Transformation of soybean Gy3 gene into Artemisaarenaria mediated by corona discharge

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Abstract. In order to improve the protein content of desert plant, a method of genetic transformation mediated by corona discharge was established. Artemisia seeds were processed in corona electric field for 120 min at 12 kV, and then soaked in 0.1SSC media that contained Soybean Gy3 gene DNA to incubate for 12 h at 26 °C. Finally the seeds were inoculated on the differentiation medium. Polymerase Chain Reaction (PCR) and Reverse Transcription Polymerase Chain Reaction (RT-PCR) detection showed that the Soybean Gy3 gene had been successfully introduced into genomic DNA of the regenerated plants of Artemisaarenaria. The study provided a new way for corona discharge in plant genetic modification.

1. Introduction
In recent years, a lot of achievements about the sand plants growing in extreme climate on Northwest China’s arid have been obtained. The responses of net photosynthetic rate (Pn), chlorophyll II fluorescence parameters and trehalose content to drought stress were studied [1]. Water parameters of different parts of Artemisia ordosica and their roles in water transfer of SPAC system were studied by using the plant pressure-volume technique [2]. Leaf and young stem were used as the explant to study callus induction and proliferation in the desert plant Reaumuria soongorica [3]. But there are very few reports using transgenic methods to improve psammophytes protein content.

Artemisaarenaria are subshrub belonging to genus Artemisia of Compositae, which is grown in China’s Junggar basin of xinjiang, irtysh river basin, Hexi corridor in gansu sand, inner Mongolia alashan desert, the qaidam basin in Qinghai province (China); Mongolia and southern parts of Europe, the caucasus and central Asia. Artemisaarenaria, with strong deep roots and low feeding value, is good at defending the breeze sand and have abilities of keeping water and soil to grow. Its seeds are edible, and can also extract oil for food, paints, or preparation of food conditioner. At the same time, they can be used as medicine, the efficacy of anti-inflammatory scattered swollen and Chest profit gas [4]. Currently, only the tissue culture, chemical composition analysis and its food value were studied towards Artemisaarenaria. The study of molecular biology, which will take an important role in improving crop drought-tolerant quality and is good for recovering the mechanism of Artemisaarenaria’s drought-tolerant, however, has no reports in Artemisia transgenic. The
Experimental results were found by Li Yu-hua [5]. Results show that the anthers of two types of strawberry cultivate Mingling and Minglei, which were treated with high-voltage electrostatic field before inoculation, could significantly increase the percentages of induction, differentiation and differentiated shoot clumps of strawberry anther-derived callus, as well as increase the ratio of granular crowed callus. Song etc successfully observed the phenomenon that the power of the plasmid transfection, when E. coli (JM105) and scale ratio of DNA is 50~75 on low-frequency electric field’s intensity is \((5~20) \times 10^3 \text{ V} \cdot \text{m}^{-1}\) [6]. Liu Chang-jun [7] etc proved that low-intensity \((2 \times 10^3 \text{ V} \cdot \text{m}^{-1})\) (Ensure a high survival rate of cells) and fast electromagnetic pulse (The pulse width of \(10^{-7} \text{ s}\), the rise time is \(1.2 \times 10^{-9} \text{ s}\)). Irradiated cells about 20-100 min by 300 Hz can lead to cell electroporation. Based on the above research, to better understand the biological effects of high-voltage corona field, expanding the application and study a new means of transgenic [8]. A new experimental device which can produce the high-voltage corona electric field is developed. The first time the pBI121 plasmid, which contains a copy of GUS gene and kanamycin resistance gene, was introduced into Artemisaarenaria by corona discharge, and regenerated plants were obtained.

2. Experimental materials and measuring method

2.1 Experimental material
Artemisaarenaria seeds are provided by NingXia BIG West Agriculture Seed Ltd. The soybean “Heinong 37” is provided by the Heilongjiang Province, Dragon Seed Industry. The pMD19-T is provided by the Takara Biotechnology (Dalian) Co. Ltd. PBI121 which contains GUS gene and kanamycin resistant gene is provided by Li Jing Shandong University.

