PRODUCTION OF MUTANT PLANTS
CONDUCIVE TO SALT TOLERANCE

by

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PRODUCTION OF MUTANT PLANTS CONDUCIVE TO SALT TOLERANCE

Completion Report

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by

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ABSTRACT

Much successful work has been done on altering the environment by
irrigation to increase the amount of land in agricultural production,
however the costs may increase as suitable water becomes more difficult
to develop. The alternative may be to adjust the plants to accept lower
quality water through selection for appropriate spontaneous mutants.
Methods for the selection of desirable spontaneous mutants require the
application of environmental pressure to give the mutant a competitive
advantage and also require the examination of millions of individual
plants. To accomplish this in field trials is time consuming and costly.
Instead this project employs individual cells in tissue culture where
ten million cells occupy 100 ml and each cell effectively is a plant,
because a plant can be regenerated from an individual cell. Plant
properties such as salt tolerance which is exhibited on the cellular
level can be selected in tissue culture at much less cost due to the
small scale required.

The focus of this project has been toward the selection of salt
mutants. Two lines of tobacco cells, tolerant to NaCl levels of 8000 ppm,
have been selected while normal tolerance to NaCl in this species is 800
ppm. Plants have been regenerated from the tissue cultures at many levels
of NaCl tolerance between these extremes, and these plants are currently
being tested for NaCl tolerance and for mutation inheritability. Although
practical tolerance needs may not exceed 2000-3000 ppm, the higher ranges
of salt tolerance may yield mutants with better yields at the moderate
tolerance levels. Considerable progress has been made toward tissue culture
mutant selection systems for oats, wheat, soybean, corn, and sugar beets.
Selection for salt tolerant mutants is currently underway for both oats and
wheat.

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I. ORIGINAL OBJECTIVES OF THE PROPOSED RESEARCH

Our principal objective is to utilize tissue culture procedures to select several new, mutant varieties of useful agricultural plants. These mutants will be chosen so that they can utilize water supplies which are currently agriculturally unsuitable due to undesirable ionic concentrations.

A breakdown of our main objective into its several parts delineates the need for the following types of mutant plants.

A. <u>Varieties which are specifically resistant to NaCl.</u> Such plants could grow in NaCl contaminated irrigation water, partially desalinated sea water, or on unirrigated land on which NaCl concentrations have remained at inhibitory levels due to a climate with insufficient rainfall and leaching. NaCl (particularly the Na^+ ions) often act as rather specific inhibitors of plant growth at very low concentrations (often only a few hundred ppm). The presence of excess NaCl in otherwise agriculturally suitable soils and waters is a common enough problem to necessitate the generation of tolerant varieties.

B. <u>Varieties which are highly efficient in taking up calcium from the soil.</u> Calcium is a vital element for the proper functioning of plants and maintenance of cell membranes; calcium deficiencies cause plants to be noticeably more susceptible to NaCl poisoning (1). The problem is compounded by the fact that many sources of irrigation water contain insufficient calcium and that sodium, when present in high enough quantities, tends to displace calcium from the soil (2), resulting in Ca^{++} deficient, NaCl damaged plants, and Ca^{++} deficient animals. Production on most irrigated land could be markedly improved

and much non-arable land could be brought into production if new varieties of plants, more efficient at calcium uptake, were available. Using these plants, low calcium conditions could be better tolerated.

C. **Varieties which can take up water in the face of high ionic strength in the soil (osmotic effect).** Between periods of irrigation the ionic strength of soils frequently becomes very high as a result of pure water consumption due to evaporation and transpiration, with an accompanying decrease in total water supply due to drainage. Under such conditions plant growth is frequently inhibited because the roots can no longer uptake water from the salt coated soil particles. The solution to this problem is the use of new varieties of plants with a heightened ability to take up water.

D. **Varieties which can utilize irrigation water of inhibitory ionic strength and composition.** Water from major drainage systems is frequently inhibitory to plant growth due both to ionic strength and to a particular combination of ionic species. For these major rivers new plant varieties, specifically adapted to tolerate their ionic pattern, are needed. In addition, varieties are needed which are selected in anticipation of future increases in ionic strength or changes in ionic composition.

II. ACHIEVEMENT OF THE OBJECTIVES

The original proposal envisioned a longer time frame (2 years instead of 1½ years) and larger funding level ($54,163 instead of $25,168). The current achievement of the objectives as described originally is limited to creating varieties which are specifically resistant to

NaCl (A). An objective not stated but nevertheless necessary in the performance of this proposal is the development of tissue culture and plant regeneration techniques for the plant varieties involved. Although techniques for these procedures are published in the literature for tobacco, oats and wheat, this laboratory had to work out modifications and adjustments to obtain satisfactory results. For soybean and sugar beet, tissue culture procedures are published but the regeneration procedures do not exist. This laboratory is currently engaged in research to obtain regeneration procedures for these plants. Thus once the unstated objective is met work proceeds on the stated objectives. Of these it was felt that objective A was the most important. Accordingly, tobacco cells, with which this laboratory has considerable tissue culture experience, have been selected in a ten stage process to a NaCl tolerance of 8000 ppm, a tenfold increase over the normal tolerance of 800 ppm. Plants have been regenerated from the tobacco tissue culture cells at many levels of salt tolerance between the extremes of the cells selected. Some of the plants are getting large enough to produce seed in the next two months. In addition, wheat and oat callus tissue cultures are entering the salt tolerance selection phase.

Objectives relating to calcium uptake, water uptake, and custom selected ionic tolerance (objectives B, C, and D) will have to be addressed in further continuation of the project now funded for future work.

III. RESEARCH PROCEDURE

   A. Select plant species to be used.

       The main criterion which influenced the selection of varieties were:

         ● a species which has proved satisfactory in this laboratory

         ● a species which seems to have well worked out procedures in

           the literature for tissue culture and plant regeneration

         ● a major food crop

         ● a species widely cultivated throughout the world, and

           particularly in the western United States

       Although tobacco is not a food crop, work has proceeded on this

   variety because of the experience with tissue culture and regeneration

   procedures already available in this laboratory. Other crops now

   being utilized in the laboratory include wheat, oats, soybeans, corn,

   and sugar beets. Although peas, barley, tomatoes, dry beans, sugar

   cane, alfalfa, and rice were considered in the original proposal, work

   has not yet begun on these species.

   B. Obtain tissue culture and regeneration procedures. The procedures for

       tobacco are published in the literature. However, this laboratory had

       to modify and make adjustments to these procedures to obtain successful

       results. The adjustments refer principally to modification of hormone

       types and concentrations employed to obtain tissue cultures and

       regenerated plants. Experience is required to learn the most satis-

       factory parts of the plants to create the tissue cultures, the normal

       time progression and appearance of the cultures, and media and

       cultural conditions necessary to regenerate plants. Procedures for

       oats and wheat in the literature also had to be modified, and

procedures for regeneration of plantlets from tissue cultures of
soybean, corn, and sugar beets are under development since none are
published in the literature.

C. Institute selection procedures. The first requirement of selection
of spontaneous mutants in tissue culture is that the property being
selected is exhibited at the cellular level. The original objectives
were all chosen with this restriction in mind (see attached review
article for discussion of other cellular properties which may be
selected in tissue culture).

The general procedure in tissue culture is first to establish
a rapid growing pure tissue culture. Then environmental selection
pressure (such as NaCl) is applied. This slows the growth of the
normal cells giving the desired spontaneous mutants a growth advantage.
As the mutants overgrow the culture the growth rate of the culture
returns to normal. Then the environmental pressure is increased once
again resulting in stagewise selection of mutants with increased
tolerance.

D. Regenerated plants. Once a desired mutant cell line has been established,
plant regeneration begins. This process proceeds first on a limited
scale until greenhouse tests on the plants show that the tolerance to
environmental condition is acceptable. Once the plants prove
acceptable rapid multiplication of individual plants can be secured by
returning the tolerant plants to tissue culture and regenerating
thousands of resistant plants for initial full-scale field testing.
At this point the testing will be turned over to others since the

procedures for field testing of new varieties have been well worked
out and utilized by others.

IV. PROJECT RESULTS

A. Tobacco experiments. The principle objective of the research was to
obtain NaCl tolerant plants from tissue cultures of several food
crop plants. Since tissue culture procedures are better developed
for tobacco than for more useful food crop plants, it was felt
important to demonstrate that NaCl tolerant cells could be selected
in tobacco tissue cultures, and that such cells could be regenerated
into NaCl tolerant plants which pass the trait on to offspring in a
predictable manner.

