BOT. GAZ. 139(2):180–189. 1978.

PHYSIOLOGICAL CHANGES DURING THE GERMINATION OF CATTLEYA AURANTIACA (ORCHIDACEAE)[1]

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Seeds of *Cattleya aurantiaca* grown asymbiotically on a medium containing sucrose as the sole source of organic carbon developed into leaf-bearing plants. Those sown on the same medium without sugar reached the protocorm stage but did not differentiate further. Cross transfers of seedlings between the two media indicated that following 21–30 days of growth on the sucrose-containing medium, subsequent development may proceed in the absence of sugar. Levels of chlorophyll and ribulose-1,5-diphosphate carboxylase were monitored and related to the ability of young seedlings to photosynthesize. A direct relationship between chlorophyll content and photosynthesis was not found. Increases in the activity of the enzyme were closely paralleled by increases in photosynthesis. A direct relationship was not found between chlorophyll *a*/*b* ratios and photosynthetic capacity. Germinating seeds and seedlings grown in the absence of exogenous carbohydrate were unable to convert appreciable amounts of labeled acetate to sugar.

Introduction

The orchid embryo is relatively undifferentiated when mature with neither endosperm nor cotyledon present. Food reserves are stored in the embryo proper. Consequently, in the germinating orchid seed a rigid distinction between areas of catabolic and anabolic activities cannot be made at the morphological level. During germination the small embryo swells, eventually breaking out of the seed coat. The embryo enlarges further to form a top- or cone-shaped seedling, termed a protocorm (BERNARD 1909). Under natural conditions, the seedling remains at this developmental stage until infected in its basal half by an appropriate endophytic fungus (BURGEFF 1932, 1936). After infection the first leaves are formed at the apex of the protocorm, followed later by roots. Although the infection may persist in the roots of the mature plant, it is not certain what benefits, if any, this mycorrhizal relationship provides. In protocorms, however, the presence of the fungus is clearly needed for further growth under natural conditions (BERNARD 1899; BURGEFF 1932). When grown aseptically, developing seedlings must be supplied with a suitable carbohydrate source in order to advance beyond the protocorm stage.

Many investigations have been conducted with the intent of defining the factor(s) provided by the fungi to initiate the phase change from a protocorm to a leaf-bearing seedling (for review, see ARDITTI 1967). Comparative studies have found that the germination requirements within the family vary. It is clear, however, that there is a general requirement for an exogenous source of suitable carbohydrate.

In the present study, a number of physiological changes occurring during germination and growth are examined in orchids as they progress from seeds to leaf-bearing plants.

Material and methods

SEEDLING CULTURE.—Seeds of self-pollinated *Cattleya aurantiaca* (Batem. ex Lindl.) P. N. Don (Orchidaceae) were collected from dehisced capsules, air-dried 8 h at room temperature, and stored over calcium chloride at 5 C. All seeds were used within 6 mo of collection.

One group of seeds was germinated and grown on culture medium (KNUDSON 1946) modified by the inclusion of microelements and the substitution of potassium phosphate buffer, pH 5.3, for potassium phosphate (ARDITTI 1967; HARRISON and ARDITTI 1970). A second group was cultured on KC-SUC. All seedlings were grown aseptically on solidified medium (HARRISON and ARDITTI 1970). Seeded flasks were maintained at room temperature under 16-h photoperiods. Light was supplied by two 40-W Gro-Lux lamps 45 cm above the cultures. Seedlings were transferred onto fresh medium every 2 mo.

GROWTH INDEX.—A growth index (CURTIS 1947; SPOERL 1948; MARIAT 1952; ARDITTI 1967, 1968) was used to follow seedling development quantitatively. Ungerminated seeds were assigned a class value of 1; swollen seeds, 2; protocorms, 3; seedlings with a single leaf, 4; those with two leaves, 5; and plantlets with at least one root, 6. To calculate the growth index, a total of 250 seedlings were observed for each flask. The frequency of each size class was determined as a percentage of the total number of plants

[1] Abbreviations: DW = dry weight; FW = fresh weight; GSH = glutathione; KC = Knudson C medium (KNUDSON 1946); KCF = Knudson C medium in which the carbohydrate is fructose instead of the usual sucrose; KCG = Knudson C medium with glucose instead of sucrose; KC-SUC = sugar (sucrose)-free KC; RuDP = ribulose diphosphate; RuDP carboxylase = ribulose diphosphate carboxylase.

[2] A portion of this work was submitted by C. HARRISON in partial fulfillment of the requirement for the Ph.D. degree in biological science, University of California, Irvine. Please address reprint requests to J. ARDITTI.

Manuscript received December 1976; revised manuscript received June 1977.

counted. These percentages were then multiplied by the value of their respective size classes and summed. Multiplication of the total by 100 provided the growth index of each flask. Averages of 10 replicate flasks were used in plotting growth indices against age.

WEIGHT DETERMINATIONS.—Culture flasks were flooded with water, and loosened seedlings were poured onto a filter-paper disk in a Buchner funnel under suction. We determined FW as soon as the plantlets separated freely from each other and the filter disk. The DW was recorded after drying for 24 h at 80 C.

Since many of the experiments required the use of homogenates or samples of very small size, it was necessary at times to calculate DW from a curve of FW versus DW.

