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MACROMOLECULAR PHYSIOLOGY OF PLASTIDS XIV. *VIRIDIS* MUTANTS IN BARLEY: GENETIC, FLUOROSCOPIC AND ULTRASTRUCTURAL CHARACTERISATION

by

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A sample of 42 *viridis* mutants of barley has been localised by diallelic crosses to 32 nuclear genes. They have been grouped into five different categories on the basis of their fluorescence induction kinetics; this was used as a rapid method for the determination of their photosynthetic capacities. The mutants within each category were further characterised by low temperature fluorescence emission spectroscopy, chlorophyll content, visible fluorescence under UV light, viability, and chloroplast ultrastructure as seen by thin-section electron microscopy. The photosystem I-type mutants have a high initial fluorescence, variable fluorescence, but no fluorescence decline. They fluoresce under UV light and are seedling lethals, but in general have well-developed thylakoid systems. Photosystem II-type mutants, which have a high initial fluorescence, no variable fluorescence, and no fluorescence decline, fluoresce brightly under UV light and are all seedling lethals. Their chloroplast ultrastructure is characterised by giant grana, but as a group, their chlorophyll content is low. The nature of the photosynthetic defects of mutants in the third category is not known, but they are unusual in having a steady-state fluorescence lower than the initial fluorescence. Many of these mutants have a near-normal chloroplast ultrastructure, high chlorophyll content, and are viable under favourable conditions. The fourth group of mutants have fluorescence induction kinetics similar to those of the wild-type, and they are not considered to have major photosynthetic defects, as might be expected from the fact that many will survive under suitable growth conditions. The fifth category contained mutants with extremely low levels of chlorophyll under the growth conditions used.

The low temperature fluorescence emission peak at long wavelengths is in part due to antennae chlorophyll of photosystem I. In the mutants deficient in photosystem I, this peak was present, but its wavelength was shifted from 739 nm (wild-type) to 729–731 nm. However, photosynthetically competent mutants with reduced amounts of chlorophyll also showed similar or greater wavelength shifts, and it is concluded that at present, fluorescence emission spectra can not be used to predict the nature of the photosynthetic defects. Similarly, it was not possible to discern a usable relationship between chloroplast ultrastructure and specific photosynthetic deficiencies.

Abbreviations: F_o = initial fluorescence; F_m = maximum fluorescence; F_s = steady state fluorescence; F_v = variable fluorescence.

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1. INTRODUCTION

Chloroplast mutants in barley are classified according to seedling leaf colour using the system of GUSTAFSSON (17). Those mutant seedlings which can be distinguished from the parental wild-type by their paler green colour, are designated *viridis,* and in barley, 42 mutants have been localised by diallelic crosses to 32 genes (46; and Table I). All the mutations are recessive and show mendelian inheritance. In most cases the mutation is lethal and the seedling dies when the endosperm reserve is exhausted, so that seed stocks are maintained in the heterozygous condition. Some mutants will live longer under favourable conditions, and a few reach maturity and produce viable seeds (10).

The selection and characterisation of such higher plant mutants contribute to our understanding of the structure and function of the thylakoid membrane. Mutants provide uniform and reproducible preparations of thylakoid membranes in which one or more polypeptides have been genetically removed. They are thus invaluable in studies correlating membrane structure, composition and function (18, 37, 41, 47, 48). Probably the most useful of such mutants are those in which a single polypeptide is changed in its primary structure (11), or missing altogether from the membrane (40), since they allow a precise determination of the function and location of a polypeptide in the thylakoid. Although the *viridis* mutants of barley were selected on the basis of their paler colour, the primary effect of the mutation may be on the synthesis of a thylakoid protein whose absence results in a reduction in chlorophyll accumulation.

This paper examines *viridis* mutants of barley using techniques which are rapid and non-destructive, require a small amount of leaf material, and are suitable for the screening of large numbers of mutations. They are grouped into different classes on the basis of their fluorescence induction kinetics and characterised according to chlorophyll content, fluorescence under UV light, low temperature fluorescence emission spectra and chloroplast ultrastructure. Many mutants are shown to have photosynthetic defects and in some cases, the approximate nature of the lesion can be determined from the fluorescence induction kinetics. Such photosynthetic mutants, particularly those with a high chlorophyll content and a well-developed thylakoid system, will be characterised in greater detail using more time-consuming techniques. Thylakoids can be isolated from the seedling leaves, their polypeptide composition analysed (30, 41), the precise nature of the photosynthetic lesion elucidated by partial electron transport reactions (30), and their freeze-fracture ultrastructure examined (40, 41).

2. MATERIALS AND METHODS

2.1. Genetic analyses

The *viridis* mutants have been isolated from M_2 progenies after various mutagen treatments in the years 1951 to 1960 as detailed in Table I. The M_2 progenies were grown either in the greenhouse at the Royal College of Forestry in Stockholm or in the fields of the Swedish Seed Association at Svalöv. Seed stocks were routinely kept as single plant progenies in field plots at the Swedish Seed Association at Svalöv from 1951 to 1962, and at the Danish (Atomic) Energy Research Establishment, Risø from 1963 to 1979 where all the diallelic crosses were carried out. The mutants are now propagated at the Carlsberg Laboratory. The progeny from the diallelic crosses were tested in the greenhouse under natural light-dark cycles during the winter months. Two mutants are designated as allelic when the F_1 of crosses between heterozygous plants segregates for *viridis* seedlings. A minimum of 10 wild-type seedlings in a F_1 cross is required before allelism is considered to be excluded. The mutants are numbered consecutively upon isolation and this number is retained as the allele designation. The locus is identified by small letter(s).

2.2. Plant material

Seeds were germinated in plastic trays containing vermiculite moistened with tap water. Wild-type and mutant seedlings were analysed after growing under standard conditions of 7 days at 20 °C in continuous white light, as previously described (39).

2.3. Electron microscopy

Seedling leaves were cut transversely 2–3 cm from the tip while submerged in 2% glutaraldehyde, 10 mM-$MgCl_2$ in 0.06 M-phosphate buffer, pH 7.4 and fixed in this solution for 2 h. This was followed by two washes in phosphate buffer and post-fixation in 1% OsO_4 in 0.06 M-phosphate buffer, pH 7.4 for 1 h. Samples were then dehydrated in a graded ethanol series and embedded in Spurr's resin. Thin sections were cut with a diamond knife and post-stained for 30 min with 5% aqueous uranyl acetate at 60 °C followed by Reynolds' lead citrate for 30 min at 20 °C and examined with a Siemen's Elmiskop 102.

2.4. Fluorescence induction kinetics

Leaf material 2–3 cm from the tip of 7-day old, dark-adapted seedlings was placed in a light-tight holder at the end of a quartz cylinder attached to a bifurcated light pipe (Dolan-Jenner Industries, Inc. Melrose, Mass.). The end of the quartz cylinder nearest the leaf was masked with a slotted disc so that a constant area of leaf was illuminated. Broad band blue light from a voltage stabilised quartz-halogen lamp passed through a Corning 4–96 filter (transmitting wavelengths between 350 and 600 nm) was used for fluorescence excitation. Onset of illumination was controlled by a photographic shutter (opening time 1.6 msec). Chlorophyll fluorescence from the leaf was detected through a Corning 2–64 red filter passing wavelengths greater than 660 nm using a Photops UDT-500 photodiode (United Detector Technology, Inc., Santa Monica, California), attached to the other arm of the bifurcated light pipe. Transient signals were recorded on a Nicolet Explorer II digital oscilloscope (Nicolet Instruments, Madison Wisconsin) and permanent copies were plotted on an x-y recorder.

Values for initial fluorescence (F_o), maximum fluorescence (F_m) and steady state fluorescence (F_s, measured at 20 sec) were read directly from the oscilloscope screen and were corrected for the baseline signal, and the background fluorescence of the equipment without a leaf sample in the holder (about 110 mV).

2.5. Fluorescence emission spectra

Fluorescence emission spectra were recorded from whole leaves at liquid nitrogen temperature (77 K) using a fibre optic system (Applied Photophysics, London, England). The leaf sample was placed in a light-tight holder at the end of a quartz cylinder attached to a bifurcated light pipe. The sample holder was plunged into liquid nitrogen and excited with broad band blue light (Corning 4–96 filter) through one arm of the light pipe. Fluorescence emission from the illuminated surface of the leaf was detected through a high radiance monochromator by a Hamamatsu R928 photomultiplier tube attached to the other arm of the light pipe. The emission spectra were recorded on the digital oscilloscope and plotted on an x-y recorder. No correction was made for the light source or photomultiplier response, and spectra were recorded with 5 nm slits and a 100 msec time constant on the amplifier.

2.6. Chlorophyll determination

The in vivo absorption spectra of whole leaves were recorded on an Aminco-Chance DW-2a dual wavelength spectrophotometer. An approximate value for the chlorophyll content was obtained by expressing the value of the absorbance at the 678 nm peak for each mutant as a percentage of wild-type leaves.

2.7. Fluorescence under UV light

This was assessed by illuminating the seedlings in their plastic trays with long UV radiation from two Osram-L 20W/73 tubes placed 30 cm above the plants in the dark. Plants were then examined for fluorescence by visual inspection through a red plexiglass filter and scored according to their brightness (+ to ++++). Under these conditions, fluorescence from wild-type leaves could not be detected visually.

3. RESULTS

3.1. Genetic characterisation of the mutants

The 42 *viridis* mutants were assigned by diallelic crosses and complementation analysis to 32 different loci designated as *vir-a* to *vir-zd* and

Table I

Data on *viridis* mutants in barley

Mutant	Year of isolation	Mutagen	Dose	Condition[a]	Variety	Marker genes	Special remarks
vir-b [10]	1954	X-rays	4500 rad	resting	Bonus	*ert-a*[23]	
vir-c [11]	1954	neutrons[b]	250 rad	resting	Bonus	*ert-a*[23]	
vir-e [12]	1954	X-rays	2700 rad	resting	Bonus	*ert-a*[23]	
vir-f [13]	1954	X-rays	2700 rad	resting	Bonus	*ert-a*[23]	
vir-g [14]	1954	X-rays	5400 rad	resting	Bonus	*ert-a*[23]	homozygous stock
vir-h [15]	1954	X-rays	5400 rad	resting	Bonus	*ert-a*[23]	
vir-i [17]	1954	X-rays	2700 rad	resting	Bonus	*ert-a*[23]	translocation linked, cross-over observed
vir-a [18]	1954	X-rays	2700 rad	resting	Bonus	*ert-a*[23]	
vir-j [19]	1954	X-rays	2700 rad	resting	Bonus	*ert-a*[23]	
vir-b [21]	1954	X-rays	5400 rad	resting	Bonus	*ert-a*[23]	
vir-k [23]	1954	X-rays	16000 rad	resting	Bonus	*ert-a*[23]	
vir-d [24]	1954	X-rays	16000 rad	resting	Bonus	*ert-a*[23]	
vir-d [25]	1954	X-rays	16000 rad	resting	Bonus	*ert-a*[23]	
vir-l [27]	1955	neutrons[c]	140 rad	resting	Maja	*ert-c*[14], *-d*[14]	
vir-m [29]	1955	neutrons[c]	140 rad	resting	Bonus	*ert-a*[23]	
xan-q [75]	1955	neutrons[c]	140 rad	resting	Bonus	*ert-a*[23]	(*vir* 30)[d]
vir-a [33]	1955	X-rays	13500 rad	resting (20 % H_2O)	Bonus	-	
vir-n [34]	1955	X-rays	13500 rad	resting (20 % H_2O)	Bonus	-	
vir-o [35]	1955	X-rays	13500 rad	resting (20 % H_2O)	Bonus	-	
vir-p [38]	1955	8-ethoxycaffeine	0.2% x 24 hr	soaked	Bonus	-	
vir-a [39]	1955	spontaneous			Bonus	-	
vir-a [41]	1955	theophylline	0.1% x 48 hr	soaked	Bonus	-	
vir-q [42]	1956	triethylenemelamine	0.0004% x 24 hr	soaked	Bonus	-	
vir-r [43]	1956	1,4-bis(methanesulphonyloxy) butane	saturated sol. x 24 hr	resting	Bonus	-	anthocyanin-rich
vir-s [44]	1956	1,4-bis(methanesulphonyloxy) butane	saturated sol. x 12 hr	resting	Bonus	-	
vir-t [45]	1955	nebularine	0.025% x 24 hr	soaked	Bonus	-	
vir-u [46]	1951	X-rays	2250 rad	resting	Bonus	-	
vir-v [47]	1951	X-rays	18000 rad	resting	Bonus	-	dwarf seedling
vir-ze [49]	1952	X-rays	9000 rad	resting	Bonus	-	
vir-w [50]	1952	X-rays	9000 rad	resting	Bonus	-	dwarf seedling
vir-x [51]	1957	ethyleneimine	0.25% x 2 hr	soaked	Bonus	-	
vir-a [52]	1956	γ-rays	2700 rad	resting (8.5% H_2O)	Bonus	-	
vir-e [55]	1956	triethylenemelamine	0.01% x 24 hr	resting	Bonus	-	
xan-q [76]	1956	*p*-N-di(β-chloroethyl) phenylalanine	0.1% x 24 hr	resting	Bonus	-	(*vir* 56)[d]
vir-y [59]	1956	ethyleneoxide	0.18% x 10 hr	resting	Bonus	-	
vir-z [60]	1956	ethyleneoxide	0.18% x 10 hr	resting	Bonus	-	
vir-za [61]	1956	spontaneous			Bonus	-	
vir-zb [63]	1948	X-rays	9000 rad	soaked	Bonus	-	
vir-e [64]	1949	X-rays	9000 rad	resting	Bonus	-	
vir-zc [65]	1949	X-rays	18000 rad	resting	Bonus	-	
vir-e [68]	1960	ethyl methane sulphonate	0.3% x 24 hr	resting	Bonus	-	homozygous short, sterile
vir-zd [69]	1960	ethyl methane sulphonate	0.35% x 24 hr	resting	Bonus	-	

