Recent Advances of Genetic Transformation in Camellias

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Abstract

Most camellia species have high ornamental and economic values, such as making beverages and planting in parks for ornamental purposes. Although traditional breeding technology has made great contributions to the improvement of Camellia varieties, the application of genetic transformation technology has become a new way due to the limitations of traditional breeding and the requirements of low production cost and high productivity. In this paper. This paper will provide valuable information for breed improvement workers.

Keywords: Camellia, genetic transformation, *Agrobacterium rhizogenes, Agrobacterium rhizogenes*, biolistic gun method.

Introduction

Since the first transgenic tobacco was created in 1983, great achievements have been made in plant genetic transformation. At present, the commonly used genetic transformation methods are biolistic gun method, Agrobacterium mediated method, etc. Because of the unique advantages of Agrobacterium mediated method -- such as easy operation, low cost, high efficiency, good determination of inserted fragments and low copy number of transgenes -- it has become the preferred method in the transgenic strategy. About 80% of the nearly 200 kinds of transgenic plants that have been obtained are mediated by *Agrobacterium tumefaciens*. *Agrobacterium tumefaciens* is a kind of gram-negative plant pathogenic bacteria, which can induce the infected plant cells to form tumors, namely crown galls. *Agrobacterium tumefaciens* can introduce part of its genetic material into the chromosomes of the host plant, thus changing the genetic characteristics of the host.

Research progress of *A.tumefaciens* mediated genetic transformation of camellia

In the study of *A.tumefaciens* mediated genetic transformation of camellia plants, Yingying Luo(2000) cut Bt gene from pGA471 plasmid by restriction endonuclease Hind □ and Bgl II and transferred it into vector pCAMBIA2301. The constructed plasmid contains B gene, GUS gene and NPT □ gene. The constructed plasmids were transformed into E.coli and introduced into the *Agrobacterium tumefaciens* strains LBA4404, EHA105 and pRi15834 by three parent mating. Gus transient expression was obtained by Agrobacterium mediated transformation of tea leaves and calli. Using hygromycin as the selective reagent of callus screening, the effect is better than kanamycin, and the suitable concentration is 20mg·L^{-1}; using kanamycin as the screening reagent of tea leaf material, 50mg·L^{-1} is suitable.

Mondal (2001) established the production plan of transgenic tea by Agrobacterium mediated somatic embryo genetic transformation, and constructed two kinds of *A.tumefaciens* (EHA 105 and LBA4404) carrying dual vector p35SGUSINT plasmid containing neomycin phosphotransferase gene (NPT) and β - glucosidase gene (Gus) as a carrier system, in order to maximize the efficiency of transformation, several parameters were adjusted. It was found that pre-culture, injury and acetosyringone treatment all had inhibitory effects on transformation, while the appropriate bacterial growth period (OD_{600} = 0.6), co-culture time (5 days) and pH value in co-culture medium (5.6) promoted transformation. Finally, transformation was obtained transgenic plants.

Zhijin Tang (2013) cultured the leaves of the tea plant Longjing 43 on the medium containing different concentrations of kanamycin, and found that the best screening concentration of kanamycin was 100 $mg \cdot L^{-1}$. Then, the leaves of Longjing 43 were transformed by *A.tumefaciens*. After 3 days of pre-culture, the leaves were infected with activated Agrobacterium EHA105 (including RNAi vector pCAM-RNAi-GS) for 15 minutes, cultured in YMB medium for 4 days, and then cultured in screening medium after de bacteria. Finally, 8 resistant calli were obtained. Three of them were positive by Gus histochemical staining and PCR detection. The content of L-theanine in the three calli decreased by 85.09% compared with the control.

Singh (2015) constructed pCAMBIA 1301 vector of potato (*Solanum tuberosum*) class I chitinase gene (AF153195) and hygromycin phosphotransferase (HPT) gene, which had the function of resistance to hospital fungi. After they were introduced into agricultural bacterium LBA 4404, 12 transgenic plants were obtained by Agrobacterium mediated transformation into tea tree, and the transgenic plants were verified by pathogenic fungi The leaves of transgenic plants had obvious inhibitory effect on pathogenic fungi.

Jiangying Wang (2015) established xylose screening system by inducing callus from leaves, stem segments and cotyledons of *Camellia azalea* with handle, and then screening callus with xylose of different concentrations. It was found that the growth condition of the selected calli was good, the start-up date of callus induction was about 10 days earlier than that of the control group, and the growth amount was 1.5-2.5 times of that of the control group. It was found that the xylose concentration of 2.5$g \cdot L^{-1}$ was suitable for callus induction and growth of *C.azalea*.

Qianru Lv (2018) conducted effective plant regeneration and genetic transformation of tea plants by Agrobacterium mediated method. The calli from cotyledons of tea plants were used as receptors, and *A.tumefaciens* EHA105 containing PS1aG-3 plasmid was used for transformation. The factors influencing *A.tumefaciens* mediated transformation were studied. The best parameters of Agrobacterium mediated transformation of tea were: cotyledon callus was pre-cultured on yeb medium containing 150 $\mu mol \cdot L^{-1}$ acetobutanone for 3 days, then infected with EHA105 for 15 minutes, and then cultured in darkness for 3 days. The transient expression rate of GUS gene was 62.6%. After 3 days of selective culture,

the infected calli were transferred into differentiation medium and rooting medium, respectively, with the addition of 100 mg·L^{-1} spectinomycin, and the transgenic tea seedlings were obtained. The transformation rate was 3.6%.

