NASA CONTRACTOR REPORT 166306

NASA-CR-166306
19820013002

Genetic Engineering Possibilities For CELSS: A Bibliography and Summary of Techniques

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CONTRACT NASA P.O. A 73308B (JEP)
March 1982

CELSS

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Genetic Engineering Possibilities For CELSS: A Bibliography and Summary of Techniques

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Prepared for
Ames Research Center
under Purchase Order P.O. A 73308B (JEP)

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CELSS

N82-20876#

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Part III

Resume' of the State of the Art in Plant-Bacterial Genetic Engineering and its Potential Application in the CELSS Program

STATEMENT OF WORK

It is a goal of the CELSS (Controlled Ecological Life Support System) Program to develop the capability to recycle materials within a closed system containing people. To accomplish this, it will be necessary to grow food on nutrients generated within the system. Such nutients would come from wastes generated by humans and by other biological components in the system. The nutrients would be used to grow some primary producer, that is, one or a group of photosynthetic organisms.

One group of photosynthetic organisms that are of primary interest are the higher plants. To a significant extent, the characteristics of the plants within the system will determine the general size of the system, and consequently the weight and volume of such a system. Because it is anticipated that such a closed system will serve to support people in space, it is desirable to decrease the ratio of the plants - to - man volume and weight. Two ways in which this could be accomplished would be to increase the productivity of the plants, and to simplify their nutrient requirements.

For the purposes of CELSS, it is of interest to investigate the potentialities of genetic engineering of plants. The goals of such engineering might be directed toward increased productivity, or toward the development of mechanisms for nitrogen fixation within the organisms. It is also conceivable that genetic manipulation might alter the ratio or the quality of plant products.

The contractor is requested to provide the background information necessary to allow decisions on whether extensive investigation of genetic engineering should be considered in the context of CELSS.

Given the stated goals of the CELSS program as expressed above, the following condensed review summarizes succinctly the state of the art with respect to genetic engineering possibilities particularly in regard to plant and bacteria-plant interactions.

It would seem that the following considerations are generally regarded as high priority,and any genetic engineering approaches to plants as food, whatever the system, including closed biological systems, must include them.

I. Plants as food sources - some of the essentials.

a) N_2 fixation

Nitrogen is of course a major requirement of all plants. The usualcrop plants can be divided into two major classes. The cereal crops such as rice, wheat, corn, etc. require fixed forms of nitrogen such as NH_4^+, or NO_3^- and cannot utilize atomspheric nitrogen. The other class, legumes, such as soybeans, pea and clover, to name only a few, are capable of utilizing atmospheric nitrogen (N_2) by virtue of a symbiotic relationship with certain bacteria which form root nodules on these plants. The genes responsible for this are the nif genes (for nitrogen fixing genes), a cluster known to contain at least 14 genes. The cluster can be transferred to a plasmid, and therefore is mobilizable and transferable.

There are considerations beyond N_2 fixation however, which deal with maximum efficiency of the process, and must be considered in the overall genetic engineering decisions.

Some of these considerations are as follows.

1) Energy demand of the process of N_2-fixation:

An important consideration here is the <u>hut</u> genes, involved in enhancing the energy efficiency of N_2-fixation. The mobilization on plasmids for transferability has been achieved.

b) CO_2-fixation

This is of course an essential event in the metabolism of all plants and photosynthetic bacteria. The responsible genes are the <u>cfx</u> genes. There is no reason to believe they could not be mobilized although, as yet, they have not been engineered in that way.

c) Water-splitting enzymes for generation of reductant

These are the <u>lit</u> genes and are critical to production of reducing power which together with ATP fulfill the energy requirements of photosynthesis.

Of lesser importance, but significant, are such things as the <u>osm</u> genes for osmoregulation, the <u>cut</u> genes for cellulose utilization, and the <u>den</u> genes for denitrification. All of the aforementioned genes are discussed in detail by Andersen <u>et. al</u> (1980 see bibliography Part I).

There is no reason to believe the mobilization and transfer of these sets of genes by plasmid vectors is not realizable. The general technology is well worked out. The specifics in each case must be separately analyzed, and successful realization of the suggested potential may require a substantial committment in brainpower, manpower and financial support.