Glossary: a refers to condition of the treated plant material
resting: resting seeds with a water content of 11%, if not otherwise stated
soaked: seeds which have been soaked in water for 12 to 24 hours

b fast neutrons from cyclotron, Be(d,n); 15 MeV d; thick Be target, cf. EHRENBERG 1954 (13)

c neutrons in reactor centre, cf. AHNSTRÖM and EHRENBERG 1961 (1)

d provisional name of mutant used in earlier publications

Table II

Segregation data of *viridis* mutants in barley

Mutant	Wild-type seedlings		*Viridis* seedlings	For 3:1 segregation χ^2	P	Wild-type seedlings AA		Aa	For 1:2 segregation χ^2	P
vir-b [10]	281	:	93	0.004	0.95	557	:	1057	1.007	0.32
vir-c [11]	1765	:	565	0.701	0.40	444	:	943	1.087	0.30
vir-c [12]	788	:	302	4.258	0.04	637	:	1201	1.446	0.23
vir-f [13]	387	:	139	0.570	0.45	267	:	549	0.138	0.71
vir-g [14]	10	:	6	1.333	0.25					
vir-h [15]	485	:	161	0.002	0.96	537	:	873	14.327	3×10^{-4}
vir-i [17]	592	:	188	0.335	0.57	450	:	759	8.222	0.005
vir-a [18]	1804	:	403	53.470	$<10^{-10}$	716	:	1075	35.580	3×10^{-9}
vir-j [19]	1113	:	360	0.246	0.62	271	:	484	2.227	0.15
vir-b [21]	2113	:	718	0.198	0.66	563	:	1121	0.008	0.94
vir-k [23]	767	:	269	0.515	0.48	554	:	1048	1.124	0.29
vir-d [24]	1309	:	455	0.593	0.45	247	:	483	0.083	0.78
vir-d [25]	1317	:	426	0.291	0.59	257	:	504	0.066	0.80
vir-l [27]	1092	:	365	0.002	0.96	641	:	1107	8.759	0.004
vir-m [29]	1706	:	472	12.871	4×10^{-4}	742	:	1028	58.739	$<10^{-10}$
xan-q [75]	1370	:	313	36.792	2×10^{-9}	750	:	790	163.674	$<10^{-10}$
vir-a [33]	229	:	72	0.187	0.67	345	:	643	1.118	0.29
vir-n [34]	634	:	201	0.384	0.54	709	:	1110	26.078	5×10^{-7}
vir-o [35]	324	:	109	0.007	0.94	750	:	952	88.224	$<10^{-10}$
vir-p [38]	1123	:	464	11.199	8×10^{-4}	247	:	811	47.493	$<10^{-10}$
vir-a [39]	485	:	163	0.008	0.93	409	:	622	18.629	2×10^{-5}
vir-a [41]	420	:	169	4.284	0.04	214	:	438	0.077	0.79
vir-q [42]	522	:	167	0.213	0.65	450	:	1013	4.365	0.04
vir-r [43]	480	:	142	1.563	0.21	463	:	851	2.140	0.15
vir-s [44]	626	:	95	53.759	$<10^{-10}$	320	:	359	58.150	$<10^{-10}$
vir-t [45]	439	:	160	0.935	0.34	465	:	885	0.750	0.39
vir-u [46]	498	:	219	11.753	6×10^{-4}	7	:	565	265.393	$<10^{-10}$
vir-v [47]	409	:	123	1.003	0.32	469	:	816	5.792	0.02
vir-ze [49]	471	:	179	2.234	0.14	389	:	691	3.504	0.07
vir-w [50]	252	:	82	0.036	0.86	293	:	184	169.396	$<10^{-10}$
vir-x [51]	520	:	161	0.670	0.42	185	:	365	0.023	0.89
vir-a [52]	566	:	80	54.838	$<10^{-10}$	273	:	238	92.829	$<10^{-10}$
vir-e [55]	595	:	187	0.493	0.49	582	:	1091	1.592	0.21
xan-q [76]	698	:	74	97.831	$<10^{-10}$	873	:	630	414.323	$<10^{-10}$
vir-y [59]	985	:	333	0.050	0.83	192	:	345	1.416	0.24
vir-z [60]	551	:	172	0.565	0.46	424	:	638	20.763	5×10^{-6}
vir-za [61]	420	:	158	1.682	0.20	240	:	345	15.577	2×10^{-4}
vir-zb [63]	578	:	158	4.899	0.03	801	:	1358	13.787	3×10^{-4}
vir-e [64]	598	:	188	0.490	0.49	813	:	1320	21.949	3×10^{-6}
vir-zc [65]	468	:	154	0.019	0.89	563	:	1023	3.344	0.07
vir-e [68]	2065	:	559	19.124	2×10^{-5}	447	:	655	25.919	5×10^{-7}
vir-zd [69]	2177	:	709	0.289	0.59	765	:	1232	22.233	2×10^{-6}

xan-q (Table I; 46, 47). It is apparent that mutation in a large number of genes can give rise to pale green or conditionally pale green seedlings. In only six of the 32 genes were multiple alleles found, namely *vir-a* (5 alleles), *vir-b, vir-c, vir-d* (2 alleles each), *vir-e* (3 alleles) and *xan-q* (7 alleles). Two mutant alleles in the latter locus, i.e. *xan-q*^{75} and *xan-q*^{76} were originally designated as *viridis* 30 and *viridis* 56, respectively (Table I; 18, 47). It was subsequently found that these were allelic to *xan-q*^{51} and they are therefore re-symbolised *xantha-q*^{75} and *xantha-q*^{76}, respectively. Other alleles in this locus are designated *xan-q*^{77} to *xan-q*^{80}. The seedling phenotype of the seven allelic mutants in locus *xan-q* varies with the growth condition from yellow to pale green and frequently the tip of the leaf is green with the base being paler, even whitish.

All of the mutants behaved as mendelian recessives and with the exception of *vir-g*^{14} they are lethal or sublethal under field conditions. Segregation data are compiled in Table II and include segregations of selfed heterozygotes for *viridis* seedlings as well as ratios of wild-type plants with the genotype AA and Aa.

Among the 42 mutants, 23 segregated with the expected ratio of 1 *AA* to 2 *Aa* to 1 *aa* whereas 7 mutants segregated with a deficit of homozygous mutant seedlings (aa) as well as a deficit of viable heterozygotes (Aa). This is observed for *vir-a*^{18}, *-a*^{52}, *-e*^{68}, *-m*^{29}, *-s*^{44}, *xan-q*^{75} and *xan-q*^{76} (Table II). Such deficits are attributed to gametic selection, a phenomenon common among chloroplast mutants (16, 26, 27, 32) and implying poor viability or competitive ability of the mutant allele-carrying gametes. Unequal transmission of mutant and wild-type alleles is also the most likely cause for the deficit of heterozygous mature plants in the 10 stocks comprising *vir-a*^{39}, *-e*^{64}, *-h*^{15}, *-n*^{34}, *-o*^{35}, *-w*^{50}, *-z*^{60}, *-za*^{61}, *-zb*^{63} and *-zd*^{69}. The amount of gametic selection is dependent on the environment and varies therefore considerably from year to year as is evident, when the distorted segregations of different years are compared in the material computed for Table II.

The two remaining mutant stocks, for *viridis-u*^{46} and for *viridis-p*^{38}, segregate with a significant excess of recessive mutant seedlings. A great majority of the mature green plants of the line *vir-u*^{46} segregate for *vir-u*^{46} seedlings upon selfing and are therefore heterozygous (Aa). In this respect the line is a balanced lethal stock (46) and similar to the mutant stock *tigrina-o*^{34} analysed by O. F NIELSEN (32) and the pollen lethals 9 and 11 studied by L. LEHMANN, P. HAGBERG and G. HAGBERG (19) indicating that a pollen lethal linked in repulsion to *vir-u*^{46} prevents functioning of pollen grains carrying the wild-type allele. Occasional crossing-over would produce functional pollen grains with the wild-type allele and give rise to non-segregating green plants (AA). Seven such plants out of a total of 572 (Table II) were found giving a map distance of 2.4 units between *vir-u*^{46} and the pollen lethal. We would expect a 1:1 segregation of green (Aa) and *viridis* (aa) seedlings, if the macrospore nuclei carrying the mutant (*vir-u*^{46}) allele and those with the wild-type allele have an equal chance of becoming the egg nucleus. This is not the case as only 30.5 % of the seedlings are *viridis* and preferential formation of ovules carrying the wild-type allele is therefore indicated. Further genetic studies are required to support this hypothesis.

The cause for the excess of recessive *viridis* seedlings in the stock for *viridis-p*^{38} as well as the excess of heterozygous green plants (Aa) in this stock has not been analysed.

3.2. Fluoroscopic characterisation of wild-type and mutants

The *viridis* mutants in barley were grouped into five main categories on the basis of their fluorescence induction kinetics, and the properties of each group of mutants will be described in the following sections.

The fluorescence induction transient from dark-adapted wild-type barley grown under the standard conditions described in section 2.2. was characterised by a rapid rise from the baseline to an initial level (F_o) measured at 1.6 msec after the onset of illumination. There was then a gradual rise to a maximum value (F_m), followed by a slow decrease to a steady state level (F_s) (Fig. 1). For convenience, the value at 20 sec after illumination was used for F_s, although the final value was not reached until 40 sec or more. The difference between F_m and F_o is the variable fluorescence (F_v). The actual values, in arbitrary

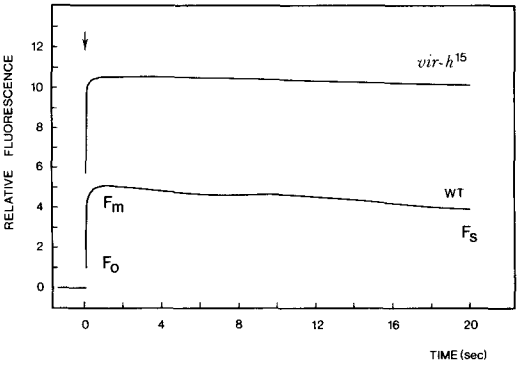


Figure 1. Fluorescence induction kinetics of dark-adapted seedling leaves of wild-type (WT) and *viridis-h*^{15}.

The initial fluorescence (F_o) is shown at 1.6 msec after onset of illumination (arrow), after which there is a rapid rise to a maximum value (F_m). The wild-type leaves show a decline in fluorescence yield with time, finally reaching a steady-state value (F_s), which for convenience has been measured after 20 sec, although a further slow decline continues for another 20 sec. The absence of the fluorescence decline in *viridis-h*^{15} and other mutants in Table III indicates a deficiency in photosystem I activity.

units (mV) are given in Table III, as well as the ratios F_m/F_o and F_s/F_o. For wild-type, the ratio F_m/F_o was between 4 and 5.5, and the value for F_s/F_o was less, usually between 3 and 4,

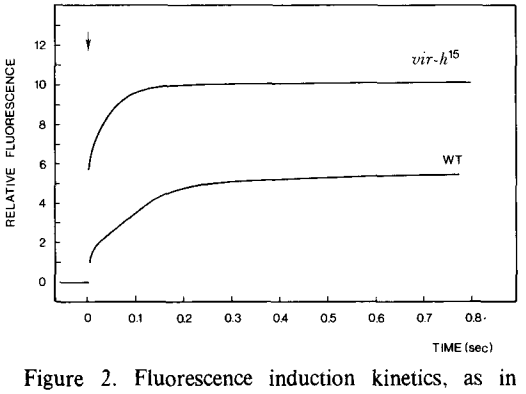


Figure 2. Fluorescence induction kinetics, as in Figure 1, with an expansion of the time scale to demonstrate the sigmoidal nature of the fluorescence rise for wild-type leaves.

In contrast the photosystem I mutants showed monophasic rise kinetics and reached the F_m value more rapidly, reflecting a slow decline in fluorescence with increasing time of illumination of dark-adapted leaves.

3.3. Photosystem I-type mutants

The six *viridis* mutants in Table III, arising from mutations in five different genes, were characterised by a high initial fluorescence (F_o) and only a small fluorescence decrease from F_m to F_s (Fig. 1). The rise from F_o to F_m was more rapid than in the wild-type and monophasic, in contrast to the sigmoidal rise of wild-type (Fig. 2). This type of fluorescence induction transient has been found for photosystem I defective mutants of Chlamydomonas (3, 4, 14) and maize (22–25). The low temperature fluorescence emission spectra of these barley mutants were characterised by a shift in the long wavelength peak from 739 nm (wild-type) to 729–731 nm (Fig. 3 and Table IV). The fluorescence intensity at 685 nm was always less than that at 730 nm. All six mutants showed moderate visible fluorescence under UV light and all were lethal under field conditions (Table IV). The chlorophyll content varied between 25 to 76% of wild-type, the mutant *vir-n*^{34} being the greenest and the most difficult to distinguish from the wild-type by eye.

The chloroplast ultrastructure of these *viridis* mutants (Figs. 4–7) reveals the presence of both grana and stroma lamellae. The grana of *vir-q*^{42} (Fig. 5) had a larger diameter and fewer discs than those of wild-type, while *xan-q*^{75} (Fig. 6) had many grana, which were larger than wild-type and had reduced intrathylakoidal spaces. Chloroplasts of *vir-h*^{15} (Fig. 4) were characterised by few stroma lamellae, while in both *vir-n*^{34} and *vir-zb*^{63}, apparently normal grana were connected by stroma lamellae which occasionally appeared to be collapsed (Fig. 7).

3.4. Photosystem II-type mutants

The seven *viridis* mutants in the second group, classified as photosystem II-type mutants (3, 5, 14, 23), arose from mutations in four nuclear genes. They were characterised by almost no detectable variable fluorescence (F_v) and little or no decrease in fluorescence with time ($F_m = F_s$) (Table V, Fig. 8). With the exception of *vir-ze*^{49},

Table III

Photosystem I-type *viridis* mutants: fluorescence induction data

Mutant	F_o	F_v	F_m	F_s	F_m/F_o	F_s/F_o
wild-type	424	1834	2258	1608	5.33	3.79
viridis-h^{15}	2210	1866	4076	3904	1.84	1.77
xantha-q^{75}	2824	914	3738	3686	1.32	1.31
viridis-n^{34}	2542	2824	5366	4944	2.11	1.94
viridis-q^{42}	1778	1490	3268	3048	1.84	1.71
xantha-q^{76}	2309	938	3247	3209	1.41	1.39
viridis-zb^{63}	1496	1704	3200	3195	2.14	2.14

Table IV

Properties of photosystem I-type *viridis* mutants of barley

Mutant	Chlorophyll content	Long wavelength fluorescence peak	Viability	Fluorescence under UV light
wild-type	100	739	viable	–
viridis-h^{15}	45	729	lethal	++
xantha-q^{75}	29	729	lethal	++
viridis-n^{34}	76	730	lethal	++
viridis-q^{42}	25	731	lethal	++
xantha-q^{76}	26	729	lethal	++
viridis-zb^{63}	64	731	lethal	+++

Table V

Photosystem II-type *viridis* mutants: fluorescence induction data

Mutant	F_o	F_v	F_m	F_s	F_m/F_o	F_s/F_o
wild-type	424	1834	2258	1608	5.33	3.79
viridis-c^{11}	2370	6	2375	2338	1.00	0.99
viridis-c^{12}	3116	10	3126	3058	1.00	0.98
viridis-l^{27}	2060	26	2086	2036	1.01	0.99
viridis-ze^{49}	397	19	416	416	1.05	1.05
viridis-e^{55}	4434	52	4486	4363	1.01	1.01
viridis-e^{64}	3356	32	3388	3372	1.01	1.00
viridis-e^{68}	3606	20	3626	3520	1.01	0.98

Table VI

Properties of photosystem II-type *viridis* mutants of barley

Mutant	Chlorophyll content	Long wavelength fluorescence peak	Viability	Fluorescence under UV light
wild-type	100	739	viable	–
viridis-c^{11}	32	730	lethal	+++
viridis-c^{12}	25	728	lethal	+++
viridis-l^{27}	28	699	lethal	++
viridis-ze^{49}	10	724	lethal	–
viridis-e^{55}	31	724	lethal	++++
viridis-e^{64}	30	723	lethal	++++
viridis-e^{68}	30	723	lethal	++++

Table VII

Fluorescence induction data for *viridis* mutants with $F_s/F_o \leqslant 1$

Mutant	F_o	F_v	F_m	F_s	F_m/F_o	F_s/F_o
wild-type	424	1834	2258	1608	5.33	3.79
viridis-a^{18}	3364	2012	5466	3024	1.62	0.90
viridis-m^{29}	3480	2186	5666	3830	1.63	1.10
viridis-a^{33}	2861	960	3821	1731	1.34	0.61
viridis-p^{38}	2036	2560	4686	2142	2.30	1.05
viridis-a^{39}	1564	664	2228	1560	1.42	1.00
viridis-a^{41}	2683	941	3624	2030	1.35	0.76
viridis-t^{45}	3954	1248	5202	2978	1.32	0.75
viridis-u^{46}	3670	1258	4928	3224	1.34	0.88
viridis-v^{47}	1761	944	2705	1450	1.54	0.82
viridis-a^{52}	2270	1037	3307	1634	1.46	0.72
viridis-z^{60}	3222	782	4004	2164	1.24	0.67
viridis-za^{61}	2101	591	2692	1666	1.28	0.78
viridis-zc^{65}	1442	859	2301	1226	1.60	0.85
viridis-zd^{69}	2415	257	2672	2421	1.11	1.00

Table VIII

Properties of *viridis* mutants characterised by $F_s/F_o \leqslant 1$

Mutant	Chlorophyll content	Long wavelength fluorescence peak	Viability	Fluorescence under UV light
wild-type	100	739	viable	–
viridis-a^{18}	59	730	sub-lethal	++
viridis-m^{29}	61	737	sub-lethal	+++
viridis-a^{33}	77	732	lethal	++
viridis-p^{38}	75	736	sub-lethal	+++
viridis-a^{39}	47	730	lethal	+++
viridis-a^{41}	73	733	sub-lethal	+++
viridis-t^{45}	34	733	sub-lethal	++
viridis-u^{46}	40	730	lives 6–10 wk	+++
viridis-v^{47}	37	733	lethal	++
viridis-a^{52}	44	733	lethal	++
viridis-z^{60}	40	732	lethal	+++
viridis-za^{61}	42	731	lethal	++
viridis-zc^{65}	57	731	lethal	++
viridis-zd^{69}	25	731	lethal	+++

they have an initial fluorescence (F_o) much higher than wild-type, and an extremely high visible fluorescence under UV light (Table VI). All seven mutants die when the endosperm reserve is exhausted. Chlorophyll content varied between 10 and 32% of wild-type, so that the segregating mutant seedlings were readily distinguishable from the wild-type phenotypes.

