FRONTIERS
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Mutant Plants From Cells
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THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION NEW HAVEN

Producing mutant plants to increase food production

By Joseph C. Polacco

Each day there are 250,000 new mouths to feed in the world. As the ultimate source of food for these and the other 4 billion people on Earth is the green plant, we must increase crop production if these people are to be fed. However, increasing crop yields by more extensive use of fertilizers, pesticides and mechanization results in a high and sometimes prohibitive energy cost.

While the energy (calories) stored in plant food is derived from an essentially limitless source, the sun, there is a finite land area in the world upon which crop plants can productively capture sunlight. Thus, it behooves us to seek ways of improving the energy conversion of plants without increasing consumption of fossil fuels.

The reaction to the right summarizes the photosynthetic energy conversion of sunlight to energy rich carbon compounds.

$$\text{energy} + 6CO_2 + 6H_2O \underset{\text{respiration}}{\overset{\text{photosynthesis}}{\rightleftarrows}} C_6H_{12}O_6 + 6O_2$$

A six carbon sugar represents the *photosynthate,* the food produced. Ultimately, 95% of the dry weight of plants is derived from photosynthate. The *total* photosynthate, which bears heavily on plant yield, is the net product of the above reaction. Thus, food energy is produced by photosynthesis, but some of this food energy is lost by respiration.

Agriculturalists seeking to improve plant yield by improving photosynthetic efficiency could never eliminate respiration because it is as essential to plants as it is to animals. However, there is much evidence that respiration can be reduced with no ill effects for the plant and at a net gain in the amount of food produced.

Plant respiration, unlike animal respiration, exhibits a cyanide-resistant component. The levels of cyanide-resistant respiration for some plant species are shown in Table 1. Most or all of this residual respiration is specifically inhibited by salicylhydroxamic acid (SHAM); the reason for this inhibition is not yet clear. Cyanide-resistant respiration oxidizes the same materials as normal respiration and shares some, but not all, features with the normal respiration found throughout nature. Importantly, this alternate respiratory mechanism is wasteful in that it is only one-third as effective as normal respiration in converting the sunlight energy captured in photosynthate into utilizable ATP. ATP may be likened to a hot water tank in a solar energized house. When the sun isn't shining, the calories stored (in ATP or hot water) can be utilized for energy requiring processes. Should the respiratory production of ATP be made more efficient by eliminating the inefficient cyanide-resistant component, greater net photosynthesis would likely be the result.

There is much evidence that respiration can be reduced with no ill effects for the plant and at a net gain in the amount of food produced.

I am seeking means to eliminate cyanide-resistant respiration (the alternate oxidase) by introducing the appropriate mutations to block it. We can conservatively estimate that the chances of finding a mutation which eliminates the alternate oxidase are one in a million. As this mutation would most likely be recessive, only plants homozygous for the recessive mutation would lack the alternate oxidase. These would occur randomly at a frequency of one in one trillion plants!

To find these few unique blades of grass in the great agricultural plain, the plant breeder must find a simple, inexpensive way to screen for the alternate-oxidase deficient trait. To do this, I am exploring tissue culture techniques (see Frontiers Vol. 26 #1, 1973), which allow

Table 1. Levels of cyanide resistant respiration in various crop plants.

	% Inhibition		
Material	Cyanide	SHAM	Cyanide & SHAM
Tobacco Cell Suspension	25%	77%	100%
Soybean Cell Suspension	38%		100%
Tobacco leaf disk	13%	1%	77%
Corn Leaf Disk	69%	0%	87%

SHAM is salicylhydroxamic acid, a specific inhibitor of cyanide-resistant respiration.