The callus of tea plant was induced by Ying Gao (2019). Some 7,193 differential expression genes (DEGs) were screened from 42,417 single genes. In the process of callus growth, more DEGs were observed. Through the analysis of KOG and KEGG, it was found that the transcription factors MYB15 and RAP 2-12 were closely related to callus regeneration. These findings will help to optimize the regeneration system by regulating gene expression patterns.

Progress in *A.rhizogenes* mediated genetic transformation of Camellia

Guanghui Zhang (2006) established a high-frequency induction system for hairy roots of tea plants, with the highest induction frequency of more than 30%. He found that the best induction system for hairy roots was: OD_{600}=0.5-0.8 of *A.rhizogenes* infected the stem segments of sterile seedlings which had been cultured for 60-70 days for 10-50 minutes, and co-cultured on YMB solid medium with 100 mmol·L^{-1} acetosyringone for 2 days, and 500 mg·L^{-1} of cephalocytes, a large number of lateral branches and root hairs were produced on MS medium of thioxime sodium. PCR and GUS histochemical staining confirmed that the foreign gene had been inserted into genomic DNA and expressed.

Yaping Yang (2015) studied the inhibitory effect of cefotaxime, carboxybenzylpenicillin and Timentin on *A.rhizogenes* in MS medium and their effects on the clustered buds of tea plant tissue culture seedlings. The results showed that both cefotaxime and Timentin could effectively inhibit the growth of Agrobacterium LBA9402 and 15834, while 15834 grew on the plate of carboxybenzylpenicillin within 17 days; for the explants, both cefotaxime and carboxybenzylpenicillin significantly reduced the rate of clumping bud proliferation of tea tissue culture seedlings, and the rate of abnormal seedlings was high; when the concentration of Timentin was less than 400 mg·L^{-1}, the effect on tea tissue culture seedlings was small, compared with the normal effect There was no significant difference in light, Timentin had the potential to be used as an antimicrobial agent in the genetic transformation system of tea.

Rana (2016) studied the effect of different additives on *A.rhizogenes* mediated transformation of tea plant. It was found that the co-culture medium and post co-culture medium made of acetosyringone (150 μM) and 30 g·L^{-1} sucrose, 0.1 g·L^{-1} L-glutamine and 5 g·L^{-1} cross-linked povidone (PVPP) were added to the MS medium before the callus was inoculated. The rate of hairy root formation in cotyledon callus of tea was 16.7%, which was significantly higher than that in control group (MS + 30 g·L^{-1} sucrose).

Alagarsamy (2018) in order to reduce the impediment of phenolic compounds in the genetic transformation of tea trees, infected the hypocotyls of the 2-month-old tea cultivar seedlings with *A.rhizogenes*. It was found that the hypocotyls were kept moist after infection, and were continuously watered with A4 suspension medium with density of OD_{600} = 0.6. In

this way, the maximum transformation efficiency could be obtained. After two months, 88.3% of the infected parts came out Hairy roots appear. Finally, PCR identification and GUS staining were used to confirm the occurrence of transgenic hairy roots.

Progress in genetic transformation of camellia mediated by biolistic gun method

Shan Wu (2003) studied the method of biolistic gun transformation of tea tree. The optimized procedure was: 1.6μl plasmid DNA (1 $\mu g \cdot \mu L^{-1}$) was added into 10μL tungsten powder suspension (60$mg \cdot mL^{-1}$), then 0.1M spermidine 4μL, 2.5M $CaCl_2$ 15μL were added respectively, and finally the volume was fixed to 48μL; the amount of samples was 8-10μL each time. Under the condition of bombardment pressure of 7MPa, range of 5cm and vacuum degree of -0.095Mpa, bombardment once is suitable for GUS expression and callus regeneration. The survival rate of resistant callus was 5.0% - 12.1% after 2 months of resistance screening.

Sandal (2015) bombarded the plasmids containing Gus and nptII genes into the tender leaves of test tube seedlings of tea with PDS1000-HE biolistic gun. Among the 500 bombarded leaves, 217 (43.4%) showed callus on the screening medium containing 1.71μM kanamycin for 5 weeks. 15 of them had adventitious buds. PCR analysis showed that only 7 of the 15 callus with adventitious buds were positive. It was found that the genetic ability of transgenic seedlings cultivated in greenhouse decreased significantly.

Conclusion

At present, genetic transformation of camellia plants only succeeds in cultivating transgenic plants on tea trees, and transformation rate is very low. The research into genetic transformation of camellia plants used only cultivated callus, hairy root and somatic embryo, but no complete plant. Therefore, the case of genetic transformation of camellia is mainly faced with difficulties in the differentiation of adventitious buds. It is necessary to find the mechanism of the effect of antibiotics on the formation of adventitious buds on the success of genetic transformation of Camellia.

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