The low temperature fluorescence emission spectra of these mutants were all different from wild-type and had few features in common (Fig. 9). The three mutants at the *viridis-e* locus and *vir-ze*^{49} had high fluorescence intensity at 683 nm and low levels of fluorescence at longer wavelengths, where the peak was shifted from 739 nm (wild-type) to 723 nm. The two mutants at the *viridis-c* locus both had a maximum at about 730 nm and shoulders at 695 and 709 nm. The emission spectrum of *vir-l*^{27} was different again, with a single broad peak at 699 nm (Fig.

Table IX

Fluorescence induction data for *viridis* mutants with $F_m/F_o > 3.5$

Mutant	F_o	F_v	F_m	F_s	F_m/F_o	F_s/F_o
wild-type	424	1834	2258	1608	5.33	3.79
viridis-b^{10}	834	404	1238	820	1.48	0.98
viridis-f^{13}	462	1527	1989	1044	4.31	2.26
viridis-g^{14}	612	2002	2614	1732	4.27	2.83
viridis-i^{17}	692	2154	2846	2394	4.11	3.46
viridis-j^{19}	386	1674	2060	1212	5.34	3.14
viridis-b^{21}	918	608	1526	1204	1.66	1.31
viridis-k^{23}	138	588	726	562	5.26	4.07
viridis-d^{24}	350	1223	1573	755	4.49	2.16
viridis-d^{25}	326	1001	1327	785	4.09	2.41
viridis-o^{35}	651	1745	2396	1250	3.68	1.92
viridis-s^{44}	143	909	1036	800	7.26	5.61
chlorina-f2	372	900	1272	1007	3.42	2.71

Table X

Properties of *viridis* mutants characterised by $F_m/F_o > 3.5$

Mutant	Chlorophyll content	Long wavelength fluorescence peak	Viability	Fluorescence under UV light
wild-type	100	739	viable	–
viridis-b^{10}	36	738	lethal	–
viridis-f^{13}	26	737	lethal	–
viridis-g^{14}	86	738	viable	–
viridis-i^{17}	88	739	sub-lethal	–
viridis-j^{19}	77	739	sub-lethal	–
viridis-b^{21}	24	739	sub-lethal	–
viridis-k^{23}	27	720	lives > 13 wk	–
viridis-d^{24}	46	738	sub-lethal	–
viridis-d^{25}	24	736	lethal	–
viridis-o^{35}	95	738	lethal	–
viridis-s^{44}	52	723, 732	lethal	–
chlorina-f2	77	732	viable	–

Table XI

Fluorescence induction data for *viridis* mutants with low chlorophyll content

Mutant	F_o	F_v	F_m	F_s	F_m/F_o	F_s/F_o
wild-type	424	1834	2258	1608	5.33	3.79
viridis-r^{43}	254	24	278	256	1.09	1.01
viridis-x^{51}	81	6	87	86	1.07	1.06
viridis-y^{59}	33	0	33	33	1.00	1.00

Table XII

Properties of *viridis* mutants with low chlorophyll content

Mutant	Chlorophyll content	Long wavelength fluorescence peak	Viability	Fluorescence under UV light
wild-type	100	739	viable	–
viridis-r^{43}	2.0	729	lethal	–
viridis-x^{51}	0.6	678	lethal	–
viridis-y^{59}	0.2	–	lethal	–

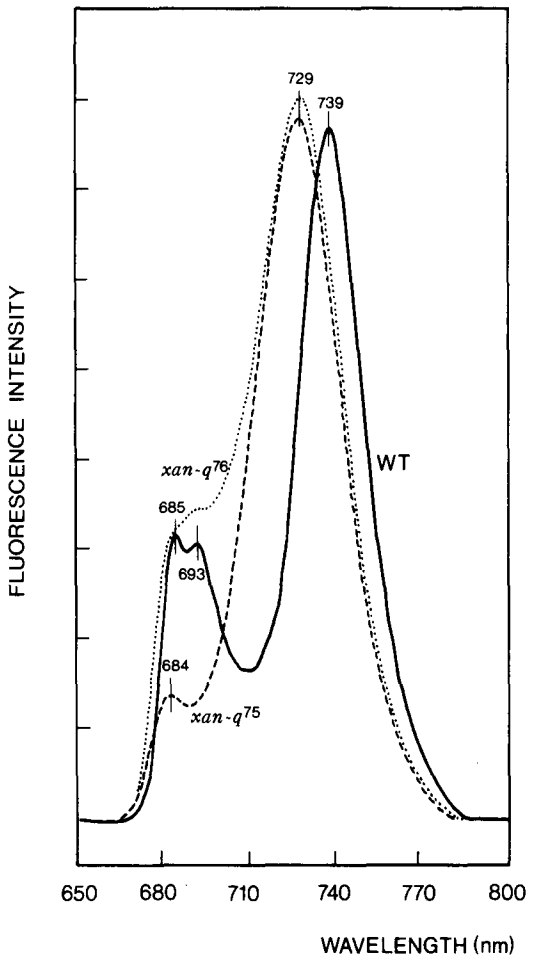


Figure 3. Low temperature (77 K) fluorescence emission spectra of seedling leaves of wild-type (WT) and photosystem I mutants of barley.

The wild-type spectrum is characterised by three peaks, at 685, 693 and 739 nm, with a higher fluorescence yield at 739 nm compared with the other two peaks. The photosystem I mutants examined all showed spectra similar to one another, with the major peak at 729–731 nm (see Table IV) but varied in the relative size of the peaks at shorter wavelengths. These spectra were obtained by exciting whole leaves with broad band blue light and no corrections were made for the light source or the photomultiplier response.

9). The chloroplast ultrastructure of these mutants, however, was similar in that they all contained very large diameter grana consisting of approximately ten discs, with reduced intrathylakoidal spaces (Figs. 10–12). Mutants *vir-c*^{11}, *-c*^{12} and *-l*^{27} also contained considerable amounts of stroma lamellae (Figs. 10 and 11), while *vir-e*^{55}, *-e*^{64}, *-e*^{68} and *-ze*^{49} almost completely lacked stroma lamellae (Fig. 12).

3.5. Mutants with $F_s/F_0 \leqslant 1$

The third group of mutants had the following fluorescence induction features in common: high F_0 and $F_m > F_s$. There was, however, considerable variation in the size of the variable fluorescence (F_v, Table VII) and in the exact shape of the induction transients (Figs. 13 & 14), so that several different types of photosynthetic mutants may have been grouped together. With the exception of *vir-m*^{29}, *-p*^{38}, *-a*^{39} and *-zd*^{69},

Figure 4. Thin-section electron micrograph showing the fine structure of the internal membranes of the chloroplasts of the mutant *viridis-h*^{15}.

The thylakoid membranes are organised into stroma and grana lamellae and appear similar to wild-type chloroplasts examined by the same technique except that stroma lamellae are few. It should be noted that in this, and most other micrographs, the chloroplast ribosomes are well-preserved owing to the inclusion of 10mM-$MgCl_2$ in the fixative. All micrographs have been printed at the same final magnification. (Bar = 1.0 μm). × 50,000

Figure 5. Electron micrograph showing the thylakoid organisation of a typical chloroplast from *viridis-q*^{42}.

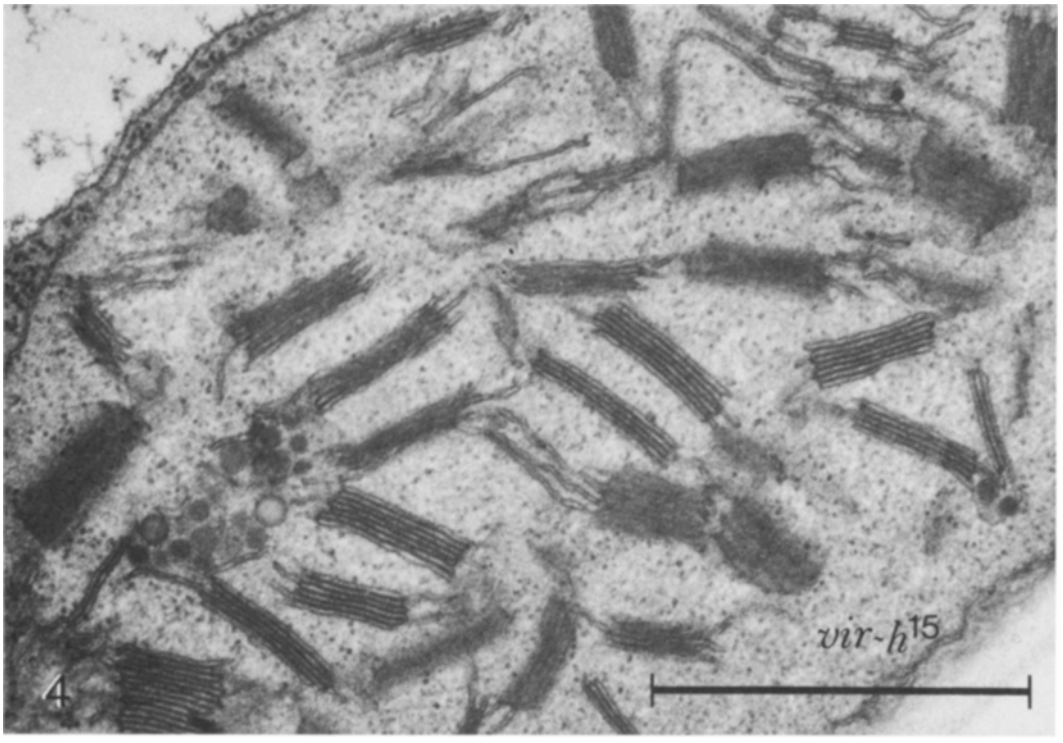
The grana tend to have a larger diameter than those of wild-type, with fewer discs per granum, and the stroma lamellae are few. (Bar = 1.0 μm). × 50,000

Figure 6. Mutant *xantha-q*^{75} (formerly *viridis*-30) and the allelic *xantha-q*^{76} (formerly *viridis*-56) are both characterised by large grana comprising many discs, and a relatively low amount of stroma lamellae.

(Bar = 1.0 μm). × 50,000

Figure 7. Thin-section of seedling leaf of *viridis-n*^{34} revealing an ultrastructure almost identical to that of wild-type, especially for the grana membranes.

In some plastid profiles the stroma lamellae appear collapsed (arrowhead), but other stroma lamellae in the same chloroplast are of normal structure. Similar features were found for *viridis-zb*^{63}. (Bar = 1.0 μm). × 50,000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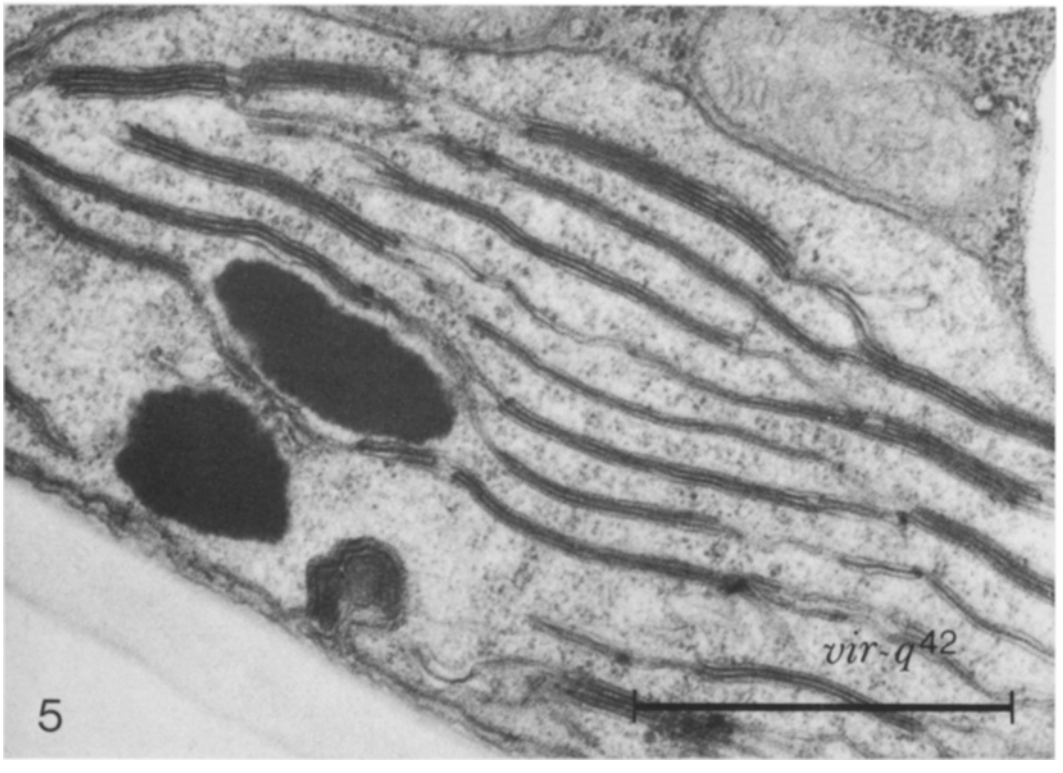
4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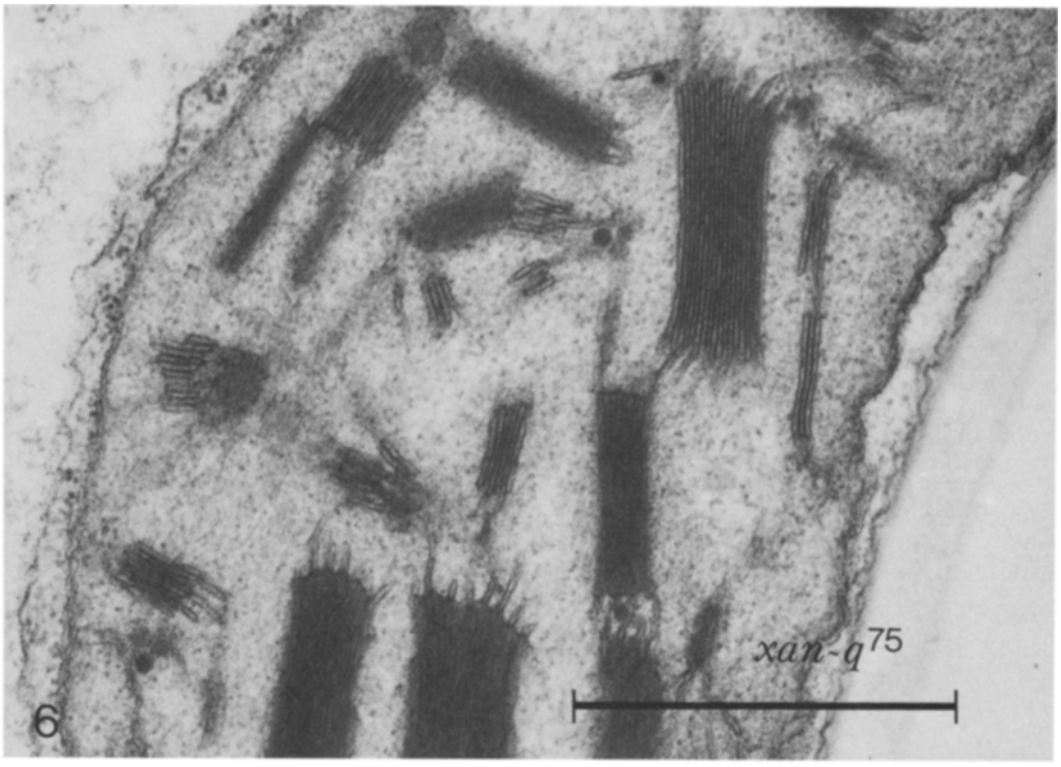
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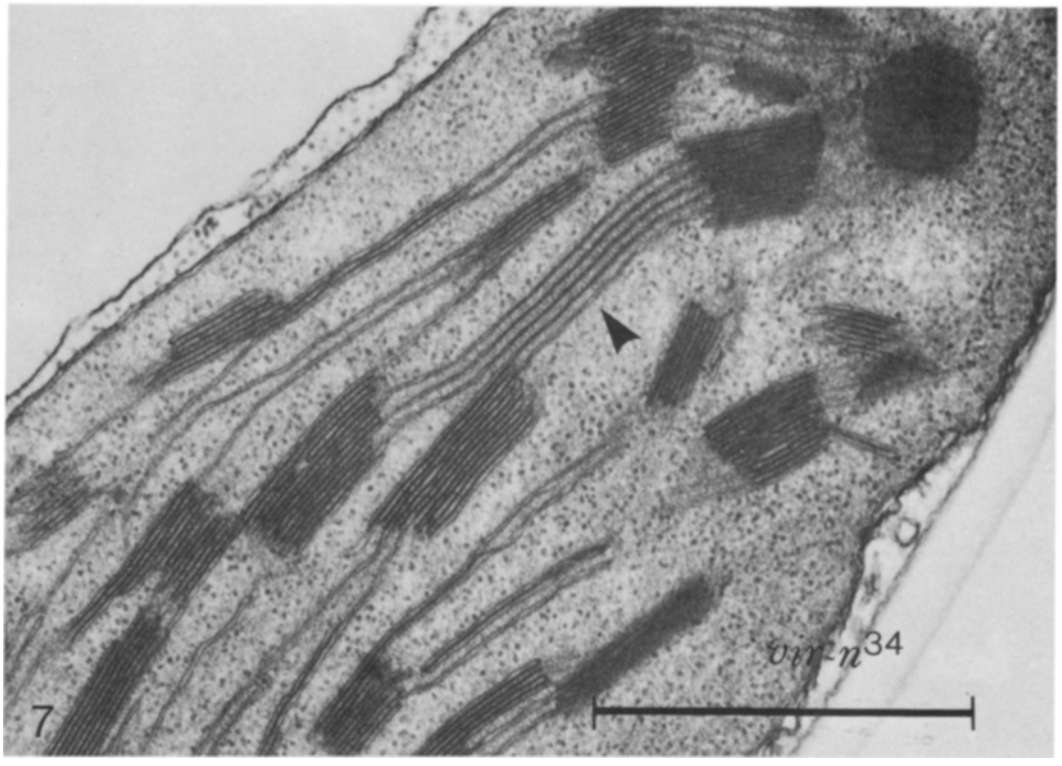
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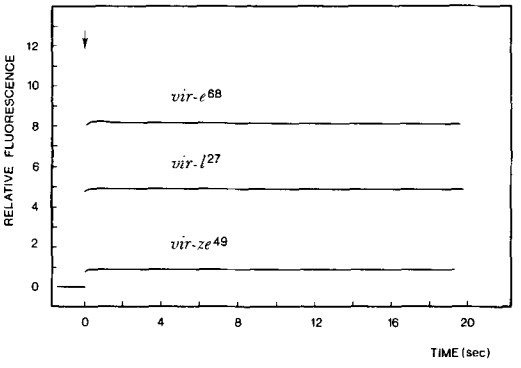