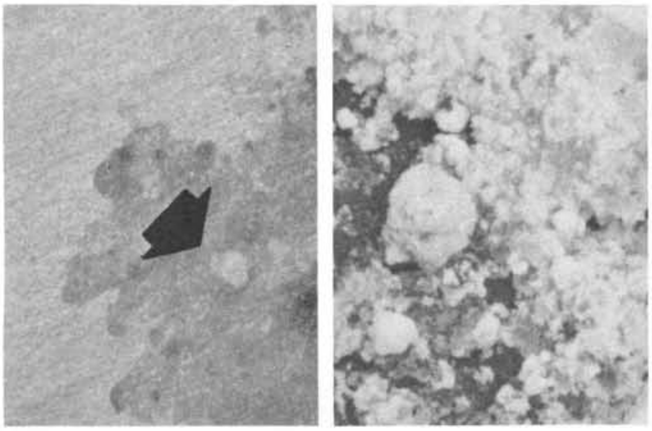

Fig. 1 (Above). Appearance of carboxin-resistant callus. The photo at the left shows a colony of mutant tobacco cells growing in the presence of inhibitory carboxin concentrations. The control plate at the right represents uninhibited growth. Fig. 2 (Right). The author with a carboxin-resistant mutant. This is the same plant that is pictured on the cover and in Fig. 1.

enormous numbers of single cells to be used in the selection process. This speeds up the selections and allows the initial screening to be carried out from among millions of small cells in the laboratory rather than from hundreds of large plants growing in a farm plot or greenhouse.

I have been using cells of tobacco because it is the easiest crop plant to manipulate in culture. Once the alternate oxidase is eliminated in tobacco, it should be possible to do it with food crop plants such as wheat and soybeans. The simplest means to select for the alternate oxidase-deficient trait is to use a chemical to kill cells which have an alternate oxidase. This would allow mutants that lack it to form a visible clone.

A Greek scientist working with the corn smut fungus, *Ustilago maydis,* has found that several mutants of the fungus that are resistant to the fungicide carboxin lack the alternate oxidase. Carboxin is a respiratory inhibitor which blocks the enzyme succinic dehydrogenase in intact mitochondria.

I thought that it could be possible that the same sort of inhibition could occur in tobacco. As I expected, it was difficult to kill tobacco cells with the fungicide. However, at high concentrations, I was able to kill almost all tobacco cells. It appears that the death of these cells is indeed due to inhibition of succinic dehydrogenase as it is with the smut fungi.

So far, I have isolated three carboxin-resistant lines of tobacco from large numbers of cells treated with ultraviolet light to increase mutation frequency. One such mutant is shown in Fig. 1 as it appeared among approximately one million carboxin-killed tobacco cells.

I have observed that these carboxin-resistant mutants grow at least as fast as the cells from which they were derived. When enough material is available, I will test the succinic dehydrogenase in these mutants and measure levels of the alternate oxidase.

The plant shown regenerating from callus (cover photo) comes from the mutant cell line of Fig. 1. Should test tube assays show that this mutant lacks the alternate oxidase, this plant will be doubled in chromosome number, selfed, and its progeny field tested and compared with the non-mutant plant from which it was derived.

The research described in this report is an outgrowth of my hope that the "new biology" and classical plant breeding will become a productive partnership in the business of feeding the world.

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NOTICE TO READERS

We have recently completed a change in the mailing plates for *Frontiers.* Please let us know if your name and address appear incorrectly on the back page.

Fortifying fermented foods using mutant bacteria . . .

By Lester Hankin and David Sands

Foods may be nutritionally deficient for a variety of reasons, such as being low in proteins or lacking in vitamins. Some grains, for example, are deficient in one or more of the ten essential amino acids. As the body cannot make its own, these amino acids must be supplied in food.

Where eating habits are well established, attempts made to introduce new foods to overcome inadequacies may be resisted. Thus, improving the nutritional value of foods already being eaten would appear to be the best approach because there should be little change in taste or appearance.

There are two methods now used to supplement essential amino acids in foods. One is to add amino acids, such as lysine, which have been synthesized chemically or recovered from fermentation procedures. An example is lysine added to rice. The other way is to find or genetically improve plants so that their proteins are naturally enriched with amino acids. An example is the high lysine mutants of corn that contain up to twice the lysine as the wild-type.

This approach allows current food preferences to remain, while nutritional values are increased.