EFFECTS OF LIMITED EXPOSURE TO SUCROSE.—Seeds or seedlings from KC were transferred onto KC-SUC at selected ages (3, 5, 9, 14, 21, 30, 39, 41, 47, 55, and 60 days). The further development of seedlings in the absence of a carbohydrate source was noted, and the percentage of protocorms that eventually produced plantlets with one or more leaves was recorded.

In a similar experiment, seeds were first sown on KC-SUC. After 15, 30, or 60 days, the swollen seeds and protocorms were transferred to KC. Then, at intervals (2, 8, 12, 18, 25, 42, and 55 days) the plants were returned to KC-SUC and the percentage that eventually formed one or more leaves was recorded.

GROWTH IN DARKNESS.—Five flasks containing KC and another five with KC-SUC were seeded. All were placed in the dark and observed once only after 90 days.

CHLOROPHYLL DETERMINATIONS.—Chlorophyll was extracted from whole seedlings and leaf sections (BRUINSMA 1963). The extracts were filtered through a Millipore filter (pore size 0.22 μm) to remove residual particles (presumably lipid bodies) that could not be removed by low-speed centrifugation. Chlorophyll *a*, chlorophyll *b*, and total pigment were determined by standard procedures (MACKINNEY 1941; ARNON 1949).

PROTEIN DETERMINATIONS.—Aliquots of filtered extracts were assayed for soluble protein (LOWRY 1951). A final concentration of 5% cold trichloroacetic acid was used for precipitation and 0.1 M sodium hydroxide for resuspension. A standard curve was constructed using egg albumin. Colorimetric measurements at 525 nm were made with a Bausch and Lomb Spectronic 70.

CARBOXYLASE ASSAY.—Preweighed groups of whole seedlings or leaf sections (ca. 200 mg FW) were homogenized in 5 ml of 40 mM Tris-HCl, pH 7.8, containing 10 mM $MgCl_2$, 0.15 mM EDTA, and 5 mM reduced GSH. The homogenate was centrifuged 20 min at 30,000 *g*, and the supernatant was assayed for enzyme activity without further purification. All preparatory steps were performed at 4 C (this is a modification of the assay of BJÖRKMAN [1968]).

Each assay was begun within 40 min of the start of homogenization. Crude enzyme extracts were warmed to 37 C for 5 min just prior to the assay, and 0.2 ml was added to 0.3 ml of a freshly prepared solution containing 2.5 μmol $NaH^{14}CO_3$ (sp act 0.5), 0.15 μmol Tris-HCl (pH 7.8), and 2.5 μmol $MgCl_2$. After 15 min at 37 C, the reaction was ended by adding 0.1 ml of 6 M acetic acid. Aliquots were placed into scintillation vials, oven dried 2 h at 90 C, and resuspended in 0.5 ml water. Ten milliliters of fluor (BRAY 1960) were added to each vial. Radioactivity was determined using a Tracerlab Model 401 DSF liquid scintillation counter. Three replicates and one blank (RuDP omitted) were used for each group of plants examined.

LIGHT AND DARK FIXATION OF CARBON DIOXIDE.—Seedlings grown on KC or KC-SUC were tested for their ability to fix $^{14}CO_2$ in the light or dark. Standard scintillation vials served as reaction vessels, each containing 2.0 ml of solidified KC or KC-SUC and a smaller (1 × 3 cm) vial which held 0.2 ml of an aqueous solution containing 0.27 μmol $NaH^{14}CO_3$ (sp act 2.21). Residual volume of the outer vial (including the unused volume of the smaller vial) was approximately 20 ml.

Immediately before the start of each test, groups of seedlings (ca. 20–50 mg FW) were transferred to reaction vessels containing the same medium as the one on which they had been growing. Inoculated vials (three replicates per test) were then flushed with nitrogen, sealed quickly with Parafilm, and incubated 15 min in a 25-C water bath under light or dark conditions. Aluminum foil was used to shield the dark vials. Incandescent light was provided at 8,700 $\mu W/cm^2$.

Each reaction was started by injecting 0.5 ml of 6M HCl with a syringe through the Parafilm cover into the smaller vessel, releasing $^{14}CO_2$. Lanolin was quickly applied to seal the puncture. After 15 min the seedlings were killed by placing them in 5 ml boiling 80% ethanol. They were homogenized 1 min with a Potter Elvehjem tissue grinder, and a 1.0-ml aliquot was used for radioactivity determination (see Carboxylase assay).

FATE OF LABELED ACETATE.—Seedlings (ca. 100 mg FW) were incubated with 25 μmol of potassium phosphate, pH 5.0, and 0.25 μmol acetate-2-^{14}C (sp act 39.0) in a total volume of 1.0 ml.

Incubations were carried out in plastic petri dishes with 3-cm-diameter center wells. Medium was pipetted into the center well and covered with a 2.5-cm-diameter Millipore filter disk. Disks were not immersed in the solution, however, but rested on a rim running around the inner wall of the center well. The underside of each disk came into contact with the solution, thus providing a moist platform onto which

the plants were placed. One milliliter of hyamine hydroxide (Rohm and Haas) was pipetted into the outer ring of the dish to absorb respired carbon dioxide. Dish halves were sealed with lanolin, and assembled units were placed in the dark at 25 C for the duration of each experiment.

Each dish was opened once only after 4, 8, 12, or 24 h. Plants were collected on a filter disk, rapidly rinsed with 50 ml water, and placed in 5 ml boiling 80% ethanol. The tissue was then successively extracted for 10-min periods on a steam bath with 5 ml of 80% ethanol, 5 ml of 20% ethanol, 5 ml of water, and again with 5 ml of 80% ethanol. Extracts were filtered, combined, and dried at 40 C under vacuum.