Figure 8. Fluorescence induction kinetics of dark-adapted leaves of mutants from Table V, showing almost no variable fluorescence, indicating inactivity of the photosystem II reaction centres.

The fluorescence yield is high for most mutants, except where the chlorophyll content is particularly low (as for *viridis-ze*^{49}). There is virtually no fluorescence decline for any of these mutants.

they all have F_m/F_o ratios between 1.2 and 1.6, and F_s/F_o ratios of less than 1.0 (between 0.67 and 0.90: Table VII). Mutant leaves showed moderate to high fluorescence under UV light. Not all the mutations are invariably lethal and, under favourable conditions, some live beyond the depletion of endosperm reserves, and may produce viable seed (Table VIII). Many have a high chlorophyll content with values ranging from 25 to 77% of wild-type.

Again, there was a variety of low temperature fluorescence emission spectra recorded for the different mutants. The long wavelength fluorescence peak was located between 730 and 733 nm for most mutants in this class, with *vir-p*^{38} and *-m*^{29} being closer to wild-type at 736 and 737

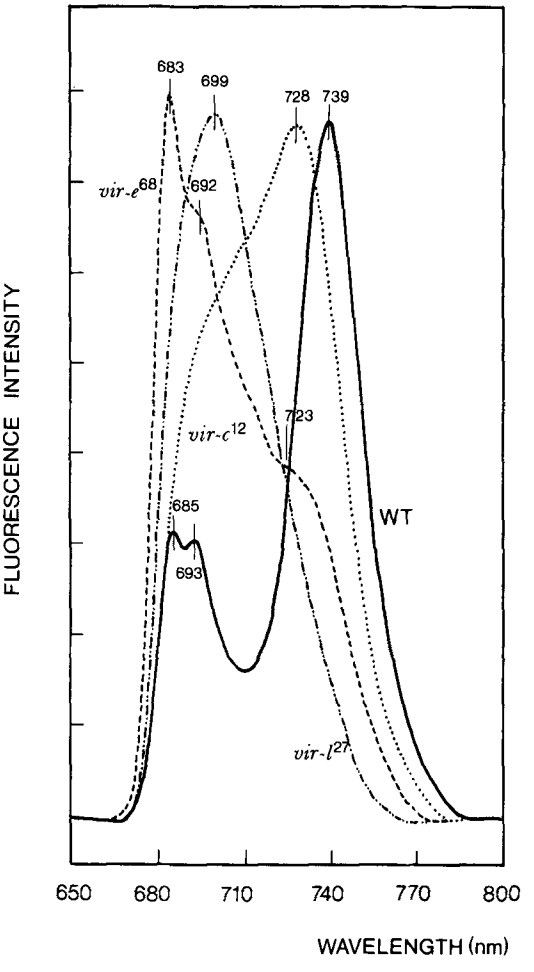
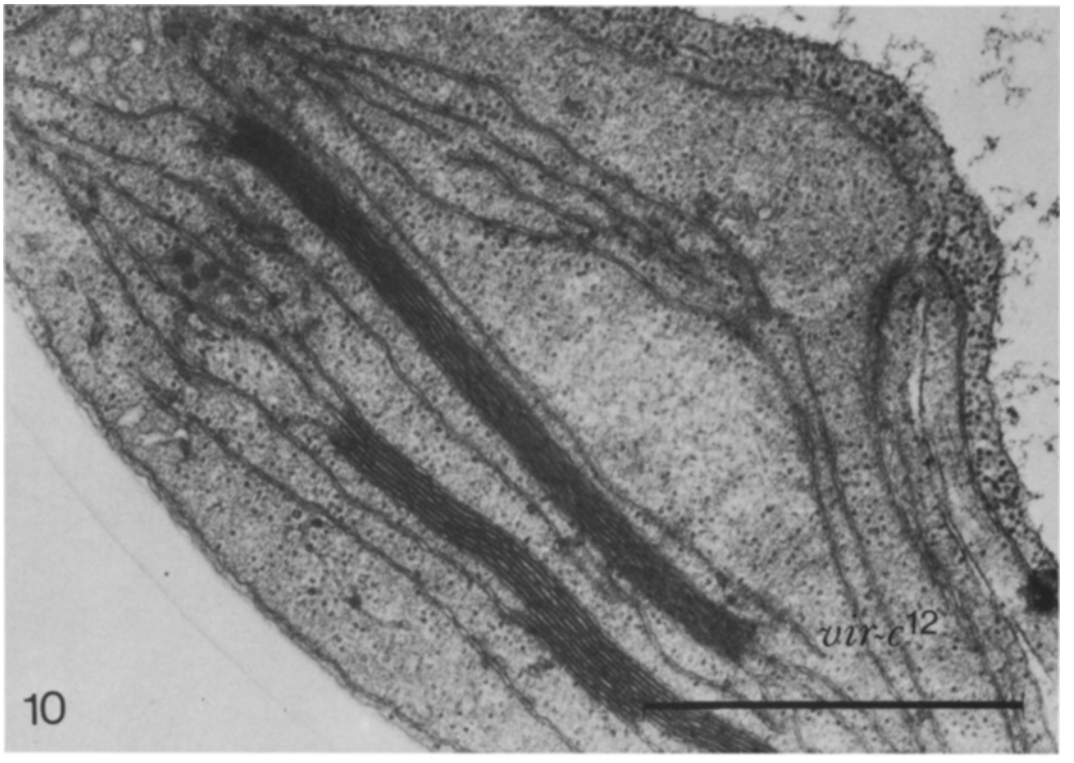


Figure 9. Low temperature (77 K) fluorescence emission spectra of photosystem II mutants (see Table V) compared with wild-type (WT).

In all cases the long wavelength peak was shifted to shorter values, but it is difficult to correlate these spectra with the known photosynthetic properties of these mutants. There are striking differences between the spectra from mutants at the *vir-c*, *-e* and *-l* loci.

Figure 10. The ultrastructure of mutants *viridis-c*^{11} and *-c*^{12} is similar, with thylakoids organised into a few extremely large diameter grana consisting of several discs, in addition to stroma lamellae oriented parallel to the grana.
(Bar = 1.0 μm). × 50,000

Figure 11. The chloroplasts of *viridis-l*^{27} are similar to those of *vir-c*, containing large diameter grana composed of several thylakoid discs with a reduced intrathylakoidal space, and parallel stroma lamellae.
(Bar = 1.0 μm). × 50,000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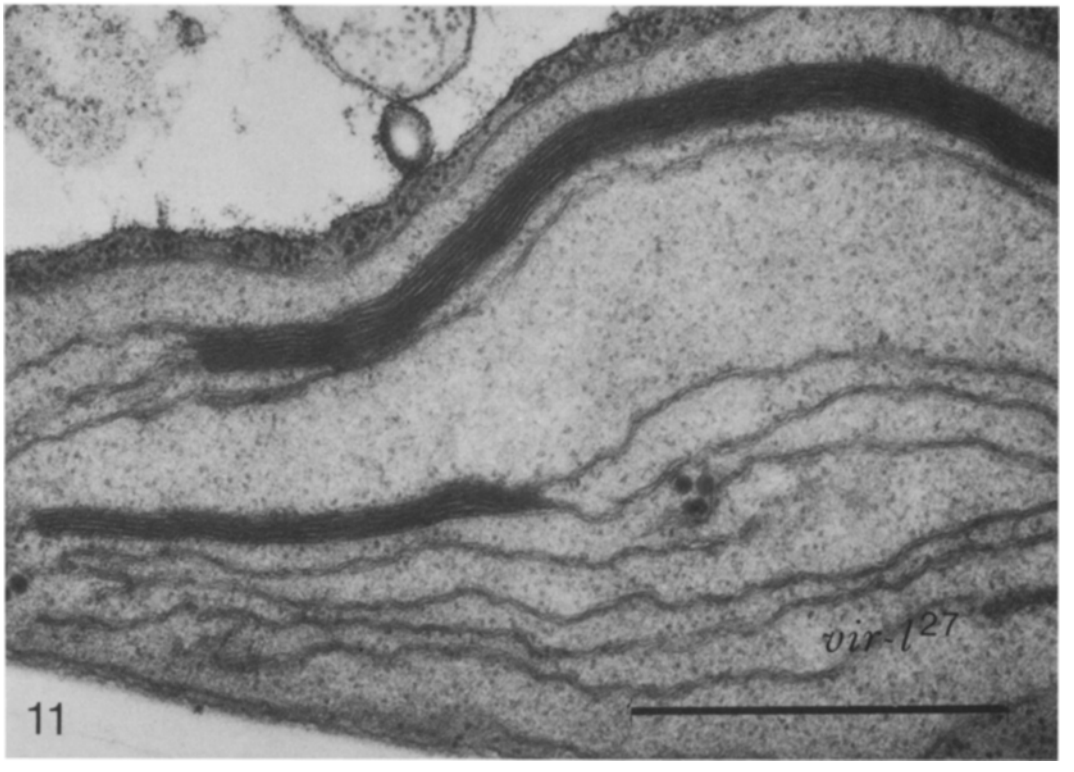
10


vir-l27
11

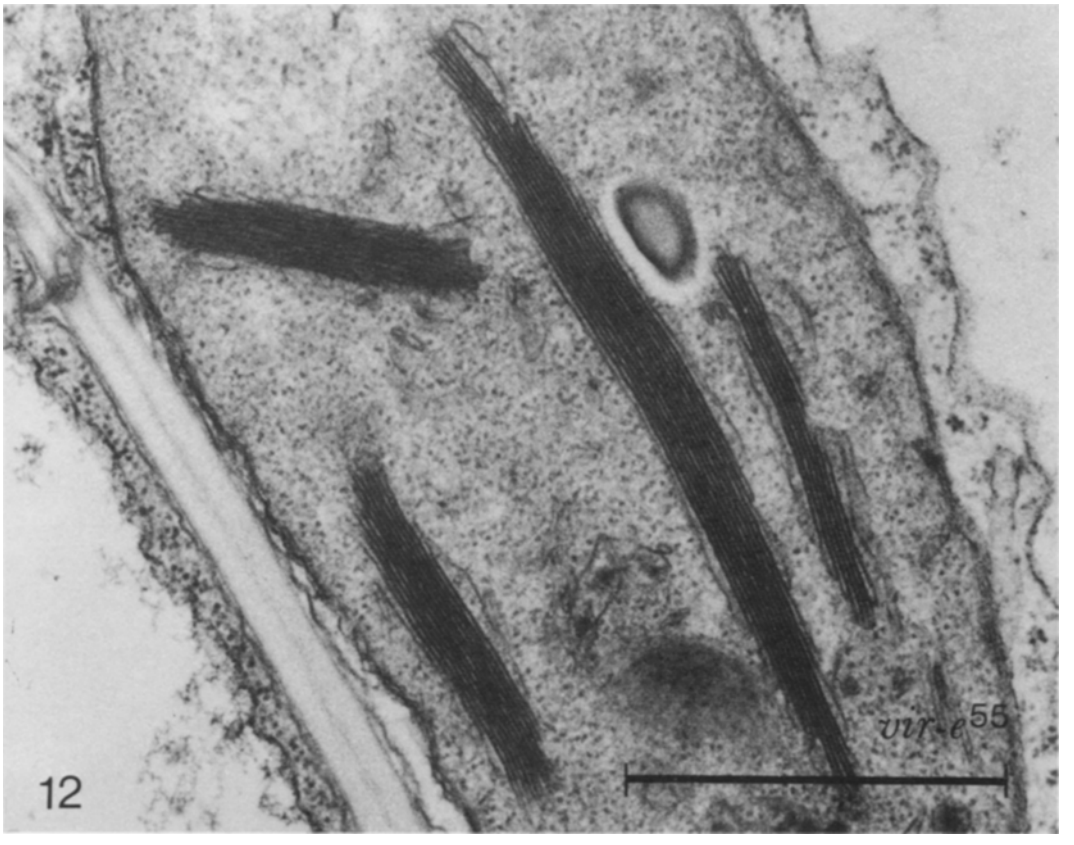


Figure 12. The mutants *viridis-e*^{55}, *-e*^{64} and *-e*^{68} have the same chloroplast ultrastructure.

They contain grana with a large diameter and normal intrathylakoidal spaces, but almost no stroma lamellae. (Bar = 1.0 μm). × 50,000

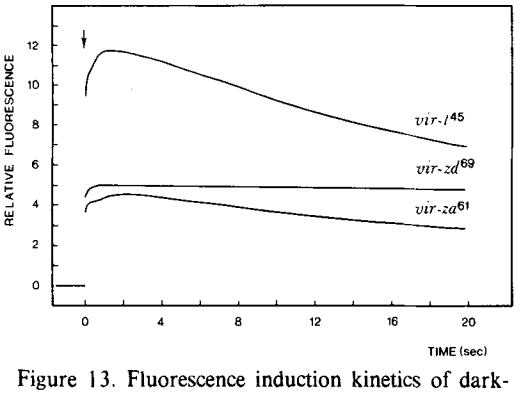


Figure 13. Fluorescence induction kinetics of dark-adapted barley mutant leaves (Table VII).

