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Original Contribution

EXPRESSION OF HUMAN GROWTH HORMONE ALTERS THE SHOOT MORPHOLOGY IN TRANSGENIC POTATO PLANT

M. Akhshik[1, 2], H. Hashemi Sohi[2*], E. Jourabchi[2], D. Forouzan Boroojeni[2]

[1] Department of Nanobiotechnology, Faculty of Life Science, Tarbiat Modares University
[2] National Institute for Genetic Engineering and Biotechnology

ABSTRACT

Plants could be used as bioreactors today, although many aspects of this process are known there are also many physiological changes in plants which remain unknown. In this research *Solanum tuberosum* L. cv. Kardal used as expression host via *Agrobacterium* mediated transformation to produce human growth hormone as a model protein. Although expression level was not high but potato plants represented some morphological changes. These plants were thicker and shorter than wild type and transgenic plants expressing different genes or transformed with an empty construct. It seems that human growth hormone could change plant morphology.

Key words: *Agrobacterium*-mediated transformation, Human growth hormone (hGH), Morphological change, transgenic Potato

INTRODUCTION

Using plants as bioreactor show many advantages over other expression systems like Bacteria, Yeast and transgenic animals (1, 2). Some of the most important advantages are: using available agricultural infra structure, ability for cultivation of wild surface of the land, scalability with low costs in large-scale, absence of human pathogens and moreover the ability of plant to fold proteins correctly (1, 3, 4). There are some good articles on this topic elsewhere (1-4). In this filed expression level as high as 7% of total soluble protein has been reported for (hGH) human growth hormone (5). The human growth hormone (hGH) is a 22 KD (191 amino acid long) polypeptide that interconnect with two disulfide bonds (6). Not only this peptide is used as drug in many cases (6, 7) but also because of simplicity of expression and no post translational modification (6) it is also a favorable model protein for developing the technology of plant made pharmaceuticals. Potato on the other hand could store expressed protein for several weeks which make it a good host for expressed protein and peptides (10).The main purpose of the study was production of a model protein hGH in plant expression system via *Agrobacterium*-mediated transformation, to set up a large-scale system of plant producing pharmaceuticals and in this research a strange effect observed which is indicating that even after about 30 years of expression in plants, many parts remain unknown in molecular farming. To our knowledge there is only one report about physiological change by expressing bovine growth hormone in tobacco plant roots and morphological change in potato shoot with the expression of human growth hormone has not been reported before.

***Correspondence to**:*Haleh Hashemi Sohi , Phone: 0098 912 345 7058, Fax: 0098 21 44580436, Email: akhshik@modares.ac.ir*

MATERIALS AND METHODS

Potato tuber provided from a local Potato tuber provider, primer set was produced by Eurofins MWG Operon, Germany and PCR kit provided from Qiagen Inc. USA. The enzymes and buffers were obtained from Fermentas Inc. USA and all other materials provided from Sigma-Aldrich Chemie GmbH, Germany.

Bin19/pRTL/GH plasmid was used as an expression vector which was a Bin19 Vector that uses *Cam*V35s promoter and *nos* terminator of pRTL and containing our Gene constructs (TEV enhancer + hGH Gene) as it is on **Figure 1**. The hGH gene derived from

digestion of pSK plasmid containing hGH with *Bam*HI whole plasmid was tested with PCR and transient expression in plants. The Bin19/pRTL/GH construct was transferred to *Agrobacterium tumefaciens* (GV3850) by direct transformation (8, 9). *Solanum tuberosum* L. cv. Kardal tuber disks were transformed essentially as described (10). The presence of the hGH gene in the genomic DNA (400 ng) of the putative transgenic plants was detected by PCR amplification using the specific primer set for the hGH gene; the forward primer used was 5' TTCGGATCCTATTAGAAGCCACAGC 3' and the reverse primer used was 5' TTCGGATCCATATGTTCCCAACTATACC 3' The PCR amplification program proceeded as follows; 5 min of denaturation at 94°C followed by 30 cycles of 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min, with a final 5-min extension step at 72°C. The PCR products were then separated via electrophoresis on 1.0% agarose gel. All plants were analyzed as mean ± Standard Deviations (SD) to test the significant differences among measured sizes. An unpaired student's t-test with statistical significant value of $P<0.05$ was applied. Each group of plants (transformed with hGH, transformed With empty construct and wild type) had five replicates.

Given the required time to plant to produce recombinant hGH total protein was extracted from plants as described (11). Total amount of protein determined as described via Bradford assay (12). The concentration of biological active hGH was determined by Active Immunofunctional hGH ELISA kit (13) as it was described by manufacturer's. Using the bioactive ELISA kit, leaf extracts was tested for the presence of material that reacts specifically with monoclonal antibody from potential transgenic plant. As expected like other plant expression systems low levels were observed, ranging from 0.6 to 3 ng/mL of extraction. The reaction was specific as wild-type potato, and also empty construct transformed potato showed no detectable hGH.

Transgenic plants were harvested and homogenized by grinding with a mortar and pestle at 4°C in extraction buffer as described by Lammeli et al.(11). 1 mM cocktail of protease inhibitors, which was solved in 0.05% Tween-20. The homogenate was centrifuged at 17000 g in a Beckman GS-15R centrifuge for 15 min at 4°C to remove insoluble cell debris. The sample aliquots were separated by 15% sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and the separated bands were blotted on the PVDF filters. After blocking with 5% skim milk, the filter was incubated with mouse monoclonal anti-human growth hormone antibody (16), followed by binding to an anti-mouse IgG conjugated to horseradish peroxidase as a secondary antibody. A 4-chloro-1-naphthol reagent was applied as a colorimetric detection substrate by horseradish peroxidase, as described by Sambrook et al. (9) Plants for this study measured 30 days after cultivation.