The dry residues were extracted with diethyl ether and then water to yield lipid- and water-soluble fractions, respectively. The water-soluble material was further partitioned into acidic (mostly organic acids), basic (mostly amino acids), and neutral (mostly sugars) fractions by the use of ion-exchange resins (CANVIN and BEEVERS 1961). Eluates were dried at 40 C under vacuum and brought to volume (5 ml) with water. A 1.0-ml aliquot from each sample was added to 10 ml of fluor (BRAY 1960) and assayed for radioactivity. An internal standard was used to determine quenching. Results are presented as the averages of three replicates.

EFFECTS OF SELECTED CARBOHYDRATES ON SEEDLING GROWTH.—Instead of sucrose, KC was prepared with glucose (KCG) or fructose (KCF). In each instance the substitute carbohydrate was supplied at the same molarity as sucrose in KC. Germination and growth on these media were compared with that on KC.

Results

GERMINATION AND DEVELOPMENT IN CULTURE.—Newly flasked seeds were noticeably swollen (growth index 200) after 12 days on KC-SUC (fig. 1). Greening commenced at 15–20 days, and at approximately 30 days the protocorm stage (growth index 300) was reached. In general, no leaves or roots were ever produced, and the seedlings remained at the protocorm stage until they became senescent.

Seeds sown on KC were swollen at 8 days; they reached the protocorm stage and began greening by day 15. The first leaf usually appeared between 40 and 47 days and the second after 60–90 days (fig. 1).

GROWTH IN DARKNESS.—Seeds sown on KC-SUC and held in the dark germinated but did not develop beyond the protocorm stage. In contrast, 73% of dark-grown seeds flasked on KC had produced their first leaf by 90 days. However, no roots or additional leaves were formed, and all seedlings were completely white.

EFFECTS OF LIMITED EXPOSURE TO SUCROSE.—It was not essential to keep developing seedlings constantly on KC. Seedlings with one or more leaves continued to develop when transferred to KC-SUC. After 28–30 days on KC, 50% of the protocorms transferred to KC-SUC produced leaves and developed into larger plantlets (fig. 2). The remaining half failed to develop further. When swollen seeds and protocorms were transferred onto KC-SUC after 21 days on KC, only 13% formed plantlets with leaves. After 47 days on KC, 92% of transferred protocorms continued their development. In general, the percentage of transferred protocorms that formed leaves was directly proportional to the length of time they were grown on KC (fig. 2).

Seeds germinated and grown for 15, 30, or 60 days on KC-SUC required 21–30 days of growth on KC before 50% would form plantlets upon their return to KC-SUC (fig. 3).

CHLOROPHYLL.—Seedlings grown on KC had detectable chlorophyll after 15 days. At that time the levels of chlorophyll *a* and chlorophyll *b* were nearly equal (fig. 4). The levels of the two pigments were slightly higher but still equal at 45 days. After 45 days, however, the concentration of chlorophyll *a* increased and reached a maximum by 180 days. Chlorophyll *b* concentration remained unchanged. The increasing chlorophyll *a* to chlorophyll *b* ratio (fig. 5) reflects an increase in the concentration of the *a* component alone.

Chlorophyll content in seedlings cultured on KC-SUC was measurable at day 25. Again, the levels of chlorophylls *a* and *b* were equal. Unlike seedlings sown on KC, however, there was no subsequent increase in the concentration of chlorophyll *a* in seedlings grown on KC-SUC. Indeed, the levels of both chlorophylls remained constant throughout day 150. No determinations were recorded for KC-SUC seedlings after 150 days as yellowing occurred and pigment concentrations fell below detectable limits. The steady 1.0 ratio of chlorophyll *a* to chlorophyll *b* (fig. 5) represents equal and constant amounts of the pigments in the KC-SUC protocorms.

CHANGES IN SOLUBLE PROTEIN LEVELS.—Ungerminated seeds contained 25 mg soluble protein per gram DW. During the first 5 days of germination on either KC or KC-SUC medium, there was a sharp decrease in measurable protein in 5–10 mg/g DW. After this initial drop, protein concentration began to rise.

In seedlings grown on KC-SUC, the soluble protein concentration reached a maximum of 48 mg/g DW at day 60. This level remained unchanged through day 100 and was followed by a gradual decline in senescing protocorms (fig. 6).

