STADLER SYMP. VOL. 7 (1975) University of Missouri, Columbia-135-

GENETIC ANALYSIS OF PHOTOSYNTHESIS

(photosynthesis mutants, electron transport, phosphorylation)

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SUMMARY

It is now possible to select mutations of nuclear genes controlling various steps of the light reaction of photosynthesis in higher plants. Forty-nine such mutants, which were not previously available for the study of photosynthesis, have been isolated on the basis of their high level of chlorophyll fluorescence or their ability to withstand treatment of the photodynamic inhibitor, diquat. Diquat requires photosynthetic activity in order to kill plants and therefore mutants of photosynthesis survive. Full green seedling lethal photosynthesis mutants of Zea mays have been isolated which have near normal chloroplast ultrastructure. Using standard measurements of photosynthetic reactions we have characterized the first series of these mutants to the single protein involved. Among those now identified, one is missing the NADP+ reductase enzyme (hcf-1); another has lost cytochrome f(hcf-2); and one has no high potential cytochrome b_{559} (hcf-3). There is also indication that hcf-4 does not have the ATP synthesis enzymes for non-cyclic photophosphorylation. These maize mutants are now being applied to basic studies of the pathway of photosynthetic electron transport and phosphorylation. Unique information gained in this way may have application to our understanding of photosynthetic control in an important food plant species.

INTRODUCTION

The selection and use of mutants to elucidate complex biochemical problems is now a well established technique of modern biology. A prime example comes from the use of auxotrophic mutants (BEADLE and TATUM 1941) in microorganisms to sequence enzymes in numerous biosynthetic pathways. Mutants have been similarily applied to an analysis of photosynthetic reactions. Since it is necessary to handle large numbers of individuals during screening and isolating procedures, the

photosynthesis mutants thus far isolated have been confined to a few species of green algae. In this paper I will describe the selection and characterization of some mutants of photosynthesis in higher plants.

The early work of GRANICK (1949) on the pathway of chlorophyll biosynthesis utilized mutants strains of *Chlorella* which were chlorophyll deficient. This work was followed by the isolation of fully pigmented photosynthesis mutants in *Chlorella* (DAVIS 1952) and established the usefulness of genetic techniques in algae for the study of photosynthetic pathways.

PRESENT CONCEPT OF PHOTOSYNTHETIC ELECTRON TRANSPORT AND PHOSPHORYLATION

The scheme of the light reactions of photosynthesis has been recently reviewed by TREBST (1974), BISHOP (1971), and others. The relationship of the chloroplast photochemical pigment systems to electron carriers and to photophosphorylation sites was originally suggested by HILL and BENDALL (1960). The so called Z-scheme for electron transport has undergone modification to the generally accepted present day concept as outlined in Figure 1.

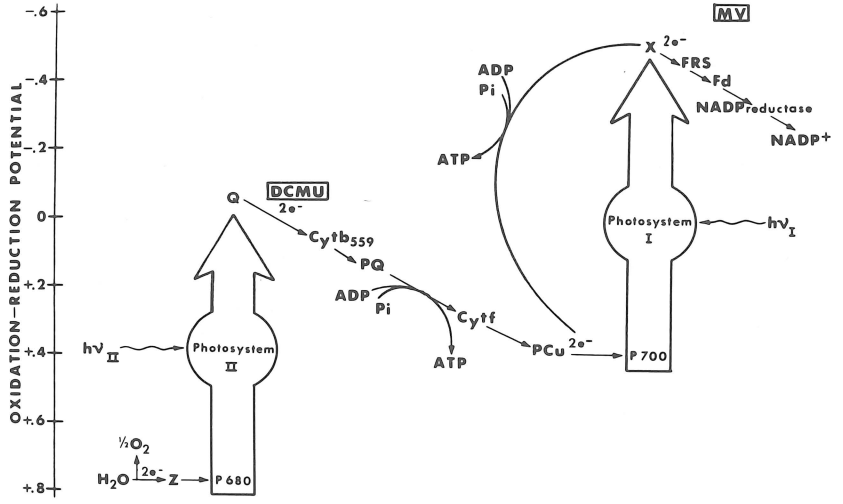

Figure 1. "Z-scheme" for the arrangement of electron transport carriers and sites of ATP synthesis. The scale of oxidation-reduction potential of the various components are indicated at the left.

Abbreviations: DCIP: 2,6-dichloroindophenol
DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethylurea
MV: methyl viologen
Q: electron acceptor of photosystem II.

