and Biale (8) and Huffaker and Wallace (4) have shown that citrus fruit will fix CO_2 in the organic acids and this may be an area in which the sweet and sour lemons differ.

Summary

Active mitochondrial preparations were obtained from the peel of young Faris sweet lemons. The high buffer capacity of the grinding solution and the maintenance of a slightly alkaline reaction while grinding the tissues directly in the solution obviated inactivation of the mitochondria usually associated with a low pH.

Citric acid occurred in a lower concentration than malic acid in the Faris sweet lemon. Oxidative phosphorylation was obtained with citrate, α-ketoglutarate, succinate, and malate as substrates. When citrate-1, 5-C^{14} was used as a substrate, labeled α-ketoglutarate was found after 60 minutes and labeled malate after 120 minutes. When succinate-1, 4-C^{14} was used as a substrate, malate and fumarate were found after 60 minutes and also citrate after 120 minutes. When pyruvate-3-C^{14} was used as a substrate together with a sparker acid, citrate, α-ketoglutarate, and malate were found after 120 minutes. These findings, together with the inhibitory effects of cyanide and malonate, indicated the citric acid cycle was operative in this variety of lemon.

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Effect of Light Quality, Light Intensity and Temperature on Pigment Accumulation in Barley Seedlings [1, 2]

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Introduction

The first action spectrum for chlorophyll accumulation was obtained by Schmidt (Smith and Young, 19) using etiolated corn seedlings. His results indicated 3 peaks of effectiveness at 640, 567, and 450 mμ. Since the early work of Schmidt there have been a number of reports concerning the effect of wavelength on chlorophyll accumulation and on the conversion of protochlorophyll to chlorophyll. Differences in the action spectra for these 2 phenomena (19) indicate that they should be considered independently, and only the former is pertinent to the work reported in this paper.

Several reports indicate that the red region is most effective in chlorophyll accumulation (12, 13, 19), while others report the blue to be most effective (1, 5). The same disparity exists for carotenoid accumulation (14, 18, 19). In addition Kakhnovitch (7) points out that wavelength has no differential effect on accumulation if the incident energy is greater than 20,000 ergs/cm.^{2} per second.

The initial production of chlorophyll in etiolated plants is directly proportional to light intensity at relatively low incident energy (19). This relationship is not extended over long time studies or at high intensity illumination (16, 19). In long-term experiments the determination of the most effective in-

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tensity is complicated by differences in the accumulation of photosynthate (21) or by differences in leaf expansion caused by different intensities. Thus, Sargent (15) found that *Chlorella* grown at high light intensity contained one-half the chlorophyll per cell of those grown at one-seventh the intensity, but that a culture as a whole would produce 2.5 times as much chlorophyll at the higher than at the lower intensity.

Shirley (17), using a number of species grown under 74% to 1% of the intensity of sunlight, noted an increase in chlorophyll content per unit weight of leaf through the intensity range 74% to 8%. One percent appeared to be below the optimum intensity.

Using Marquis wheat Friend (6) observed an increase in chlorophyll content in the first 3 leaves through the range 200 to 2500 ft-c provided the temperature was 20 to 30°. This pattern was obtained whether the chlorophyll content was calculated per leaf or as a percentage of the fresh leaf weight. At lower temperatures maximum accumulation took place at intermediate intensities. There was, however, a consistent increase in chlorophyll content at all temperatures when the intensities were increased from 200 to 1000 ft-c. The optimum conditions for chlorophyll accumulation on the basis of leaf weight were at 2500 ft-c and 30°, although little difference existed in the range 20 to 30° at 2500 ft-c.

Sachs (Smith and Young, 19) first reported that a threshold temperature exists for chlorophyll formation and that the rate of greening increases with temperature. Virgin (22) obtained similar results for protochlorophyll production in etiolated barley seedlings over the temperature range 0 to 30°. Lubimenko and Hubbenet (10) found the optimum temperature for chlorophyll accumulation per g fresh leaf weight of etiolated wheat seedlings to be about 26° for long-time exposures. Friend's data (6), however, indicated an optimum of at least 30°. Also, he obtained a significant interaction for light × temperature on chlorophyll accumulation.