RESULTS AND DISCUSSION

Plants (hGH transgenic, wild and empty vector transgenic) cultivated and plants with putative hGH gene was separated with PCR amplification reaction, followed by separation via electrophoresis on 1.0% agarose gel. DNA fragments were visualized by staining with ethidium bromide as described elsewhere (9). Figure 1.a shows the results for the best transgenic lines. Western blot analysis result for human growth hormone proteins which were separated by SDS-PAGE (15%) that depicted in Figure 1.b shows detail of the best transgenic lanes and it also prove that our transgenic plants could produce hGH. Interestingly most of hGH expressing potato's had a common morphological change in comparison with transgenic potato's with an empty construct, wild plant or even transgenic potato expressing GUS. Stem of these plants were thicken and shorten in comparison with control plants ($p<0.05$) and this phenotype was also observed in the offsprings **(Figure 2)**. Transgenic Plants which could produce hGH had stems with mean diameter of 0.98 ± 0.09 centimeter whereas non transgenics mean was 0.46 ± 0.05. This difference was significant ($p<0.05$) as it could be seen in Figure 2. Mean plant height for hGH producing plants at the measurement time where 13 ± 1.2 centimeter and for non hGH producers where 29.8 ± 0.6 Centimeter at the same time and the difference was also significant ($p<0.05$) here **(Figure 2)**.

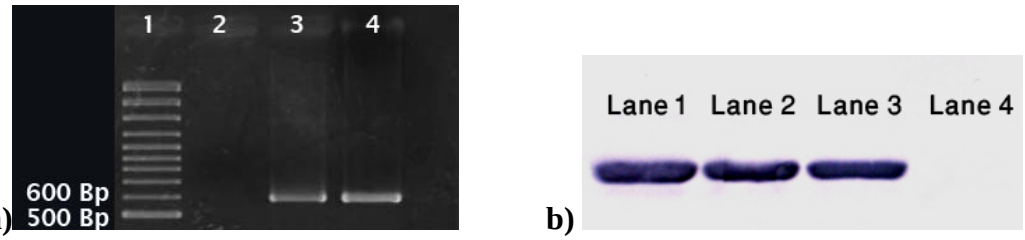

Figure 1. (A) PCR amplification of the best transgenic line, Lane1: Marker, Lane2: Wild plant, Lane3: Transgenic Plant, Lane4: Positive control. (B) Western blot analysis of the best transgenic lanes was done on 15% Gels. Each gel well was loaded with 50 mgr of Sample as follows: lane1: (hGH NOVO), lane2: Transgenic Plant, Lane3: Transgenic Plant, Lane4: Wild plant

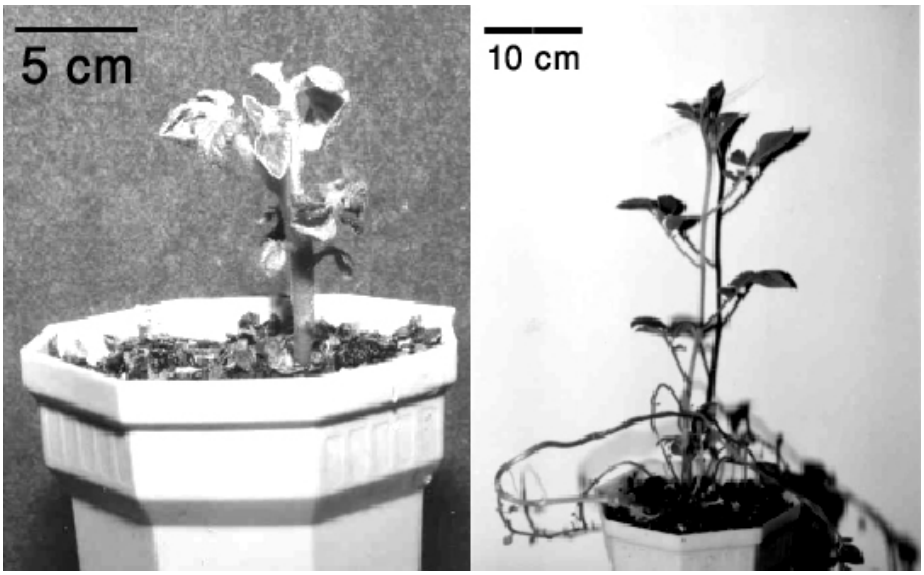

Figure 2. Right transgenic potato expressing hGH, left wild type potato

Growth hormones are protein-based peptide hormones that stimulate growth, cell reproduction and regeneration in humans and other animals but as in this study we have seen that it could also alters potato plant morphology. Here with we report for the first time effect of Human Growth hormone on plant life form. The results of the three experiences, Expression of the human growth hormone in transgenic potato plants and Expression of the bovine growth hormone in transgenic tobacco plants (14) and also expression of another growth hormone in potato (15) suggest that Growth hormone might change plant morphology and mechanism of this action remains unknown. This effect was not seen in the expression of *GUS* gene which is a reporter gene system. Further study is necessary for any conclusion. Although transgenic plant is a normal event for today's life many parts remain unknown within this field and it is necessary to understand this event more because plants relate to human in many ways whether it is food, clothing or even shelter, Any change in our environment could have a sever effect in future and there are lessons to be learned from the past disasters like DDT (dichlorodiphenyltrichloroethane) pesticide.

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