The driving forces of electron transport are photochemical pigment systems which convert light energy into oxidation-reduction energy. These are referred to as photosystem I and photosystem II. These systems consist of reaction centers; P-700, P-680 which become oxidized in light and primary electron acceptors X, Q which are reduced in response to light. Electron flow takes place from Q to P-700 through a carrier system consisting of cytochrome b_{559}, plastoquinone (PQ), cytochrome f, and plastocyanin (PCu). From the acceptor X,transport is through the ferredoxin reducing substance (FRS), ferredoxin (Fd), to the flavoprotein, NADP oxidoreductase. The oxidizing potential of photosystem II is satisfied by electron flow from the secondary electron donor (Z) and eventually from H_2O with the evolution of oxygen. ATP synthesis can be coupled to a site near cytochrome f and is associated with an overall non-cyclic flow of electrons. In the presence of the appropriate cofactor such as pyocyanin (PYO), cyclic electron flow occurs around photosystem I. This electron flow is also coupled to ATP synthesis and is termed cyclic photophosphorylation. The overall products of the light reaction are oxygen, ATP, and reducing potential in the form of $NADPH_2$. The latter two products are used to drive dark, carbon reduction reactions. The electron transport and phosphorylation reaction should be amenable to study through mutation just as a variety of other biochemical pathways have been studied.

TYPES OF ALGAL PHOTOSYNTHESIS MUTANTS

Since the earlier work on *Chlorella* a number of photosynthesis mutants (*i.e.* those blocked only in reaction of photosynthesis) have been isolated from *Scendesmus* by Bishop's laboratory (BISHOP 1962). One such mutant, Bishop's #8 lacks the activity of photosystem I and another, #11, is missing photosystem II function (PRATT and BISHOP 1968). These mutants have been made available and widely used in studies of primary photochemical reactions, of various electron transport pathways, and photosynthetic phosphorylation (BISHOP 1966). Similarly, Levine and his associates (1961) have isolated green mutants of *Chlamydomonas* which no longer fix CO_2 and are acetate-requiring. These strains have genetic blocks at a number of different sites in the light-dependent electron transport reactions of the chloroplasts (LEVINE 1969). Some of the recent work using these mutants has been described at these Symposia (LEVINE 1973).

Though studies of *Euglena* have primarily centered around the genetics of development (SCHIFF 1971), mutants with altered photosynthetic electron transport have also been described (RUSSEL et al. 1969). A comprehensive review of most mutants previously used in the study of photosynthesis has been prepared by GARNIER (1965).

THE PHOTOSYNTHETIC APPARATUS IN LOWER AND HIGHER PLANTS

Though there is some doubt that the photosynthetic pro-

cesses in algae and the higher land plants are precisely analogous, the information gleaned from work with algae has been used in many instances to explain photosynthetic reactions of crop plants. Moreover, some of our current photosynthetic dogma is entirely based on such information from green algae. This is a fact that has always been somewhat bothersome since indeed, many dissimilarities have been recognized between algae and higher plants. For example, most higher plants have granular chloroplasts containing stacks of lamellae or grana. Algae, by comparison, have simpler structured lamellae or agranular chloroplasts in which the membranes may be oppressed but not organized into large stacks (MÜHLETHALER 1966). Other differences include the ability of many algal species to adapt to hydrogen in place of water as the electron donor source (GAFFRON 1940) and variations in the photosynthetic cytochromes. Generally the c-type cytochrome of algae is weakly bound to the membrane and can be very easily extracted whereas higher plant cytochrome is firmly bound. The algal cytochrome also has a lower molecular weight and a lower oxidation reduction potential (BOARDMAN 1968). Algal plastocyanin has also been shown to be chemically different (LIGHTBODY and KROGMANN 1967).

MUTANTS OF HIGHER PLANTS

A few years ago when there were practically no photosynthesis mutants available in higher land plants, my laboratory began a program to also apply genetics techniques in this field. We hoped to learn whether information previously gained from algal mutants was truly applicable to photosynthesis in higher plants. In addition, the genetic analysis would complement a larger amount of work on photosynthesis in higher plants which was obtained by other approaches. Sites in electron transport or photophosphorylation could be revealed through mutation which may not be recognized by analysis through chemical inhibitors or physical disruption and separation of subchloroplast systems.

A survey (Table 1) of the photosynthesis mutants of higher plants available or which have been studied reveals very few types (GARNIER 1965, LEVINE 1966).

One problem common to all of these mutants is that pigment content or type is altered. Most often there are gross changes in pigment which are associated with changes in chloroplast structure. Such mutants were used since they were selected by the phenotypic color difference from normal type. Before our recent work, this was the sole means for isolating photosynthesis mutants in higher plants. An added problem with these mutants is that most represent incomplete or partial blocks of photosynthesis.

In addition to these true photosynthesis mutants (those altered in the reaction of photosynthesis) there are a large number of higher plant mutants with considerable alteration in pigmentation, or those of the virescent phenotype, or a larger group which have altered photosynthetic unit size. One example

is olive necrotic 8147 of *Zea mays* (BAZZAZ et al. 1974). These plants are not useful for studies of photosynthetic pathways since they represent developmental changes involving a number of components.

The approach we have taken is to search for ideal mutants which would have full, normal pigmentation, would have normal chloroplast ultrastructure, would be blocked in only one step of the light reaction, and would possess a complete block. The mutation which comes closest to this description is the full green seedling lethal. During the remainder of this paper, I will describe the methods used by our laboratory for induction, selection, characterization and the application of full green seedling lethals of *Zea mays*.