2.2 Design of experimental device
The developed experimental device is shown in figure 1.

![Figure 1. Sketch map of equipment for corona discharge](image)

The voltage supplied by DC high voltage power can be continuously adjusted from 0 to 30 kV. An ammeter whose resolution is 10 μA and accuracy is 0.8% is used to detect the discharge current. The applied voltage is tested by Q-3V voltmeter. The determined optimum parameters are the following: the cone tip electrode is made by nickel-iron alloy. The needle tip’s diameter is 0.05 mm, the needle tail’s is 1.5 mm and the length of needle is 2.6 mm. There were 100 needles \((n=100)\), the distance between the needle tips is 4 mm, and adjustable range is 4~32 mm, the distance between the adjacent needle plates is in the range of 0~60 mm.
2.3.1 Preparation of plasmid pBI121 [9] According to literature reports [10], PBI121’s registration number in gene sequences is M36686. The specific primer (RT-PCR) is designed by software Oligo6.0. The upstream primer is introduced into the XbaI restriction site. The gene sequences are: F1 (5'-tctaga AACACTCATCAGTCATCACC-3'). The downstream primer is introduced into the BamHI restriction site. The gene sequences are: F2 (5'-ggatcc CTAAGCCACAGCTCTCTTC-3'). The primers are synthesized by Dalian TaKaRa Biotechnology Company. The first strand of cDNA that is reversed transcription as a template, the target fragment is amplified after PCR reacted. Connecting the target fragment is amplified by PCR and pMD19-T vector. The positive clone expression vector that connect product is named pBI121/ Gy3.

Connecting the cDNA of the Gy3 subunit and pMD19-T carrier, the positive clones is sequenced by Dalian TaKaRa Biological Engineering Company. The results show that there are 1523bp in the gene fragment and reversing into the T vector. Then doing Homology comparison with corresponding sequence in Genbank (M36686), the results show that the homology of the corresponding sequence between the soybean Heinong No.37 Gy3 subunit gene nucleotide sequences and Gy3 subunit in GenBank is 99%. On pBI121 plasmid vector and the target fragment that connected product are identified by PCR. The target fragment has been successfully constructed into pBI121 plasmid of the vector. This phenomenon shows that the cDNA of Gy3 subunits expression vectors have been constructed successfully.

2.3.2 Transforming Of plasmid DNA into Artemisaarenaria by Needle - plate corona electric field
Select Artemisia’s vernalized seeds, stocke on a glass slide with double sided tape, and then process by Corona electric field (voltage 12 kV and time 120 min). After processing, the seeds are immediately soaked into 0.1 × SSC import media that containing 200 μg·ml⁻¹ DNA. They are incubated for 12 h at 26 °C, and then removed and washed 3 times with 0.1 × SSC solution repeatedly. After that the seeds are sucked up the remnants of solution by sterile filter paper, and are inoculated on the differentiation medium. The above operations are performed in sterile ultra-clean workbench.

2.3.3 PCR detection of transgenic plant regeneration Genome of the leaf of Artemisaarenaria is extracted by Broad Tektronix genome mention kit, PCR is reacted by the specific primers which has designed, the PCR products are used as negative control. The cDNA sequence of Glycinin Gy3 subunit which has been cloned used as positive control. PCR is reacted by DNA of transgenic regenerated plant leaves which is extracted by Broad Tektronix genome mention kit.

2.3.4 RT-PCR detection of transgenic plant regeneration The leave has been detected by PCR, and the total RNA of leave were extracted. RT-PCR reaction was conduct by using the synthesized specific primers and the optimal reaction condition which have been found out. The cDNA sequence of Glycinin Gy3 subunit which has been cloned was used as positive control. The experiments were conduct according to plant RNA extraction kit and RT-PCR kit which purchased from Dalian TaKaRa Biotechnology Company.

3. Results and Analysis

3.1 Cultivation transgenic plants
The exogenous DNA was introduced into vernalized seeds of Artemisia by corona discharge. Then they were inoculated in differentiation medium at 26 °C, cultured for 15 days till began to sprout roots. They grew into small plants about 7 days, and it was shown in figure 2.