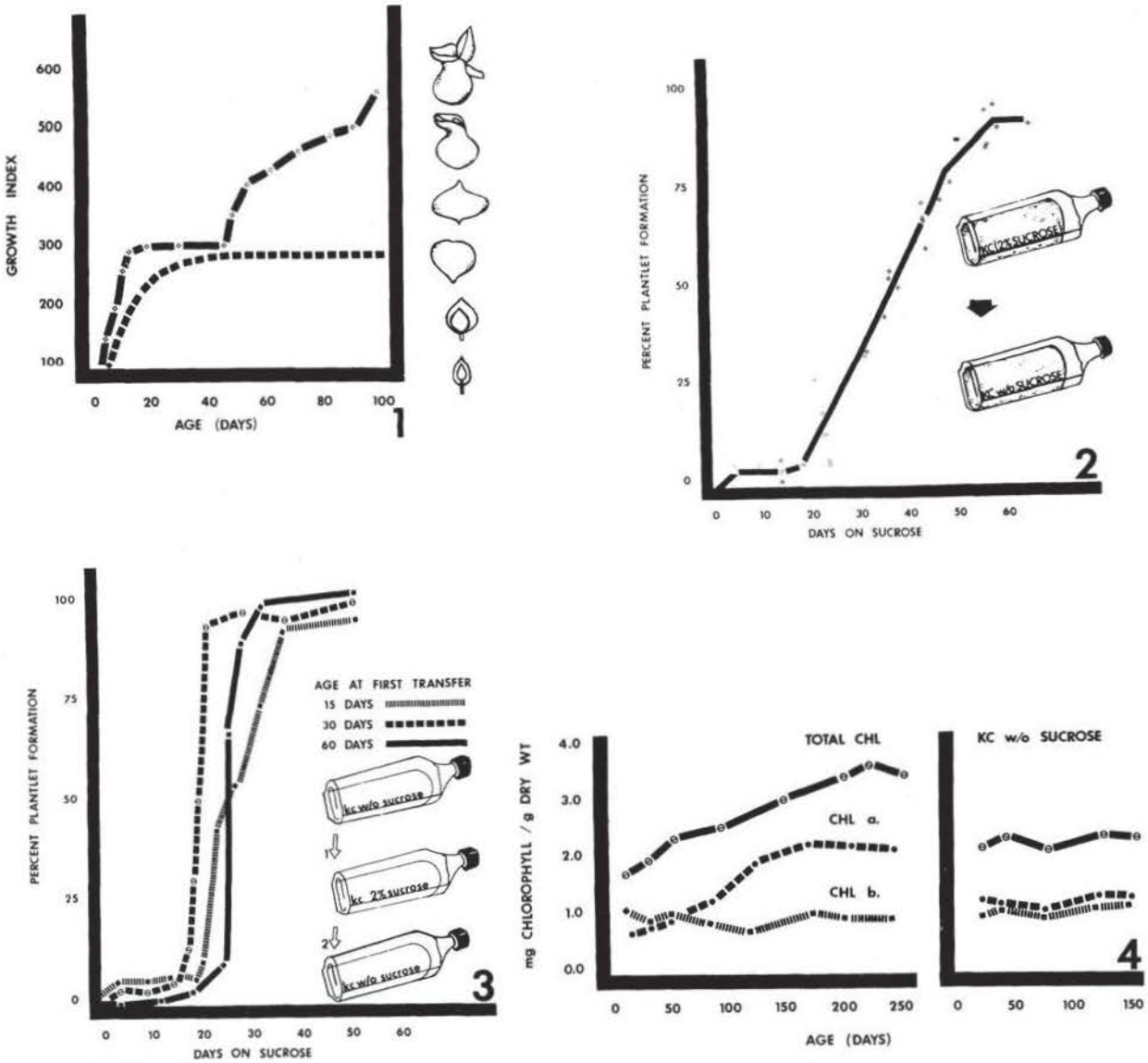
The soluble protein concentrations in seedlings raised on KC were considerably higher than those of seedlings grown on KC-SUC (fig. 6). A plateau was not reached by day 230 in KC seedlings.

RuDP CARBOXYLASE.—Activity of RuDP carboxylase increased rapidly between the ages of 20 and 60 days in seedlings grown on KC (fig. 7). Specific activity of the enzyme (units of enzyme ac-

tivity/mg protein) also rose sharply during this period and was highest at day 35 (fig. 8). After day 60 the activity level of the enzyme remained constant. On a DW basis it equaled the activity in leaves of mature plants. The specific activity of the enzyme declined somewhat after day 60 but at day 140 was still 75% of the maximum value recorded at day 35.

Enzyme activity rose slowly through day 60 in seedlings grown on KC-SUC and increased only slightly thereafter (fig. 7). In this instance, the final activity of the enzyme was only about 25% of that found in plants grown on KC. The specific activity of the enzyme was also considerably lower in seedlings flasked on KC-SUC (fig. 8). There was a more gradual increase of the specific activity through day 60, followed by a leveling off. At day 140, the specific activity in KC-SUC seedlings was only 35% of that found in those grown on KC (fig. 8).

LIGHT AND DARK FIXATION OF CARBON DIOXIDE.—Seedlings grown on KC exhibited little or no capacity for CO_2 fixation at day 10 (fig. 9). At day 15, however, there was a low but appreciable level of fixation in the light that persisted through day 30. Dark fixation was still negligible. A large, 10-fold increase in the level of light incorporation was recorded at day 40, accompanied by a corresponding increase in



FIGS. 1–4.—Fig. 1, Growth index of seedlings raised on KC with (solid line) and without sucrose (broken line). Fig. 2, Percentage of protocorms forming plantlets as a function of the length of time grown on KC before transfer to medium without sucrose (drawing denotes transfer sequence). Fig. 3, Percentage of protocorms forming plantlets after an initial period on KC-SUC, transfer to complete KC, and return to KC-SUC. Fig. 4, Levels of chlorophylls *a* and *b* and total pigment in seedlings grown on KC with and without sucrose.