Many of these mutants are characterised by a high initial fluorescence (F_o) and a steady-state value (F_s, at 20 sec) which is lower than F_o (e.g., *viridis-l*^{45}). The variable fluorescence (F_v) varies greatly among the different members of this heterogeneous group.

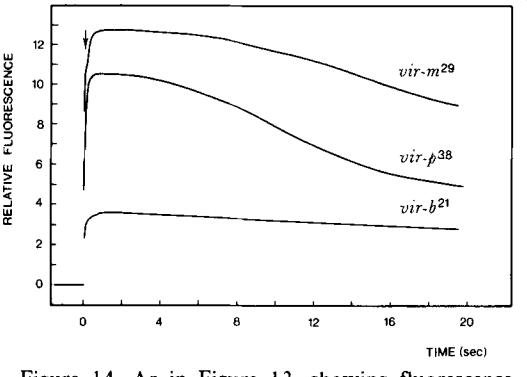


Figure 14. As in Figure 13, showing fluorescence induction curves of mutants *viridis-m*^{29} and *-p*^{38}, which for convenience have been placed in Table VII.

Both have a high F_o, high F_v, and a rapid fluorescence decrease, so that F_s is approaching the F_o value. These mutants are sub-lethal, as is *viridis-b*^{21}, but the reason for the high F_o is not known.

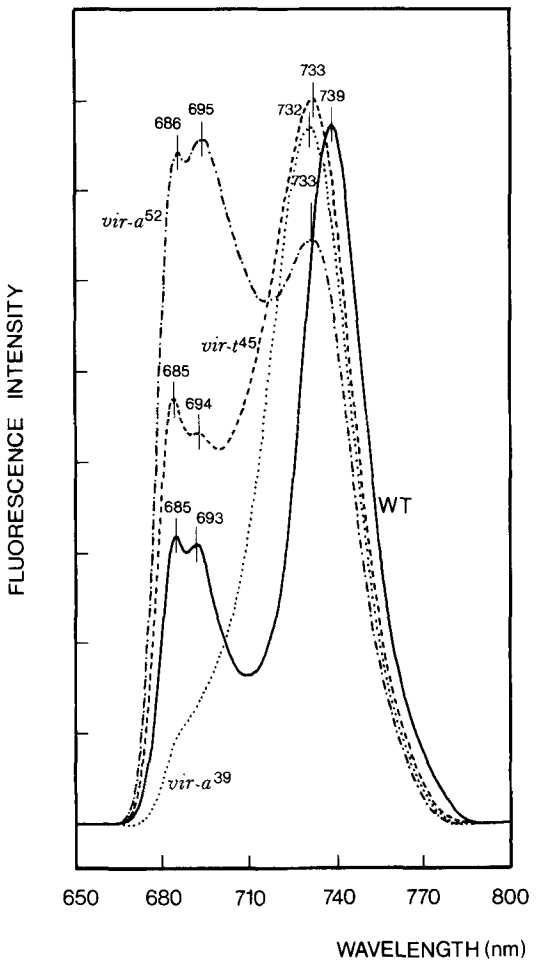


Figure 15. Low temperature (77 K) fluorescence emission sepctra of representative mutants of Table VII.

The long wavelength maximum is generally between 730 and 733 nm and there is among these mutants a high variability in the ratio of the fluorescence yield at 685 and 693 nm to that at 730 nm.

nm respectively. The ratio of the fluorescence emitted at 685 to that at 732 nm varied enormously (Fig. 15). Chloroplast ultrastructure also differed from one mutant to another. Those with mutations in the *viridis-a* gene (-a^{18}, -a^{33}, a^{38}, -a^{41} and -a^{52}) all exhibited the same ultrastructural features, with many apparently normal grana (Fig. 16). They also frequently contained highly curved grana consisting of discs with a reduced intrathylakoidal space, and often terminating in a network of membranous tubules at the granal margins (Fig. 17).

The organisation of the internal membranes of chloroplasts of *vir-m*^{29} and -p^{38} was very similar to wild-type, except that *vir-m*^{29} contained some very large diameter grana (Figs. 18 and 19). Some plastids of *vir-t*^{45} looked normal, but others in the same cell were full of vesicles, making this mutant less suitable for further characterisation than others of this group. Mutants *vir-u*^{46} and -v^{47} contained both giant grana and normal grana (Fig. 20), while the chloroplasts of *vir-z*^{60} and -za^{61} (Fig. 21) were very similar to wild-type in ultrastructural appearance. Both *vir-zc*^{65} (Fig. 22) and *vir-zd*^{69} (Fig. 23) contained extensive thylakoid systems mainly organised into grana which were larger than wild-type in both diameter and in the number of discs.

3.6. Mutants with $F_m/F_o > 3.5$

The fourth category of mutants consisted of those with relatively low initial fluorescence (F_o) and moderate variable fluorescence (F_v) and with the exception of *vir-b*^{10} and -b^{21}, all have F_m/F_o ratios greater than 3.5 and show an appreciable F_m to F_s decline (Table IX). The fluorescence induction kinetics were therefore similar to those of the wild-type and in all cases the rise from F_o to F_m was sigmoidal (Fig. 24). The low fluorescence intensity of some of these mutants (*vir-k*^{23}, -d^{24}, -d^{25} and -s^{44}) can be attributed to their low chlorophyll content, in combination with apparently normal photosynthetic capacity (Table IX). The chlorophyll content of the mutants of this group ranged from 24 to 95 % of wild-type and several are viable under favourable conditions (Table X).

Representative low temperature fluorescence emission spectra of these mutants are shown in Figures 25 and 26. Many were similar to wild-type, with a blue shift in the long wavelength peak of 0–3 nm and a relative increase in the fluorescence intensity at 695 nm (*vir-b*^{10}, -f^{13}, -g^{14}, -i^{17}, -j^{19}, -b^{21}, -d^{24}, -d^{25} and -o^{35}). Other mutants (*vir-k*^{23} and -s^{44}) showed a large decrease in the value of the long wavelength peak (Fig. 26). The low temperature fluorescence emission spectrum for the viable mutant *chlorina-f2*, which lacks chlorophyll *b*, is given for comparison (Fig. 25).

The ultrastructure of the chloroplasts of many of these mutants (*vir-g*^{14}, *-i*^{17}, *-j*^{19}, *-d*^{24}, *-o*^{35} and *-s*^{44} (Figs. 27 and 31) was very similar to that of wild-type chloroplasts. However, mutants *vir-b*^{10}, *-f*^{13}, *-b*^{21} and *-d*^{25} had fewer internal membranes with grana containing a smaller number of discs than those of wild-type (Figs. 28 and 30), while *vir-k*^{23} has mainly stroma lamellae, with very limited membrane appression (Fig. 29).

3.7. Low chlorophyll mutants

The fifth group of *viridis* mutants was characterised by very low initial fluorescence, very little variable fluorescence and extremely low chlorophyll content (Tables XI and XII). Such seedlings appeared almost white when grown under the present standard conditions. This was reflected in their plastid ultrastructure, which was typical of *albina*-type mutants. The internal membranes consisted of isolated vesicles and tubules, with no recognisable chlorophyll-containing membranes present (Figs. 32 and 33). These mutants accumulate higher levels of chlorophyll under other growth conditions, for example light/dark cycles with a 15°/10 °C thermoperiod. Mutants *vir-x*^{51} and *vir-y*^{59} were previously shown to have abnormal features of porphyrin metabolism (18). The mutant *vir-w*^{50} was not investigated owing to its extremely slow growth and low chlorophyll content under the present condition.

Figure 16. Thin-section of a chloroplast from the mutant *viridis-a*^{52}, which is typical of all mutants at the *vir-a* locus (*vir-a*^{18}, *-a*^{33}, *-a*^{39}, *-a*^{41} and *-a*^{52}).

Many of the grana are apparently normal in both size and structure, although they tend to be oriented at different angles to one another. (Bar = 1.0 μm). × 50,000

Figure 17. Section through a cylindrical granum, typical of the *viridis-a* mutants.

The thylakoid discs have a reduced intrathylakoidal space and the grana terminate in a series of close-packed tubules. These structures are also commonly seen in plastid profiles with normal grana organisation as that in Figure 16. (Bar = 1.0 μm). × 50,000

Figure 18. Chloroplast from *viridis-m*^{29}, showing a well-developed thylakoid system, with grana and stroma lamellae.

Many of the grana are apparently normal, but there is usually present one or more giant grana with extremely large thylakoid discs. (Bar = 1.0 μm). × 50,000

Figure 19. The thylakoid system of chloroplasts of *viridis-p*^{38} is similar to that of wild-type, with well-developed grana interconnected by stroma lamellae.

(Bar = 1.0 μm). × 50,000

Figure 20. The plastids of *viridis-u*^{46} were difficult to fix and have a poorly organised thylakoid system, consisting of small, randomly oriented grana, and some giant grana with normal intrathylakoidal spaces.

The mutant *viridis-v*^{47} has a similar plastid ultrastructure. (Bar = 1.0 μm). × 50,000

Figure 21. The chloroplasts of *viridis-z*^{60} and *-za*^{61} cannot be distinguished from those of wild-type with regard to their thin-section ultrastructural appearance.

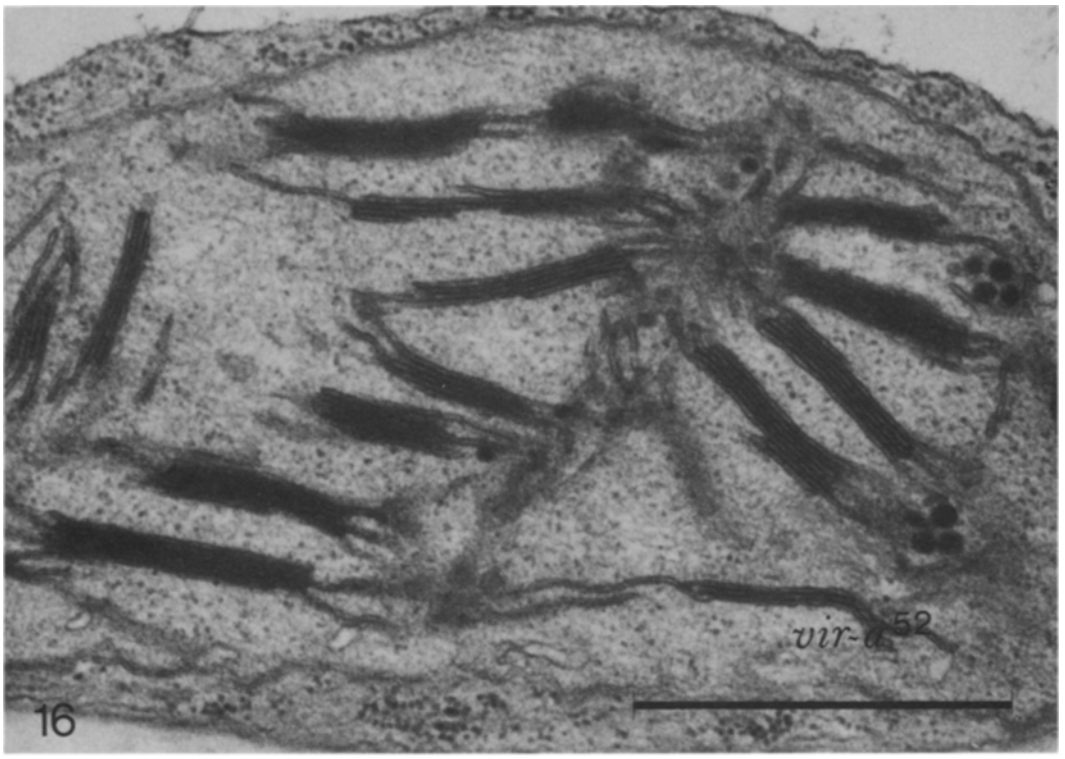
(Bar = 1.0 μm). × 50,000

Figure 22. The thylakoids of *viridis-zc*^{65} are organised into well-developed grana which tend to overlap one another extensively and consist of many discs characterised by a reduced intrathylakoidal space.

The amount of stroma lamellae is limited. (Bar = 1.0 μm). × 50,000

Figure 23. The ultrastructural appearance of *viridis-zd*^{69} chloroplasts is characterised by large grana containing several thylakoids with narrow intrathylakoidal spaces, and a severely reduced stroma lamellar system.