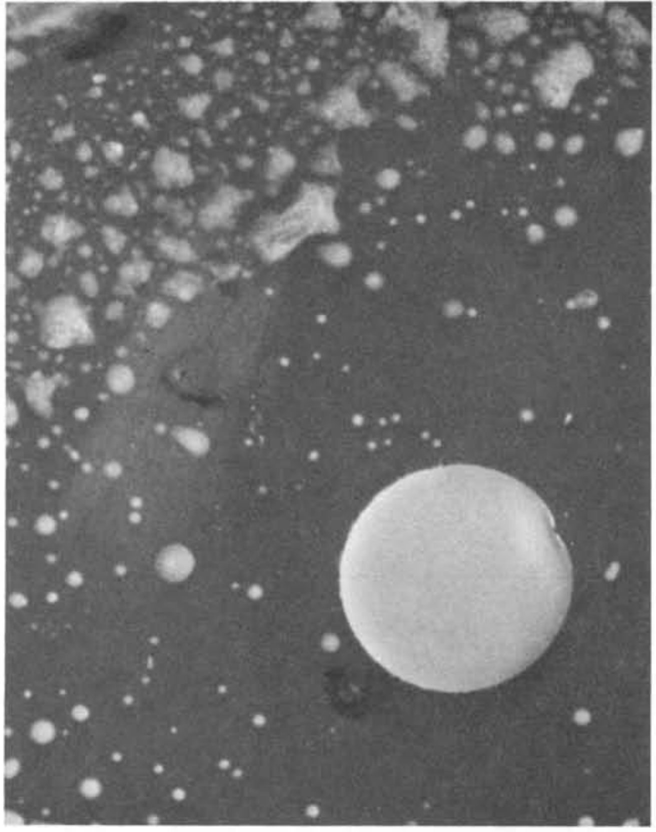


Fig. 1. Lactobacillus colonies growing closest to the analog-impregnated disc are lysine-excreting mutants.

A new way to enrich foods with amino acids occurred to us. We decided to do it by altering fermentation bacteria now in use so that they would produce increased amounts of lysine.

Everyone is familiar with fermented foods such as buttermilk, yogurt, cottage cheese, pickles, etc. Fermentation may be used to improve the taste, or to make unpalatable raw ingredients edible. However, if a material to be fermented is inadequate in nutritional value, the fermented food thus produced is probably also inadequate in nutritional value.

We worked to get organisms normally used in fermentation to excrete an essential amino acid. This approach allows current food preferences to remain, while nutritional values are increased.

Two of the lactobacilli from which we selected high lysine-excreting mutants had already been used to ferment soybean milk to yogurt, and a third lactobacillus had been used experimentally to ferment chopped corn to silage.

Our tests showed that these mutant bacteria increased

Table 1. Examples of fermented foods.

Place	Product Fermented	Product
United States	Milk	Cheese-Yogurt
Orient	Grain	Soy sauce
Japan	Rice	Tamari sauce
Indonesia	Soybeans	Tempeh
Orient	Fish	Sauce
Hawaii	Taro	Poi
China	Eggs	Pidan
Java	Peanuts	Ontjon
Java	Coconuts	Bongkrek
Indonesia	Rice or Cassava	Tapé
World-wide	Grain	Beer, Bread

the lysine content of soy milk yogurt up to 32% over that obtained using unselected lactobacilli. Similar results were obtained with silage.

Some food companies have written for more information and for cultures. We do not expect our cultures to be used commercially because these companies would prefer to select from organisms already being used. However, by using our methods, food processors should be able to increase the nutritional value of their product, while still retaining the flavor and other attributes of their fermented products.

In countries where many people depend upon fermented plant products for nutriment, foods could be enriched by introducing into the process organisms that produce excess lysine. In this manner, diets will be supplemented by the additional lysine produced without additional cost or requiring a change in eating habits.

We are now attempting to find mutants that will excrete other essential amino acids. Our new technique might also be used to increase the nutritional value of other food products which employ yeasts and molds in fermentation processes.

. . . how it is done

Bacteria and yeasts usually produce only enough amino acids for their own growth. If amino acids are present in their food supply, they use these first.

