

PD-AAW-321
52162

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 6/26/87

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18
FROM: AID/SCI, Victoria Ose *VO*
SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 4. 506
3

Attachment

2 cys

4. 506

AID Project 936-5542 Ob1. No. 3641294

"Use of Agrobacterium plasmid vectors to modify nutritive value of potatoes"

Progress Report No. 3 (18 month)

J. H. Dodds and J. Jaynes

Progress on this project has continued over the last 6 months. In December 1985 Dr. Jaynes visited CIP to present a progress paper to our Annual Internal Review.

The Annual Report of the project (copy attached) indicated experiments that were still in progress. This report will show how much progress has been made in these areas.

(1) Use of Ti plasmid and Leaf Discs.

The synthetic DNA sequences coding for enriched essential amino acid protein have been inserted into a plasmid of Agrobacterium tumefaciens that has had the tumor genes removed ("disarmed" plasmid). This plasmid has had inserted a gene for resistance to spectromycin as a selectable marker. These plasmids offer a more reliable and controllable transformant selection system and also they remove the tumorous gene from the regenerated plants which may affect adversely the developmental physiology of the plants.

Rec'd in SCI: JUN 26 1987

3

We have now optimised a medium that allows the regeneration of plants from cultured leaf discs. The leaf discs form a small amount of callus along the cut edge and small numbers of plantlets regenerate from this callus. We are now beginning to use this regeneration system coupled to the selective antibiotic in the culture medium.

(2) Induction of in vitro tubers.

We have now been able to optimise general methods for the induction of in vitro potato tubers (see attached manuscript). This method has been successfully applied in transformed plants and we have obtained transformed tubers to use for DNA, RNA and protein analysis.

(3) Analysis of DNA, RNA and Protein of the synthetic sequence.

As shown in the Annual Progress Report (see Fig. 1) we have successfully detected the insertion of the synthetic DNA sequence (Southern blot). The next step is to detect the presence of mRNA transcribed from the synthetic gene. Detection of mRNA is again by use of a P32 probe using what is known as a Northern blot. Figure 2 shows a Northern blot from transformed potato tubers clearly demonstrating the production of mRNA from the synthetic gene insert.

The final stage is to detect in the tubers the presence of the synthetic protein. Although we have preliminary positive results we intend to spend the next few months optimising an immunological quantitative detection system for the synthetic protein.

Encl.: Annual Progress Report
Manuscript in vitro tuberisation

27