Kakhnovich (8) has reported a variation of 2.5 to 7 in the ratio of chlorophyll a to b in cucumber leaves due to varying light intensities, while Smith and Young reported (19) that variation in temperature had no effect on this ratio in barley seedlings.

The purpose of the present study was to determine the effect of light quality, light intensity, and temperature on the pigment accumulation in normal Gateway barley and a chlorotic mutant of this variety.

Materials and Methods

The characteristics of the cabinets and filters used for the light quality experiments have been reported earlier (4, 23). The intensity of illumination in all light quality treatments was adjusted to 2900 ergs cm^2 per second using a pyroheliometer (4).

Two of the cabinets were modified for use in the temperature and light intensity studies. The cabinets were divided vertically into 2 compartments. Four intensities of 320, 1020, 1800 and 2600 ft-c were obtained by varying the distance between the shelves and the light source (300 w incandescent lamps). Baffles were arranged in the cabinets so that the plants were essentially illuminated only from the top. The intensities were measured with a Weston light meter approximately 3 cm above the soil surface. To a refrigerated air supply, sufficient hot air was added to give mean temperatures of 14.0, 19.5, 24.5 and 29.0°. These temperatures, based upon the temperature of water in stoppered erlenmeyers placed on each shelf, were read 3 times each day.

The virescent barley mutant used in this study has been described earlier (11). The parent variety, Gateway, was used for comparison. Seeds were spread on trays of vermiculite, watered, covered with absorbent tissue, and placed in a germinating cabinet in the dark at 20°. After 24 hours they were selected for uniformity of development and transferred to pots containing California mix. After an additional 2 days at 20° in darkness they were placed in the various treatments. The timing of this transfer was such that most of the coleoptiles had broken the soil surface. Continued selection was carried out during the experiment to eliminate very early and late emerging seedlings.

In the light quality experiments the seedlings were allowed to develop for 5 cycles of 8 hours light and 16 hours darkness at 20°. In the light intensity and temperature studies the seedlings were given 4 cycles of 16 hours light and 8 hours darkness. The seedlings were always harvested at the end of the dark period. Exposure to light during harvesting was such that protochlorophyll synthesized during the last dark period would be converted to chlorophyll. Three to 10 primary leaves constituted a sample. Three samples were taken for each replication in the light quality studies, and 4 samples in the temperature and light intensity studies. The studies were replicated after a time lapse. The leaves were cut into approximately 3 cm lengths, placed into glass vials and freeze-dried for 3 days. The leaf tissue was then stored over P_2O_5 at −28° for a minimum of 3 days before analysis.

The dry leaf material was weighed and then ground with sand using a mortar and pestle. Pigments were extracted with 80% acetone and the absorbency of the extract read at 633, 645 and 440 mμ in a Beckman DK-1 spectrophotometer. The content of chlorophylls a and b and carotenoids were calculated using the equations given by Maclachlan and Zalik (11).

Results and Discussion

Light Quality. The differences between the normal and mutant seedlings in accumulation of chlorophyll were highly significant. Normal seedlings

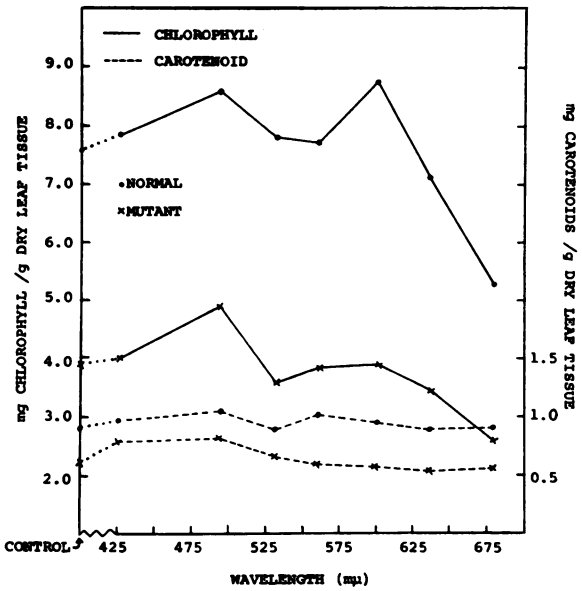

FIG. 1. Effect of light quality on the pigment content of normal and mutant barley seedlings. The 50 % transmittance levels are given in table I for the filters which are represented as points on this graph. Each point on the graph represents the mean of 6 readings.

