Application of a transformation method via the pollen-tube pathway in agriculture molecular breeding☆

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Abstract

The transformation method via pollen-tube pathway has great function in agriculture molecular breeding. This article is to introduce the mechanism, molecular evidence, technique details and the latest achievement applying this method. And we compare the advantages and shortages of this method with other transformation pathways. [Life Science Journal. 2007;4(1):77-79] (ISSN: 1097-8135).

Keywords: pollen-tube pathway; molecular breeding

1 Foundation and Its Theoretical Evidence of the Transformation Method via the Pollen-tube Pathway

According to Chinese scientist Zhou's hypothesis in 1979, the normal distant hybridization existed the hybridization of DNA segment[1], and a new plant transformation method was put forward, it was called the pollen-tube pathway transformation technique. And heterologous DNA was introduced into cotton successfully in 1983, the new anti-wilt cotton cultivars was obtained[2]. The technique invented by Chinese scientists was paid attention to broadly.

After angiosperms' blooming, pollen bourgeoned on the stigma and the pollen tube grew. Before pollen tube entered the ovule, nucelli and embryo sac were closing entity culture. In process of pollen tube extending, some cells of nucelli began degenerate and became pollen tube pathway through which pollen tube could enter embryo sac by nucelli. The pathway was larger than pollen tube, and then between pollen tube formation and closing, heterologous DNA could enter embryo sac and integrate into the zygote cell and the forepart embryo cells.

2 The Molecular Evidence of the Pollen-tube Pathway Transformation Technique

The mechanism of the pollen tube pathway transformation was confirmed by isotope tracer method. Gong[3] labeled cotton total DNA using ^{3}H, and injected the labeled DNA into cotton ovary at 24 hours after the self pollination. After 30 minutes, ^{3}H-DNA was found in some embryos. Between 2 - 4 hours ^{3}H-DNA was found in more than 80% of embryos. Except the pathway from micropyle to embryo, there was no isotope trace autoradio graphy spot in other part of nucelli and pollen tube which entered the embryo. In addition, it was observed that micropyle was close state before pollen tube arrived, and when pollen tube arrived at ovule, micropyle opened. Experiments confirmed that the nucelli pathway of pollen tube after self pollination was the only pathway that heterologous DNA entered the embryo from micropyle.

Huang[4] transferred *GFP* gene into cotton and obtained transformed young embryo plants through measurement of flurescence microscope and handled ultraviolet purple and molecular hybridization. The results provided dependable cell and molecular biology proofs for the possibility of pollen-tube transformation technique.

In a report[5], the gramineous expression vectors pGU4AGBar and pGBIU4AGBar were used, respectively. The s *gna* gene, a synthetical agglutinin gene of Galanthus navies, had been transferred into the winter wheat varieties Xinong 2208 and Xinong 132 by pollen-tube pathway. The PCR and Southern blotting analysis showed that 20 transgenic plants with s *gna* gene were obtained. Western blotting analysis revealed that the target protein was expressed in the transgenic plants. The transformation frequency was 0.28% - 0.84%.

The *cryIa* gene, a synthetical insecticidal crystal protein gene of *Bacillus thuringiensis*, was transferred into the wheat varieties Xinong 2208 and Xinong 132 using the gramineous expression vector pGU4ABBar by pollen-tube pathway. By PCR and Southern blotting analysis 27 transgenic plants with *cryIa* gene were obtained. Western blotting analysis showed that the protein was expressed in the transgenic plants. The transformation frequency was about 1.13% - 1.21%[6].

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Wang[7] transformed *bar* gene into indica cultivars E32 and obtained transformed plants which had resistance to herbicide basta. The result confirmed *bar* gene had integrated and expressed in transformed plants.

15 seeds of D0 were obtained by pollen-tube pathway transformation method, using plasmid pBI121 with *NPTII* gene and *GUS* gene as donor, and using rice breed of Teqing NO. 2 as transgenic acceptor[8]. 1500 seeds of D1 were obtained by breeding D0 seeds. 18 green seedling were obtained when the seeds haired in the Kana water. Distill the DNA of the green seedling, using the probe with the *NPTII* gene segment, the southern result reported heterologous DNA had integrated into the rice genome.

The leaf senescence inhibition gene P_{SAG12}-*IPT* was used to improve wheat varieties that had disadvantage of leaf presenility[9]. With wheat cultivar Xinong 1376 as material, 5 transgenic plants were obtained through pollen tube pathway mediated transformation. By means of PCR amplification, GUS histochemical analysis, Dot and Southern blot hybridization, the target gene with specific promoter was demonstrated to integrate into the wheat genome already. The P_{SAG12}-*IPT* gene could inherit steadily in the most transgenic plants. The leaf cytokinin, chlorophyll, senescence development and agronomical character of transgenic plants were discussed. The results indicated that P_{SAG12}-*IPT* gene might specifically express in the senescent leaf of some transgenic plants, and the leaf senescence was obviously delayed.