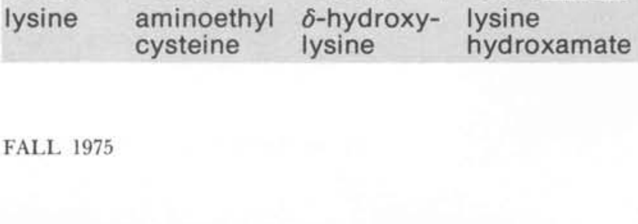
Several complicated biochemical mechanisms prevent excess amino acid from being produced. We had to select bacteria that would over-produce lysine and excrete it into the fermentation mixture.

In our experiments we used *Lactobacillus acidophilus* and *L. bulgaricus,* which are used to ferment soy milk to yogurt, and *L. plantarum,* which in experiments has fermented corn to silage.

Many wild-type lactobacilli routinely used to ferment foods were tested and none was found able to over-produce lysine. Therefore, we had to develop a method to find bacteria that would produce excess lysine.

We started by spreading the test lactobacillus on the surface of bacteriological media in a petri dish. We placed a disc of paper that contained a lysine analog on the media. A lysine analog is a compound similar to lysine but different in at least one constituent. Examples of the analogs we used are in Fig. 2. The portion of each molecule which differs from lysine is not shaded in the figure.

Fig. 2. Structural Similarity of Lysine and Lysine Analogs



A zone of inhibition, within which only a few bacterial colonies are growing, surrounds the disc.

As the lactobacillus grows, it uses the analog as if it were lysine. However, the analog kills all bacteria except those able to excrete lysine. Thus, a zone of inhibition, within which only a few bacterial colonies are growing, surrounds the disc. The bacteria growing within this zone are the spontaneous mutants that we seek.

Over-production of lysine serves as a defense mechanism to insure survival. The more lysine these bacteria excrete, the more dilute the analog in the medium becomes. Thus, these cells have less chance of using the analog, which would kill them.

We tested many spontaneous mutants growing within the zone of inhibition against increasing dosages of various lysine analogs to select for those able to excrete the highest amount of lysine.

Under laboratory conditions, wild-type lactobacilli excrete less than 1 ppm lysine. The high lysine selections excreted 72 ppm. Selections that have been kept in culture for over a year retain their ability to over-produce lysine.

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Nematodes in the home garden

By Patrick M. Miller and Saul Rich

Many articles about gardening problems mention nematodes, but most of the problem plants brought to the Experiment Station by troubled backyard gardeners are not affected by these microscopic worms.

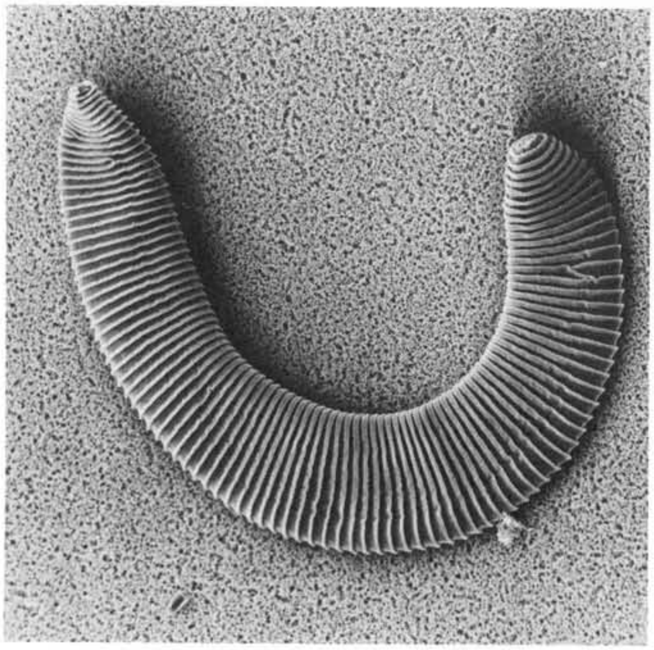
Still, the burgeoning interest in growing vegetables at home made it important for us to find out if nematodes could become troublesome in new gardens because many are planted in turf areas that harbor nematodes. Although these nematodes usually do little damage to grass, they can feed on the roots of other plants.

