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Minireview

LIGHT- AND TEMPERATURE-SENSITIVITY OF CHLOROPHYLL-DEFICIENT AND VIRESCENT MUTANTS

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Abstract: Chlorophyll-deficient and virescent mutants are nonlethal and are similar in great redution in chlorophyll concentration, higher chlorophyll a/b ratio, low protein composition on thylakoid membrane, immature development of thylakoid membrane, high frequency of whorl lammellae, great change of chlorophyll-protein complexes and sensitivity to temperature, light intensity and photoperiod. The light- and temperature sensitivity of these two mutants may be the results of the above deficiencies. According to the response to environmental alterations, chlorophyll-deficient mutants can be placed into two groups: one just adjusts the chlorophyll content, whereas the other simultaneously adjust both chlorophyll content and a/b ratio.

INTRODUCTION

The utilization of mutants as research tools in the analysis of metabolic pathway is a well-established approach. The study of mutants deficient in chlorophyll was instrumental in the elucidation of chlorophyll biosynthetic pathway, in observing the structure-function relationship between pigments and polypeptides that associate with the pigments, and in studying the chloroplast biogenesis. There are about 100 separately designed loci which directly or indirectly affect photosynthetic pigmentation in higher plant chloroplast (Somerville, 1986). In this minireview, we briefly compare the light- and temperature-sensitivity of the nonlethal chlorophyll-deficient and virescent mutants in higher plants and predict the possible use of the two mutants in the future.

Chlorophyll-deficient mutants

Chlorophll-deficient mutants are classified into two major phenotypes, one with no detectable chlorophyll b and another with a reduced amount of the pigment. Both mutants are capable of completing their life cycle and their color varies from pale-yellow to yellow green (King, 1991). Mutants deficient in chlorophyll b have been reported in barley, maize, pea, rice, soybean, sweetclover, wheat, *Arabidopsis thaliana*, and *Chlamydomonas reinhardtii* (see ref. of Yang, 1989; Yang et al., 1990 and Bevins et al., 1992). Among these species, the spontaneous chlorina f2 chlorophyll b-lacking mutants of barley which has normal total chlorophyll content, PSI and PSII activities, is the most intensely used for studying photosynthesis. Characteristics identified for chlorophyll b-deficient mutants include reduction in chlorophyll content, higher chlorophyll a/b ratio, immature ultrastructure of thylakoid membrane, marked changes in chlorophyll-protein complexes, and sensitivity to temperature, light intensity, and photoperiod. Higher temperature causes higher level of total chlorophylls in either wild type or some mutants, but causes no change in chlorophyll a/b ratio. However, *ch*10 and *ch*11 mutants of sweetclover, *CD*3 mutant of wheat, *Oy-Yg*

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mutant of maize, and *ch*2 mutantof *Arabidopsis thaliana* exhibit great change in both chlorophyll content and a/b ratio between 17°C and 26°C (Table 1). Therefore, chlorophyll-deficient mutants can be classified into two groups according to their response to the growth temperature (Table 2). The group I mutants, as well as all wild types, response to temperature alterations by only adjusting their chlorophyll content, while group II mutants are able to simultaneously adjust both chlorophyll content and a/b ratio. As far, no examples of higher plants have been found in the nature to adapt temperature alterations by just changing their chlorophyll a/b ratio (Group III) or by changing neither chlorophyll content nor a/b ratio (Group IV). It seems that all wild type plants and some mutants are capable of adapting environmental alterations by only adjusting their chlorophyll concentration, while some mutants should adjust both chlorophyll content and a/b ratio.

In general, the decrease in chlorophyll content of chlorophyll-deficient mutants result in the decrease in the size of photosynthetic unit rather than in the number of photosynthetic units. Chlorohpyll-deficient mutants also exhibit substantially higher rates of photosynthesis per unit of chlorophyll than the wild type at saturating, but not subsaturating, light intensity, due to the reduced photosynthetic unit size (Boardman, 1977). The chlorophyll-deficient mutants do not compensate for their loss of photosynthetic capacity by increasing the number of chloroplasts (Keck et al., 1970). The plastoquinone per unit chlorophyll and the half-time for plas to quinone oxidation are much higher in the chlorophyll-deficient mutants than in the normal plants (Kyle and Zalik, 1982). Most chlorophyll-deficient mutants are prabably not blocked in the biosynthesis of chlorophyll (Nelson, 1967).