(Bar = 1.0 μm). × 50,000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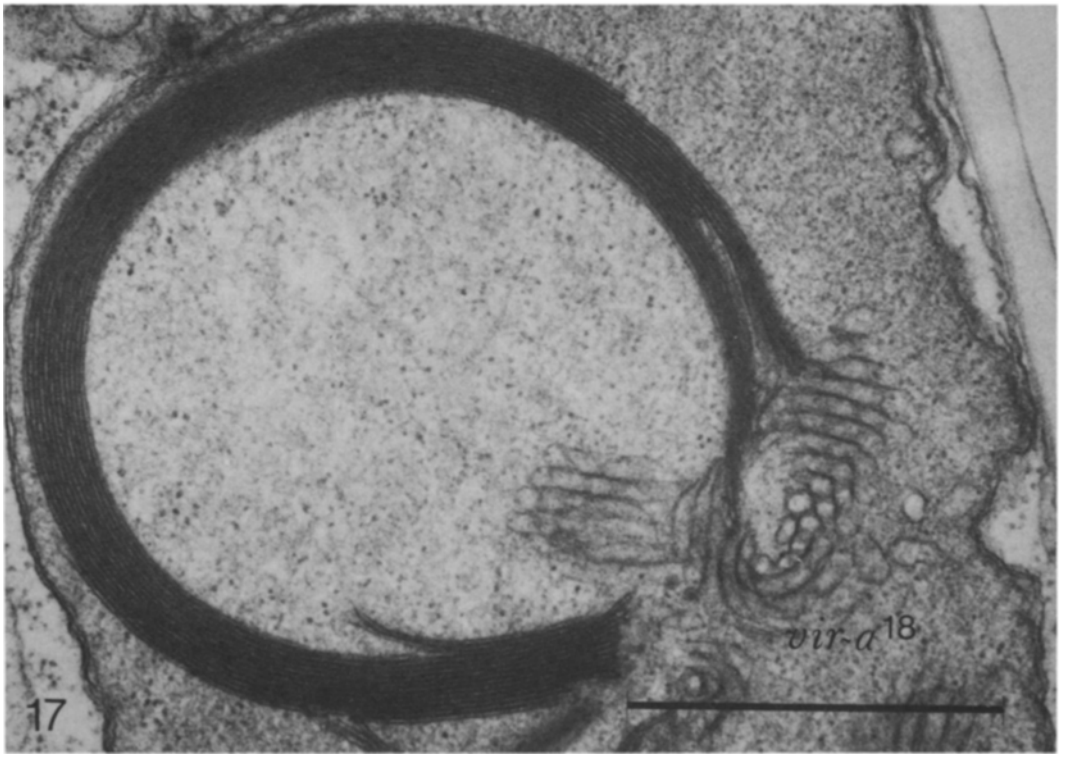
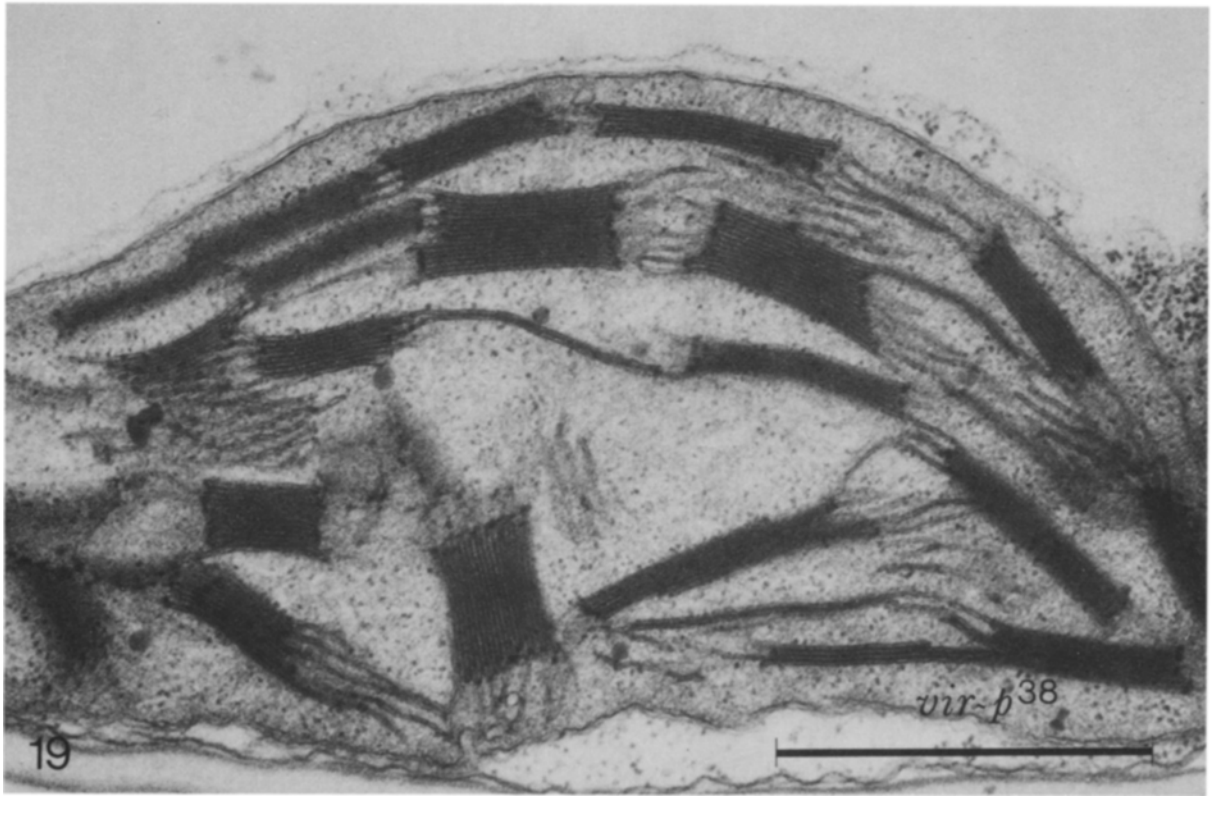
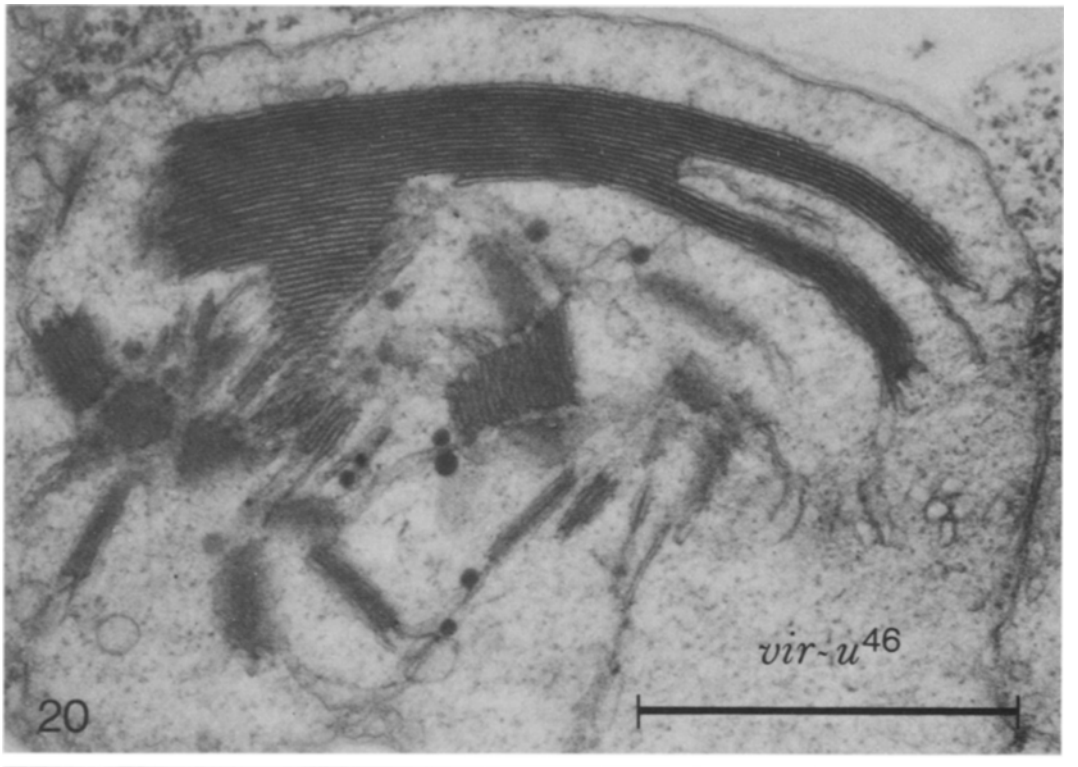
16


vir-a 18
17


vir-m29
18


vir-p38
19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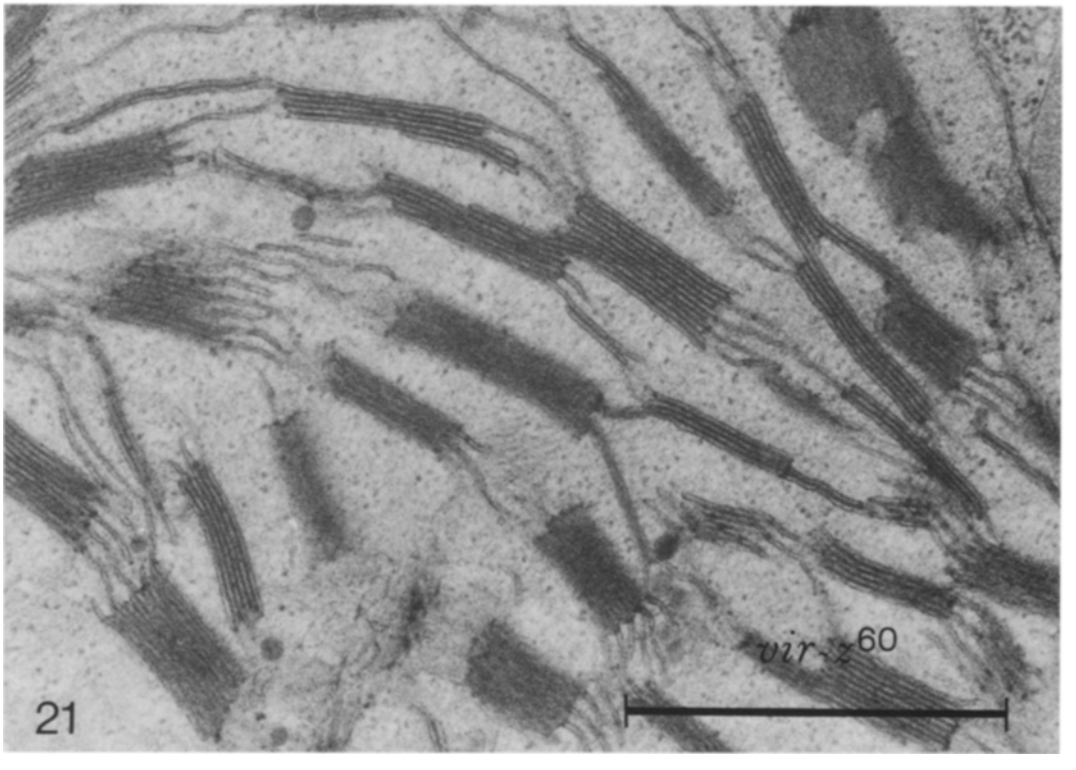
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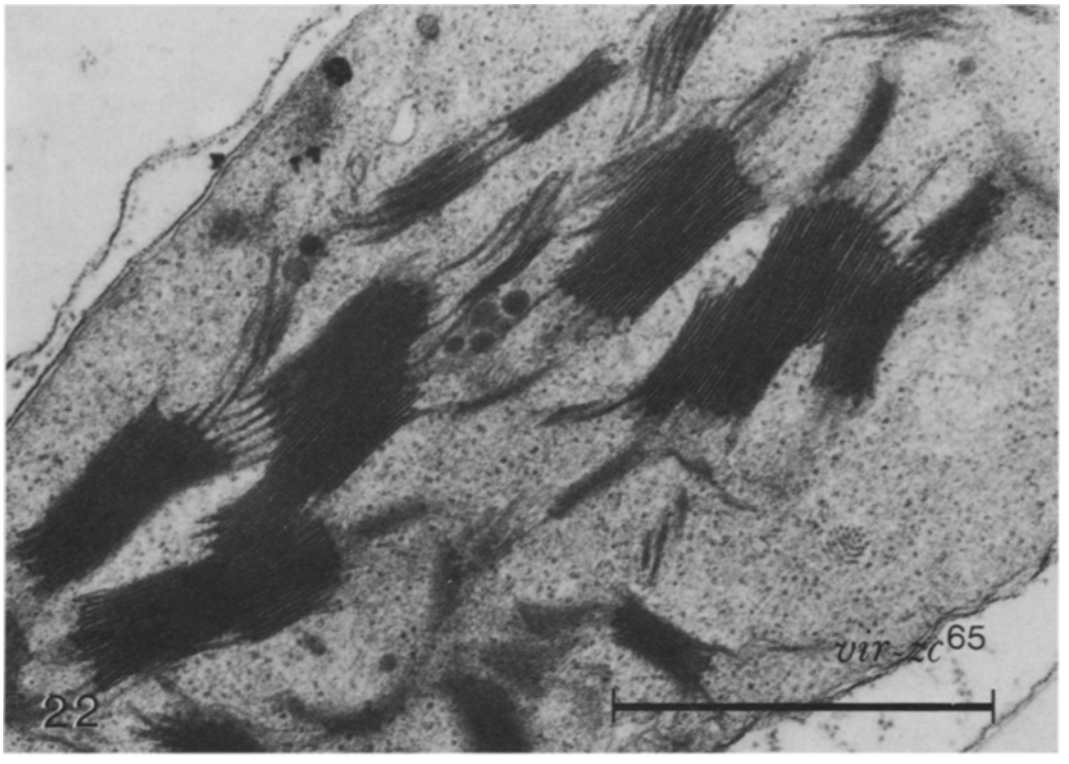
21


vir-zc 65
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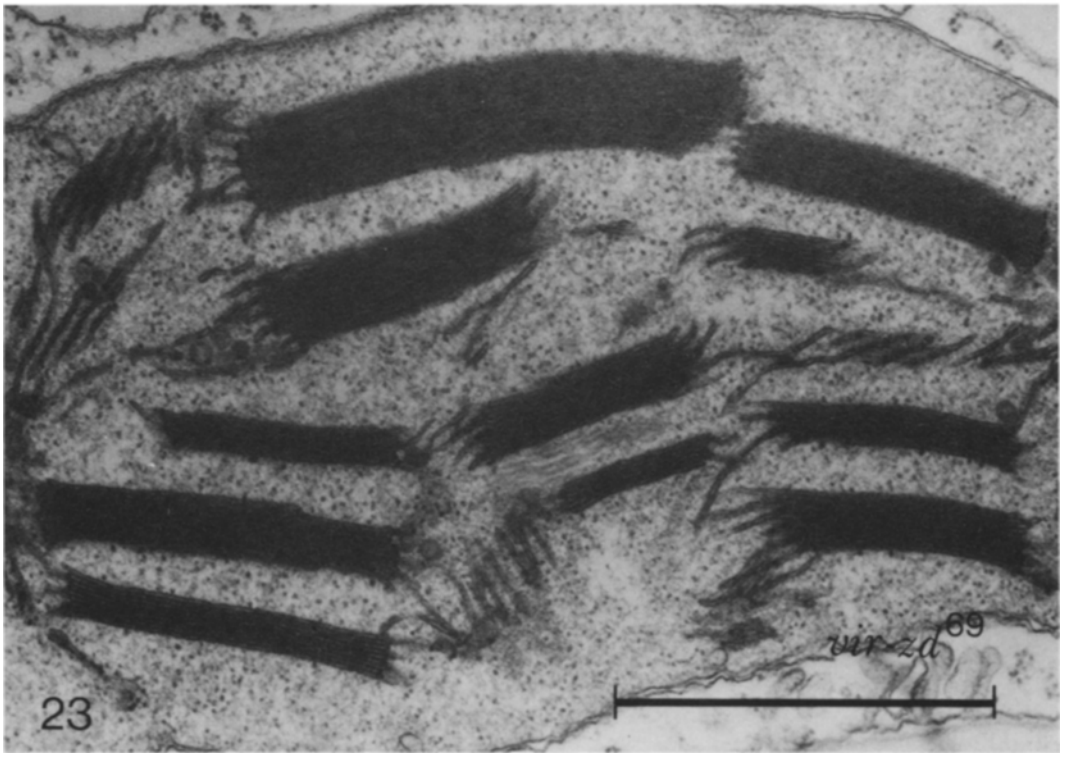

vir-zd 69
23

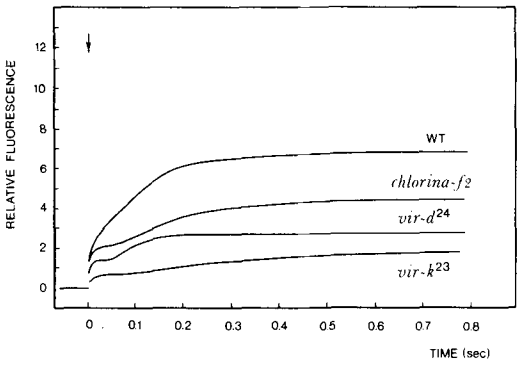


Figure 24. Fluorescence induction kinetics of dark-adapted seedling leaves of barley mutants from Table IX.

The time scale is expanded (see Fig. 2) to show the sigmoidal rise in fluorescence after the onset of illumination. Since equal leaf areas were illuminated with saturating light, the differences in fluorescence yield may be related to the different levels of chlorophyll in the mutants, and specifically to low levels of the light-harvesting chlorophyll *a*/*b*-protein in some mutants (*viridis*-k^{23} and *chlorina*-*f2*).

4. DISCUSSION

4.1. Fluorescence induction kinetics

Whole leaf fluorescence induction kinetics has been used to analyse the nature of photosynthetic lesions in Chlamydomonas (3–5, 12, 14), maize (22–25) and pea (44), all of which are genetically well-characterised species. Such fluorescence induction transients can be interpreted by correlating them with photosynthetic defects, as determined by partial electron transport reactions.

It is generally agreed that the rise in fluorescence with time, from the initial value (F_o) to maximum fluorescence (F_m) is associated with normal photosystem II activity, involving electron transport from oxygen to plastoquinone (15, 33). The decline in fluorescence from F_m to steady state fluorescence (F_s) indicates the normal functioning of electron transport from plastoquinone to ferredoxin NADPH-reductase in combination with photophosphorylation (15, 33). High values of the initial fluorescence are indicative of antennae chlorophyll absorbing energy that cannot be transferred to photosynthetic reaction centres. Thus, in principle, photosystem I defective mutants show little or no F_m to F_s decline, and photosystem II defective mutants are characterised by little or no variable fluorescence (F_v). The interpretation is in fact more complicated since photosystem I affects the F_o to F_m rise, and many factors, such as the proton gradient, temperature, anaerobiosis and electron transport between the two photosystems will affect the F_m to F_s decline.