Chalcone synthase-A (CHSA) was a key enzyme in the biosynthesis of all classes of flovonoids, and ariation of its expression might affect the colour of flowers. *CHSA* gene was cloned from flowers petals of *petunia* (*Petunia hybrida*) just coming into bloom, and was inserted into expression vector – pBI121 and pWM101, which contains CaMV 35S promoter in a sense-orientation[10]. Frist transferred *Cyclamen persicum* via pollen-tube pathway of germ line transformation. More than 4400 seeds were gotten. Among them the transgenic plants had altered the flower colour. Yellow or light yellow spots took on the edge of some petals in 8 plants with white flowers, even the whole petal turned yellow. Half petal of a few turned peach (Er qiao) among 3 plants with white flowers, even the whole flower turned peach. PCR assay of the transgenic Cyclamen plant is positive.

The above molecular biology evidence found the theoretical basis for application of pollen-tube pathway transformation in genetic breeding.

3 Optimization of Pollen-tube Pathway Technique

When heterologous DNA was sent into ovary in certain time after pollination, the heterologous DNA could enter into embryo sac by itself. Recombinant plasmid with target gene or donor total DNA that the target gene wasn't separated, could use the technique to transform. The technique operation was simple, but some condition need explore and optimize, for example weather term while transformation, different flower structure, pollination time, the density of DNA and teach melting agent.

Maize and cotton had the bigger flower structure than others', and could adopt the ovary injection. But small flower botany such as rice adopted instilling method. Both right method and transformation time were needed to notice. Cotton pollen tube arrived at ovary 8 hours after blooming[11]. While 20 hours, the pollen-tube pathway became. While 20 – 24 hours, bolt structure became from neck outer edge to ovary, and affected the DNA enter ovary. While 1 – 3 hours after rice self pollination, 1/3 hull could be removed, and distilled the heterologous DNA[12]. Also some people thought that 2 – 3 hours after rice self pollination was fitter. While 0.5 – 3 hours after wheat blooming, feather-like neck could be cut and distilled DNA solution immediately[13]. Because 50 hours was needed to complete the fertilization process, the fitter transformation time should be chosen at 10 – 20 hours after blooming[14]. The soybean was cleistogamy pollination crop. While 6 hours after self pollination fertilization started. So 6 hours after self pollination was a right time to distill DNA solution. The best period of pollen-tube pathway transformation technique was 6 – 20 hours after pollination[15]. Anyway, it was necessary to choose the right time to transformation, and couldn't cut neck too early and led to fertilize disfully and the fruit fall.

The density of the target DNA was also a very important parameter in transformation. The most suitable density of different crop was different, for example the wheat use 100 – 300 μg/ml, also use 700 μg/ml and obtained better transformation result; soybean was 300 – 500 μg/ml; the rice was 100 μg/ml, cucumber was 1000 μg/ml. the certain DNA pure degree was necessary, OD260/OD280 > 1.8 and OD260/OD230 > 2 could meet the request.

4 The Advantages and Shortages of Pollen-tube Pathway Transformation Technique

The operation of pollen-tube pathway transformation technique was done at receptor plant, and didn't touch with the receptor cell directly. It had no dependence to species and cultivars. Theoretically, it could apply in any flower botany. Recombinant plasmid with target gene or donor total DNA that the target gene wasn't separated, could be used in this technique. And it wasn't necessary to know the genetic back of the donor and the acceptor plant. It wasn't restricted by

genotype.

It needn't the tissue culture process, and overcame the technique obstacle of transformation receptor regeneration. Seeds could be obtained directly, reduced the variety possibility in process of vitro transformation, especially for the plant that it was difficulty to regenerate *in ivtro* culture; it had the great application value in heterologous DNA transformation.

It could be operated in field experiment environment. Transformation frequency was higher, but need to be aimed at the flower structure, blooming behaviour, fertilization process, temperature and humidity while transformation. The stability time of transformation offspring was shorter than traditional breeding process. For example, cotton and rice need 3 – 4 generation to obtain stability variety using the technique. The shortcoming of the technique was that it could be used only in blooming botany, and that it could be operated only in flowers period. If using total DNA to transformation, some unwanted gene DNA segment would be transformed. And it was reported the transformation offspring would bring variety.

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