Progress in planta transformation without tissue culture

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Abstract With the development of planta genetic engineering, more emphases have been laid on convenient and high efficient genetic transformation methods. And transformation without tissue culture is a prospective direction of it. In this paper, traditional transformation methods and the methods of non-tissue culture were summarized. With the exploration and application of A*rabidopsis* transformation mechanism, with the use of ion beam-mediated transformation invented by Chinese scientists and the development of other transformation methods, transformation methods without tissue culture and planta genetic engineering could be improved rapidly.

Keywords Planta transformation without tissue culture, Genetic transformation, A*rabidopsis* transformation, Ion beam-mediated transformation

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Gene transformation is a key step in plant genetic engineering. Now there are many kinds of reported gene transformation methods. With development of these methods, it is not difficult to transfer foreign DNA into many dicotyledons and some monocotyledons, and these foreign DNA can express stably. But these traditional methods have some disadvantages such as time consumption of preparing the recipient system, expensiveness and low transformation efficiency. More and more researchers are engaged in looking for easier and more efficient transformation methods. Recently, the study on transformation of non-tissue culture is emerging in the world. And there are more and more inspiring reports about this. The study and application of these new transformation methods maybe become a revolution of planta transformation.

1 Traditional transformation methods

According to the transferring procedure, transformation methods can be devided into two types including direct transformation systems and indirect transformation systems. Direct transformation systems are through physical and chemical methods without vectors. Physical methods and chemical methods have their own features. (a) The receptor range of physical methods is smaller than that of chemical methods, but chemical methods usually use protoplast as the receptor. (b) In physical methods, the molecular weight of foreign DNA should be smaller than that in chemical methods because in process of physical transformation the foreign DNA is easy to be deleted or rearranged. In direct transformation systems, there are PEG transformation,[1] liposome,[2] electroporation,[3] gene gun,[4] microinjection,[5] sonication transformation,[6] pulse electrophoresis,[7] laser microbeam,[8] and silicon carbon fiber-mediated transformation.[9] Indirect transformation system transfers the target gene into recipient cells through the vector. Agrobacterium[10] and virus[11] transformations are indirect transformation systems. The study and application of agrobacterium transformation was earlier. It has become the most popular transformation method in dicotyledons. Monocotyledons agrobacterium transformation is rather difficult. With building the efficient agrobacterium-mediated rice transformation system, a new research climax of agrobacterium-mediated monocotyledons transformation is coming. Virus vector can be divided into DNA virus vector and RNA virus vector. In DNA virus vector Caulimovirus (CaMV)[12] is researched most deeply. In RNA virus vector Gemini-

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viruse (GeNV)[13] is potential. GeNV has more popular most plants than CaMV.

Receptors of all the above transformation method are usually callus, suspension cells and other tissue in vitro, even protoplast. Preparing recipient system, specially protoplast，is rather difficult. And some apparatus are needed. This leads to expensive cost.

2 Transformation methods of non-tissue culture

Many laborataries are exploring the transformation methods of non-tissue culture. The receptors of these methods are meristems or other tissues that ultimately produce seeds. Chee and Slighton[14] used agrobacterium or tungsten particles in a number of species to transform cells in or around the apical meristems that are subsequently allowed to grow into plants and produce seeds. “Pollen tube pathway”[15] was forwarded by Zhou firstly according to the mutation of foreign pollen in wide cross. Foreign DNA was brought into embryo sac in process of pollen tube elongation and entering into embryo sac. Following the success on cotton, this method was used in wheat, pepper and other plants.[16] A variety of pollen transformation procedures and electroporation-mediated gene transfer into intact meristems in planta have been reported. Unfortunately, these methods have a common disadvantage of low reproductive rate.

3 *Arabidopsis* transformation of non-tissue culture

As a model plant, *Arabidopsis* transformation is relatively easy compared with other species. And this is a breakthrough in transformation of non-tissue culture.

In 1987, Feldmann and Marks[17] used agrobacterium to transfer germinating seeds. They obtained transformed plants in progeny through antibiotic screening.

Chang *et al*. reported “floral dip” transformation method in Vienna Arabidopsis Conference firstly, and described the procedure in the article in 1994.[18] Having cut the flowering shoot and all visible axillary buds, they applied a droplet of overnight culture of agrobactrium tumefaction on the wounded area. The secondary flowering shoots were dealt with as the first flowering shoots. So they got stable transformed plants.

Arabidopsis transformation by vacuum infiltration was born in 1993.[19] It was a revolution in *Arabidopsis* transformation. The procedure is as follows. Emerging bolts close to rosette were clipped off to encourage growth of multiple secondary bolts. 7 to 9 days later, the plants were inverted into agrobacterium culture solution and the air was exhausted for 20~30 min (according to the extracting velocity). A plastic bag was then placed over the plants overnight. The plants were transformed back to soil, grown to seed, and in the next generation stably transformed lines could be selected using the antibiotic screening. This method is simple, rapid and reliable. And a large number of transformation plants can be obtained. This method is used in process of creating *Arabidopsis* mutants using various traps and tags.

These methods were tried in other species in many laboratories but failed. So exploration of the mechanism of *Arabidopsis* transformation and other more popular transformation methods have become more and more important.