grown in the green and red treatments did not differ in chlorophyll accumulation, but had significantly more chlorophyll than the other treatments. In the mutant there was significantly more chlorophyll accumulation in the green, and in both lines markedly less chlorophyll in red-far-red than in any other treatment (table I, fig 1). That there was a differential response of the normal and mutant lines to light of different quality was borne out by a highly significant interaction of lines × light quality in the analysis of variance. The calculation of chlorophyll content as a percent of the control (white light) for each line indicated that the major difference in response occurred in the green, yellow and red treatments. Under green light the mutant was relatively more efficient than the normal, whereas under yellow and red the reverse was true.

There was little difference in the ratios of chlorophyll a to b which for the normal ranged from 2.4 in the red to 2.8 in the green, and for the mutant ranged from 2.4 in the red-far-red to 2.9 in the blue.

In all treatments the normal seedlings accumulated significantly more carotenoids than the mutant. Under both blue and green light the mutant accumulated significantly more carotenoids than under the other treatments. In the normal, however, there was no significant difference in carotenoid concentration between the blue, green, orange, and red treatments.

Light Intensity and Temperature. The data (table II) indicates that the same conditions of light intensity and temperature used in this study were optimal for both the mutant and normal line. These conditions, on the basis of chlorophyll per unit dry weight, were 24.5° and 1020 ft-c, and on the basis of chlorophyll per cm leaf length, 24.5° and 1800

Table 1. *Pigment Concentrations of Normal and Mutant Barley Seedlings Exposed to 5 Cycles of Light of Several Spectral Bands*

Numbers in each column that are not followed by the same letter are significantly different from each other at the 5 % level of significance as judged by Duncan's Multiple Range Test(3).

At 50 % transmission (mμ)	Color designation	Avg leaf wt (mg)		mg Chlorophyll /g dry leaf		Chlorophyll as % of control		mg Carotenoids /g dry leaf	
		N	M	N	M	N	M	N	M
393–463	Blue	8.3	8.0	7.8b	4.0b	103	103	0.97abc	0.80a
406–532	Green	8.2	7.6	8.6a	4.9a	113	125	1.04a	0.82a
500–577	Yellow	8.3	7.4	7.8b	3.6b	102	92	0.88c	0.66b
540–605	Orange	8.5	7.7	7.7bc	3.8b	101	98	1.01ab	0.59bc
580–655	Red	8.4	7.8	8.8a	3.9b	115	100	0.95abc	0.58bc
610–705	Deep-Red	8.5	7.7	7.1c	3.5b	93	89	0.89c	0.53c
643–738	Red-Far-red	8.7	7.5	5.3d	2.6c	69	66	0.90bc	0.57bc
346–890	White***	8.7	7.3	7.6bc	3.9b	100	100	0.92bc	0.61bc

Analysis of Variance for Pigment Concentrations

Source	df	Chlorophyll ms	Chlorophyll f	Carotenoid ms	Carotenoid f
Lines	1	351.67	313.99**	2.193	95.35**
Light quality	7	8.25	7.37**	.067	2.91
Lines × light quality	7	1.12	4.31**	.023	2.55*
Error	78	.26		.009	

* Significant at 5 % level.
** Significant at 1 % level.
*** Control.