The procedure consists of five steps 1) utilizing a small section
of stem or leaf tissue to obtain a rapidly growing, undifferentiated
cell mass (a "callus") on solid medium; 2) placing the callus in
liquid medium on a shaking machine to obtain "a cell suspension";
3) increasing the NaCl concentration of the cell suspension to select
for salt tolerant mutants; 4) regenerating plants from tolerant
suspension cells; 5) testing whole plants for salt-tolerance and
inheritability.

These experiments had already been under way for two years when
this grant took effect. During the tenure of this grant, selection
for salt tolerant mutant cells and plants continued in tobacco.
Sodium chloride tolerant strains were selected at levels varying by
steps of 800 ppm. To date tolerance to 8000 ppm NaCl level has been
reached in suspension culture using two lines of cells. The stepwise

progression of tolerance selection permits spontaneous mutant cells with increased NaCl tolerance to overgrow the less tolerant cells. Also the stepwise progression has created suspension cultures with sodium chloride tolerances of 800, 1600, 2400, 3200, 4000, 4800, 5600, 6400, 7200 and 8000 ppm. This variation and range will permit cell permeability studies to proceed at many levels of salt tolerance. It should be possible to determine the relationship between cell permeability and salt tolerance helping to determine cell physiological changes which may occur with the increased tolerance to salt.

It may be that the desirable salt tolerance level for some agriculture species need not exceed 2000 to 3000 ppm in order to be successful from a practical standpoint. This could be due to sufficient water resources available at that salt level. It could also be due to possible soil damage with higher salt concentrations in irrigation water. However, the tobacco cells were pushed to the 8000 ppm salt tolerance level for two reasons: 1) To determine the maximal tolerance level obtainable in this species; 2) Because cells and plants tolerant to very high salt levels might have higher than normal growth rates in lower salt concentrations.

Small regenerated plantlets have been produced at salt tolerance levels of 800, 3200, 4000, 4800, 5600, and 7200 ppm. This choice of levels covers the ranges thought important for practical reasons. The regeneration procedure consists of removing small groups of suspension cells to a solid medium which encourages further growth and incipient regeneration. Then the solid cell mass ("callus") is

subdivided and placed on a second solid medium in which shoot formation actually occurs. Regenerated shoots are transferred to yet a third medium which elicits root formation. At this point plantlets are transferred to pots and placed in a growth chamber or in the greenhouse.

A scanning electron microscope (SEM) examination was made of a tobacco plantlet developing from callus cells. The purpose was to look for the site of developing buds. This particular sample did not show the site optimally, possibly because the leaves were already well developed and obscured their point of origin (Figures 1, 2, and 3). However, an intermediate stage was discovered. Trichomes or leaf hairs were found emanating from callus cells directly (Figure 4). Since the sample is necessarily killed in preparation it is not known if this site would proceed further into the development of a leaf bud. Only SEM examination of more samples may shed some light on this phenomena.

Experimentation is just now moving soldily from the plant cell suspension to the plant stage. Subjects still to be studied include tolerance of the plants to salt, plant growth rates and yields, and inheritability of the mutant traits. As mentioned earlier, even though the practical desired tolerance may be 2000 to 3000 ppm, best yields may come from plant strains selected in tissue culture at much higher salt concentrations. This phenomena could arise both from growth rate characteristics of tolerant plants and from the fact that evaporation and transpiration between irrigation periods raises soil salt concentrations considerably above levels found in irrigation water.

Figure 1. An overview of the callus leaf bud of tobacco made
with a scanning electron microscope at magnification
(80X) Leaves (L) and callus (C) are designated by
arrows. B refers to the location of larger detailed
Figure 2, and A refers to the location of Figure 3.

Figure 2. A higher magnification view (544X) taken from above
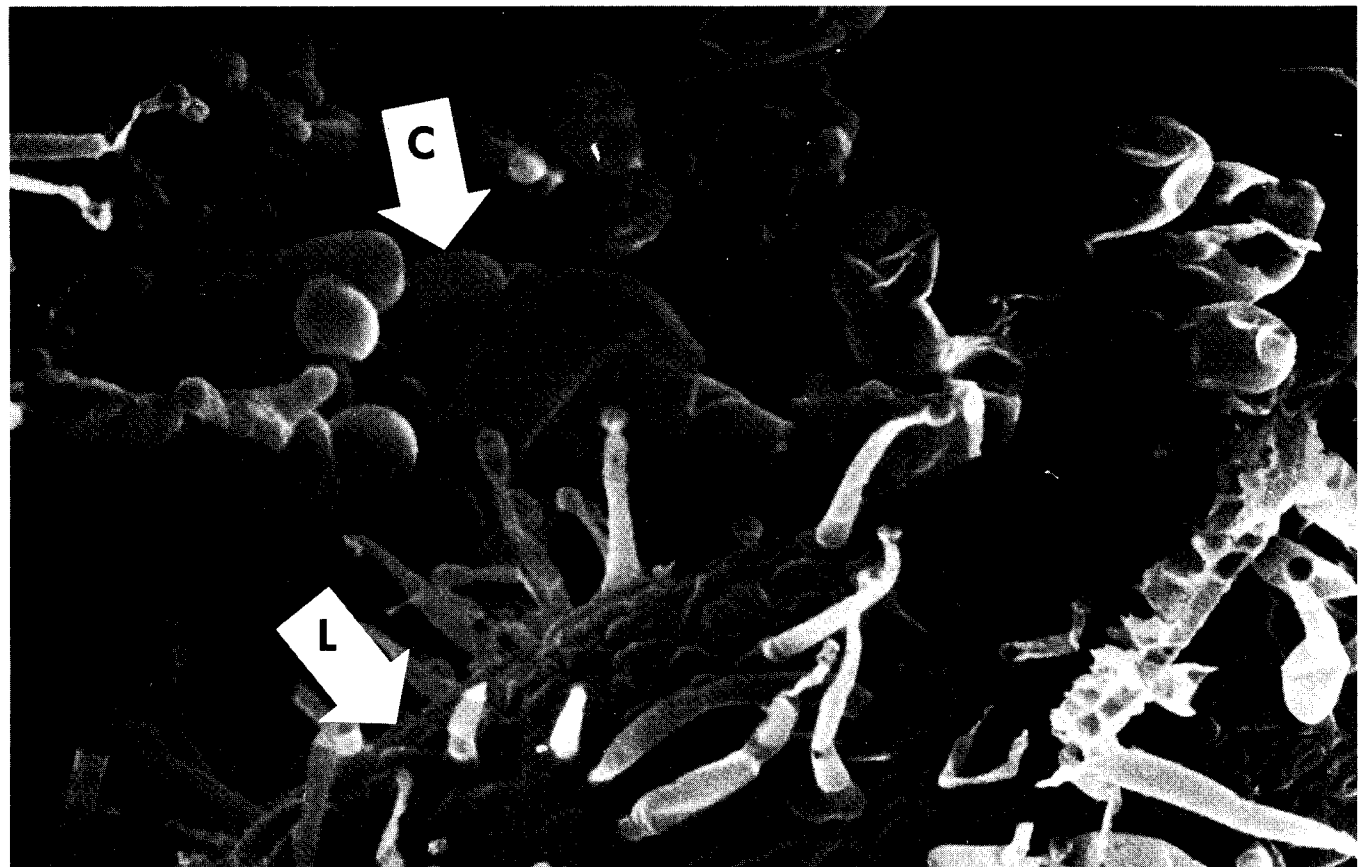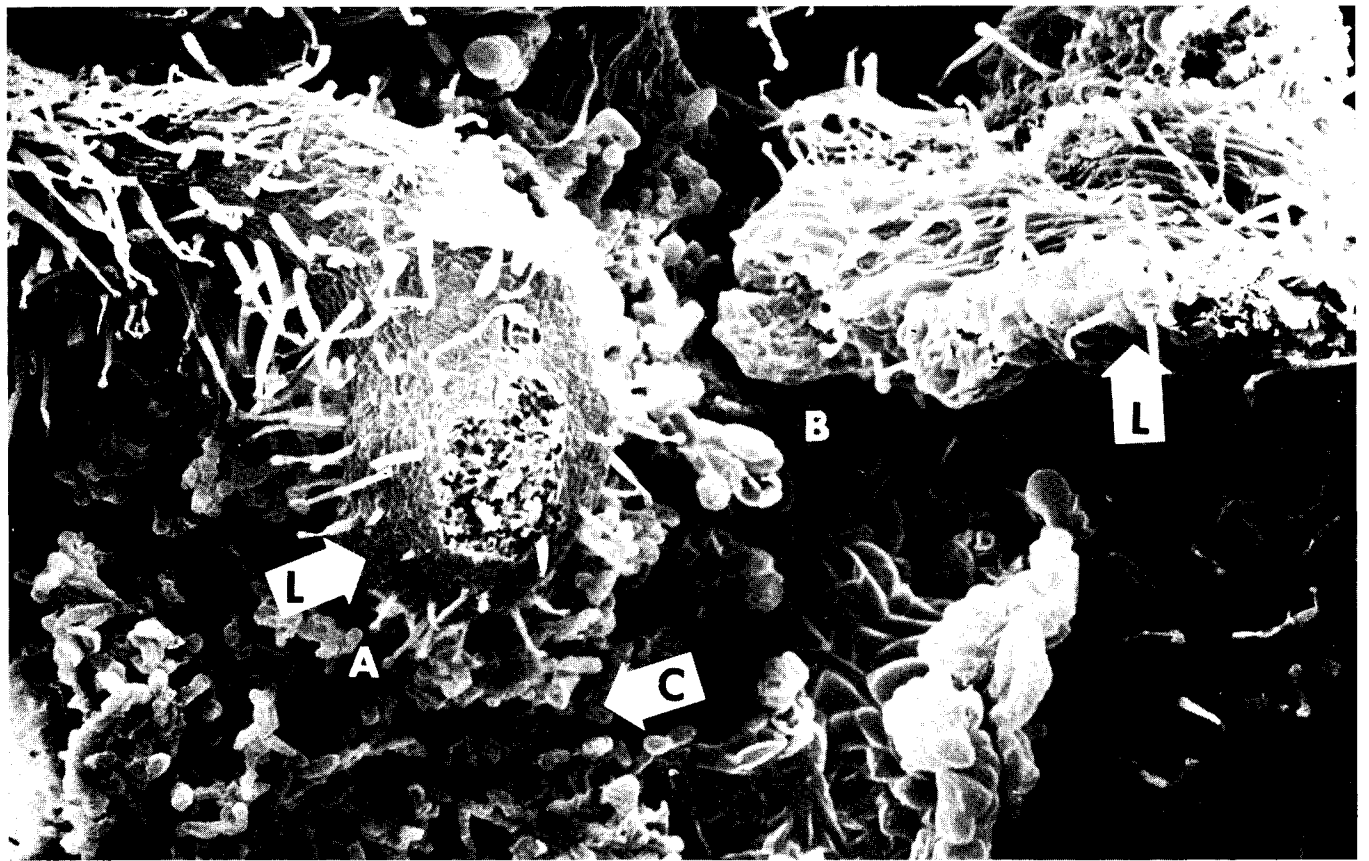showing leaf (L) and callus (C) cells.