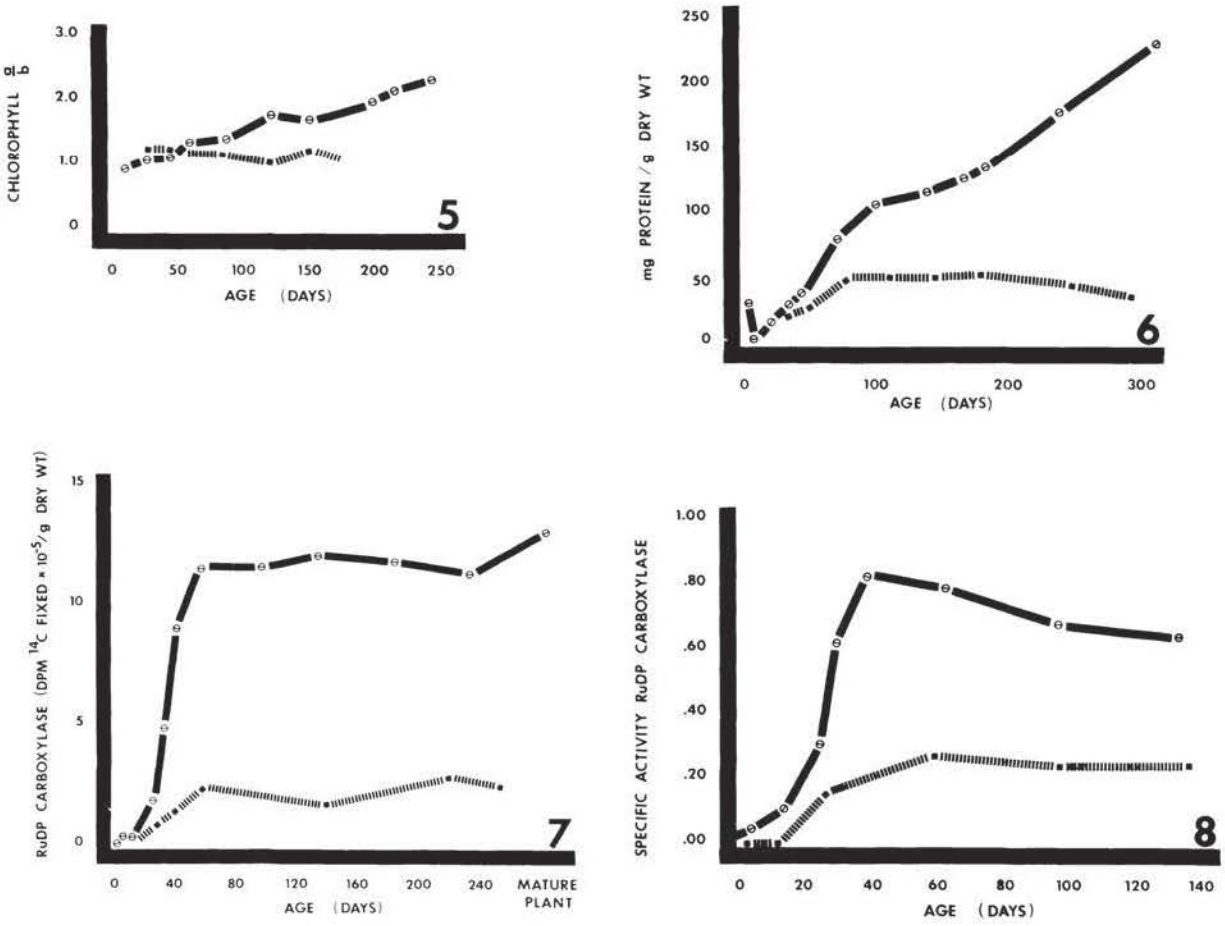
the capacity for dark fixation. By day 60, the rates of both light and dark fixation of CO_2 doubled again but remained constant thereafter.

In seedlings sown on KC-SUC there was negligible CO_2 fixation until day 20, when the rate of dark fixation exceeded the rate of photosynthetic CO_2 incorporation (fig. 9). Total CO_2 fixation exceeded the rate of photosynthetic CO_2 incorporation (fig. 9). Total CO_2 fixation increased steadily through day 60 and was unchanged thereafter, as was also the case for seedlings grown on KC. In contrast to KC seedlings, final levels of CO_2 fixation by seedlings grown on KC-SUC were 2–3 times lower on a DW basis. Also, dark fixation represented a larger percentage of total CO_2 fixation in the KC-SUC seedlings. It is apparent that protocorms are capable of at least limited photosynthesis even in the absence of an exogenous carbohydrate source during germination and early growth.

FATE OF LABELED ACETATE.—The label from acetate-2-^{14}C was readily detectable in both the lipid- and water-soluble fractions of all extracts (table 1). In general, younger seedlings (10 and 19 days) grown on KC-SUC accumulated more label per gram FW than older ones (26 and 84 days) on the same medium. The percentage of ^{14}C in the lipid-soluble fractions increased with seedling age. However, in all cases the level of radioactivity was higher in the water-soluble fraction (table 1).

When the water-soluble portion of extracts was further partitioned, the majority of label was found in the acidic (organic acid) fraction (table 2). The basic (amino acid) fraction contained the next highest percentage of counts. Only a very small share of counts was present in the neutral (sugars) fraction. As incubation time increased, the percentage of ^{14}C in the acidic fraction generally decreased, accompanied by a corresponding increase in the basic fraction. The amount of label in the neutral fraction remained low (3.1% or less) regardless of the incubation time.