Table 1. Photosynthesis mutants of higher plants previously studied

Species	Designation	Step affected	Reference
Vicia faba	-	NADP+ reductase	Heber and Gottschalk 1963
Nicotiana	NC95	Photosystem II	Homann and Schmid 1967
Antirrhinum	-	Light reaction	Debuch 1961
Oenothera	I alpha	Photosystem II	Fork and Heber 1968
	I gamma	Photosystem II	
	I delta	Photosystem II	
	II gamma	Q	
	II alpha	Photosystem I	
Glycine max	LY	Altered PQ pool	Keck 1970

SCREENING AND SELECTION OF PHOTOSYNTHESIS MUTANTS

Zea mays stocks used for these experiments were treated with the mutagen, ethyl methanesulfonate which was applied to pollen grains as described by NEUFFER (1968). After pollination, each resulting kernel was treated separately and the progeny was selfed. Screening was carried out on M_2 seedlings.

In the past, photosynthesis mutants have been detected in three ways; 1) surveyed for a change in chlorophyll, 2) surveyed for the fixation of radioactive carbon (LEVINE 1960), and 3) surveyed for the level of chlorophyll fluorescence (BENNOUN and LEVINE 1967). We wanted to avoid changes in pigment and and following ^{14}C uptake would have been too time-consuming,

therefore, we have screened the population for chlorophyll fluorescence under ultraviolet illumination.

Comparative fluorescence can be used because the level of chlorophyll fluorescence is controlled by the oxidation state of the primary electron acceptor of photosystem II. This acceptor is termed the quencher of fluorescence or Q (DUYSEN and SWEERS 1963). When Q is oxidized the level of fluorescence is low but as photosystem II reduces Q the fluorescence level goes to a much higher level (see Figure 1). If photosynthetic electron transport is blocked by mutation or chemical inhibitors, such as DCMU, the leaves show a high level of fluorescence. It has been shown that this technique which was previously used only on algae could be successfully applied to the leaves of land plants (MILES and DANIEL 1973). In this procedure, plants were illuminated with a long-wavelength ultra-violet lamp (peak at 365nm) and fluorescence viewed directly or through a red cut-off filter (cut at 610nm) or through goggles equipped with these red filters. When maize seedlings are treated with photosyn-

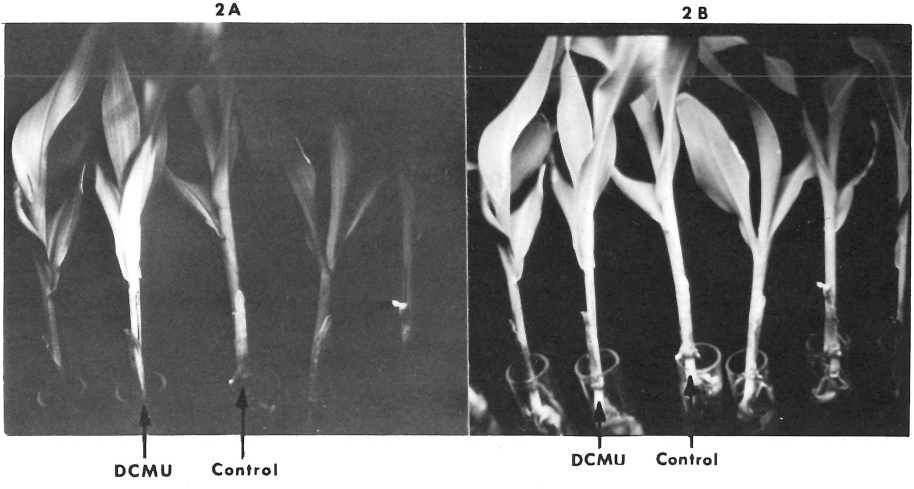

Figure 2. The *in vivo* fluorescence of normal maize seedlings treated with DCMU. Plants on the right are viewed directly in the UV light and on the left the same plants are viewed through a red filter.

thesis inhibitors by suspending roots in a test tube, containing either water or a 10μM solution of DCMU, photosynthesis can be stopped and the level of visible fluorescence increased (Figure 2). These photographs show the appearance of plants viewed under a UV lamp with (2A) or without (2B) a red filter covering the leaves.

Using this technique we have isolated a number of high chlorophyll fluorescence mutants, designated *hcf* of *Zea mays* (MILES and DANIEL 1974). Figure 3 shows a typical example of what is seen in white light, or in UV-light with or without a

red filter when these mutants are examined.