3.2 transformers were tested by PCR
Artemisia seeds were transformed and inoculated in differentiation medium, and then they grew into small plants. Differentiated Artemisia seedlings were detected by PCR and RT-PCR. According to the steps that it was provided by broad Tektronix genome miniprep kit, the genomic DNA was extracted
from the leaves of Artemisaarenaria that were untransformed or transformed, PCR reaction was conducted by using the specific primers which has been designed. In the conducted electrophoresis experiment, PCR products about genomic DNA of Artemisaarenaria which have been transformed were used as the negative control, the cloned cDNA sequence of Glycinin Gy3 subunit were used as the positive control.

3.3 PCR experimental results of transformnts
Genomic DNA of Artemisaarenaria seedlings for PCR reaction, through the agarose gel electrophoresis, the PCR results were showed in figures 2 and 3.

Figure 2. Genomic DNA M:Marker(λ-HindIII) 1,2,3,4: transformants.

Figure 3. PCR Results M: Marker 1,2: negative control 3:positive control 4,5,6: transformants.

Figure 2 showed that the genomic DNA of Artemisaarenaria seedlings were extracted through the agarose gel electrophoresis. Figure 3 showed that PCR was reacted by specific primers heap of genomic DNA. It can be seen from figure 3, PCR test of transformants in lane 4, 5, 6 and positive control in lane 3 have the same size of 1520 bp fragments, which is cDNA sequence of Glycinin Gy3 subunit, but the negative control in lane 1, 2 can not amplify the expected fragment.

3.4 RT-PCR experimental results of transformants
The experimental were conduct according to plant RNA extraction kit and RT-PCR kit which purchased from Dalian TaKaRa Biotechnology Company. The total RNA was extracted from Artemisaarenaria seedlings which has been introduced the exogenous gene and not intrduced foreign gene, RT-PCR reaction was conduct by using the synthesized specific primers and the optimal reaction condition which have been found out, and then the extracted samples were identified by agarose gel electrophoresis. In the electrophoresis experiment that was conducted, RT-PCR products of the total RNA in Artemisaarenaria seedlings which have been transformed were used as the negative control, the cloned cDNA sequence of Glycinin Gy3 subunit were used as the positive control. The RT-PCR results were showed in figure 4.
3.5 Determination of crude protein content in transgenic plants
The roots of the seedling and the above part were dried at 105 °C, 10 min to 30 min, and then dried at 80 °C 3 h to 10 h until the plant water was evaporated completely. The dried plant was crushed to determine the crude protein. The crude protein content was 3.50% in the control group, but it was higher in the transformed plants than in the control group, in which the average increases to 4.67%.

4. Conclusion
Soybean DNA was introduced into Artemisaarenaria seeds mediated by corona discharge, and then transformed seedlings were obtained. Experimental results showed that the indexes of physiological and biochemical of desert plant were affected by corona discharge. The method used was a genetically modified one that can obtain efficient expression of transgenic plants.

References
[1] Song W M, Zhou H Y, Jia R L, et al.2008 J. Desert Res. 28 449
[2] Li X J, Tan H J, Zhang Z S and Li X R. 2007. J. Desert Res. 27 448
[3] Li Y B, Zhao X and Tan H J. 2008 J. Desert Res. 28 254
[4] Bai S N, Yong T W and Yun X F. 2000 J. Packaging & Food Mach. 18 17
[5] Li Y H, Deng J G, Lei J J, et al. 1995 J. Shenyang Agric. Univ. 26 136
[6] Song T Y 1990 Biophys. J. 58 897
[7] Liu C J, Wang B Y and Zhang H 1999 Chin. Sci. Bull. 44 1157
[8] Xu X, Na R, Yang J and Liu J J 2009 J. Inner Mongolia Univ. 40 600
[9] Maniatis T, et al. 1982 Mol. Cloning Lab. Manual. (New York :Cold Spring Harber Laboratory) p 75
[10] Utsumi S, Kohno M, Mori T and Kito M 1987 J. Agric. Food Chem. 35 210