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Mini Review

Review on Genetic Engineering in Castor Bean

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Introduction

Castor (*Ricinus communis L.*) is a special industrial oil crop. Yearly, about 900 million pounds of castor oil and its derivatives are used in the manufacture of soaps, lubricants, waxes and polishes, nylon, hydraulic and brake fluids, paints, dyes, drugs, and perfumes. Castor is also widely used as a bio energy source. Research has shown a 90% reduction in greenhouse gas emission using biodiesel from castor compared to petroleum diesel and other oils. Because the main problems restricting the spread and development of castor bean are diseases, insect pests, harmful toxin of castor bean and so on, so it is critical to breed varieties with resistance to disease, insect and low-toxic protein content, which is very difficult to realize by conventional method. With the development of biotechnology, it has become possible to create such varieties through introducing exogenous or endogenous genes into the castor genome to express exogenous genes directly or regulate the expression of endogenous gene. In recent years, primary progress on genetic engineering in castor has been made. Researches on genetic transformation on disease resistance, insect resistance and low toxicity protein content has been conducted in castor through pollen tube pass way, *Agrobacterium tumefaciens* mediated transformation, particle bombardment and other methods, transgenic castor bean has been achieved successively.

Mckeon et al. [1] transferred target gene into castor using agro bacterium-mediated method from the injury of buds. Sujatha et al. [2] transferred genes hpt and gus into castor by agro bacterium-mediated method and obtained transgenic castor for the first time, with a transformation rate of 0.08%. Based on the genetic transformation system established by Sujatha et al. [2], Malathi et al. [3] introduced the target gene cry IAb into the castor receptor using cotyledonary node as explants and acquired transgenic plant with resistance to inchworm larvae, with a transformation rate of 0.42%. Sailaja et al. [4] transferred genes GUS and hpt II into castor using particle bombardment for the first time and gained transgenic plants again with a transformation rate of 1.14%. Sujatha et al. [5] used hypocotyls as explants again and transferred gene cry 1EC into castor by agro bacterium mediated method and particle bombardment, getting the transgenic plants with resistance to prodenia litura and inchworm, with a transformation rate of 0.82% and 0.69% respectively. Also with cotyledonary node as explants, Ganesh et al. [6] transferred the gene GUS and cocoa chitinase gene into castor by agro bacterium mediated method, getting the resistant transgenic plants to wilt and a transformation rate of 1.17% and 1.06% respectively.

Although the genetic transformation system of castor has been preliminarily established, there are still problems that need to be improved urgently. The transformation method used by Mckeon et al. [1] is simple and feasible, and it does not require complex tissue culture process. However, most of the transgenic plants obtained are chimera, which is not conducive to future breeding research. Sujatha et al. [2,5] used hypocotyls as receptor of exogenous gene in his transformation system, but the castor embryo is very small and it is difficult to distinguish the hypocotyls, germ and radical by visual observation, further, it is also hard to make wound freehand on hypocotyls in the process of transformation, which is not conducive to large-scale transformation experiment. In general, the preliminarily established regeneration and transformation systems cannot be popularized and industrialized due to the difficulties in regeneration system establishment, long cycle, tedious steps, low transformation, poor repeatability, heavy workload and high cost; therefore it is urgent to develop the transformation method independent of tissue culture to break down that obstacle.

Although agro bacterium-mediated method has unique advantages such as low copy number of inserted gene, good expression effect, relatively less gene silencing and good integrity of gene fragments, plant cell wall is one of the main obstacles to *agrobacterium*-mediated plasmid transfer. Many transgenic methods have focused on how to damage the cell walls of plants. On one hand, the wall damage results in the generation of phenolic substances such as acetosyringone, etc, which can induce the expression of agro bacterium-toxic protein and promote the transfer of T-DNA, on the other hand, it, will directly reduce the physical barrier of T-DNA transfer. If the ultrasonography, vacuum negative pressure treatment, ion beam and other method are used to assist agro bacterium-mediated transformation, its efficiency will be greatly improved. Bechtold et al. [7] applied vacuum infiltration, a method used in plant pathology to assist the entry of plant viruses

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into plants, to the *agrobacterium*-mediated genetic transformation in Arabidopsis, which opened up a new way for the transgenic work of plants. Afterwards, it was successfully used in rice, wheat, cotton, soybean, rape and tobacco. Supartana et al. [8] established a agro bacterium-mediated in situ transformation method assisted by acupuncture in rice, which possessed the advantages of low cost, no tissue culture, shorter transformation cycle, good repeatability and high reliability, and could gain transgenic seeds directly. Lin et al. [9] established the acupuncture-vacuum infiltration assisted agro bacterium transformation method in rice by modifying the above method, increased the transformation rate greatly.

Our research group performed the acupuncture-vacuum infiltration assisted agro bacterium transformation on castor. The results proved that this method has the advantages of high transformation efficiency, simple operation, and good repeatability, wide range of receptors and small variation of exogenous genes. We have established a relatively mature genetic transformation system successfully, created a batch of transgenic lines and gained a transformation rate of over 75%. This method has been patented by the state [10]. We transferred the plant expression vector pCAMBIA1305.1 into castor HY1 through seed culture, acupuncture, vacuum infiltration, co-cultivation, cephalosporin's drug sterilization, potted plants, hygromycin resistance selection and PCR detection, screening out the optimum vacuum infiltration pressure, vacuum infiltration time, *agrobacterium* culture time and the titromycin concentration for resistance selection. Using this transformation system, we studied the function of phenylalanine ammonialyase gene (RcPAL) and phosphoenolpyruvate carboxylase gene (RcPEPC) and got good results.

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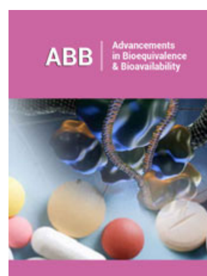

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