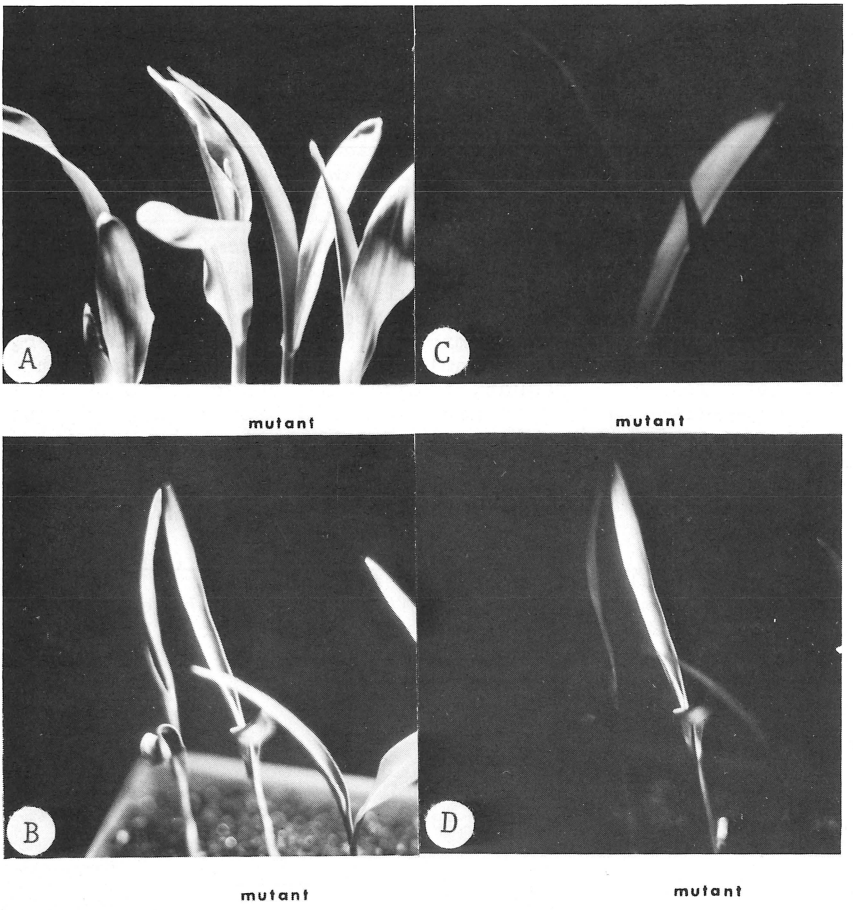

Figure 3. Fluorescence emission from normal and mutant maize seedlings in: (A,B) white light, (C) UV light, (D) UV light with a red filter over the plants.

Though fluorescence screening works well, it has two limitations. First, it is a screening procedure as opposed to a selective enrichment which means we must screen thousands of individual plants to find the desired mutations. Second, since the technique depends on the oxidation state of Q, it can detect only mutants blocked from that point on through photosystem II and I (Figure 1). Genetic blocks on the oxidizing side of photosystem II will not be detected. In *Chlamydomonas* such photosystem II mutants have been revealed by the lower than normal fluorescence yield of the mutant colonies (EPEL and LEVINE 1971). Lower than wild-type levels of fluorescence would be very diffi-

cult to observe in whole leaves, therefore, we have gone to other techniques.

We have been testing various chemical agents as positive selectors for non-photosynthesizing individuals. To be successful the selective agent should eliminate all normal plants which are carrying on photosynthesis and select out only plants, solely dependent on starch reserves for growth. TOGASKI (1972) has successfully used inorganic arsenate as a selective agent for non-photosynthesizing colonies of *Chlamydomonas*. The arsenate technique has been applied to *Euglena* with good selection results (SHNEYOUR and AVRON 1975).

There are a series of known photodynamic (light requiring) inhibitors of photosynthesis (MORELAND 1968) which have been studied. These include the viologen dyes and N-ethylmaleimide which block electron transport between the two photosystems in a light dependent manner (McCARTY 1974). However, the most promising of these inhibitors are the bipyridylium herbicides, diquat, paraquat, triquat and other viologen dyes. These compounds have a very negative oxidation-reduction potential and are reduced by electron transport of photosystem I (see Figure 1). Once reduced they become oxidized which initiates a series of reactions ultimately leading to the formation of reactive peroxide radicals or hydrogen peroxide which is the poison.

We have applied as a foliar spray a series of photodynamic inhibitors some of which are listed here and have thus far found a commercial preparation of diquat (Chevron Chemical Co.) to be most effective. The active agent in this product is 1, 1'-ethylene-2,2'-dipyridylium dibromide.

Figure 4. Diquat selection of photosynthesis mutants.

Figure 4A shows the effects on 10 day old maize of application of 10mM diquat followed by 12 hour incubation in the

light. Treated plants in the light first show isolated necrotic spots followed by complete drying and browning of the leaves. During the process, breakdown products of the protoplasm are released on the leaf surface in brown droplets. Only later are the leaf sheaths and stem affected. When a known family segregating photosynthesis mutant individuals (verified by high fluorescence) is sprayed with diquat and illuminated, normal sibs develop large necrotic areas and eventually die while the mutant looks normal, at times surviving the wild type plant by several days (Figure 4B). Therefore this technique appears to be selective for plants which are not photosynthesizing. The exceptions to this would be a mutation of the NADP+ oxidoreductase or ferredoxin which are past the site of diquat or MV reduction. These plants would also be killed by diquat. The recessive lethal mutants selected are maintained through the heterozygotes. In the future this technique will be applied to select additional types of mutants in maize, and should nicely complement the fluorescence procedure, since with both the entire series of photosynthetic reactions can be screened.

