OCCASION

This publication has been made available to the public on the occasion of the 50th anniversary of the United Nations Industrial Development Organisation.

DISCLAIMER

FAIR USE POLICY

CONTACT

Please contact publications@unido.org for further information concerning UNIDO publications.

For more information about UNIDO, please visit us at www.unido.org

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

21289

CUBA

"Transformation of sweet potato (*Ipomoea batatas L.*) to increase its nutritional value as human food and animal feed"

Principal investigator: Dr. Guillermo Selman Houssein, Center for Genetic Engineering and Biotechnology, Ave. 31 e/190 y 158. Havana, Cuba.
Tel:+53-7-218466, Fax:+53-7-218070, Télex:512330 INGENCU.

UNIDO Contract No. 91/050
ICGEB Reference No. CRP/CUB90-02.

Keywords: Sweet potato, sporamin, plant transformation, aminoacid content.

Abstract: Results obtained in the genetic modification of sporamin A gene in the previous year supported our expectations. Two of three variants of sporamin A gene, both containing the synthetic DNA fragment, were chosen to transform plants through *Agrobacterium tumefaciens*. Criteria took into account for selecting the variants came from the results of sequencing and *E.coli* expression. Tobacco, as an easier molecular plant model, was selected to be transformed with the quimeric sporamin A variants. Conventional conditions for tobacco transformation were used. For sweet potato, transformation conditions were the same as those established in 1993. Km resistant plants, both tobacco and sweet potato, were evaluated by molecular methods, including Southern, Northern and Western blotting. Also P.C.R., to amplify the genetic modification of sporamin A from a total DNA extraction of transgenic plants, was performed. Results allowed us to select some few sweet potato transgenic clones to be evaluated for its nutritional quality.

Background: Sporamin is the major storage protein in tuberous roots with 60-80% of total soluble protein, has an apparent molecular weight of 25 000 daltons (1). Sporamin is considered a kind of storage protein, and during sprouting of the next generation it is specifically degraded (1,2), but it may have protease inhibitor activity, considering also the homology between aminoacid sequence of sporamin and Kunitz type trypsin inhibitors (3,4) Sporamin is encoded by a multigene family and it is possible to distinguish two subfamilies coding for sporamin A and B. Both types of proteins are immunologically very similar(3). Isoelectric points for sporamin A and B are 5.1 and 5.2 respectively(5). The Northern blot test demonstrates the tissue specific expression of these genes, detecting sporamin mRNAs in tubers but not in other organs(5). The improvement of the storage protein nutritional value is an important target in plant genetic engineering (6,7,8). One important aspect in this kind of work is the choice of insertion site in the storage protein gene, because a drastic change in the spatial structure could provoke low protein yields or protein degradation(9). Using appropriate softwares, different genetic versions of sporamin were analyzed. The nucleotide sequences and some molecular and stuctural predictions were compared with the natural protein. the modifications included the insertion of a synthetic DNA fragment at different positions of the nucleotide sequence of sporamin A gene. the synthetic fragment, similar to that of HEAAE-DNA(10), was prepared according to the established methodology(11). Sporamin A genetic variants were checked in *E.coli*. Tobacco and sweet potato were transformed with the previously selected constructions. Transgenic plants have been obtained.

Objectives:

Three main objectives were proposed for the third year of the project:

1-Transformation of sweet potato with the required plasmid and obtention of transgenic plants expressing the modified sporamin.

2-Preliminary analysis of transgenic plants by Southern blot and P.C.R.

3-Evaluation of nutritional value of tubers from transgenic sweet potato plants.

Work progress:

The current status of the project includes the following outcomes:

Obtainment of transgenic plants: Using NPTII as reporter gene, tranformation of both tobacco and sweet potato plants was performed. The constructions carried the genetic variants of sporamin A under regulation of 35S CaMV promoter and Ω fragment. As it has been described in the previous report last year, MS basal medium and a combination of BAP and NAA hormones were used to achieve the regenaration of plants belonging to the Jewel variety. However, combinations of Zeatin and 2,4D were necessary for regeneration of plants of the Cuban variety CEMSA 78-354. For tobacco plants, standard regeneration conditions (12) were applied. The concentration of Kanamycine in the selective medium was as high as 50 mg/L. Kanamycine resistant plants were chosen as potential transgenic plants.