Nematodes can damage plants by sucking the sap from roots, causing root malformations, permitting the entry of pathogenic fungi, and transmitting virus diseases.

Generally, the larger the number of nematodes feeding on the roots, the more severe the injury. However, some plants can support large populations and show no obvious symptoms.

To see if nematodes could become a problem in newly-planted gardens, we ran an experiment at Lockwood Farm using a former bluegrass test plot that had been roto-tilled in the spring. The turf contained large populations of stylet and ring nematodes, two plant-parasitic forms not usually found in vegetable gardens. Part of the plot was treated with a chemical that kills nematodes. Such a chemical is called a nematicide.

Fig. 1. A scanning electron micrograph of a ring nematode. The specimen is about 0.8 mm long.



It quickly became evident that gardens newly-formed from turf had the most nematodes.

Some soil samples from this area were taken to the greenhouse for planting with beans, radish, cabbage, and two varieties of corn. The beans, radish, and one variety of corn grew much better in the soil that had been treated with nematicide. The cabbage and the other variety of corn grew just as well in treated or untreated soil.

At the farm, we planted string beans, peppers, and tomatoes. Some of the plants were mulched with thin, black plastic sheeting. As the growing season progressed, we checked the kinds and numbers of nematodes in each section of the garden. We also weighed and measured the plants at maturity.

We found that beans grew better in the soil that had been treated with nematicide or was mulched, as compared to soil that had not been treated. Beans grew best in soil that received both treatments. Peppers grew equally well in all plots, regardless of treatment. In some plots, growth of tomatoes was improved if the soil had been treated with nematicide and mulched.

In our experiment, nematode populations were reduced by surface mulching with black plastic sheeting or decaying organic matter. Covering the garden with salt hay did not reduce the number of nematodes.

Although a mulch of grass clippings is effective against nematodes, using clippings from a lawn that has been recently treated with a weed-killer could be disastrous to many plants

As an unexpected result of this farm experiment, we also observed that rabbits destroyed some of the bean plants growing on bare soil, but did not touch bean plants mulched with black plastic.

To gather further information, we asked colleagues and neighbors to bring us soil and root samples from new and old vegetable gardens, and from lawn or turf surrounding the gardens.

This request brought in about 240 samples from 20

Table 1. Effect of nematicide treatment and black plastic mulch on plant-parasitic nematodes and plant growth. The beans were planted on May 27; the data were taken on July 11.

Treatment	Nematodes per 100 grams of soil	Fresh weight of 12 bean plants (kilos)
Nematicide	26	3.7
Plastic	151	3.6
Both	65	6.1
Neither	114	1.4

gardens in the New Haven area. Forty-five vegetable varieties, and six common flowers were growing in these gardens. Some of the gardens had been mulched. When the soil samples were checked for seven genera of plant-parasitic nematodes it quickly became evident that gardens newly-formed from turf had the most nematodes. Generally, older gardens had few nematodes, although an occasional older planting would have many.

Dagger and ring nematodes (turf dwellers) usually were absent from the older gardens. Stylet nematodes were most commonly found around beans, chives, dahlias, muskmelons, parsley, potatoes, soybeans, squash, and tomatoes. Occasional high populations of lesion nematodes, which are common in Connecticut, were found around broccoli, summer squash, lettuce, rhubarb, and sweet peas.

We often found that soil from around one kind of vegetable in a garden would contain many nematodes, while there would be few or none in the soil from around other vegetables in the same garden. As this was true in many cases where the particular nematode is known to feed on both kinds of vegetables, we concluded that the nematode-free vegetable was resistant, or that nematodes had been brought in on some of the transplants and had not yet spread.

The results of these experiments indicate that nematodes are most abundant in gardens freshly-dug from lawns, and appear to become scarcer as the garden is used year after year.

Table 2. Effect of various soil additives on plant-parasitic nematodes. The additives were mixed with soil containing nematodes and stored in the greenhouse for 6 weeks before the counts were made.