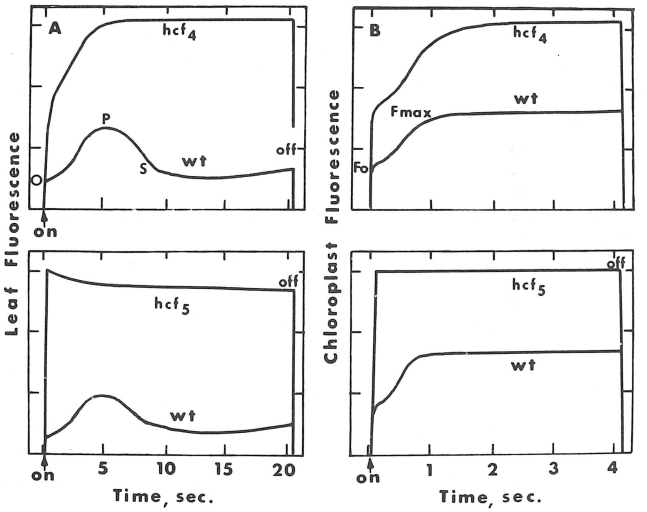

Figure 5. Leaf (A) and chloroplast (B) fluorescence for two mutants as compared to low fluorescence sibs, wt (wild type).

CHARACTERIZATION OF HIGH FLUORESCENCE MUTANTS

Techniques which are now standard have been applied in this analysis and are detailed elsewhere (MILES and DANIEL 1974). Where practical, non-destructive techniques have been favored. To show that the selected plants had indeed an alteration of photosynthesis, the fixation of CO_2 was measured by

infrared gas analysis. None of the five mutants described here was able to reduce CO_2 to carbohydrates to an extent which would compensate for CO_2 release by respiration. Following this measurment, the mutant plants were saved for further characterization.

One of the first parameters we studied in more detail was fluorescence since we know it to be altered in these plants. The time course for the induction of fluorescence in dark-adapted leaves or in isolated chloroplasts is an important monitor of electron transport function and does not involve destructive techniques. Typical results for two high fluorescence mutants are shown in Figure 5.

Upon illumination fluorescence yield rose rapidly to an initial point (O), then increases more slowly to a maxiumum yield (P in leaves and F_{max} in chloroplasts). This second slow rise is associated with normal photosystem II activity (DUYSENS and SWEERS 1963). Therefore, *hcf 4*,which has retained the slow rise, appears to have photosystem II function while *hcf 5* does not. In leaves, photosystem I function is associated with the decline in fluorescence yield from P to the S level (MUNDAY and GOVINDJEE 1969). *hcf 4* has lost the P-S decline while *hcf 5* has retained a portion of it. After a first examination of fluorescence kinetics we would tentatively classify *hcf 4* as a photosystem I mutant and *hcf 5* as a photosystem II mutant. We have previously published fluorescence traces for *hcf 1*, *hcf 2*, and *hcf 3*; *hcf 1* is similar to *hcf 4* while *hcf 2* and *hcf 3* look like *hcf 5*.

To pinpoint the effect of a mutation more closely the rates of well-known partial reactions of the photosystems (TREBST 1974 general reference) were examined and the results were compiled in Table 2.

In reaction 1. the evolution of oxygen with a Clark-type oxygen electrode is followed using $K_2Fe(CN)_6$ as the electron acceptor while reaction 2. measures the same segment of electron transport with DCIP as acceptor. In 2. the reduction of the acceptor was followed spectrophotometrically. In reaction 3. both photosystems are required and methyl viologen reduction is measured through an uptake of oxygen from the medium. Photosystem I is measured in reactions 4. and 5. when ascorbate-reduced $DCIPH_2$ is used as an electron donor adding electron between the two photosystems. If methyl viologen is the acceptor (4.) only a portion of the system is involved (see Figure 1.) but when NADP+ is the acceptor (reaction 5) the entire system I chain is measured. NADP+ photoreduction is also followed by an absorbance change this time at 340 nm.

Reactions 7. and 8. represent a measurement of the light induced pH change resulting from function of the proton pump of the grana membrane. The pH change as well as ATP synthesis can be coupled to cyclic (PYO) or non-cyclic (MV) electron transport. Photophosphorylation was measured by the technique of (LILLEY and WALKER 1973). Mutants *hcf 1* and *hcf 4* appear to have reasonable rates of photosystem II electron transport ac-

tivity but *hcf 1* has lower than normal NADP+ reductase activity while *hcf 4* has lost non-cyclic phosphorvlation ability. To support this observation for *hcf 1* chloroplast the NADP+ reductase enzyme was isolated from *hcf 1* membranes and again it showed 60% lower activity (MILES and DANIEL 1974). *hcf 4* is very interesting since it possesses good rates of non-cyclic electron transport, proton pumping and pH gradient formation but has no non-cyclic ATP synthesis. The significance of this observation will be discussed later but a tentative explanation for this mutant is that it has lost the phosphorylating enzyme system or coupling factor for non-cyclic phosphorylation.