Figure 3. A higher magnification view (708X) showing callus (C) and leaf (L). Greater detail of the leaf is visible showing origin of trichomes (T) or leaf hairs from the leaf cells and stomates (S) present.

Figure 4. Detailed view of callus (424X) from opposite side from Figure 1. This callus (C) shows trichomes (T) originating from callus cells, an unexpected finding.
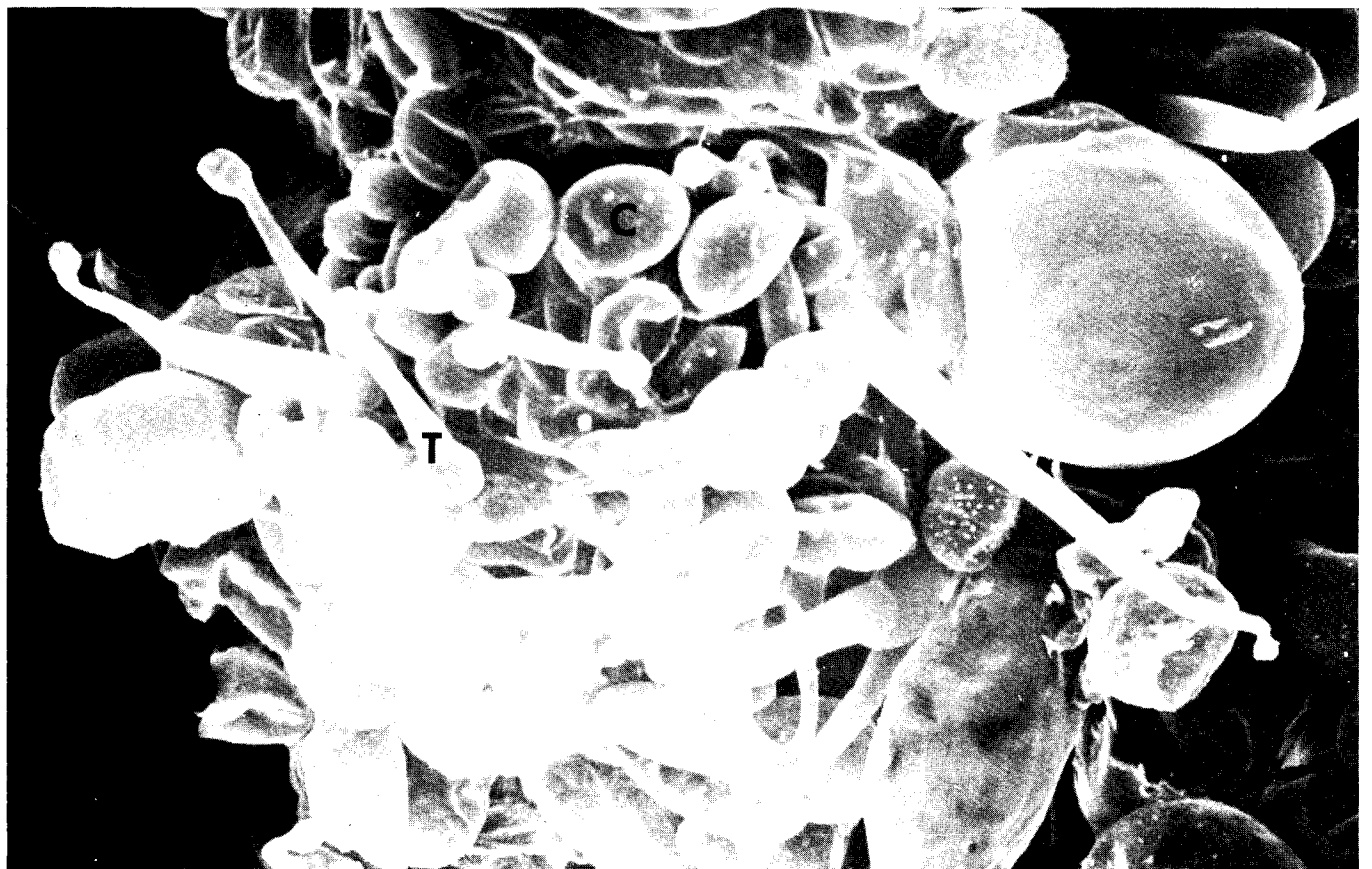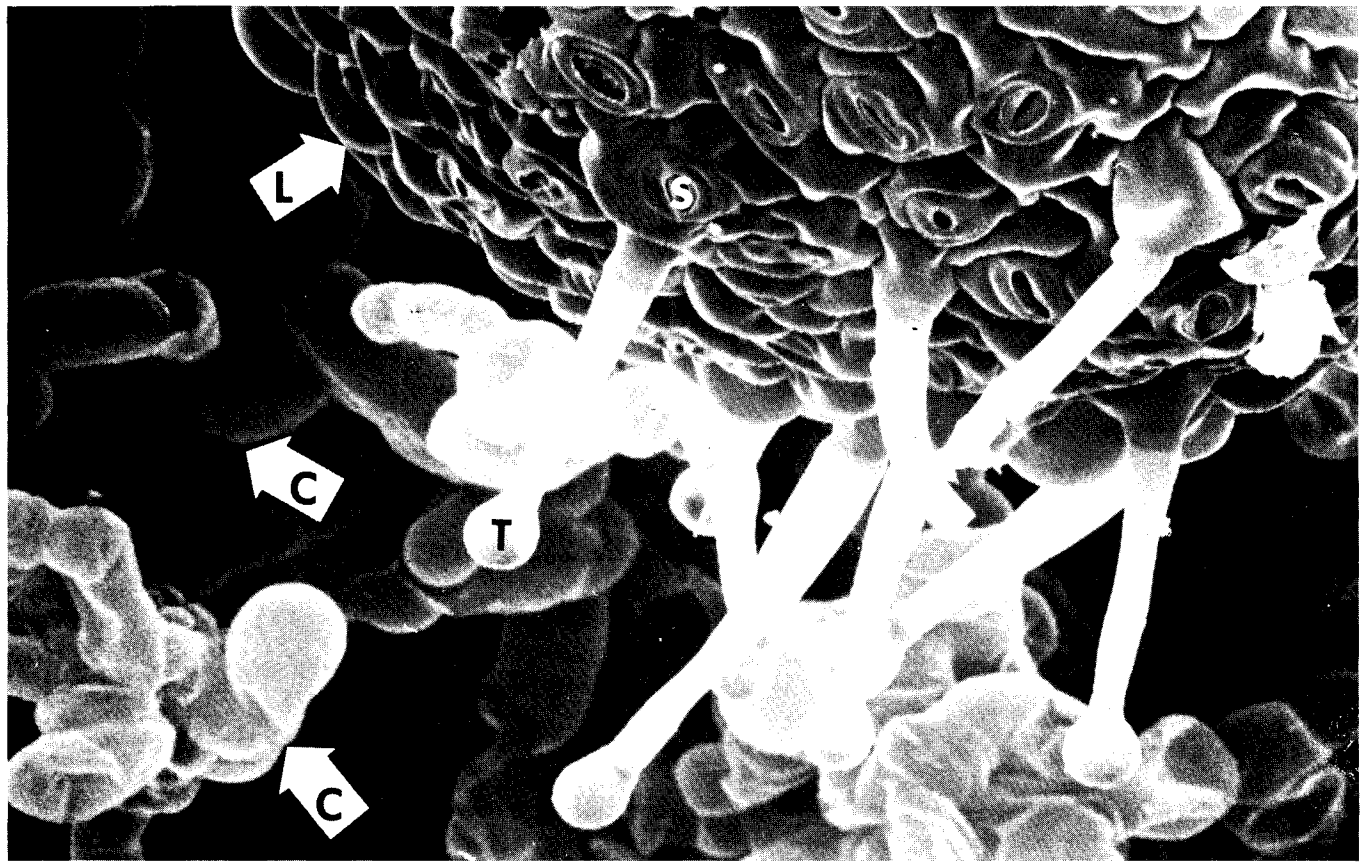
Selection for increased salt tolerance in tobacco has not stopped at the 8000 ppm level. Currently one line of these resistant cells is being attempted. Preliminary results indicate that 8000 ppm may represent a plateau level of resistance which is difficult to increase. This may well result from a requirement for two or more simultaneous mutations in one cell to achieve resistance beyond 8000 ppm NaCl. Again experience gained by solving a potentially multi-gene selection problem with tobacco may help with other species which may have a similar selection threshold at a lower salt tolerance level.