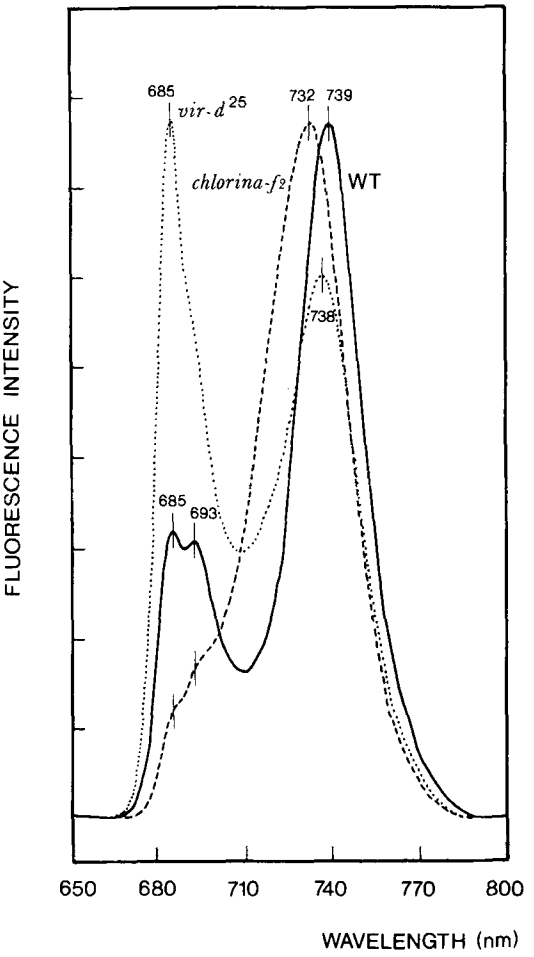


Figure 25. Low temperature (77 K) fluorescence emission spectra of leaves from mutants of Table IX.

Many of these mutants had a long wavelength maximum close to that of wild-type (739 nm), although the relative fluorescence yield varied considerably. The in vivo fluorescence emission spectrum of the *chlorina-f2* mutant is included for the purposes of comparison, and shows a low fluorescence yield at 685 and 693 nm compared with wild-type, and has a long wavelength maximum at 732 nm, which is higher than reported from studies on chloroplasts isolated from this mutant.

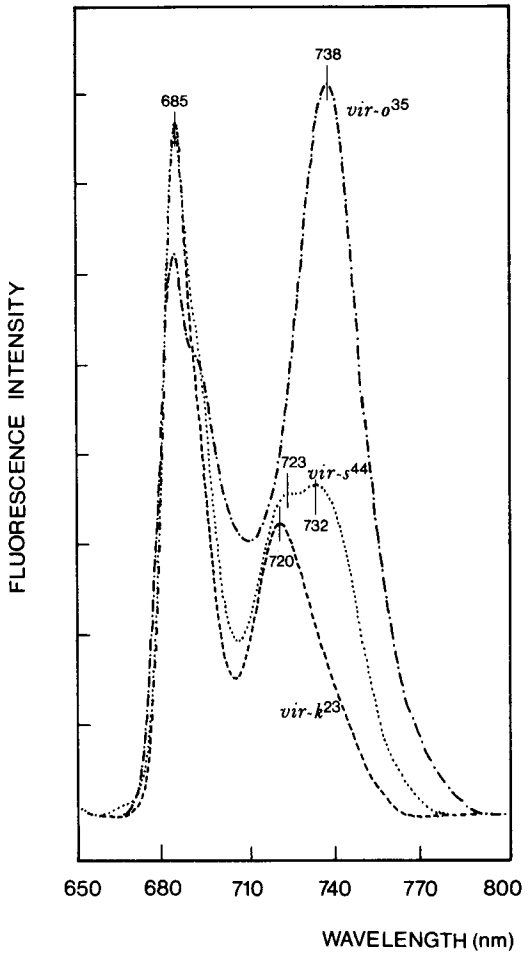


Figure 26. As in Figure 25, showing additional low temperature fluorescence emission spectra.

The mutant *viridis-k*^{23}, like *chlorina-f2*, is photosynthetically competent, but is deficient in chlorophyll *b*, and has a long wavelength maximum at 720 nm. The mutant *viridis-s*^{44} is unique in having a double maximum in this region, at 723 and 732 nm.

The first group of mutants (Table III) show fluorescence induction kinetics typical of photosystem I deficiency (3, 23). When the P700-chlorophyll *a*-protein 1 is completely absent, the initial fluorescence of such mutants is close to the wild-type (3, 5). However, when there is simply a deficiency of this reaction centre chlorophyll-protein, the initial fluorescence is much higher than in the wild-type (5). Lesions in electron transport on the reducing side of photosystem I produce similar transients, except that the initial fluorescence is somewhat lower (24). Photophosphorylation mutants of Chlamydomonas, which lack the chloroplast coupling factor 1 (CF_1), show a similar type of transient if grown under high light intensity, but can be distinguished by their high F_o and small amount of variable fluorescence (5).

The mutant *vir-n*^{34} has been studied in some detail (30). It has about 10 % of the photosystem I electron transport activity of wild-type and is deficient in P700 and chlorophyll *a*-protein 1, while having near normal photosystem II activity (30). The residual photosystem I activity may be responsible for the slight F_m to F_s decline observed (Table III), and it might be predicted that the mutant *vir-zb*^{63} is an even more stringent photosystem I defective mutant. The photosystem I activity for this mutant is reported to be 8 % of the wild-type on a chlorophyll basis (31). Mutants *xan-q*^{75}, *-q*^{76} and *vir-q*^{42} have a much lower chlorophyll content than that of wild-type and their photosynthetic defects may result indirectly from a lesion preventing chlorophyll accumulation.

The second class of mutants (Table V) are almost totally lacking variable fluorescence and show a very high initial fluorescence (except for *vir-ze*^{49}). This type of transient is characteristic of photosystem II defective mutants of maize (23) and Chlamydomonas (14) deficient in cytochrome b_{559}, a polypeptide synthesised on chloroplast ribosomes (49). Since these *viridis* barley mutants are nuclear gene mutants, the primary lesion is likely to affect the synthesis of a polypeptide made in the cytoplasm, but functioning close to the reaction centre of photosystem II. Mutants *vir-c*^{12} and *-e*^{64} have very low photosystem II electron transport activities (0.25 % and 2 % of wild-type, respectively) (42), and it seems likely that the other mutants in Table V will prove to have similarly low photosystem II activity. The level of photosystem I activity cannot be predicted from the induction curves, although partial electron transport measurements of *vir-c*^{12} and *-e*^{64} give lower values than wild-type on a chlorophyll basis (42). The chlorophyll content of these mutants is generally less than one-third that of wild-type, which make them less suitable for making correlations between function and composition.

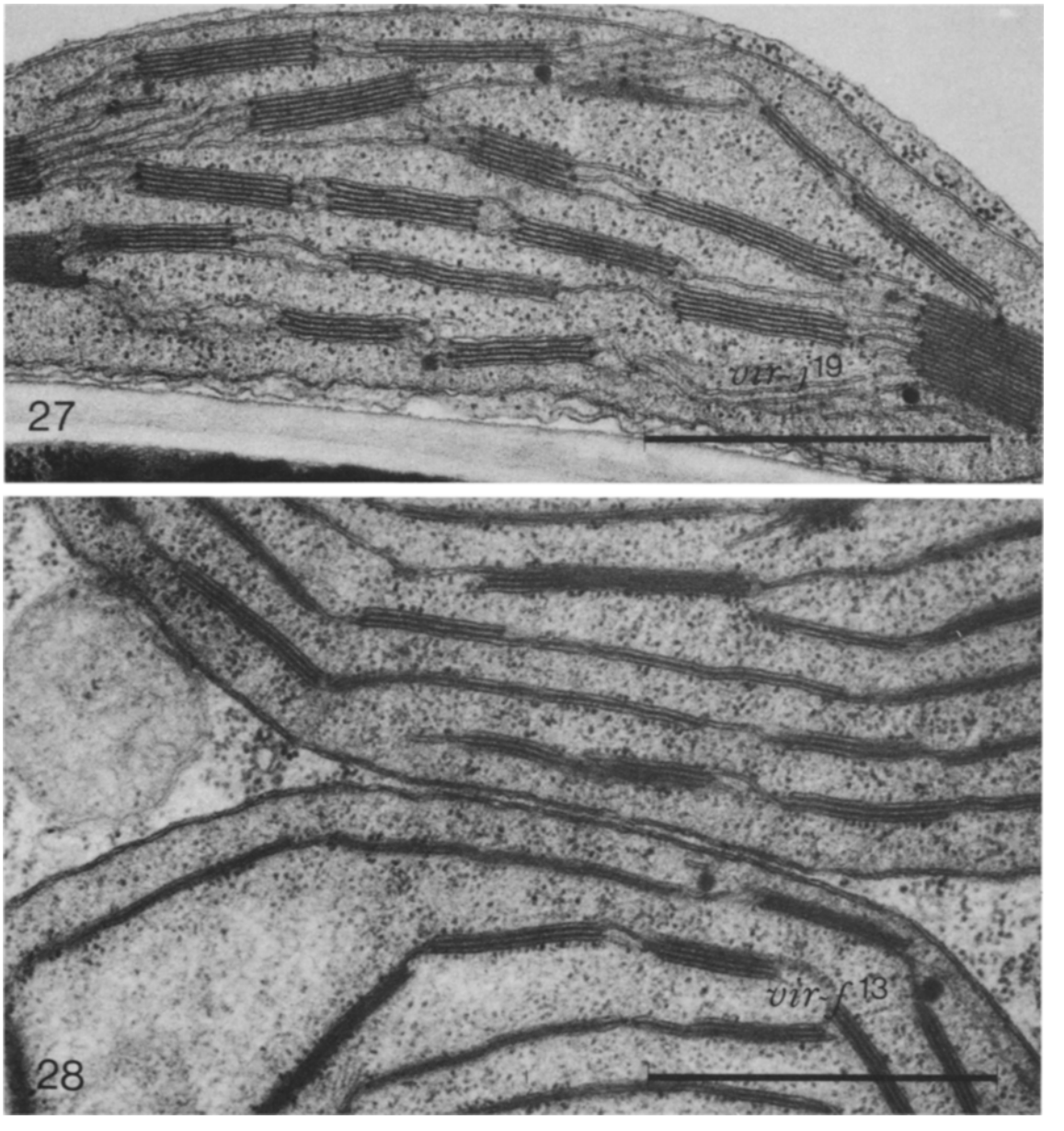
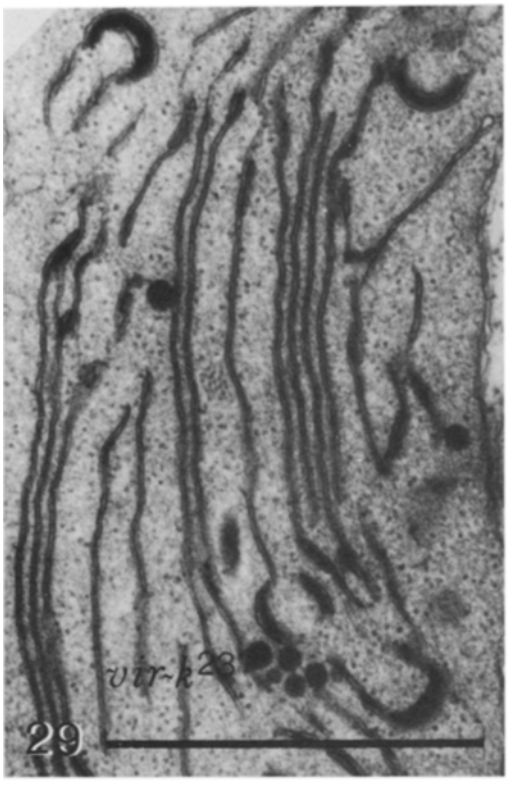


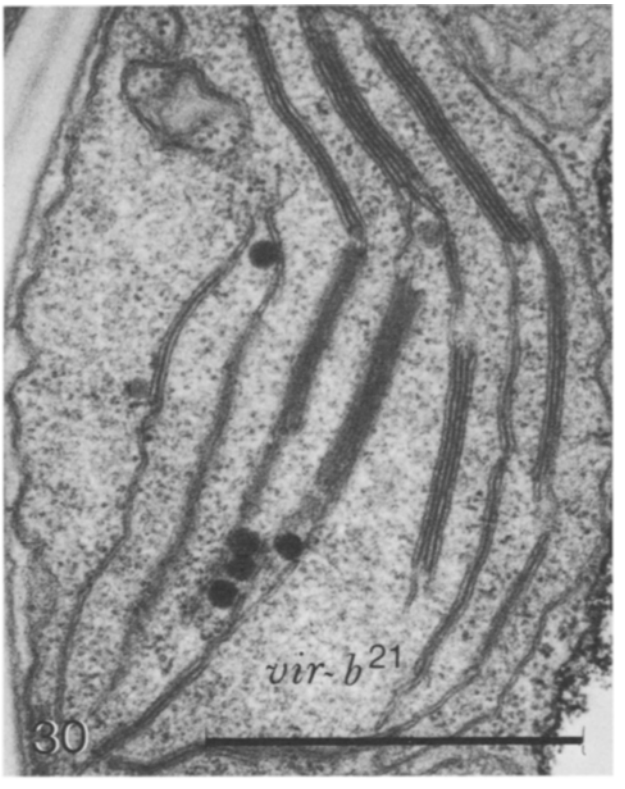
Figure 27. Thin-section electron micropgraph of the mutant *viridis-j*^{19}, which cannot be distinguished from wild-type on the basis of chloroplast ultrastructure.

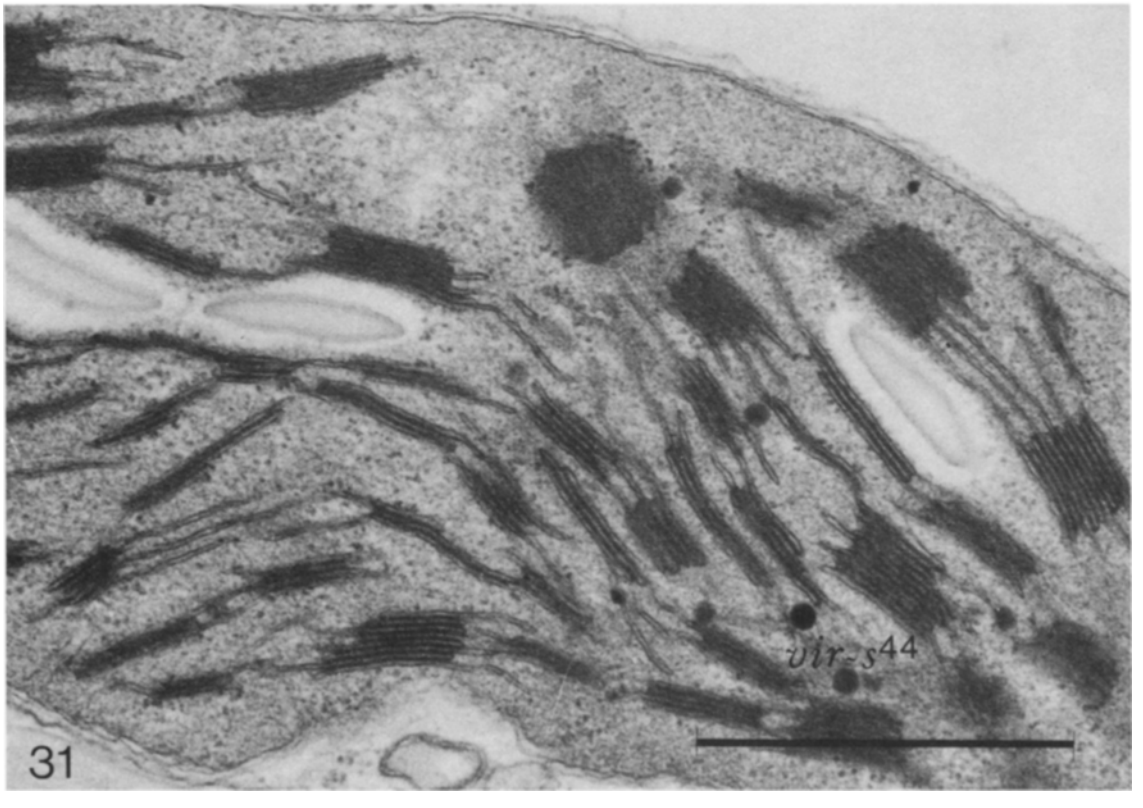
The same is true for *viridis-g*^{14}, *-i*^{17}, *-d*^{24} and *-o*^{35}, all of which are characterised by a well-developed thylakoid system consisting of grana and stroma lamellae. (Bar = 1.0 μm). × 50,000