Chlorophyll-deficient mutants have been widely used to study the biogenesis of the photosynthetic apparatus, and to demonstrate the stabilizing effect of chlorophyll b on the accumulation of light-harvesting chlorophyll-protein complexes in the thylakoid membrane (Bennett, 1981). In the absence of chlorophyll b, polypeptides that bind this pigment turn over more rapidly than their normal type counterparts, causing these chlorophyll-binding proteins to not accumulate in the mutant thylakoid membranes. It appears that chlorophyll b is a stabilizing helper for effective accumulation of its binding polypeptides, but not involved in the synthesis, transport, processing and insertion of proteins into thylakoid membrane (Bellemare et al., 1982). The stabilizing effect of chlorophyll b appears to differ among various apoproteins in the core components of PSI and PSII. It is still unknown why chlorophyll b produces different stabilizing effect on various apoproteins of pigment-protein complexes; the apoproteins of pigment-protein complexes prabably possess different affinities for chlorophyll a and b. The differential stabilizing effect of chlorophylls may also be affectcd by the affinity between the apoproteins and the lipid bilayer of thylakoid membranes. In higher plants, it is not known whether chlorophyll a or b has a more stabilizing effect on the polypeptides of the pigment-protein complexes, or whether both of them have equal importance. A decrease in abundance of one pigment is always accompanied by a decrease in the other pigment, indicating that the synthesis of chlorophyll a and b may be closely interrelated. The fixed ratio of chlorophyll a and b biosynthesis imply that a regulatory mechanism may exist to control chlorophyll a/b ratio at a fixed number.

Virescent mutants

Virescent mutants which are genetic variants in delay in pigmentation of large-seeded higher plants appear albino, pale yellow or yellow green at seedling stage but eventually grow as normal as wild type, although in mostcase the chlorophyll biosynthetic pathway is unimpaired (King, 1991). The virescent mutants have been reported in alfalfa, barley, bean, bromegrass, cotton, maize, muskmelon, oat, petunia, rice, soybean, tobacco, and wheat (see ref. of Yang, 1989). Characteristics identified for individual virescent mutants include: great reduction in chlorophyll content, higher chlorophyll a/b ratio, low protein composition on thylakoid membrane, immature development of thylakoid membrane, high frequency of whorl lammellae, great change of chlorophyll-protein complexes during

greening, sensitive to temperature, light intensity and photoperiod during chloroplast biogenesis, and deficient in 70S chloroplast ribosomes (Archer and Bonnett, 1987; Hopkins and Elfman, 1984; Lemoine et al., 1987).

Except for the deficiency in 70S chloroplast ribosome, the above phenotypes of virescent mutants are also discovered in the chlorophyll b-deficient mutants of higher plants. However, these characteristics just appear for a short period in virescent plants but are present during the entire life of mutants deficient in chlorophyll b. It is possible that the genetic expression of virescent mutants at their seedling stage is similar to that of chlorophyll b deficient mutants in their whole life. Furthermore, a natural mechanism responsible for initiating the greening of leaf of virescent mutants is lacking or totally inhibited or totally pulsed by certain factors in the mutants deficient in chlorophyll b. The virescent mutants may provide a research tool to explore the biochemical regulation of chloroplast development and thylakoid grana stacking.

Light- and temperature-sensitivity

The effect of light (i.e. light quality, light intensity or photoperiod), temperature and other enviromental factors on the photosynthetic apparatus of normal plants has been widely studied, but relatively less information exists about the effects on mutants. Most normal plants can grow in a range of temperatures and produce leaves with normal pigment content and normal appearance; growth outside the temperature range can result in decreased pigmentation and abnormal leaf appearance (Berry and Bjorkman, 1980).

Chlorophyll-deficient mutants have been found to be sensitive to their growth conditions (Table 1). The biogenesis of thylakoid membranes of a wheat CD3 mutant, deficient in chlorophyll b, is controlled by light intensity. The light-sensitive CD3 mutant is modulated by light intensity to dramatically change its chlorophyll content, the accumulation of chlorophyll b-binding protein complexes, the degree of thylakoid stacking, and the overall thylakoid morphology. When grown at low light intensity, the CD3 mutant reverses all the effects caused by high light intensity, i.e. chlorophyll b and chlorophyll a/b-binding proteins accumulate to a near wild-type level, and thylakoid stacking and its morphology are more nearly wild-type in appearance (Allen et al., 1988). The chlorophyll b-deficient oil yellow-yellow green (Oy-Yg) mutant of maize is also light-sensitive and exhibits differential reductions in the accumulation of the three major chlorophyll b-containing antenna complexes (CP29, CP43 and CP47) and sizable changes in thylakoid morphology under high light conditions (Greene et al., 1988). These characteristics may be based on differential incorporation of chlorophyll b into different complexes to stablize its associated polypeptides.