4 Ion beam-mediated transformation method

During the study of interaction between low energy ion beam and cells’surface, Yu *et al*.[20] found that ion beam could etch the cell wall. So they produced the idea of ion beam-mediated transformation firstly in the world.[21] The ion beam-mediated transformation method has some features compared with other methods.[22] First, etching by ion beam with energy and dose makes holes in the region providing microway to foreign DNA entering the cells. Secondly, implanted positive particles will decrease the negative charge on cell surface. This stimulates the foreign DNA to enter into the cells. Last, both direct and indirect function of ion beam can damage the DNA structure. This helps the foreign DNA integret into recipient genome. The receptor of ion beam-mediated transformation can be suspension cells or mature embryos. And in non-tissue culture transformation system, it is suitable. Through ion beam-mediated transformation, the total DNA of maize was transferred

into rice Zaoxian 213.[23] With many years screening, stably transformed plants were obtained.[24] They have stronger root system and exhibit purple red character of maize in short, glumal tips, stigma. The primary molecular evidence had been got. After transferring soybean total DNA into wheat, WU Li-Fang and YU Zeng-Liang screened and obtained two stains whose protein content was raised to 20.46% and 25.35%.[25] Gus gene was transferred into wheat mature embryos directly. And the analysis of PCR and Southern-blot indicated that gus gene integrated into wheat genome. The rate of positive plants of M0 was 3.9%.[26] All the above shows that as a non-tissue culture transformation, the ion beam is available, efficient and universal in many species.

5 Expectation

Transformation of non-tissue culture can be completed in a smaller laboratory, costing less and avoiding difficult technique. With the exploration and application of *Arabidopsis* transformation mechanism, with the use of ion beam-mediated transformation and the development of other transformation methods, planta genetic engineering can be improved rapidly. And these methods avoid somaclonal variation in process of tissue culture. It provides convenience of mapping clone, inserting mutation and other things related to transformation.

References

1 Paszkowski J, Shillito R D, Saul M *et al*. EMBO J, 1984, **3**(12): 2717-2722

2 Fukunaga Y, Nagata T, Takeba I *et al*. Exp Cell Res, 1983, **144**: 181-189

3 Fromm M, Callis J, Taylor L P *et al*. Methods Enzymol, 1987, **153**: 351-366

4 Sanford J C, Klein T M, Wolf E D *et al*. J Part Sci Technol, 1987, **6**: 559-563

5 Crossway A, Oakes J V, Irvine J M *et al*. Mol Gen Genet, 1986, **202**: 179-185

6 Zhang L J, Chen L M, Xu N *et al*. Bio/technol, 1991, **9**: 996-997

7 Ahokas H. Theor Appl Genet, 1989, **77**: 469-472

8 Weber G, Monajembashi S, Greulich K O *et al*. Eur J Cell Biol, 1989, **49**: 73-79

9 Kaeppler H F, Gu W, Somers D A *et al*. Plant Cell Rep, 1990, **8**: 415-418

10 Zambryski P, Tempe J, Schell J. Cell, 1989, **56**: 193-201

11 Elmer S, Rogers S G. Nucl Acids Res, 1990, **17**: 2391-2403

12 Viaplana R, Turner D, Covey S. J Gen Virol, 2001, **82**: 59-65

13 Bashir A M, Shadnam S, Mansoor S *et al*. Evidence for the presence of two new germinivirus species infecting cotton in Pakistan, 5th internetional congress of plant molecular biology, 21-27 September 1997, Singapore

14 Chee P P, Slighton J L. Methods mol boil, 1995, **44**: 101-19

15 Zhou G Y, Weng J, Zeng Y S *et al*. Introduction of exogenous DNA into cotton embryos, In: Wu R, Grossman L, Molddave K (eds), Methods in enzymology, Recombination DNA, Part C. New York: Academic Press, 1983, **101**: 433-481

16 Hu C Y, Wang L. In vitro cell Dev boil-plant, 1999, **35**: 417-420

17 Feldmann K A, Marks M D. Mol Gen Genet, 1987, **208**: 1-9

18 Chang S S, Park S K, Kim B C *et al*. Plant J, 1994, **5**: 551-558

19 Bechtold N, Ellis J, Pelletier G. Mol Biol Gen, 1993, **316**: 1194-1199

20 Yu Z L, Deng J G, He J J *et al*. Nucl Instru Meth Phys Res, 1991, **B59/60**: 705-708

21 Yu Z L, Yang J B, Wu Y J *et al*. Nucl Instru Meth Phys Res, 1993, **B80/81**: 1328-1331

22 Yu Z L. Introduction of ion beam bio-technology (in Chinese), Anhui Science Technology Press, 1998, 251

23 Wu J D, Wang X F, Wu Y J *et al*. Anhui Agric Sci (in Chinese), 1997, **25**(2): 112-113

24 Li H, Wu L F, Song D J. Acta Laser Biology Sinica (in Chinese), 1999, **8**(4): 261-265

25 Wu L F, Yu Z L. Acta Agric Nucl Sinica (in Chinese), 2000, **14**(4): 206-211

26 Wu L F, Yin R C, Gu Y H *et al*. Acta Biophysica Sinica (in Chinese), 2001, **17**(4): 724-730