Molecular analysis of transgenic plants: From a very small amount of tobacco and sweet potato leaves (5-10 mg), total DNA was purified by a procedure previously reported (13). P.C.R. was carried out using specific oligonucleotides to isolate the complete nucleotide sequence of genetic sporamin A variants. Conditions used were the reported (14). The results were as follow: in transgenic tobacco, bands around 1.1kb appeared for both variants. In transgenic sweet potato, 2 bands were present in the electrophoresis of P.C.R. products for both variants. The first migrating band had a molecular size of 0.9kb aproximately, and the second one. 1.1kb. It coincided with the expected behavior, because the smaller band corresponded to the natural genetic information for sporamin A, and those of the 1.1 kb suppossed to be the recombinant quimeric sporamin A genes. P.C.R. results were the first molecular evidence of transgenic character of transformed plants. Because of tobacco transgenic plants were obtained sooner, they were tested by Southern, Northern and Western blotting before sweet potato plants. Synthetic DNA fragment was used as probe in Soutern and Northern tests. For Southern blotting DNA was digested EcoRI-HindIII . The results of this technique were not conclusive neither for tobacco nor sweet potato plants, even in transgenic plants selected as positive by P.C.R. Nevertheless, Northern blotting results fullfilled the expectations, some transgenic tobacco and sweet potato clones showed bands according to the expected size. It indicates the presence of the synthetic fragment inserted in an expressed mRNA. The strongest evidence of the desired features in transgenic plants were those that came from Western blotting results. Some few clones, 3 of transgenic Jewel and 2 of CEMSA 78-354 transgenic plants, expressed the modified sporamins. It was detected by using rabbit polyclonal antibodies specific for sporamin. In the case of plants carrying either sporamin variants, 2 bands were observed. One of them at the size of 24 KDa, and the other one at 33 KDa aproximately. It was not possible to differenciate the exact size between the two sporamin variants because they migrated similarly in this conditions. In general, the expression levels were high. Transgenic plants expressing the desired sporamin are being propagated in tissue culture conditions. Despite of this important results, the third objective proposed for this year of the project was not performed. It is necessary to increase the number of transgenic plants by the current propagation, to adapt them to at

least greenhouse conditions, and then to start with the evaluation of nutritional quality of the tubers, stems and leaves. We suppose it will take us a year more.

References:

1-Maeshima, M.; T. Sasaki and T. Ashashi (1985). *Phytochemistry* **24:** 1899-1902.
2-Li, H.S. and K. Oba (1985) *Agric. Biol. Chem.* (Tokyo) **49:** 773-774.
3-Hattori, T.; N. Yoshida and K. Nakamura (1989). *Plant Molecular Biology* **13:** 563-572.
4-Bradshaw Jr. H.D.; J.B. Hollick, T. Parsons, H.G.R. Clarke and M.P.Gordon (1989) *Plant Molecular Biology* **14:** 51-59.
5-Murakami, S.; T. Hattori and K. Nakamura (1987) *Plant Molecular Biology* **7:** 343-355.
6-Rao, A.S. and R. Singh (1986) *TIBTECH* **4:** 108-110.
7-Jaynes, J. and J. Dodds (1987) *New Scientist* **17:** 62-64.
8-De Clerq, A.; M.Vandeweile, J. Van Damme, P. Guerche, M. Van Montagu, J. Vandekerckhove and E. Krebbers (1990) *Plant Physiology* **94:** 970-979.
9-Hoffman, M.; D.D.Donalson and E.M. Herman (1988) *Plant Molecular Biology* **11:** 717-729.
10-Yang. M.S.; N.O.Espinoza, P.G. Nagpala, J.H. Dodds, F.F.White, L.Schnorr and J.M.Jaynes (1989) *Plant Science* **64:** 99-111.
11-Jimenez, V.; E. Guimil, E. Ferrer, G. Fontirroche, M.Vazquez, N. Arteaga and J. Gavilondo (1990) *Biotecnologia Aplicada* **7:** 142-148.
12-Uchimiya, H.; T. Handa and D.S.Brar (1989). *Journal of Biotechnology* **12:**1-20.
13-Dellaporta, S. L.; J. Wood and B. Hicks (1983). *Plant Molecular Biology Reporter* **1:** 19-21.
14-Goyenechea, B.; R. Moran, C. Gutierrez, G.de la Riva and S. Perez. *Biotecnologia Aplicada* **8**(2): 237-241.

Publications:

"Obtainment of genetic variants of sporamin A from sweet potato. Cloning; sequencing and heterologous expression in *E.coli*".
(Submitted to the Journal BIOTECNOLOGIA APLICADA).