A chlorophyll b-deficient *ch4* (U395) mutant of sweetclover demonsrates that the expression of chlorophyll in this mutant is a temperature-sensitive process, which may be modulated by photoperiod (Markwell et al., 1986). The chlorophyll accumulation of this temperature- and photoperiod-sensitive *ch4* mutant is different at early and late stages of leaf development (Markwell and Chelgren, 1988). A systematic investigation on chlorophyll-deficient mutants of sweetclover demonstrated that, except for the *ch4* mutant, the parental strain and all mutants accumulated more chlorophyll when grow at 26°C than at 17°C. The *ch5* mutant, lacking chlorophyll b under any growth condition, and the *ch2* mutant showed little temperature dependent phenotypic plasticity, where this was a marked phenomenon in the other mutants. The *ch10* and *ch11* mutants demonstrated extreme temperature sensitivity with regard to the production of chlorophyll b and the chlorophyll b-binding LHCII apoproteins (Table 1). When excised trifoliolates were supplemented with exogeneously supplied δ-aminolevulinic acid, only the *ch4* mutant was markedly impaired in the ability to produce protochlorophyllide. These data, also found in sixteen chlorophyll-deficient mutants of rice (Yang and Chen, unpublished data), indicate that temperature-sensitive phenotypic plasticity is a general phenomenon in chlorophyll-deficient mutants (Table 3) (Yang et al., 1990) and confirm that only a minority of chlorophyll-deficient mutants are impaired in the biosynthesis of chlorophyll (Nelson, 1967).

Table 1. Effect of growth temperature on chlorophyll content and a/b ratio of wild type and chlorophyll-deficient mutants. Data are the mean of triplicate determinations.

		17°C		26°C	
Strains	Genotype	Chl*	a/b ratio	Chl*	a/b ratio
Sweetclover (28 days old)					
	wt	2.28	3.01	3.41	3.17
	ch4	1.15	6.24	0.67	5.89
	ch5(U394)	1.38	∞	1.61	∞
	ch5(U374)	1.17	∞	2.30	∞
	ch5(U395)	0.89	∞	1.34	∞
	ch5(T159)	1.46	∞	1.94	∞
	ch6	0.54	3.72	2.03	3.58
	ch7	0.88	3.25	3.04	3.19
	ch8	0.64	3.29	2.45	3.31
	ch10	0.47	∞	3.55	3.43
	ch11	0.26	∞	3.26	3.19
	ch12	1.23	2.92	1.71	2.98
Wheat (10 days old)					
	wt	1.97	3.24	2.06	3.15
	CD3	0.53	10.34	1.07	5.52
Barley (10 days old)					
	wt	1.66	3.40	1.69	3.28
	chlorina 2	0.71	∞	0.64	∞
Maize (11 days old)					
	wt	2.82	3.12	3.47	3.21
	AB2	2.13	4.48	3.16	3.91
	Oy-Yg	0.37	10.08	0.57	5.22
Soybean (15 days old)					
	wt	2.29	3.17	2.92	3.04
	Y9-Y9	0.68	3.71	2.04	3.16
Arabidopsis (18 days old)					
	wt	1.27	2.98	1.62	3.08
	ch1	1.27	3.08	1.45	2.96
	ch2	0.69	17.42	0.98	22.60

*The unit of chlorophyll concentration is μmole/g of fresh leaf. Chlorophyll concentration is determined by the method of Porra et al. (1989) following extraction of fresh leaf with 80% acetone.

Table 2. Classification of chlorophyll-deficient mutants in higher plants according to their response to temperature change.

Classification	Strains	Chl[1]	a/b ratio[2]
Group I		+	-
	all wild types		
	sweetclover		
	(ch4, ch5, ch6, ch7, ch8, ch12)		
	barley*(chlorina f2)*		
	maize*(AB2)*		
	soybean*(Y9-Y9)*		
	Arabidopsis(ch1)		
Group II		+	+
	sweetclover*(ch10, ch11)*		
	wheat*(CD3)*		
	maize*(Oy-Yg)*		
	Arabidopsis(ch2)		
	Golden-leaves fig(under hight light intensity)		
Group III		-	+
	no examples		
Group IV		-	-
	no examples		

1. +, great change; -, no or very little change.
2. Chlorophyll a/b ratio higher than 30 even show significant difference between two temperatures in table 1 is defined as no change (-).

Table 3. Phenotypic plasticity in chlorophyll-deficient mutants of higher plants (see ref. of Yang, 1989; Yang et al., 1990; Bevins et al., 1992).

	Phenotypic variation caused by*		
Strain	temperature (17°C/26°C)	light intensity	light photoperiod
Sweetclover			
ch4	+	?	+
ch5	+	?	?
ch6	+	?	?
ch7	+	?	?
ch8	+	?	?
ch10	+	?	?
ch11	+	?	?
ch12	+	?	?
wheat			
CD3	+	+	+
barley			
chlorina f2	+	?	?
maize			
AB2+	+	?	
Oy-Yg			
soybean			
Y9-Y9	+	+	?
Arabidopsis			
ch1	+	?	?
ch2	+	?	?

* +, sensitive; -, nonsensitive; ?, still unknown.