Table 2. Rates of partial reactions of electron transport and phosphorylation in chloroplasts of normal and high fluorescence maize.

Reaction	Photosystem	hcf mutants (% of control rate)					wild type umoles/mg Chlorophyll/hr
		1	2	3	4	5	
1. $H_2O \rightarrow K_2Fe(CN)_6$	II	87	6	8	79	11	186
2. $H_2O \rightarrow$ DCIP	II	70	16	8	-	5	212
3. $H_2O \rightarrow$ MV	II+I	74	-	27	57	-	105
4. $DCIPH_2 \rightarrow$ MV	I	95	85	100	78	-	275
5. $DCIPH_2 \rightarrow$ NADP+	I	36	87	-	-	-	73
6. ΔpH, MV	II+I	63	0	8	95	-	104
7. ΔpH, PYO	I	75	0	52	50	-	110
8. ATP, MV	II+I	62	0	3	0	-	73
9. ATP, PYO	I	69	0	51	60	43	80

The *hcf 2*, *hcf 3* and *hcf 5* plants have clearly lost photosystem II activity but have retained some photosystem I activity (Table 2). *hcf 2* does not form a pH gradient or synthesize ATP but retains photosystem I electron transport. *hcf 3* has photosystem I electron transport and cyclic phosphorylation. This is a further indication that photosystem I is functional but photosystem II is not in *hcf 3*.

In order to pinpoint the block of these photosystem II mutants more closely we assayed for activity of the three photosynthetic cytochromes. The oxidized absorption spectrum minus the reduced spectrum for isolated chloroplasts was recorded on an Aminco DW-2 spectrophotometer using the procedure of

HENNINGSEN and BOARDMAN (1973). The chloroplasts were subjected to three different levels of oxidation and reduction in order to select for different cytochromes. When the sample cuvette of chloroplasts was treated with hydroquinone while the reference cuvette of chloroplasts was subjected to $K_2Fe(CN)_6$, an absorbance difference between the oxidized and reduced form of the cytochromes could be recorded. This treatment (column one of the Figure 6) produces an absorbance change for cytochrome f (peak wavelength at 554 nm) and cytochrome $b_{559_{HP}}$ (the high potential form of cytochrome b_{559}). Likewise the treatment in column two indicates all b cytochrome, the high and low potential b_{559} as

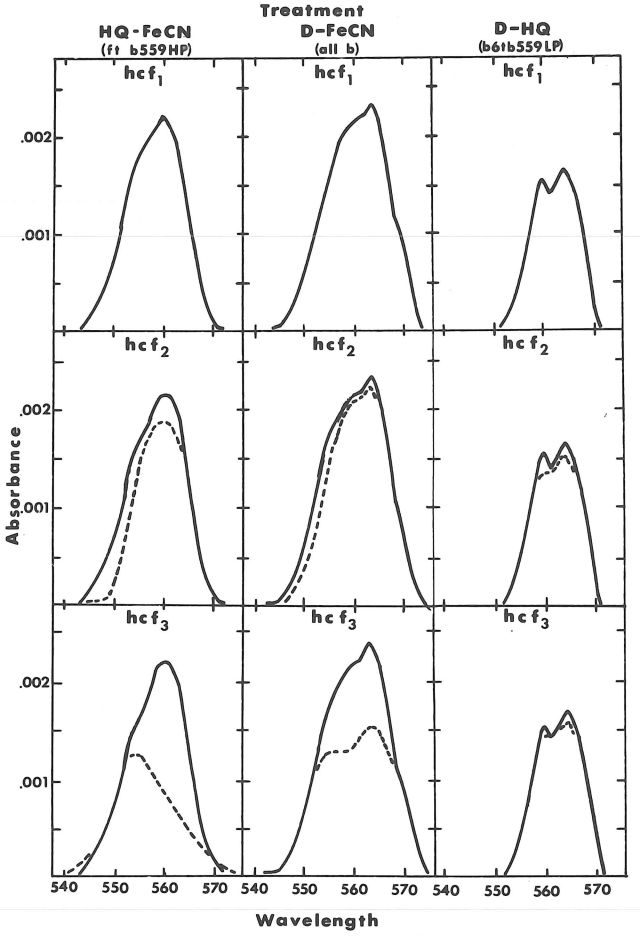

Figure 6. Base line corrected absorbance spectra for mutant chloroplasts (ca. 75µgChl) *hcf 1, hcf 2, hcf 3(--)* as compared to their low fluorescent sibs (solid).

well as cytochrome b_6 (peak wavelength 563 nm). The final column treatment (dithionite minus hydroquinone) causes an oxidized minus reduced absorbance difference for cytochrome $b_{559_{lp}}$ only.

