic transformation

1. Introduction

Cotton is an important fiber crop and is a major resource of plant protein and edible oil in the world (Zhang 2013; Chakravarthy *et al*. 2014; Guo *et al*. 2015). Because excessive weeds adversely affect the yield and quality of cotton (Awan *et al*. 2015), chemical weed control is an effective approach to reduce costs and improve economic benefits. One commonly used herbicide is glyphosate, which is non-selective and has the advantages of broad-spectrum effects, high efficiency, low toxicity, and an absence of residue (Ma *et al*. 2016). Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (EC 2.5.1.19), which is the enzyme that catalyzes the critical step of the shikimate pathway in the biosynthesis of aromatic amino acids, and its functionality is absolutely required for the survival of microorganisms and plants (Steinrucken and Amrhein 1980; Funke *et al*. 2006; Gong *et al*. 2016). However, when glyphosate kills weeds in a field, it also damages the crops. Therefore, efforts to cultivate and promote glyphosate-resistant cotton varieties *via* genetic engineering technology are the most effective approaches for controlling glyphosate weeds in cotton fields.

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There are primarily three types of strategies to achieve resistance to glyphosate, which include overexpression of sensitive EPSP synthase (Gaines *et al*. 2010), detoxification of glyphosate (Malherbe *et al*. 2003; Castle *et al*. 2004) and expression of an insensitive form of EPSP synthase (Padgette *et al*. 1995; Tian *et al*. 2010; Yan *et al*. 2011). Currently, multiple genetically modified cotton varieties, which have obtained glyphosate resistance *via* the introduction of their chromosomes to insensitive EPSPS, have been developed and are widely used in several developed countries, providing enormous economic and societal benefits (Zhang 2013; Vats 2015). Several national research groups (Xie *et al*. 2004; Zhao *et al*. 2006; Zhang *et al*. 2014; Yan *et al*. 2015) obtained glyphosate-resistant cotton cultivars. However, domestic herbicide-resistant cotton, which could be used for commercial production, has not been studied. Therefore, studies on glyphosate-resistant genes and herbicide-resistant crops are still critically needed and are of great significance in China.

In this study, a glyphosate-resistant *G2-aroA* gene was isolated and cloned from the *Pseudomonas fluorescens* strain G2 in the Prof. Lin Min's Laboratory at the Biotechnology Research Institute of the Chinese Academy of Agricultural Sciences (Zhu *et al*. 2003; Wang *et al*. 2014) *via* independent intellectual property rights. The gene was introduced into an upland cotton cultivar using K312 as a receptor *via* the *Agrobacterium*-mediated transformation. Thus, several glyphosate-resistant transgenic cotton lines were obtained. Meanwhile, resistance identification and molecular biological analysis of the cotton provides a scientific basis for breeding and commercial production in the future.

2. Materials and methods

2.1. Plant materials

Cotton seeds (*Gossypium hirsutum* L. *cv*. K312), which were stored in our laboratory, were used for this study. The seeds were manually dehusked, sterilized with 75% (v/v) ethanol for 5 min, washed three times with sterile distilled water, soaked in a 1.8% (w/v) sodium hypochlorite solution, and washed 6 times with sterile distilled water. The sterile seeds were germinated on a solidified MS medium (Murashige and Skoog 1962) that was supplemented with 1.5% (w/v) glucose and 0.8% (w/v) agar at pH 5.8. Hypocotyl segments (5–6 mm), which were excised from 5- to 6-d-old seedlings, were used for the transformation.

2.2. Cotton transformation and regeneration of transformants

The hypocotyl segments were infected with *Agrobacterium tumefaciens* LBA4404 that contained the *npt*II and *G2-aroA* genes, which were derived using a *nos* promoter and a daisy rubisco small subunit promoter (Fig. 1), respectively. Infected hypocotyls were transferred onto a piece of filter paper using co-cultivation medium. After co-cultivation, the hypocotyls were rinsed thoroughly with sterile distilled water prior to transfer to a callus induction medium. Embryogenic calli were induced for 4 wk and transferred onto fresh medium in which the concentration of kanamycin was gradually increased to 100 mg L^{-1} for further selection until they were vigorously growing and until friable, loose and white healthy calli were obtained. After approximately 3–4 wk, the embryogenic calli were excised and transferred onto embryo differentiation medium and maintained on this medium until somatic embryos developed and geminated. One subculture was performed every 4 wk, and shoot regeneration occurred within 7–9 wk. When selected shoots grew to 3–4 cm in height, they were transferred to an induced root culture medium. Selected shoots with 3–4 pieces of leaves and roots were acclimatized and then transplanted.

2.3. DNA extraction and PCR detection of the transformed cotton plant

Cotton genomic DNA was isolated from young leaves of non-transformed control plants and transgenic cotton plants using an improved cetyltrimethylammonium ammonium bromide (CTAB) extraction protocol (Paterson *et al*. 1993; Chaudhry and Yasmin 1999). The quality and quantity of DNA was analyzed using a spectrophotometer (NanoDrop

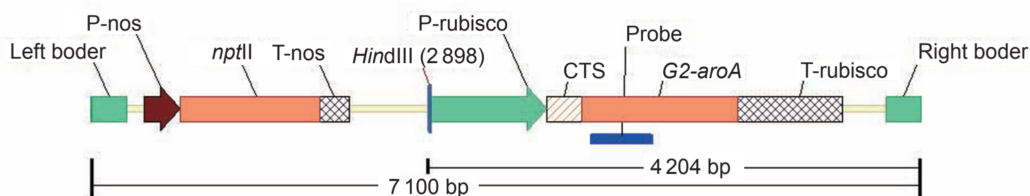

Fig. 1 Structure of the plant expression vector of p-*G2-aroA*. P-nos, nos promoter; *npt*II, neomycin phosphotransferase gene; T-nos, nos terminator; P-rubisco, Daisy Rubisco small subunit promoter; CTS, Daisy Rubisco small subunit chloroplast signal peptide; *G2-aroA*, 5-enolpyruvyl-shikimate-3-phosphate synthase gene; T-rubisco, Daisy Rubisco small subunit terminator.

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