B. Food crop plants. The transfer of an almost exclusively tobacco originated and based tissue culture technology to food crop plants-- particularly those useful in the Colorado River Basin--is the primary reason for the research we have carried out. Having established, early in the project, that NaCl tolerant mutants could be selected in tobacco tissue cultures, we began efforts to achieve the same goal in other plants. In particular we have concentrated efforts on wheat, oats, soybeans, corn, and sugar beet. During the one year operational period of this grant considerable progress has been made with each plant.

1. Wheat. Tissue culture procedures for this plant were already reported in the literature. We found, however, that some of them were unreliable. In particular suspension cultures were easily established, but continued growth of the cultures usually did not occur. After some experimentation we decided to modify our original mutant selection procedure to eliminate the cell suspension stage. In other words a culture of proliferating cells (a callus) is established on

solid medium. Then, selection for salt resistance is attempted
directly on these cells rather than on a cell suspension. Again,
we tested this procedure on tobacco and found that it worked as
well as, and in fact, more rapidly than traditional suspension
selection procedures. However the selection process may be uneven
due to difficulty in media transport across the callus cells and
difficulty in selecting desired cells from the callus mass. As of
this writing then selection for NaCl resistant wheat cells has begun.
The procedure we are utilizing is as follows: Wheat seeds are
germinated on a solid medium containing relatively high levels of a
plant hormone (auxin) which causes root cells to divide and grow in
an undifferentiated manner to form a callus. Also included in the
medium is a level of salt high enough to prevent seed germination in
about 50% of seeds. Large numbers of seeds are placed on such a
medium. In general callus formation is minimal and occurs over a
time span of many weeks. In a few cases callus formation and growth
is much more rapid. These cases may represent cell mutations for
NaCl resistant and are being further investigated and tested before
plant regeneration is carried out.

2. Oats. With respect to published results and the sorts of
experimental procedures we have utilized, this plant is identical to
wheat. We are currently in the position of having begun selection
for salt resistant callus and hope to be to the stage of plant
regeneration within about six months.

3. <u>Soybeans</u>. All tissue culture procedures are well established
for this plant except for plant regeneration. This situation is
generally encountered in legumes (with the exception of alfalfa).
We are then in the position of having the technological expertise
to establish callus and suspension cultures and to select for
resistant mutants, with no method for obtaining salt-resistant
plants. Thus, some effort has been concentrated on soybean plant
regeneration techniques. Little progress was achieved during the
first half of the year; however, in the past several months significant
breakthroughs have occurred. We have now been able to regenerate,
in one case, a plantlet from callus and hope to have the procedure
perfected by summer's end. Our procedure in these experiments has
been as follows: Tobacco callus and soybean callus are morphologically
similar in certain respects. There are a number of identifiable
characteristics which serve as solid indications that a tobacco callus
is about to form buds. We have used these indicators as guides in
soybean regeneration experiments. In such tests medium composition
and cultural conditions are permuted in a variety of ways--by adding
or subtracting components, and by varying component amount or concen-
tration--with the aim of inducing plantlet regeneration indicators in
the soybean callus. The best medium from a particular experimental
series is then used as a base for the next series. A successful
regeneration procedure would result in a methods publication relating
to this phase of the work.

4. <u>Corn</u>. Tissue culture procedures for this plant are almost
identical to those for soybean in the sense that a reliable plant

regeneration technique is not available. The one published technique relies on callus obtained from embryos in the milk stage. The specificity and difficulty of obtaining such tissue on a continuous basis encouraged us to investigate regeneration procedures from root--derived callus. Such experiments have been proceeding all year. We do not seem to be as close to a breakthrough with this plant as we are with soybean.

5. <u>Sugar beets</u>. A published regeneration technique for this plant is available, but we have found it to be unrepeatable. In addition initial experimentation on this plant determined that callus formation and cell suspension formation were not easily accomplished. Once again experimentation during the tenure of this grant has resulted in considerable accomplishment. At present we can obtain fast growing callus from several different portions of the plant, and suspension formation should be routine within a few months. With respect to plant regeneration we have several experimental results indicating that we have probably obtained the correct medium composition for this event to occur. Successful plant regeneration for sugar beets will result in a methods publication on this phase of the work.

C. <u>Summary</u>. The 18 months of this grant (which was originally conceived as a two year project) has resulted in significant achievements in the area of obtaining NaCl resistant mutant plants. We have shown that mutants can be selected repeatedly from tobacco suspensions. Plants can be regenerated from these cultures, and these plants are being tested this summer, for salt tolerance and for mutation inheritability.

Also, having established a methodological procedure for tobacco, tissue culture selection for salt tolerant mutants is now being extended to the more useful food crop plants, wheat and oats. For soybeans, corn, and sugar beets stages of the tissue culture breeding procedure, in particular plant regeneration techniques are being worked out and significant progress has been made, especially for soybeans and corn.

V. PROJECT APPLICATIONS

The applications of the research results are immense and can be viewed on several different levels. First, the results achieved so far have demonstrated the original thesis—that salt resistant mutants can be selected in tissue cultures and the indications are that they can be selected much more rapidly than with traditional agricultural methods. Second, the results have demonstrated that salt resistance can be obtained to a high level of existing salts (as demonstrated in the tobacco plant). The relatively rapid transfer of tobacco methods to more useful food crop plants indicates that a tissue culture based technology for the selection of salt-resistant, water-utilization-efficient mutants will be available in a moderate time. Our feeling is that an initial period of research totaling no more than 2-3 years should result in a methodology that can be rapidly applied to solving many sorts of water-related problems. The most important feature of tissue culture selection of spontaneous mutants is that it concentrates on the energy-efficient method of modifying the plants to suit the environment. This is to be contrasted with the energy-intensive method of modifying the environment (through irrigation, desalination, etc.) to suit the plant.