hcf 1 appears to have a full complement of cytochromes with high fluorescnece and normal chloroplasts giving identical absorbance patterns, while *hcf 2* is clearly only missing cytochrome f (the peak absorbance centering around 554 nm is lowered). *hcf 3* is very interesting in that it has lost only one form of the cytochrome b_{559}. The high potential (HP) is missing (the absorbance at 559 in column one) but the low potential form (the absorbance at 559 in column three) is present. It is interesting that the HP form is associated with the primary reaction of photosystem II (KNAFF and ARNON 1969) and the fluorescence characteristic of this mutant indicates the primary electron acceptor of photosystem II (Q) is non-functional (MILES and DANIEL 1974). These data on *hcf 3* correlate with other reports that fluorescence yield can be controlled by the oxidation-reduction state of cytochrome b_{559} (OKAYAMA and BUTLER 1972).

Further characterization of these photosynthesis mutants will involve a more complete examination of electron transport carriers such as the reaction center pigments, P-700 and P-680. These can be analyzed as cytochromes by measuring oxidized-reduced changes in absorbance. Plastocyanin may also be amenable to such quantification. This should give us a complete picture of the light reaction components in these plants.

A different type of characterization which we are now conducting is an examination of the chloroplasts proteins in mutant leaves. We will use the electrophoretic technique of THORNBER (1974) to separate the chlorophyll-protein complexes which are associated with photosystem I (CP-1) and photosystem II (CP-II). For a more detailed examination of the membrane proteins we will follow the procedure of HOOBER (1973) for SDS-acrymalide gel electrophoresis. The results for this technique of separation of specific proteins should correlate with our previous work on the function of various enzymes. Genetic characterization of mutants is also underway. Since the maize chromosome map is well known it should be possible to assign our most interesting mutants to chromosomal sites by standard procedures or through the use of the A-B translocation technique (BECKETT 1972).

Now that a series of high fluorescence maize mutants have been well characterized biochemically and the limiting step of photosynthesis identified, we are applying this information back to the *in vivo* fluorescence kinetic traces. By carefully studying the traces of known mutants we would like to look at many more newly selected mutants and make a determination of what type of a mutation we think it will be. This year we have selected 43 new high fluorescence mutants and have separated them into seven classes on the basis of their whole leaf fluorescence kinetics. For instance those that appear similar to *hcf 2* will probably be limited at or near cytochrome f. Those

which are like *hcf 3*, may be effected at Q. When a number more of these mutants are characterized we hope to know what type of *in vivo* trace results from each type of mutant. This could allow us to identify a new mutant without the detailed biochemical analysis.

APPLICATION OF *ZEA MAYS* MUTANTS TO PHOTOSYNTHETIC PROBLEMS

At this point I would like to emphasize that now full green mutants of the photosynthetic light reactions in *Zea mays* can be induced, selected, and characterized biochemically very closely, but selection and characterization cannot be the only goal of such research. They must be applied to various studies of photosynthetic mechanisms to be of value.

Earlier in this report there has been indications of how such mutants provide us with new information. One problem, in which our laboratory has special interest, is the coupling mechanism of electron transport to ATP synthesis in chloroplasts. The transfer of energy from oxidation-reduction reactions to the ATP synthesizing enzymes is thought to take place in one of two ways. The chemical theory suggests that electron transport reactions cause the synthesis of a high energy intermediate which is then utilized in ATP synthesis (SLATER 1967). The alternate theory termed the chemiosmotic theory (MITCHELL 1966), says that electron transport reactions cause an ion flux across the grana membrane and a net movement of protons into the inner space of the grana (thylakoid). The energy stored in the formation of the pH gradient is equivalent to the high energy intermediate of the chemical theory and is utilized to drive ATP synthesis. In addition to the pH gradient a membrane potential is also formed across the thylakoid membrane and contributes to the energy of the high energy intermediate.

Our data (Table 2) on *hcf 4* indicates that this plant continues to form a pH gradient with non-cyclic electron transport (MV) but does not synthesize ATP. This could indicate the loss of the ATP synthesizing enzymes associated with non-cyclic but not those for cyclic electron transport.

Table 3 summarizes the correlation between proton pumping and ATP synthesis. You can see that whenever the pH change is lost, ATP synthesis is lost. Only in one mutant is ATP synthesis lost but proton pumping retained. These data indirectly support the chemiosmotic coupling theory. A second application of these mutants is an analysis of the nature of the primary electron acceptor, Q. Since one or more of the high fluorescence mutants appear to be missing Q function (MILES and DANIEL 1974), there is a possibility of locating an oxidation-reduction carrier which is also missing. At various times, workers (BISHOP 1969) have suggested that Q was a plastoquinone. In other organisms about 50 percent of all photosystem II mutants are missing plastoquinone. Our mutant (*hcf 3*) which according to fluorescence kinetics appears to be missing Q; has normal quantities of plastoquinone. Another carrier which has been

associated with Q is cytochrome b_{559} (OYAKAMA and BUTLER 1972). As indicated in Figure 6, *hcf 3* is missing cytochrome $b_{559_{hp}}$ but not $b_{559_{lp}}$. A third membrane factor which has been correlated with Q_2 is an absorbance change at 550 nm now termed C-550 (ERIXON and BUTLER 1971). We thus far do not have clear data on the presence of C-550 in *hcf 3* chloroplasts. At this point we can say that Q may not be plastoquinone but could be a cytochrome.