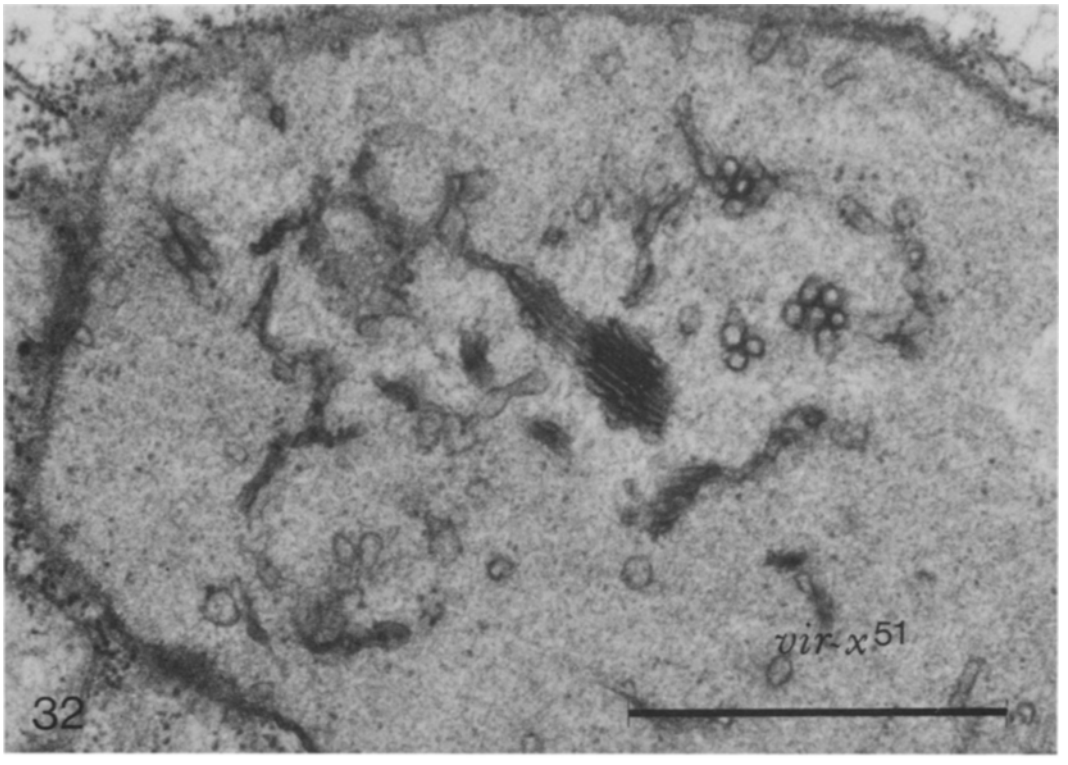
Figure 28. Chloroplasts of *viridis-f*^{13} have a relatively normal internal membrane system, except that the grana contain fewer thylakoid discs than those of wild-type.

(Bar = 1.0 μm). × 50,000


vir-k28
29


vir-b21
30


vir-s44
31


vir-x51
32

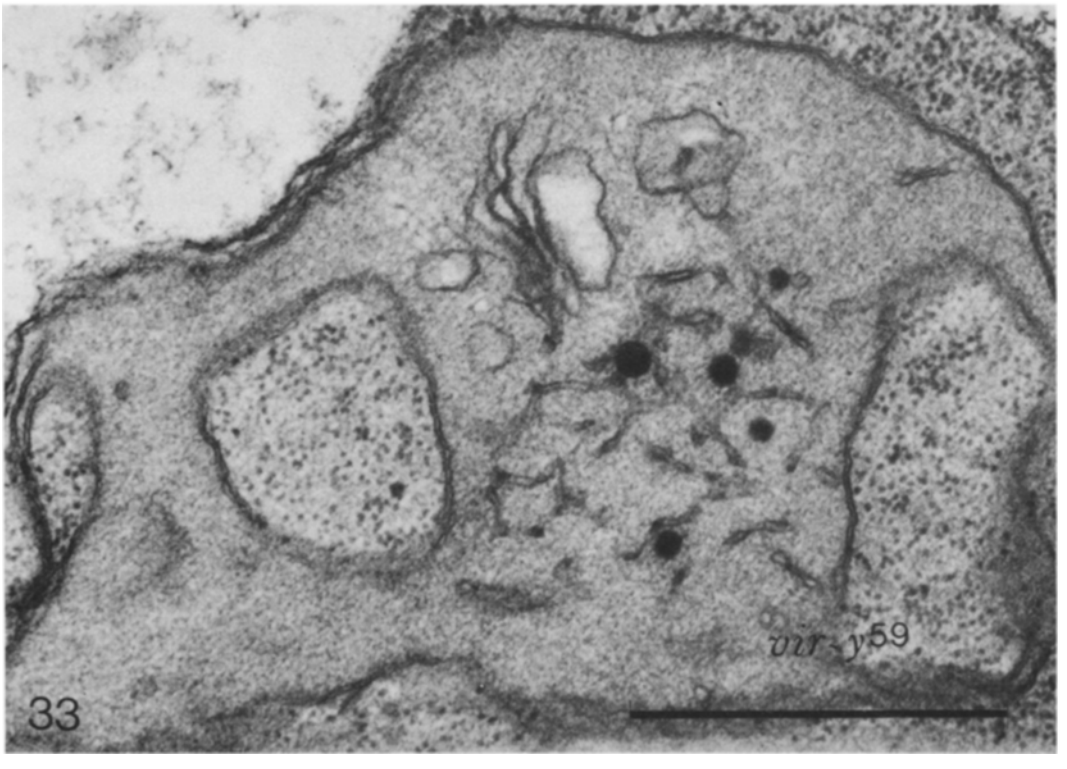

vir-y59
33

Figure 29. The chloroplasts of *viridis-k*^{23} contain long, parallel stroma lamellae, with limited regions of membrane pairing, which may not necessarily correspond to membrane appression.
Also present are electron-dense curved membrane structures which are typical of this mutant, and of *xantha-f*^{35} (47). (Bar = 1.0 μm). × 50,000

Figure 30. The mutant *viridis-b*^{21}, like *viridis-f*^{13}, contains a near-normal thylakoid system, except for a reduction in the number of thylakoid discs per granum.
This is also found for *viridis-b*^{10} and *-d*^{25}. (Bar = 1.0 μm). × 50,000

Figure 31. The mutant *viridis-s*^{44}, which is characterised by an unusual low temperature fluorescence emission spectrum (Fig. 25), has a chloroplast ultrastructure that is similar to wild-type.
(Bar = 1.0 μm). × 50,000

Figure 32. The extremely chlorophyll-deficient mutant *viridis-x*^{51} contains a primitive internal lamellar system, consisting of mainly tubules and vesicles.
(Bar = 1.0 μm). × 50,000

Figure 33. The mutant *viridis-y*^{59}, which has 0.2% of the chlorophyll content of wild-type seedling leaves, contains small plastids with a simple internal lamellar system, lacking any recognisable grana or stroma lamellae.
(Bar = 1.0 μm). × 50,000

The *viridis* mutants in the third category (Tables VII and VIII) are more heterogeneous in their properties than those of the first two groups. Their fluorescence induction curves are characterised by an initial fluorescence (F_o) at least three times that of wild-type, but the fluorescence after 20 sec (F_s) has fallen to, or is below, the F_o, and may even be lower than the F_s of wild-type. The amount of variable fluorescence of mutants of this group varies from 14 to 139% of wild-type. All fluoresce visibly under UV light, but some, particularly those with high F_v values (*vir-a*^{18}, *-m*^{29}, *-p*^{38}) will grow to maturity under suitable conditions, indicating a degree of photosynthetic competence. Mutants *vir-a*^{39} and *-v*^{47} have near normal photosystem I activity and photosystem II is about half that of wild-type (42). This, together with the low F_v values for many of these mutants, suggests that they are deficient in photosystem II to varying degrees, and electron transport is not limited by photosystem I activity. Mutants which have this type of induction kinetics (i.e., $F_o > F_s$) have not been reported before, although maize mutants defective in CO_2 fixation have $F_o \simeq F_s$ (23). Since many of these *viridis* mutants have a high chlorophyll content, they are good candidates for further characterisation.

The fourth class of *viridis* mutants (Tables IX and X) is characterised by low initial fluorescence, maximally just over twice that of wild-type (*vir-b*^{10}, *-b*^{21}) and their fluorescence induction kinetics are similar to that of wild-type leaves (Fig. 24). All mutants have a low level of fluorescence under UV light and several will produce viable seeds in a favourable environment (Table X). Some of these mutants behaving as seedling lethals in the field have not been tested for favourable environmental conditions and may need to be re-classified as sub-lethal in the future. Mutants in this group, particularly those with a F_m/F_o ratio greater than 3.5 either do not have primary lesions in the photosynthetic apparatus or have changes allowing photosynthesis to proceed. Those mutants whose photosynthetic capacities have been measured, *vir-b*^{10}, *-g*^{14}, *-b*^{21} (42) and *vir-k*^{23} (31), show activities close to, or even better than wild-type on a chlorophyll basis. Mutant *vir-k*^{23} has a leaky block in the conversion of Mg-protoporphyrin to protochlorophyllide (21).

4.2. Low temperature fluorescence emission spectra

The low temperature fluorescence emission spectra from intact leaves vary considerably from mutant to mutant. Studies on the greening

of flashed leaves (43) and on sub-chloroplast fractions (36) assigned each of the three in vivo emission peaks to a separate chlorophyll-protein complex. Thus, F-685 emission was considered due to the light-harvesting chlorophyll *a*/*b*-protein, F-694 emission to photosystem II, and F-735 to photosystem I. It has since been shown that the light-harvesting chlorophyll *a*/*b*-protein will emit at both 685 and 695 nm when it is isolated in the form of lamellar sheets (28). In addition, a F-685 peak in the emission spectrum of chloroplasts isolated from the barley mutant *chlorina-f2* (which lacks the light-harvesting chlorophyll *a*/*b*-protein) has been attributed to photosystem II (34), although prominent peaks at 685 and 693 nm are not seen in the whole leaf spectrum (Fig. 25).

There is general agreement that fluorescence emitted at long wavelengths (>700 nm) is due to chlorophyll associated with photosystem I. This has been shown for photosystem I particles isolated by digitonin treatment (36) and Triton X-100 (29), as well as for the P700 chlorophyll *a*-protein 1 band in polyacrylamide gels (2, 45). The fluorescence emission spectrum of photosystem I in pea seems to be determined by the presence of at least three different chlorophyll species absorbing at 685–90, 697 and 705 nm, and emitting at 694, 722 and 736 nm, respectively (9, 29). An increase in the wavelength of the far-red photosystem I emission peak has been observed during greening of Chlamydomonas (7). Conversely, the wavelength of this peak decreases in a step-wise manner as chlorophyll is sequentially stripped from isolated photosystem I particles by mild detergent treatment (29).

The photosystem I-type mutants of Table III have similar fluorescence emission spectra, with long wavelength emission at 729–731 nm, and there are small differences in the size of this peak relative to those at F-685 (Fig. 3). In a photosystem I-type mutant of Chlamydomonas in which chlorophyll *a*-protein 1 was completely absent, the long wavelength fluorescence emission peak was missing (6). In contrast, the photosystem I-type *viridis* mutants of barley (Table III) with reduced amounts of chlorophyll *a*-protein 1 (30, R. G. HILLER, B. L. MØLLER & G. HØYER-HANSEN, see following paper) have relative fluorescence yields at long wavelengths as high, or higher than wild-type (Fig. 3). This may indicate that the fluorescence yield of the emitting species in these mutants is increased, perhaps due to impaired energy transfer to P700 (9).

Mutations in four different genes produce photosystem II-deficient *viridis* mutants in barley, and they are characterised by three different types of fluorescence emission spectra (Fig. 9). *Viridis-e* and *-ze* mutants have high emission at 683 nm, a shoulder at 693 nm and a small peak at 723–4 nm. Enhanced fluorescence at 683 nm may be due to disconnection of the light-harvesting chlorophyll *a*/*b*-protein 2 from the photosystem II reaction centres, and lowered fluorescence from photosystem I may be attributable to reduced photosystem II to photosystem I spillover (20). This type of phenomenon may explain why the fluorescence intensities at 693 nm and long wavelengths do not accurately reflect the relative amounts of pigments associated with photosystem II and photosystem I. Low fluorescence at long wavelengths may also be the result of low photosystem I activity, which is known for mutant *vir-e*^{64} (42). On the other hand, low photosystem I activity in mutant *vir-c*^{12} (42) is compatible with high fluorescence at long wavelengths.

Although part of the fluorescence emission peak at 695 nm is thought to originate from photosystem II chlorophyll (36), it has not been possible to isolate a sub-chloroplast fraction containing photosystem II activity with a fluorescence emission maximum at 695 nm, nor to localise this fluorescence to a chlorophyll-protein in a polyacrylamide gel (2, 45). There is, however, a temperature sensitive pea mutant which loses photosystem II activity and the 695 nm fluorescence emission peak when grown at 30 °C (44). This peak can be enhanced in isolated thylakoids or photosystem II preparations by 1,10-phenanthroline (35), which may be a useful method for detecting the presence of the reaction centre of photosystem II in mutant thylakoids.

The *viridis* mutants with apparently unimpaired photosynthetic capacity are characterised by long wavelength fluorescence emission maxima close to wild-type (739 nm) or shifted to shorter wavelengths by up to 3 nm (Figs. 25, 26). The *chlorina-f2* maximum is shifted to 732 nm in vivo and that of *vir-k*^{23} to 720 nm (Table

X). This may be correlated with an absence of chlorophyll *b*-containing proteins in *chlorina-f2* and a low level of chlorophyll, particularly chlorophyll *b*, in *vir-k*[23]. As mentioned above the latter is a leaky chlorophyll biosynthetic mutant blocked at the conversion of Mg-protoporphyrin to protochlorophyllide (21). The emission maximum of chloroplast thylakoids isolated from *chlorina-f2* is lower, at 727 nm (34, 38) or 722 nm (8) and appears to be the result of the isolation procedure and length of storage of the thylakoids after isolation. Similar effects may be responsible for the changes in the relative fluorescence intensities of F-685 and F-693 compared with F-727 in isolated *chlorina-f2* thylakoids, since the fluorescence emission spectrum of wild-type leaves is similar to that obtained from isolated thylakoids (20, 34, 35, 38, 43).

The fluorescence emission spectrum of *vir-s*[44] (Fig. 26) is unique in having a double maximum at long wavelength (at 723 and 732 nm), clearly indicating the existence of several different forms of chlorophyll in photosystem I in vivo. Such *viridis* mutants are potentially useful in investigating chlorophyll organisation in vivo, and in determining the origin and significance of low temperature fluorescence emission bands. The use of fluorescence emission spectra for diagnosing photosynthetic defects in mutants is limited at present by our lack of understanding of the nature of the fluorescence emission bands.

4.3. Ultrastructure

It is not possible to discern a close relationship between chloroplast ultrastructure as visualised in thin sections and photosynthetic defects in barley *viridis* mutants. Certainly, plastids which lack an organised internal lamellar system (Figs. 32 and 33) are low in both chlorophyll content and photosynthetic capacity. Low levels of chlorophyll are usually reflected in fewer thylakoids inside the plastid (see Figs. 5, 29, 30). There is some evidence to suggest that photosystem II mutants are characterised by grana of larger diameter than those of wild-type, and a reduced intrathylakoidal space. But these mutants frequently have a lower than normal chlorophyll content, as well as other deficiencies which may affect plastid ultrastructure. It is not yet possible to predict photosystem I deficiency on the basis of the thin-section chloroplast ultrastructure, since all mutants of this type contained well-developed thylakoids organised into grana and stroma lamellae whose appearance was rather similar to those of wild-type (Figs. 4–7).

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