It is also worth noting that tissue culture technology can be utilized in another manner to solve water-related agricultural problems. The availability of plant regeneration procedures for many types of food crop plants means that salt or drought-resistant individual plants noticed in the field can be rapidly propagated in tissue culture or cloned and released to the market as a new variety in a short period of time. At present, valuable, individual mutant plants must be propagated by traditional procedures which may require several plant generations and considerable time delaying the introduction of a new variety to the market. The plant regeneration methods of tissue culture mean that an individual grower or farmer, or a water specialist anywhere in the world could identify a rare, useful mutant plant, then have it rapidly cloned into millions of individual plants for testing in many different agricultural regions. The power of tissue culture breeding in this respect is illustrated by the fact that one small flask of cell suspension (100 ml of suspension) contains 10^7 cells, each one a potential plant if tissue culture techniques are correctly applied.

We have written a "state of the art" review article on the potential usefulness of tissue culture breeding. It has been accepted for publication and a copy is attached to this report.

VI. PROJECT CONTINUATION AND LONG TERM OBJECTIVES

We anticipate that by the end of the two years proposed in the original project objectives at least several lines of salt-resistant and therefore water-utilization-efficient food crop plants will have been produced and will be in the testing stage. Also, an additional two years (beyond 7/1/76) should see stabilization of tissue culture methods for most

agricultural plants. Work would then concentrate on the selection on various different types of mutants under objectives B, C and D and on the testing of regenerated plants for mutant retention and inheritability. At present wheat and oats offer the highest probability for the first successful results; however completion of tissue culture methodology seems near for soybean and for sugar beets, and if so, rapid progress could occur with these plants as well.

Appendix A

THE USE OF SPONTANEOUSLY OCCURRING AND INDUCED MUTATIONS TO OBTAIN

AGRICULTURALLY USEFUL PLANTS

The following manuscript has been accepted for publication in BioScience

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ABSTRACT

For both spontaneous and induced mutations, traditional methods
using whole plants or seeds and tissue culture methods using cells offer
the potential for producing useful mutant plants. Tissue culture
methods have been completely tested only in tobacco. Application to food
crop plants requires (i) Cell culture and plant regeneration techniques;
(ii) Selection methods for potentially useful mutant cell types;
(iii) Demonstration that regenerated plants retain mutant traits;
(iv) Demonstration that mutant traits in regenerated plants are inheritable.

As the world population increases in the face of rising energy costs it is appropriate to evaluate prospects for rapidly and economically improving the quality and quantity of the world's food supply. For developing nations in particular, fiscal and temporal constraints are of crucial importance in attempts to increase agricultural production. Modern agriculture has increased the quantity and ameliorated the quality of agricultural production by utilizing fertilizers, irrigation water, herbicides, pesticides, machines, and improved varieties of plants. All practices but the last fall under the category of altering the environment to suit the plant. Environmental alteration requires, directly or indirectly, large amounts of fossil fuel (Pimentel et al. 1973). Altering the plant to suit the environment by the production of new plant varieties is becoming increasingly important as a means of economically augmenting production on arable land and of bringing new areas into cultivation.

The purpose of this paper is to discuss the selection and potential use of spontaneous or induced mutations by different methods. In producing new varieties the goal of plant breeders is to incorporate new useful genes into the genotypes of cultivated varieties. To achieve this end, traditional breeding programs often involve hybridizing existing domestic or wild varieties. Such methods can often add several alleles to the best existing genotype. A second procedure is that of attempting to select individuals in which the desired trait has arisen by spontaneous or induced mutation. This method avoids the several breeding seasons required to obtain a stable cross-varietal product.

Its chief usefulness is in adding one allele at a time to an existing genotype.

In general, spontaneous or induced mutants are easily utilized to improve self-fertilizing species (e.g. wheat, oats, barley, rice, soybeans, peas, beans, potatoes, and some forage grasses). A desired mutant phenotype is located in a wild or domestic variety. The mutation is established in homozygous form and the resulting plants propagated extensively to yield an altered variety. Frequently, the desirable trait is transferred to other varieties by available hybridization techniques. In the case of cross-pollinated and, therefore, heterozygous crops (e.g. corn, rye, alfalfa, many clovers, and many forage grasses), the use of spontaneous or induced mutations is more complicated. Corn seed production and breeding involves crossing highly inbred lines; so spontaneous or induced mutants can be incorporated into homozygous parents. For the allele to appear phenotypically in the F_1 heterozygote, it must be either dominant, quantitative, or incorporated into two separate homozygous parents. In many cross-pollinated crops, such as alfalfa, homozygosity is difficult to obtain due to high self sterility. When selfing does occur, severe inbreeding depression is common. For such plants, spontaneous or induced mutant alleles are useful if incorporated into a number of cross-pollinating genotypes, or if a single mutant plant is crossed to other selected plants in a specialized breeding program.

DeVries initiated emphasis on the use of mutations for solution of specific agricultural problems with speculation in the early 1900's that induced mutations would be used in the production of new plant and

animal varieties (Gustafsson 1963). Research efforts to isolate
spontaneous new mutants have been productive over long time periods and,
due to low mutation rates, have involved large land and labor commitments.
With respect to induced mutations, the publications of the International
Atomic Energy Agency (IAEA) give an idea of the expenditures of money
and time in this area. As of 1972, the verified list of useful new crop
plant varieties produced by induced mutation stood at 68.

Usually, spontaneous mutants are isolated by screening huge numbers
of seedlings or plants. Most studies of induced mutation in agricultural
plants are initiated by irradiating or chemically treating large numbers
of seeds (IAEA, 1970, 1972). All treated seeds are germinated; plants
are examined for possibly valuable new phenotypes; occasionally, selection
for specific phenotypes is imposed. Recently, it has become apparent that
plant cells or pollen grains grown in culture can also serve as mutatable
material which can be grown into entire and possibly mutant plants (Street
1973a). There have been numerous suggestions that spontaneous or induced
mutations of tissue culture materials may provide new varieties (Melchers
and Labib 1970, Brock 1971, Delieu 1972, Carlson 1973a, Street 1973b,
Sunderland 1973a, Smith 1974). To date only a few workers have produced
potentially useful results using the method.

I would like to call attention to the advantages and disadvantages
of each method for obtaining agriculturally useful mutant plants. In
particular, I shall concentrate on the potential usefulness of tissue
culture breeding and on the problems to be overcome in its development.

Spontaneous Mutants from Plants

Naturally occurring mutants are discovered by serendipity or by purposefully examining large numbers of seedlings or plants either with or without the application of selection pressure. For example, Luke, Wheeler, and Wallace (1960) utilized over 800 working hours and a large amount of space to screen about 50 million oat seedlings for resistance to Helminthosporium blight. They isolated 72 plants which appeared to be natural, resistant mutants, occurring at a frequency of 1.44×10^{-6}. Resistance to this disease is a recessive trait, and since oats are self-fertilizing plants, these mutants probably arose as a result of zygotic or gametic mutations in one to several individual plants with homozygosity following. Their method is noteworthy in that even though considerable effort was involved, the logistics were simple when compared to procedure involving selection of mature plants in the field.

Non-chimerical mutant plants must arise from either gametic or zygotic alterations. Desirable phenotypes in higher plants could result either from changes in chromosome number (ploidy change or aneuploidy) or structure (translocation, inversion, duplication, and deletion) or from point mutations causing single amino acid changes in a gene product. Examples of the first type of mutation (so called "genome" and "chromosome" mutations) would be (1) alteration or elimination of a regulatory gene which slows production of a desirable protein, or (2) disfunctional change in a gene related to production of an undesirable plant product. Examples of point mutations would be (1) increase in the number of essential amino acid residues in a storage protein or (2) modification of a transport protein's active site so that Na^+ was less readily bound.

For both dominant and recessive changes the basic assumption is that the likelihood of a particular mutation is on the order of 1×10^{-5} per gene copy per generation (Serra 1968). For instance, in corn, mutations in the gene P_r occur with a frequency of 1.10×10^{-5} per gamete. The dominant lethal mutation for retinoblastoma in humans occurs in 2.3 of every 10^5 gametes. Mutation rates are most frequently and easily measured for genes having alleles with obvious, often deleterious, phenotypic effects. Such mutation rates may actually be the sum of a number of separate events, involving one or more genes, each of which causes a particular phenotypic change—often elimination or gross alteration of a particular gene product. Agriculturally useful gene alterations might often involve more subtle structural changes—perhaps a change in one of several vulnerable nucleotides. Therefore, although I will use a standard mutation rate of 1×10^{-5} per gene copy per generation in this paper it must be understood that the mutation rate for a particular base pair is much lower. Vogel (1970) has calculated that specific nucleotide mutation rates (the rate at which a given base changes to another specified base resulting in an amino acid substitution) are on the order of 1×10^{-8} or 10^{-9} per gene copy per generation for the hemoglobin molecule. The length of a generation is difficult to specify since mutations occurring throughout the lifetime of the gamete-producing parent could contribute to the total number of mutations. Since crop plant generation times are about 0.4 years instead of 25 years, specific base mutation rates for plants may be lower than for humans. I have used the hemoglobin estimate because it is an eukaryotic system in which all mutations can be recovered, even those with no phenotypic effect.