2) Additional considerations in reference to plants as food source in closed biological systems

a) ratio of edible to non-edible components

To my knowledge, there is no directly applicable available information with regard to genetic engineering of mobilizable and transferable genetic units for plant to plant or bacteria to plant exchanges.

C. Rate of growth

Although there doesn't appear to be any specific information on mobilization of genes involved in the rate of growth of the major crop plants, there is some information on 2 major influences in this regard. Plants seem to fall into two groups with respect to the first product of photosynthesis, the C_3 group in which 3-phospho-D-glycerate is the first product of photosynthesis and the C_4 group in which malate and aspartate are the first products of photosynthesis. More importantly, however, leaves of plants of the C_4 group have high rates of net CO_2 assimilation (42 to 85 mg CO_2 per decimeter^2 per hour) and low rates of photorespiration at higher levels of irradiation in normal air at 25 to 35°C. (I. Zelitch Photosynthesis and plant productivity. C & E News, Feb. 5, 1979 p. 28-48). On the other hand, with few exceptions, leaves of C_3 crop species assimilate CO_2 at rates about half or less those of C_4 plants and have rapid rates of photorespiration. The exceptions to this rule, such as sunflower and cattail, are C_3 species with rapid rates of net photosynthesis. An understanding of the way in which these unusual species overcome the handicap of rapid photorespiration exhibited by all members of the C_3 group and the mobilization and transfer of these traits would be an invaluable advantage in improving photosynthetic efficiency.

The C_4 crop species, such as sugar cane, corn, sorghum, millet and certain weeds, have average yields and average seasonal crop growth rates (as measured by the dry weight produced per square meter of land area per week) that are two or three times greater than do C_3 species, such as wheat, soybean, spinach, tobacco and hay.

d) Altering constituents

There is to my knowledge no specific information on such things as genetic engineering to increase the protein content of potatoes or the starch content of soybeans. There is however information on recent attempts to improve potatoes by genetic engineering of potato protoplasts (Shepard, J. F., D. Bidney, and E. Shahin 1980 Potato protoplasts in crop improvement. Science 208, 17-24). The emphasis in this work was on resistance to disease, but other information which derived from this research is important to any considerations of genetic engineering including of course the possibility of increasing the protein content of potatoes.

In fact, by their single-cell nature and the relative ease with which they may be cultured, plant mesophyll cell protoplasts have simplistically been likened to microbes as objects for genetic manipulation. In theory, it is necessary only to induce a desired mutation within a protoplast population, allow ample time for expression, and then apply appropriate selection pressure to recover first a cell and, ultimately, a plant having a specified modification. Such a course of events would be possible, however, only if the cell is a true haploid, which is rare in plants, and the trait is simply inherited.

An additional finding with potatoes was that apart from the practical considerations the mere appearance of high frequency phenotypic variation for the several traits which were examined in "Russet Burbank" protoclones raised the interesting genetic question of the source of such variation particularly in the absence of intentional mutagenesis. At present, the matter is purely speculative, but it is pertinent that many vegetatively propagated plants express spontaneous mutations directly and often. Among potato plants in the field, the frequency has been placed at between 1.5×10^{-3} and 1.2×10^{-5}, depending on the character and the variety (Keiken, A. Spontaneous and x-ray induced somatic aberrations in Solanum tuberosum L. Almquist and Wikell, Stockholm, 1960). Sweet potatoes (Ipomoea batatas) are capable of even higher frequencies. In the 'Centennial' cultivar, for example, two plants per 100 differed in some morphological feature. (Hammett, H. L. 1979 Hort Science 14, 123). If genetic variation is so rampant, it would seem that with the right selection pressure the desired type should be relatively easy to obtain.

e) Other considerations

1. Plant Growth Regulators

The enormous potential of plant growth regulators to growth behavior manipulation cannot be ignored. For practical purposes plant growth regulators can be defined as either natural or synthetic compounds that are applied directly to a plant to alter its life processes or structure in some beneficial way so as to enhance yields, improve quality or facilitate harvesting. Included in this group are plant hormones (phytohormones) and herbicides when applied to induce a specific beneficial change. The four classes of hormones are auxins, giberellins, cytokinins, and inhibitors. It has been stated that giberellins probably cause changes at the gene level that result in the synthesis of new enzymes and that cytokinins also probably act at the gene level, their exact mechanism of action, however, being unknown (Nickell, L. G. 1978 Plant growth regulators - controlling biological behavior with chemicals. Chem. Eng. News, Oct. 9, 18-34). The following list of uses of plant growth regulants emphasizes their potential in the field of plant genetic and growth manipulation.