Table II. *Influence of Light Intensity and Temperature on Chlorophyll Accumulation in Normal (N) and a Mutant (M) of Gateway Barley*

Light Intensity	μg Chlorophyll/cm leaf length: Normal, Temperature 14.0	19.5	24.5	29.0	Mutant, Temperature 14.0	19.5	24.5	29.0
320	9.4	10.2	11.0	9.7	2.3	4.3	5.2	5.0
1020	10.1	10.7	13.1	10.6	2.0	5.2	6.4	5.6
1800	9.3	9.5	13.4	8.9	1.4	3.7	6.8	5.7
2500	11.4	9.9	11.4	8.8	2.7	4.5	5.8	5.7

Chlorophyll per cm leaf in M / Chlorophyll per cm leaf in N × 100, Temperature 14.0	19.5	24.5	29.0
24	42	47	51
20	49	49	53
15	39	51	64
25	46	51	65

mg Chlorophyll/g dry leaf tissue								
320	10.2	12.5	12.6	11.8	2.5	5.4	7.0	6.4
1020	10.0	11.9	14.2	11.6	2.2	6.3	7.8	6.7
1800	8.1	8.6	10.8	10.0	1.7	4.2	7.1	6.5
2600	9.1	9.0	9.3	9.0	3.1	5.2	6.4	6.7

Analysis of variance††

Source	df	ms	f
Lines (L)	1	1742.85	157.65*†
Temperature (T)	3	144.80	21.58*†
Light Intensity (I)	3	50.49	7.56*†
L × T	3	26.33	27.80*
L × I	3	26.10	27.56*
T × I	9	7.04	7.43*
L × T × 2	9	0.947	2.53*
Error	214	0.374	

* Significant at the 1% level.
† Tested against a composite error.
†† Analysis carried out on chlorophyll/g dry leaf tissue.

ft-c. However, the relative efficiency of the 2 lines differs considerably under other conditions, as shown in table II. It is evident that the mutant was more strongly affected than the normal by low temperatures at all intensities. This differential effect of temperature on the 2 lines was confirmed by the highly significant interaction of lines × temperatures in the analysis of variance.

Figure 2 presents the data on chlorophyll concentration under different light intensities at the temperatures used. Under all conditions there was significantly more chlorophyll in the normal than in the mutant line. Under 2600 ft-c, temperature had little influence on chlorophyll accumulation in the normal barley seedlings. Also, it is evident that at 24.5° and 29.0° light intensity had a stronger effect on the normal than it did on the mutant. The highly significant F value for the interaction of lines × light intensities confirmed this.

The rather high chlorophyll concentration obtained in both lines under high light intensity at low temperature may have been caused by an increase in the leaf temperature above the recorded temperature. It is generally recognized that leaves of plants may, under high light intensity, attain a temperature somewhat higher than that of the surrounding air (2). This may have occurred in spite of the 10-cm water filter. Results at lower intensities, especially for the mutant, show that a small increase in temperature would result in a sharp increase in chlorophyll content. The Q_{10} values for the mutant under the 3 lower intensities ranged from 3.1 to 4.4 over the temperature range 14.0 to 19.5° (fig 2).

Disregarding the high intensity result the trend at 14.0° was toward greater efficiency in the mutant relative to the normal as light intensity decreased. At 24.5° there was little effect of light intensity, whereas at 29.0° the low temperture trend was reversed so that the relative efficiency increased with increased light intensity. This shift in the production of chlorophyll in the mutant relative to the normal was significant as shown by the triple interaction in the analysis of variance.

Little difference existed in the ratio of chlorophyll a to b. The ratios for both lines varied from 2.4 to 2.9 in all cases except for the mutant at low temperature, where the ratio reached a maximum of 3.9.

Conclusions

It is generally recognized that high light intensity destroys chlorophyll in plants. If the expression of the mutation was due to the production of a more light sensitive form of chlorophyll holochrome in the mutant than in the normal, it would be expected that high light intensity would have a greater deleterious effect on the mutant than on the normal regardless of the temperature. As shown in figure 2, this is not the case; at high temperature light intensity had a stronger effect on the normal than on the mutant. It appears, therefore, that the mutation has not resulted in an increased rate of chlorophyll destruction by light.