EFFECT OF SELECTED CARBOHYDRATES ON SEEDLING GROWTH.—Seeds flasked on both KCG and KCF reached the protocorm stage in 20 days. Leaf



FIGS. 5–8.—Fig. 5, Chlorophyll *a*/*b* ratios in seedlings raised on KC with (solid line) and without (broken line) sucrose. Fig. 6, Soluble protein content in seedlings raised on KC with (solid line) and without (broken line) sucrose. Fig. 7, RuDP carboxylase activity in seedlings raised on KC with (solid line) and without (broken line) sucrose. The asterisk denotes the value found in mature plants. Fig. 8, Specific activity of RuDP carboxylase in seedlings raised on KC with (solid line) and without (broken line) sucrose.

formation in KCG seedlings kept pace with their production in seedlings grown on KC. However, the appearance of leaves in KCF seedlings was slower (fig. 10).

Discussion

Our purpose in this study was to gain insight into the requirement of the germinating orchid seed for an exogenous source of suitable carbohydrate before growth beyond the protocorm stage can occur (KNUDSON 1922). The possibility that seedlings grown without carbohydrate were simply unable to synthesize sucrose was checked with culture on media containing either glucose or fructose (fig. 10). Ability to utilize these sugars and grow into advanced plantlets was taken as an indication that if sucrose is a requirement for seedling growth, it can be synthesized from glucose or fructose by the seedlings. Work with other orchid species has also demonstrated the ability of seedlings to synthesize sucrose from other sugars (SMITH 1967). Several sugars other than glucose, fructose, and sucrose have been shown to be acceptable sources of carbohydrate for germination of various orchids (LA GARDE 1929; QUEDNOW 1930; WYND 1933; ARDITTI 1967; ERNST 1967; ERNST, ARDITTI, and HEALEY 1971).

The requirement for an exogenous carbohydrate source does not last indefinitely in seedlings raised asymbiotically. *Cattleya* seedlings grown to the first leaf stage on sugar-containing medium will continue to grow on a sugarless one (KNUDSON 1924). Seedlings raised 28 days on KC were moved onto KC-SUC with 50% continuing to grow into advanced plantlets (fig. 2). At day 28, however, none of the transferred protocorms had reached the first leaf stage. Thus, it appears that the presence of leaves is not a prerequisite for growth in the absence of supplied sugar. Possibly, therefore, only leaf initials or the potential to form them are required for further development. Either of these could have come into being during the initial 28 days.

To determine whether sucrose is required during the entire 4 wk or only for a shorter time toward the end of this period, 15-, 20-, and 60-day-old protocorms grown on KC-SUC were transferred onto KC and then back to KC-SUC after various lengths of time on the sugar-containing medium. The time required on KC before 50% of the protocorms returned to KC-SUC grew into advanced seedlings

TABLE 1

DISTRIBUTION OF ^{14}C FROM ACETATE-2-^{14}C INTO LIPID-SOLUBLE AND WATER-SOLUBLE FRACTIONS OF SEEDLINGS GROWN ON KC-SUC

Seedling age (days)	Incubation time (h)	dpm × 10^{-6}/g FW		Percentage ^{14}C	
		Lipid-soluble fraction	Water-soluble fraction	Lipid-soluble fraction	Water-soluble fraction
10.....	4	1.75	7.33	18.5	81.5
	8	1.59	8.26	15.5	81.5
	12	2.06	4.32	32.2	67.8
	24	6.11	13.6	31.0	69.0
19.....	4	1.96	5.46	26.6	73.4
	8	1.76	10.7	25.1	74.9
	12	2.37	8.07	22.7	77.3
	24	6.61	13.8	32.0	68.0
26.....	4	1.81	5.67	24.2	75.8
	8	2.07	6.07	26.0	74.0
	12	3.29	4.91	38.4	61.6
	24	4.46	8.80	33.7	66.3
84.....	4	2.26	3.35	40.2	59.8
	8	3.21	4.50	41.5	58.5
	12	2.69	3.84	41.2	58.8
	24	4.87	7.30	40.5	59.5