For a typical diploid organism, then, the chances of a new dominant spontaneous mutation (responsible for a specific, defined phenotype) arising at a particular gene locus are about 2×10^{-5}. If the plant is polyploid, the chances are correspondingly higher. Phenotypic expression of a recessive mutation depends on two or more independent mutational events in the same zygote or on the chance union of mutant gametes. In either case the probability of a particular new phenotype from new mutations is around 1×10^{-5y}, if y is the ploidy of the plant. The above calculations are only for plants in which outcrossing predominates and recombination of gametes occurs at random. If the plant is self-fertilizing, mating is not random and the probability that a zygote will be formed from two identical recessive mutant gametes approaches the basic mutation rate of 1×10^{-5} in the second generation following a gametic mutation.

This analysis has assumed that a particular mutation exists only because of a new event. In fact, the actual frequency of a mutation could be higher since those occurring in past generations would be maintained to an extent that depends on selective value. However, many desirable phenotypic alterations could result from point mutations which occur at a much lower frequency than the one I have used. The actual frequency of a particular mutation depends on the type of genetic alteration, forward and reverse mutation rates, selection pressure, and generations of accumulation (Dobzhansky 1970).

The point is that an investigator screening plants for a particular mutant phenotype must be prepared to examine perhaps 10^5 or 10^{5y} plants.

Actually more plants would have to be screened for 95% probability of finding at least one mutant. The time and expense involved in growing and screening large numbers of plants is considerable. Selection methods such as that of Luke et al. (1960) which can utilize seedlings rather than field-grown plants significantly improve selection economics. In some cases, however, seedlings will respond differently to a selective agent than whole plants. Certain desirable mutant phenotypes--for instance those involving altered growth habit--are easily identified by field observation. Others, such as improved amino acid profiles, cannot be identified in the field and require chemical analysis of all plants. A third group, such as herbicide-resistant or salt tolerant mutants, require that plants are subjected to selective pressure. Thus, for each specific desired mutant phenotype the potential cost and time involved in field selection should be estimated and the availability of other selection methods considered.

Spontaneous Mutants[1] from Tissue Culture

Recent developments in tissue culture techniques offer a possible method for rapidly isolating spontaneously occurring mutant phenotypes. Since a complete review of recent advances in tissue culture has already appeared (Street 1973c), I will briefly summarize: For some time, it has been possible to grow large numbers of plant cells in minimal space under sterile conditions and, by proper medium manipulation, to grow cultured cells into entire plants (Vasil and Vasil 1972, Street 1973c, Murashige 1974). Plant cells may regenerate embryos (embryogenesis), shoots or roots (organogenesis). Organogenesis of shoots is the usual

[1]Although I have used the term "mutant" it should be understood that this implies inheritability which has not been demonstrated for most variants derived from tissue culture.

regeneration method since embryogenesis is uncommon (except in carrot cultures) and since whole plants are not easily regenerated from roots. New shoots are rooted by horticultural techniques, or they form roots spontaneously in the tissue culture. Table 1 lists representative regeneration techniques for agricultural plants.

Since 100 mls of a rapidly-growing suspension culture of tobacco contain upwards of 1×10^7 cells (Nabors et al. 1975), tissue cultures allow a large number of potential plants to be grown economically in minimal space. This feature of tissue culture alone makes the method valuable for rapid propagation (cloning) or rare variants. For instance, introduction to the market of a rare, disease-resistant plant would be delayed several years by conventional means of propagation (such as cuttings or seeds). Tissue culture cloning could produce unlimited numbers of plants in a matter of months. Problems related to cloning are discussed in the following section.

Mutant cells can be selected either from calli or from cell suspensions. Considering the number of cells in a typical tissue culture and the mutation rate of single genes, it seems quite likely that a 100 ml culture would contain at least one cell possessing a given dominant mutation. A large suspension culture of 100 liters should contain more than 10^{10} cells, and a small possibility exists for recovering even recessive mutant phenotypes in diploid organisms. Use of haploid cells increases the probability of obtaining recessive mutants (see Sunderland 1973a). If the parent plant is polyploid such cultures are referred to as "polyhaploid" (Kimber and Riley 1963). Since a tissue culture is derived from a portion

of a single plant, the chance that the plant will already carry the
desired mutation is low and equal to the frequency of the mutation in
the population. Thus, in tissue culture breeding, as opposed to field
breeding, only new mutations need to be considered. When selection for
a specific phenotype is imposed, an entire culture of mutant cells is
obtained. Regenerated plants (forming from either single cells or cell
clumps) will thus carry the mutant gene, although its phenotypic
expression in whole plants is unpredictable.

Several workers have isolated naturally-occurring plant cell mutants
resistant to a metabolic inhibitor. Maliga et al. (1973a) obtained
5-bromodeoxyuridine-resistant cell lines from haploid tobacco. The same
workers (1973b) also regenerated streptomycin resistant plants from
resistant haploid callus. One mutant was isolated per 10^6 cells. Widholm
(1972a, 1972b, 1974) has isolated several types of 5-methyltrypophan-
resistant lines of carrot and of tobacco cells. In one case plants were
regenerated, and subsequent cell cultures still carried the trait.
Heimer and Filner (1970) isolated a line of tobacco cells in which
nitrate uptake was no longer inhibited by L-threonine.

Unfortunately, few workers have obtained spontaneous cellular
mutants of potential use in agriculture. Nabors et al. (1975) found that
suspension cultures of tobacco cells exposed to high levels of NaCl
gradually develop tolerance for the salt, apparently due to selection
of naturally-occurring mutants. Our mutant cells are now growing in
8000 ppm NaCl, about ten times original tolerance. Dix and Street (1975)
have also isolated NaCl tolerant tobacco cell lines. Since NaCl-tolerance

is a widespread agricultural problem (Dregne 1963, Rains and Epstein 1967, Gauch 1972, Waisel 1972) such mutants may be useful if the phenotypic trait persists in regenerated plants.

Aside from Maliga et al. (1973b) no one has estimated spontaneous mutation rates in cultured plant cells. This is an important consideration for future study since in animal cell cultures, mutation rates are sometimes abnormally high and depend on cultural conditions (Cass 1972).

Tissue culture breeding offers the possibility of rapid, economical isolation of specific mutant types with possible agricultural utility. Millions of potential plants can be grown in a single flask; selection for mutant phenotypes can occur within the flask. Thus, a mutant selection process normally involving hugh numbers of whole plants and large commitments of space and labor is tremendously simplified. The power of the technique is that it arranges the normally occurring processes of mutation formation and natural selection into a logistically simple format in which time and space requirements are remarkably compressed.

Mutant Plants from Cells--Problems

At present, four important problems pose a barrier to the utilization of tissue cultures in agricultural breeding programs.

1. Absence of Suitable Tissue Culture Techniques. Tissue culture breeding is sometimes criticized for being a tobacco-based technology. It is correct that most experiments have used tobacco and that extension of the complete method to food crop plants is at present a theoretical construct.

The method involves production of callus on solid medium, cell suspensions in liquid medium (this step is sometimes omitted), selection of mutants, and plant regeneration from mutant cells. For most agricultural plants, callus is easily produced. In dicots, stem, petiole, or cotyledon sections are easily and frequently utilized. In monocots, root or embryo callus is easily obtained. Even though callus production is usually possible it is still something of a magical art. A medium producing callus for one species may not work for a second, or for another variety of the first. Callus production from the stem may be routine whereas the root may not respond. Also, calli derived from different parts of the same plant may differ markedly in regenerative ability (for example, Doerschug and Miller 1967). Finally, a medium satisfactory for callus initiation may not support growth of callus excised from the site of formation. Problems of callus production and growth are resolved by testing as many permutations of medium constituents, their concentrations, and cultural conditions as possible (de Fossard et al. 1974). The ideal situation is rapidly forming callus with high regenerative ability.