The Q_{10} values for chlorophyll accumulation in the mutant at low temperature indicate that a highly

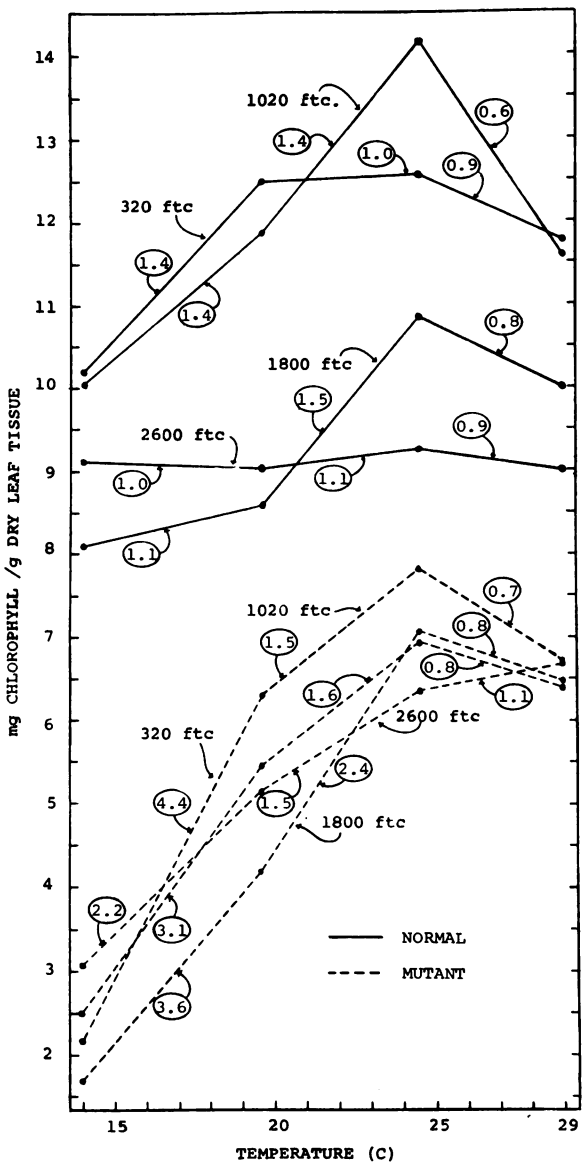

FIG. 2. Effect of light intensity and temperature on the chlorophyll content of normal and mutant barley seedlings. Each point on the graph represents the mean of 8 readings. The approximate Q_{10} values are encircled.

temperature sensitive reaction is involved in the mutation. According to Klein (9) grana formation has a large temperature coefficient.

Since the effect of light of different wavelengths must be interpreted as a difference in the qualitative utilization of light by the mutant and the normal, this effect may be interpreted as being the result of a change in the physical structure of the grana. Furthermore since the same pigments are present in the mutant as in the normal (11), the change in the structure of the grana must be due to an alternation in a moiety other than the pigment itself.

Previous results (11) have shown that photoconversion of protochlorophyll to chlorophyll a is rapid and complete in both lines, but that protochlorophyll formation is slower in the mutant than in the normal. From the biosynthetic pathway (20) it may only be deduced that it is the formation of protochlorophyllide holochrome that is affected in the mutant, since the actual existence of true protochlorophyll in leaves is questionable. Thus the slower rate of pigment synthesis could be related to the production of any one of the moieties, or to the assembling of the moieties to form the protochlorophyllide holochrome.

Considering the above results it is suggested that: A) the mutation has caused a change in the physical structure of some portion of the chlorophyll holochrome other than the chlorophyll moiety itself, and B) either a reaction leading to the formation of the altered holochrome, or a reaction incorporating the altered holochrome into the grana has a much higher temperature coefficient in the mutant than in the normal.

Summary

The effect of light quality, temperature and light intensity on pigment accumulation in normal and a mutant of Gateway barley was investigated. All 3 environmental factors had a significantly different effect on the 2 barley lines.

Little difference existed between the chlorophyll a to b ratios in either barley line under any of the treatments except in the mutant at low temperature. There was no difference in the efficiency of chlorophyll accumulation by the normal under red and green light. The mutant, however, accumulated most chlorophyll under green light. The optimum condition of temperature and light intensity for both lines was 24.5° and 1020 ft-c. The mutant was very sensitive to low temperature. At high temperature the mutant was almost insensitive to light intensity.

The results support the hypothesis that the mutation has resulted either in a reduced rate of synthesis of a moiety of the holochrome other than the chlorophyll, or a reduced rate of assembly of the moieties into grana.

Acknowledgment

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