Additive	Nematodes per 100 grams of soil	Percent reduction
None	199	—
Soil from bean root-zone	182	8
Soil from pepper root-zone	92	54
Soil from marigold root-zone	46	77
Leaf mold	66	67
Grass clippings	13	97

A Century of plant science means greater crop yields

By Paul Gough

A hundred years ago when The Connecticut Agricultural Experiment Station was founded, the science of genetics had not been put to work for man.

Many farmers grew seeds saved from the previous year which resulted from cross-pollination in the field. Seed grown under the controlled conditions that modern seed companies employ was not available.

Hybrid corn was invented by Donald F. Jones at the Experiment Station's Lockwood Farm in 1917, and constitutes virtually all of the corn grown in the United States. But, few have been able to see, beyond the cold statistics showing the rising curve of corn yields, what this difference means in the field.

Because a Centennial Celebration was planned for the annual field day at Lockwood Farm, one of the test plots was set aside for a comparison of common vegetable varieties grown in 1875 against modern counterparts. Peter Day and Diana Sattelberger of the Department of Genetics oversaw the experiment.

A search of old agricultural journals, such as *The American Agriculturist* and *The Country Gentleman,* produced the names of the varieties in use at the time the Experiment Station was founded.

Clear differences between some of the crop plants were evident.

Except for Lawrence flint corn, which came from Old Sturbridge Village in Massachusetts, most seeds were obtained from the J. Harris Company (Rochester, N.Y. 14624) and Gurney Seed Company (Yankton, S.D. 57078). The names of the varieties used and their sources are shown in Table 1.

After planting, each variety grew under the same conditions. Supplemental irrigation was used when needed, and Sevin was used to control some insects.

Since the crops were planted as a demonstration rather than a scientific experiment, yield measurements were not taken. However, clear differences between some of the crop plants were evident.

For example, the 1975 tomatoes put on less foliage, but yielded more fruit than the 1875 variety. The fruit from the 1875 variety was more irregular in shape than the rounded fruit which came from the 1975 variety. In addition, the 1875 variety fruit never seemed to redden at the top.

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A century of plant science

Continued from Page 7

Another easy-to-recognize difference was evident from gross examination of the cabbages. The heads of the 1875 variety were less full, and were cone-shaped, compared to the rounded heads from the 1975 seed.

The radishes were strikingly different. The 1875 variety radishes were much larger than the 1975 radishes, and looked more like red carrots. The 1975 radishes were the small, rounded kind that are sold in supermarkets and at roadside stands.

The results with beans showed considerably increased yields for both early maturing (Tendercrop) and late maturing (Genuine Cornfield) modern varieties as compared with their older counterparts King Horticultural and Blue Crop.

The Lawrence Flint corn was definitely the worst performer of the group. Its plants were the smallest and the ears were much smaller than any of the others in the plot. The Agway 724, (1975) plants were about as tall as the Stowell's (1875), but the plants were more uniform in size and yielded larger ears. The Sweet Sue (1975) and Sundance (1975) varieties grew larger ears of sweet corn than the Golden Bantam (1875), which also had far less leaves showing on the plants.

The results of this centennial garden, which compared varieties grown 100 years ago with those now commonly grown, show that science has produced better plants during the first century of the agricultural experiment stations.

Table 1. Varieties of vegetables grown in the century garden.

CROP	1875	1975
Pole Beans	King Horticultural (H)	Genuine Cornfield (H)
String Beans	Blue Crop (H)	Tendercrop (H)
Cabbage	Early Jersey (H)	Market Prize (H)
Melon	Nugmeg (H)	Gold Star #562 (H)
Radish	China Rose (G)	Champion (H)
Radish	French Breakfast (G)	Cherry Belle (H)
Tomato	Earliana (H)	New Yorker (H)
Sweet Corn	Golden Bantam (H)	Sweet Sue (H)
Sweet Corn	Evergreen (G)	Sundance (H)
Corn	Lawrence Flint (O)	Agway Field 724

Sources: (H) Harris; (G) Gurney; (O) Old Sturbridge Village

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