Mutant cells can be selected from callus tissue, or from cell suspensions. These are initiated from calli by mechanical or enzymatic disruption. In my laboratory, suspensions are initiated simply by placing callus tissue in a baffle-bottom Erlenmeyer flask containing liquid medium on a gyrotory shaker. Suspensions are sub-cultured when cell density surpasses a minimal value. Sub-culturing can be avoided by use of continuous culture methods (King and Street 1973). As with

callus cultures, problems are frequently encountered: Often the best

medium for callus growth may not work well--in liquid form--for

suspensions. Also a medium may allow suspension formation and some

growth, but not continued growth. In such cases perturbations of all

medium components and cultural conditions must be considered as well as

addition of new components or conditions.

The principal difficulty in adapting tissue culture breeding to

food crop plants has heretofore been in obtaining reliable plant

regeneration techniques. Many people are unaware that in recent years

such methods have been published for a number of different plants

(Table 1), especially for a number of monocots. Still, significant gaps

exist: For most legumes, in particular soybean and dry beans, regenera-

tion methods are unavailable despite extensive effort. For other plants

available methods need modification. In corn, for instance, regeneration

has been reported only from milk stage embryo-derived callus; in sugarbeet,

only from floral peduncle-derived callus (Table 1). Techniques utilizing

more easily obtainable tissue would be desirable. Another problem is

that some regeneration techniques are inefficient: rather few plants are

regenerated. In some cases this is undoubtedly due to medium composition

or cultural conditions. In other cases regenerated shoots may interfere

with the regenerative ability of nearby cells. Finally, as with other

tissue culture techniques, varietal differences are found in regenerative

ability. This is true for tobacco as well as for food crops.

All of these problems can be either resolved or lessened by continued

experimentation. Despite these problems tissue culture methodology is

complete enough for some plants (for instance wheat, oats, barley, and

differences in activity are found in all developmental stages (Schrader et al. 1966). Single cell selection could occur for mutants more efficient in utilizing available nitrate. Such mutants might have an altered nitrate uptake system or increased amounts or activity of nitrate reductase, which might in turn lead to higher protein levels. The mutants could be selected by lowering nitrogen levels in the medium until non-mutant cells could no longer grow efficiently or by adding to the medium various inhibitors of nitrate reductase induction or action (Beevers and Hageman 1969).

The goal of changing low levels of certain essential amino acids— such as lysine, tryptophan, and methionine—in grains or in grain protein also initially seems unadaptable to a tissue culture approach. Carlson's experiments (1973b) show at least that mutant cells with an enhanced amino acid level can be selected and that the trait is passed on to regenerated plants. It remains to be demonstrated that a similar mutant phenotype appears in the seed of a regenerated plant.

Increasing photosynthetic efficiency is another aim of plant breeders which seems unsuitable to the approach of selecting spontaneous mutants in cultured cells. With respect to possible breeding for structural changes in the photosynthetic apparatus (a C-4 instead of a C-3 organiza- tion, for instance) this impression is correct. However, tissue cultures are often photosynthetically active (Zelitch 1975), and one worker has produced autotrophic callus (Corduan 1970). By lowering light or CO_2 levels or by including various inhibitors of photosynthesis in such cultures, one could select for any mutants with efficient photosynthesis.

Selection of a culture utilizing glycolic acid as a carbon source
could result in elimination or reduction of photorespiration (Zelitch
1975).

In general then, clever selection techniques can probably be used
to obtain many sorts of useful mutants in the cellular stage. Table 2
gives some examples. As the physiology and biochemistry of various
mutant lines is determined, researchers will have a better idea of
initial and of secondary selection procedures which might prove useful.

3. <u>Retention of Mutant Phenotype in Regenerated Plants</u>. A third
problem with mutants from tissue cultures is that even though mutants
with altered traits such as salt or temperature sensitivity might be
selected at the cellular stage, the phenotypic characteristics may not
persist through the various stages of development to be useful in the
field. The problem can only be evaluated and if necessary resolved by
experimentation. It is encouraging that some plant diseases affect
cultured cells as well as whole plants (Ingram 1967, Helgeson et al.
1972, Ingram 1973, Gengenbach and Green 1975). Also, Carlson (1973b) has
succeeded in obtaining tobacco cells resistant to an analogue of
<u>Pseudomonas tabaci</u> toxin; regenerated plants showed increased, but not
full resistance to the disease and passed toxin-analogue resistance on
to progeny. Other workers (Maliga et al. 1973b, Márton and Maliga 1975)
selected streptomycin resistant or BUdR-resistant tobacco cells and
found the inherited trait persisted in regenerated plants.

Several other considerations are related to the problem of phenotype
persistence in regenerated plants. First the mechanism of mutation

inheritance must be demonstrated. Progress in this direction has been made by Carlson (1973b), Maliga et al. (1973b), and Márton and Maliga (1975). However, considerable work remains since several types of non-inheritable traits could be selected in cultured cells. Second, it must be shown that useful alleles do not have an deleterious "side effects" (pleiotropism) in regenerated plants.

4. <u>Appearance of Cytological Alterations in Cultured Cells</u>. Another potential problem in plant production from tissue cultures is that cultured cells frequently undergo cytological and nuclear changes (Sunderland 1973b) and suffer a progressive loss of totipotency. Such changes are usually considered degenerative in nature. In many respects, though, populations of isolated higher plant cells behave as cultures of a newly created, ill-adapted microorganism. A similar view of animal cell cultures led T. T. Puck to write a book entitled <u>The Mammalian Cell as A Micro-Organism</u> (Holden-Day, San Francisco, 1972). Spontaneous mutations or cytological changes occur in cultured cells and may confer a selective advantage or disadvantage. Cells taken into culture may be expected to undergo a long period of genetic adjustment involving phenotypic changes as each potential mutation or combination of changes occurs and is subjected to natural selection. In terms of producing agriculturally useful plants, undesired genetic changes and loss of regenerative ability are to be avoided. They can be avoided by minimizing time spent in tissue culture through use of rapid callus formation and regeneration techniques. Our tobacco suspensions contain totipotent cells after more than four years in culture. This length of time is quite sufficient for mutant selection to occur.

Induced Mutants from Plants

Besides looking for spontaneous mutant phenotypes, researchers can employ a second method: inducing mutations to increase the frequency of novel phenotypes. Mutation induction in seeds offers the possibility of increasing the mutation rate so that several desirable mutations might occur in the same seed, although probably in different cells. Statistically though, deleterious mutations are much more likely to occur than desirable ones. To induce mutations, large numbers of seeds are exposed to ionizing radiation or to mutagenic chemicals. The seeds are then germinated; sometimes selection is imposed for desirable phenotypes. For instance, Wallace, Singh, and Browning (1967) utilized cobalt-60 gamma rays and several chemicals to induce Helminthosporium resistance in oats. They found second generation mutant resistant seedlings with a frequency of about 3.0×10^{-5} and so succeeded in increasing mutant phenotype frequency to about 20 times the spontaneous rate.

The main problem with induced mutations in seeds is that the multi-cellular nature of the embryo makes it statistically unlikely to find first generation mutant plants that are not chimerical for the phenotype in question (IAEA 1970, p. 99-104; Broertjes 1972, D'Amato 1965). This point is most obvious when dealing with chloroplast mutations. Even if the entire shoot portion of the plant arises from a plumule of only a few cells, the probability that each of these cells will be mutated in the same gene is vanishingly small. An outside possibility is that the mutagenic agent could kill all plumule cells except one which then gives rise to the entire shoot. But there is no evidence that this occurs.

In many cases--such as those involving hormonal modifications or
certain types of disease resistance--the mutant chimerical phenotype may
average out to provide an apparently uniform phenotype for the entire
plant in the first generation following mutation. The problem is that
inheritance of chimerical mutants is unpredictable as well as unstable;
a few breeding seasons may be needed before pure stock can be obtained.
Establishment of homozygous stock from chimerical plants depends first
upon the chance that some mutated sectors of the plant are gamete-forming
sectors; second, on the chance that mutated gametes combine; and third on
diplontic selection (Broertjes 1972, IAEA 1970, pp. 134-137), a term
describing the fact that mutated cells may increase in number more or less
rapidly than similar non-mutated cells. Some workers (e.g. Wallace et al.
1967, Hirono and Smith 1969) have found apparently stable new phenotypes
in the generation following the mutated one. However usually two or
three generations are required for stabilization (IAEA 1970, p. 1).
Still a time-savings is frequently realized over varietal stabilization‾
from hybridization breeding.