Promote rooting and propagation of plants
Initiate or terminate the dormancy of seeds, buds and tubers
Promote or delay flowering
Induce or prevent leaf and/or fruit drop (abscission)
Control fruit set and further fruit development
Control plant or organ size
Prune the plant chemically
Increase plant resistance to pests
Enhance plant resistance to such environmental factors as temperature, water, and air pollution
Prevent postharvest spoilage

Regulate the chemical composition of plants and
the color of fruit
Influence mineral uptake from the soil
Change the timing of crop development

2) Broad spectrum nitrogen fixing root nodule formation

One last aspect which perhaps bears separate attention is the phenomenon referred to as "faithful" and "promiscuous" nitrogen fixing root nodule formation. It is recognized that nodulation genes are a composite of contributions from the symbiotic nitrogen-fixing bacteria and the host plant. Additionally it is known that some strains of Rhizobium that form nodules on leguminous plants are very selective in their choice of host, forming nodules only on a very narrow range of plants and are therefore called "faithful". In contrast, Rhizobium such as the "cowpea" of the tropics, are said to be "promiscuous", because they form nodules on many different plants including cowpea, soybean, peanut and mung bean (see Valentine, R. C. Part I). More importantly, it has now been shown that Rhizobium can form nodules on non-leguminous plants (ibid), and that there are nitrogen fixing trees, the bacterial symbiont of which is an unusual organism called Frankia which resembles soil actinomycetes, but has not yet been cultured in the laboratory. Frankia forms nodules on a variety of woody species and thus is regarded as having evolved a relatively promiscuous set of host selection genes. Frankia nodules provide fixed nitrogen to large portions of the dry eastern slope of the Sierra Nevada containing a mixed stand of Purshia (bitterbrush). Bitterbrush provides a high protein browse for deer and range animals. It is not known whether Frankia represents a diverse or closely related group of bacteria. Certainly, these bacteria possess a unique set of host-selectivity genes which might be harnessed for nodule formation in a wide variety of plant systems (Ibid).

The blue-green algae represent the most promiscuous symbionts found in nature since they work effectively with a variety of plants forming leaf nodules, root nodules, and trunk cavities on different plants, respectively. Any success in the genetic engineering of these versatile microorganisms would be invaluable to the kinds of efforts being discussed in this report.

Although the last word is far from being heard in genetic engineering of plant and bacteria-plant systems particularly with reference to "Controlled Ecological Life Support Systems", the collective body of information assembled in this report should allow rationale decisions about feasibility, and directional priorities.

1. Report No.
NASA CR-166306

2. Government Accession No.

3. Recipient's Catalog No.

4. Title and Subtitle
Genetic Engineering Possibilities for CELSS:
A Bibliography and Summary of Techniques

5. Report Date
March 1982

6. Performing Organization Code

7. Author(s)
Emmett J. Johnson

8. Performing Organization Report No.

9. Performing Organization Name and Address
Tulane University Medical School
New Orleans, LA 70112

10. Work Unit No.

11. Contract or Grant No.
P.O. A 73308B (JEP)

12. Sponsoring Agency Name and Address
National Aeronautics and Space Administration
Washington,DC 24056

13. Type of Report and Period Covered
Final Report

14. Sponsoring Agency Code

15. Supplementary Notes
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FTS 448-5573 . The first in a series of CELSS reports

16. Abstract

This report provides a bibliography of the most useful techniques employed in genetic engineering of higher plants, bacteria associated with plants, and plant cell cultures. A resume of state-of-the-art genetic engineering of plants and bacteria is presented. The potential application of plant-bacterial genetic engineering to CELSS (Controlled Ecological Life Support System) program and future research needs are discussed.

17. Key Words (Suggested by Author(s))
CELSS
Genetic Engineering
Life Support System
Nitrogen Fixation

18. Distribution Statement
Unclassified - Unlimited
STAR Category - 54

19. Security Classif. (of this report)
Unclassified

20. Security Classif. (of this page)
Unclassified

21. No. of Pages
21

22. Price*

*For sale by the National Technical Information Service, Springfield, Virginia 22161

End of Document