Table 3. Comparison of pH gradient formation and ATP synthesis in maize mutants

Mutant	Non-cyclic electron transport		Cyclic electron transport	
	ΔpH	ATP	ΔpH	ATP
hcf 1	+	+	+	+
hcf 2	-	-	-	-
hcf 3	-	-	+	+
hcf 4	+	-	+	+

CONCLUSIONS

It is now clear that photosynthesis mutants of higher plants can be obtained which have normal pigmentation and normal chloroplast ultrastructure even though they are afflicted with a blockage of the photosynthetic electron transport pathway. One of the original goals of this work was to see how higher plant photosynthesis mutants varied from algal mutants and what differences might be detected. One difference noted is the isolation of an NADP+ reductase block in maize (*hcf 1*) which has not been seen yet in algae. A second difference is that maize does not respond to the selective inhibitor sodium arsenate which was effective in screening photosynthesis mutants of *Chlamydomonas* and *Euglena*. Other than these no major differences have been noted.

One advantage of maize mutants however, in addition to being a diploid organism as compared to algae and well mapped genetically, is that maize has the C4 carbon fixation pathway (HATCH and SLACK 1970) as opposed to the C3 pathway which algae and most broadleaf plants utilize. Since plants with C4 metabolism are of major interest today because they represent the most efficient crop plants, work with photosynthesis mutants in this group will be important.

The aim of such work is to better understand the mechan-

ism and control of photosynthesis is an important crop plant. This is to be accomplished through analysis of such mutants and by the application of these mutants to the analysis of photosynthetic problems which could be attacked in no other way.

At this point I would like to pose an alternate approach to the ultimate goals of our research which we have just stated. Instead of going through the process of isolating mutants which are blocked in photosynthesis, analyzing these, and eventually applying what we have learned to the regulation of photosynthesis; an alternate access route to the problem would be to directly screen for mutants which have a single reaction step enhanced or are more productive or possess more efficient photosynthetic reactions. This type of search for hyperproduction mutants has been discussed in these symposia (CARLSON and RICE 1974). Carlson appears to have selected mutants which overproduce specific amino acids by germinating mutagen treated seed in the presence of a specific amino acid analogue. Only those mutant seeds which can overproduce the amino acid will survive. This approach has extensive validity in microorganisms, especially those used in the fermentation industry (DEMAIN 1971). Hyperproduction of a variety of metabolites and enzymes is well documented. Our laboratory has a good chance of success if the same general techniques are used to select for overproducing photosynthesis mutants. We can say this for a number of reasons. First, the details of mutagen treatment have been well worked out for maize as well as many other crop plants. Second, over the past 20 years there has been extensive, detailed work on the action of chemical inhibitors of nearly every photosynthetic reaction (IZAWA and GOOD 1972). For this reason we know how to specifically limit the activity of a variety of enzymatic steps. Third, there has been a large amount of basic research to describe the kinetics of these reactions and to pinpoint the rate-limiting steps of the light reaction. With this substantial foundation of basic research upon which to rely, we should be able to block the important rate-limiting steps in such a way that only a mutant which has a faster or larger reaction will survive. To do this screening procedure quickly, we must eliminate the protection from photosynthesis inhibitors afforded by normal respiration of stored carbohydrates. That is, we must force the developing seed to be dependent on photosynthesis at an early age. This could be done in two ways; first, respiration could be reduced by specific inhibitors. As with photosynthesis, there has been a large amount of basic work on such inhibitors in maize mitochondria. A second approach to forcing photosynthetic dependence is to make use of some well known endosperm mutants of maize. We can use stocks for screening which have decreased endosperm starch such as brittle 1 or 2, collapsed 1, shrunken 1 or 2, defective endosperm and miniature seed. These genes alone or in combinations provide extensive reduction of stored carbohydrates and causes a reduction of survival time before photosynthetic carbon fixation is required.

The time appears right to make important use of a large body of basic research information on mutagenesis; inhibition of photosynthetic and oxidative electron transport or phosphorylation and on the well studied genetics of maize to attack

the problem of photosynthetic productivity.

ACKNOWLEDGMENTS

The author wishes to acknowledge the contributions of Dennis Daniel and Kenneth Leto to this research. M.G. Neuffer kindly carried out mutagen treatment and provided the M_2 stocks used in this work. Excellent technical support was provided by Beverly Pill and Linda Cowles. The research described here is supported by a grant from the National Science Foundation, GM 32023A1.

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