To a limited extent one can avoid chimeras by mutating pollen before
fertilization (IAEA 1970, p. 134) or by utilizing plants in which various
sorts of asexual regeneration from single cells occurs (Broertjes 1968).
For instance many plants form leaf buds in this manner. The problem with
both these methods is in obtaining large numbers of potential mutant
cells. In some plants (e.g. pine or corn) large numbers of pollen grains
could be easily collected, but in others this would be difficult.
Regenerating leaf buds could be laborious to obtain in large numbers; and
in many whole plants leaf buds or similar structures originating from

single cells occur only in tissue culture.

The limited and only recent success of useful plant production from induced mutations in seeds can probably be attributed to the multi-cellular, differentiated nature of seeds, resulting in chimerical mutants, and to the physical outlay and expense involved in carrying large numbers of mutated seeds through several breeding generations to achieve phenotype stability. A 1972 International Atomic Energy Agency (IAEA) publication lists 68 useful induced mutant varieties of food crop plants released to growers between 1930 and 1971.

Induced Mutants from Tissue Culture

The production of mutant plants by tissue culture techniques and induced mutations can be divided into four steps: (i) Production of callus or suspension cultures; (ii) Mutation induction; (iii) Selection of desired mutants; and (iv) Regeneration of mutant plants from callus or suspension cultures.

Having already discussed steps i, iii, and iv in conjunction with the isolation of spontaneous mutants, I will note that recent efforts have been successful at inducing mutations in cultured plant cells, and then selecting various mutant phenotypes. Carlson (1969, 1970) has isolated various amino acid and vitamin auxotrophs in cell lines of tobacco and a fern. Also he has induced a line of tobacco cells and regenerated plants partially resistant to a Pseudomonas tabaci toxin analogue and containing increased levels of methionine (1973b). Complete resistance occurs naturally in another tobacco variety so there was reason to believe that resistance could be altered easily by mutation. A NaCl tolerant line of mutant tobacco cells has also been induced (Nabors et al.

1975) as well as a 5-bromodeoxyuridine-resistant cell line in soybean (Ohyama 1974). An auxin-autotrophic line of maple cells has been selected (Lescure 1969). The basic selection technique for spontaneous mutations is to expose cultures to conditions which slow or prevent growth of normal cells while favoring growth of the desired mutant cells.

In many cases, selection for desirable phenotypes could occur in populations of cultured cells. Alternatively mutation induction would be followed by regeneration of large numbers of plants which would then be subjected to selection. Even if the nature of the phenotype requires that selection occurs in the second manner, tissue culture methods are possibly more efficient than traditional techniques since the several breeding seasons often required for stabilization of chimeras are avoided. Chimeras are not produced for two reasons: In many and perhaps all cases plants arise from single cells. Also if selection has occurred in suspension culture all cells will carry the desired phenotype and even plants arising from cell clumps will be non-chimerical.

Mutation induction increases the frequency at which various desirable mutations appear. Mutation frequency depends on dosage of the mutagenic agent as well as various treatments preceding or following mutagenesis (IAEA 1970, pp. 44-57). One consideration for plant breeders is that most mutations are deleterious in a particular environment. It is quite possible therefore to induce a desirable mutation in one gene, an undesirable one in another gene, and to produce a mutant cell improved in one respect, but worsened in another. The ideal situation would be to obtain a mutation rate creating an average of one new mutation per cell, in a cell population large enough to insure appearance of the specific

mutation. Assuming a mutation rate of 1×10^{-5} per gene copy per

generation for a specific mutant phenotype and 10^4 genes per genome

(Strickberger 1968, p. 525), it is easily estimated that 20% of all

diploid cells contain one newly arisen spontaneous mutation.[2]

Whatever the actual percentage of cells containing new mutants, a

culture containing 10 liters (10^9 cells) of cell suspension should

have at least one cell carrying a given mutation, even if the mutation

occurs at a rate of only 1 per 10^8 cells. This means that for most

dominant or co-dominant phenotypes spontaneous mutation approaches

a suitable rate. There is no certain way of predicting whether a

particular desired mutation will be dominant, co-dominant, or recessive;

or whether the trait is under the control of several different genes

or of polygenes. It would seem advisable, if little is known about the

inheritance of the desired trait, to begin searching for spontaneous

mutants in tissue cultures and if this fails to institute mutation

induction.

[2]The chance of a mutation in a gamete is equal to the mutation frequency for single genes times the number of genes or 1 in 10. A zygote would have a chance of 2 in 10.

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Table 1. Representative procedures for plant regeneration (shoot formation) in some agricultural plants (information relating only to haploids is not included)

Amaryllidaceae: _Allium_ _cepa_ (onion)	Fridborg 1971
Chenopodiaceae: _Beta_ _vulgaris_ vars. (beet, sugarbeet)	Margara 1970
Compositae: _Lactuca_ _sativa_ (lettuce)	Doerschug and Miller 1967
Cruciferae: _Brassica_ _oleracea_ vars.(brussels sprouts)	Clare and Collin 1974
(cauliflower)	Walkey and Woolfitt 1970
(kale)	Lustinec and Horak 1970
Cucurbitaceae: _Cucurbita_ _pepo_ (pumpkin)	Jelaska 1974
Leguminosae: _Medicago_ _sativa_ (alfalfa)	Saunders and Bingham 1972
Pisum _sativum_ (pea)	Gamborg et al. 1974
Liliaceae: _Asparagus_ _officinalis_ (asparagus)	Wilmar and Hellendoorn 1968
Poaceae: _Avena_ _sativa_ (oats)	Carter et al. 1967
Hordeum _vulgare_ (barley)	Cheng and Smith 1975
Oryza _sativa_ (rice)	Nishi et al. 1968
Saccharum _officinarum_ (sugar cane)	Barba and Nickel 1969
Sorghum _bicolor_ (sorghum)	Masteller and Holden 1970
Triticum sps. (wheat)	Shimada et al. 1968
Zea _mays_ (corn)	Green and Phillips 1975
Rosaceae: _Prunus_ _amygdalus_ (almond)	Mehra and Mehra 1974
Rubiaceae: _Coffea_ _canephora_ (coffee)	Staritsky 1970
Rutaceae: _Citrus_ sps. (citrus fruits)	Murashige 1974
Solanaceae: _Lycopersicon_ _esculentum_ (tomato)	Nabors, unpublished
Solanum _tuberosum_ (potato)	Lam 1975
Umbelliferae: _Daucus_ _carota_ (carrot)	Murashige 1974

Table 2. Some agriculturally useful mutant phenotypes which might be or have been selected at the cellular stage.

Mutant Phenotype	Possible or Actual Selection Procedure	Accomplished in cells and [plants]
NaCl tolerant	(1) Add NaCl to medium	Nabors et al. 1975; Dix and Street 1975
	(2) Reduce Ca in medium (Kelley 1963)	--
Alkali tolerant	Add alkali to medium	--
Tolerant of high ionic strength	Increase ionic strength of medium	--
Resistant to temprature extremes	Grow cultures at extreme temperatures	--
Efficient utilizer of available nitrogen (possible high protein content)	(1) Reduce nitrogen levels in medium	--
	(2) Include inhibitors of nitrate utilization in medium	--
Rapid growth rate	Measure growth rate; discard slow growing cultures	--
Drought tolerant	Add non-penetrating osmoticum to medium*	--
Disease resistant	Add toxin or pathogenic organism to culture (this procedure will only be successful for selected diseases)**	--
Inhibitor or herbicide tolerant	Add normally inhibitory amounts of compound	--
Efficient at photosynthesis	(1) Omit carbon sources from medium (see Zelitch 1975)	--
	(2) Add photosynthetic inhibitors to medium	--
Decreased photorespiration	Supply glycolate as the carbon source (Carlson and Polacco 1975)	--
Increased levels of certain amino acids	Add amino acid analogues to medium	Widholm 1972a, 1972b; [Carlson 1973b]

*
  See next page.
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  See next page.

Table 2 (continued)

*In an osmoticum, the water potential of plant cells (which is a negative quantity) will be raised toward zero. Thus selection will occur for cells which have a lower water potential and can grow more rapidly. Many osmotica leak slowly into cells and soon cease to be effective. Polyethylene glycol 4000 or 6000, or Ficoll (MW=400,000) are examples of usually nonpenetrating osmotica.

**Plant diseases can be divided into two types: those caused by pathogenic toxin and those, for which no pathogenic toxin has been isolated, requiring presence of the pathogenic organism itself. In both cases some diseases will prove infective at the tissue culture level and others will not.