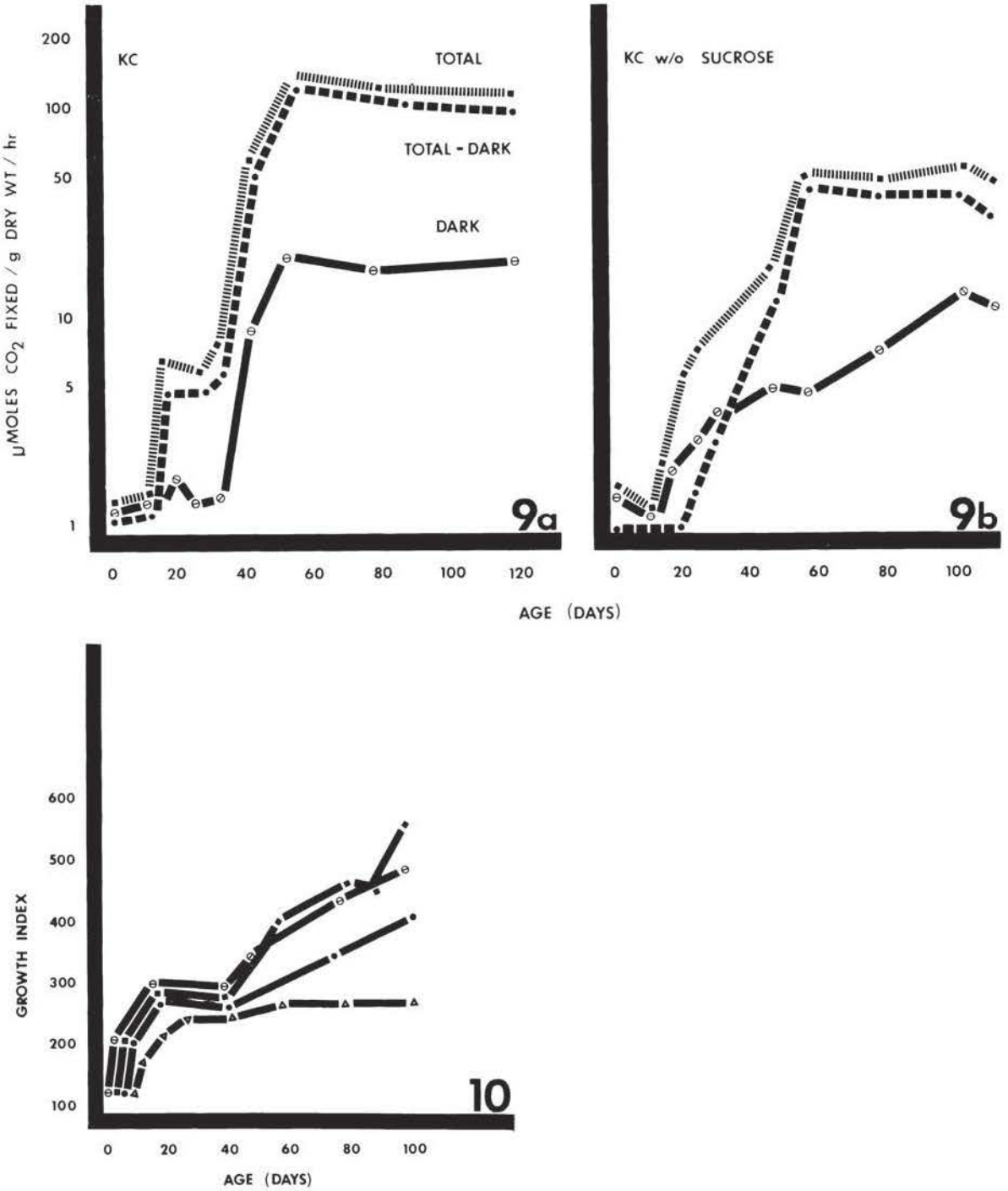
TABLE 2

DISTRIBUTION OF ^{14}C FROM ACETATE-2-^{14}C WITHIN THE WATER-SOLUBLE FRACTION COLLECTED FROM SEEDLINGS GROWN ON KC-SUC

Seedling age (days)	Incubation time (h)	Percentage ^{14}C		
		Acidic fraction	Basic fraction	Neutral fraction
10.....	4	94.9	5.1	.0
	8	92.0	7.3	.7
	12	83.5	15.5	1.0
	24	79.4	18.6	2.0
19.....	4	93.2	6.5	.3
	8	85.7	14.3	.0
	12	84.0	14.4	1.6
	24	81.1	16.8	2.1
26.....	4	90.2	7.3	2.5
	8	91.5	5.5	3.0
	12	88.6	10.0	1.4
	24	85.7	12.5	1.8
84.....	4	72.0	27.5	.5
	8	63.3	36.5	.2
	12	63.4	33.7	2.9
	24	63.5	33.5	3.0

varied from 21 to 20 days (figs. 2, 3). Clearly, sucrose is needed for an extended period of time (21–60 days) and does not act solely as a short-time stimulus.

The major enzyme involved in the initial incorporation of CO_2 during C_3 photosynthesis, RuDP carboxylase, is located in the chloroplasts of higher plants (FULLER and GIBBS 1959; SMILLIE 1963) and may represent 5%–10% of the soluble protein in photosynthetic tissues (WEISSBACH, HORECKER, and HURWITZ 1956). Therefore, a close correlation should exist between the amount of this enzyme and the rate of photosynthesis, which is true in *C. aurantiaca* seedlings. Both photosynthetic capacity and RuDP carboxylase of seedlings grown on KC increased



FIGS. 9–10.—Fig. 9, Photosynthetic and dark fixation of carbon dioxide by seedlings raised on KC medium with (*a*) and without (*b*) sucrose. Fig. 10, Growth of *Cattleya aurantiaca* seedlings on KC (asterisks), KCG (filled circles), KCF (triangles), and KC with *myo*-inositol rather than sucrose (squares).

steadily during the first 60 days of growth, reaching a plateau at that time (figs. 7, 8). In contrast, enzyme levels and photosynthetic CO_2 fixation were lower in seedlings grown on KC-SUC. The amount of RuDP carboxylase in seedlings raised on KC-SUC reached roughly one-fourth of the level found in those grown on KC. At the same time, the capacity of KC-SUC seedlings to fix CO_2 photosynthetically was only one-third that of plantlets cultured on KC. Generally, then, the level of enzyme activity paralleled the increase in photosynthetic capacity. These findings are in accord with those reported for growing pea leaves (SMILLIE 1963), greening bean leaves (BRADBEER 1969), and developing leaves of *Populus deltoides* (DICKMANN 1971).