Grana stacks are the predominant ultrastructural characteristics of thylakoid membranes in higher plants and green algae. Grana stacking has been proposed to be mediated by the surface charge density of thylakoid membrane (Barber, 1982), which is regulated by LHCII apoprotein phosphorylation and dephosphorylation in the thylakoid membrane (Allen, 1992). If this proposal is true, the chlorophyll b-deficient mutants should exhibit poorly-developed thylakoid grana stacks, like the thylakoid structures of etiolated chloroplasts and the chlorophyll b-lacking mutants should have no grana stackings. However, a chlorophyll b-lacking *ch5* (U374) mutant of sweetclover (Nakatani and Baliga, 1985) and a chlorophyll b-less mutant of barley (Burke et al.,1979) exhibit grana stacking to the same extent as the wild-type and require only a slightly higher than normal concentration of divalent cations to stack in vitro, suggesting that certain component or factor other than LHCII may be involved in the mechanism of grana stacking of thylakoid membranes.

Both *ch10* and *ch11* mutants appear to thrive and produce viable seeds at restrictive temperature. Under these growth conditions, they produce no detectable chlorophyll b and LHCII apoproteins. The biochemical phenotypes of *ch10* and *ch11* are readily reversible between the restrictive and nonrestrictive temperatures. The unique ability of being able to turn the mutant phenotype on and off by changing the growth temperature suggested that the two mutants may be good candidates for solving the following problems in higher plants: (i) the chlorophyll b biosynthetic pathway between protochlorophyllide and chlorophyll b; (ii) the processing of chlorophyll-binding apoproteins and the assembly of chlorophyll-protein complexes; (iii) the coordination of chlorophyll and apoprotein biosynthesis; and (iv) the mechanism of grana stacking and unstacking of thylakoid membranes. In short, the collection of chlorophyll-deficient mutants of sweetclover and rice, both demonstrating unique and plural characteristics to growth temperature, may provide a useful tool for studying many problems pertinent to chloroplast biogenesis.

When Golden-leaves fig (*Ficus microcarpa* cv. Golden-leaves) is grown under PPFD higher than 100 μmole $m^{-2}s^{-1}$ for ten days, green leaves start turn into yellow or yellow-green and the chlorophyll a/b ratio also changes from normal level to more than 4. When grown under the PPFD of 400 μmole $m^{-2}s^{-1}$, chlorophyll content of Golden-leaves fig gradually increases to the same level as normal plant and their chlorophyll a/b ratio dramatically increases to 7 or more and then gradually declines to around 3 as the Golden-leaves fig grows older, while those of normal fig leaves always keep constant. This is the first case that a higher plant possesses leaves with various chlorophyll content and a/b ratio on the same shoot. The phenotypic plasticity of chlorophyll content and a/b ratio of Golden-leaves fig are able to dramatically reverse between restrictive and nonrestrictive light intensity, also suggesting that Golden-leaves fig may be a good tool to study the photobiochemistry and photomorphogenesis of higher plant chloroplast by controlling its growth light intensity (Yang and Chen, 1993; Yang et al., 1994)

Conclusion and prospects

The biosynthetic pathway of chlorophyll a is relatively well-established. However, the biosynthetic pathway of chlorophyll b between protochlorophyllide and chlorophyll b is still uncertain. Chlorophyll-deficient mutants of algae or higher plants have been used to study the structure and function of the photosynthetic apparatus, chloroplast development, and the biosynthetic pathway of chlorophyll a and b with mutants results from the inherent limitations of mutants deficient in chlorophyll, many such mutations should be lethal to the organism. Both chlorophyll-deficient and virescent mutants share many similar biochemical deficiencies. It will be interesting to observe if they also have similar genetic or biochemical mechanism regulating the greening of leaf above the temperature threshold. The systematical investigation of chlorophyll-deficient mutants of sweetclover and rice, and chlorophyll-deficient leaves of Golden leaves fig, all demonstrating unique and plural characteristics by taking advantage of light- or/and temperature-sensitivity, may have great potential for studying many problems pertinent to chloroplast photobiochemistry.

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葉綠素缺失和淡綠色突變種的光線及溫度敏感性

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葉綠素缺失和淡綠色突變種屬非致死性，其具共同性有葉綠素濃度的大幅減低、葉綠 a/b 比值較高、類囊膜上的蛋白質組成較少、不完全的類囊膜發育、輪狀囊膜重疊現象的出現機率較高、葉綠素－蛋白質復合物的大量減少及對溫度、光強度及光週期具敏感性。上述缺失導致兩類突變種對光線及溫度的敏感性。依其對環境改變的反應而言，葉綠素缺失突變種可區分爲兩大類：一爲單純調整葉綠素含量的突變種；另一爲同時改變葉綠素含量及 a/b 比值的突變種。