It is interesting to note the relationship between the length of time a seedling must be grown on KC before it can be transferred and grown on KC-SUC (fig. 2) and the development of RuDP carboxylase activity (fig. 7). As the seedlings are left for longer periods on KC, the enzyme level and photosynthetic capacity increase. Seedlings with higher rates of photosynthesis were better able to continue development when transferred to KC-SUC.

Specific activity of RuDP carboxylase (fig. 8) rises sharply between days 15 and 40 of growth on KC but increases only slightly in seedlings grown on KC-SUC. This indicates that in plants maintained on KC, there is a preferential increase in the level or activity of the enzyme compared with the overall level of protein. The increase in the level of this enzyme might be part of a series of events initiated in seedlings when an adequate supply of carbohydrate exists.

The relationship between chlorophyll content and photosynthesis has been the subject of several investigations (IRVING 1910; SMILLIE and KROTKOV 1961; ŠESTÁK 1963; DICKMANN 1971). Some of these studies found a direct relationship between chlorophyll concentration and photosynthesis, but the plant material used did not include very young non-photosynthesizing tissues. Both chlorophyll levels and photosynthetic rates in seedlings raised on KC increased between day 20 and day 50 (fig. 4). After day 50, total chlorophyll content increased while the photosynthetic rate leveled off. There was no further increase in photosynthesis with rising chlorophyll levels. A direct relationship between chlorophyll levels and photosynthesis, therefore, was not found. On the other hand, the high degree of correlation between RuDP carboxylase levels and photosynthesis suggests that protein metabolism might be a more important, if not limiting, factor in photosynthetic rates.

In seedlings sown on KC-SUC, the chlorophyll level did not increase above that first detected at day 25 (fig. 4). However, the photosynthetic rate continued to rise through day 60 (fig. 9), indicating the absence of a direct relationship between chlorophyll content and photosynthesis.

Studies on expanding leaves of *P. deltoides* (DICKMANN 1971) and young leaves of radish and spinach (ŠESTÁK 1969) revealed a close relationship between net photosynthesis and increasing chlorophyll *a*/*b* ratios. In both investigations the increase in the ratio was a reflection of increasing levels of the *a* component. In *C. aurantiaca*, the increasing *a*/*b* ratio in seedlings grown on KC was also due solely to a rise in chlorophyll *a*. However, the photosynthetic capacity of the seedlings remained unchanged after 60 days, while the chlorophyll *a* level continued to rise. The *a*/*b* ratios for seedlings on KC-SUC remained at 1.0, while the photosynthetic rate was increasing (figs. 5, 9). Neither a direct relationship between chlorophyll *a*/*b* ratio and photosynthesis nor a close relationship between RuDP carboxylase activity and chlorophyll content was found in *C. aurantiaca* and other plants (BENEDICT and KOHEL 1969).

Dark carbon fixation levels by the seedlings are high (fig. 9), but the pathway being utilized by the seedlings is as yet unclear. Several orchid species, including members of *Cattleya*, possess the crassulacean acid metabolism carbon fixation pattern in their leaves (KNAUFT and ARDITTI 1969; WITHNER 1974). It is possible, therefore, that the same pathway may be operative in the protocorms and/or seedlings. In young seedlings grown on KC-SUC and fed acetate-2-^{14}C, most of the label is found in the organic-acid and amino-acid fractions (tables 1, 2) and only very small amounts in sugars (3.0% or less). This contrasts sharply with the high conversion of acetate to sugar in germinating castor bean (CANVIN and BEEVERS 1961), peanut, sunflower (BRADBEER and STUMPF 1959), and other fatty seeds (CHING 1972).

For the conversion of fat to carbohydrate, two important biochemical pathways must be operative in germinating seeds. First, the glyoxylate cycle (CANVIN and BEEVERS 1961) must function. The net result of this pathway is the conversion of two acetate units released from the lipid reserves into one molecule of malate. Then, there must be a reversal of the glycolytic pathway so that malate is used for the production of sugars. If either the glyoxylate cycle or the reversal of glycolysis or both are absent, acetate units cannot be converted to carbohydrates, and acetate could pass through the Krebs cycle without the production of sugar. This seems to be the situation in germinating seeds of *C. aurantiaca*. While it has not been determined which part(s) of the biochemical pathway may be inoperative, it does appear that the conversion of acetate to sugars is, at best, severely limited. To some degree this may be a reflection of the fact that neither cotyledons nor an endosperm is present in orchid seeds. In non-

orchidaceous seeds, only these structures, but not the embryo axis, are able to convert acetate to sugars (CHING 1972). Hence, it seems that the disappearance of endosperm and cotyledon in the orchids may have led to the loss of certain biochemical capabilities. Glyoxysomes have not been found in germinating orchid seeds (HARRISON 1977).

The inability to convert stored lipids into carbohydrates, which are not stored in the seed, would explain the requirement of the germinating seed for an exogenous supply of carbohydrate. In the laboratory, seeds are raised on culture media containing suitable sugars (KNUDSON 1921, 1922), while in nature a symbiosis must be established with an endophytic fungus (BERNARD 1899, 1903, 1909; BURGEFF 1932, 1936, 1959). It is widely held that carbohydrates (and other substances) are obtained from the fungus (ARDITTI 1967; SMITH 1967).

The loss of the ability to synthesize sugars and the resulting dependence on fungal symbionts for these metabolites may have had both advantages and disadvantages during the evolution of orchids. Most infections of the orchid seed result in parasitism of the fungus on the orchid (BERNARD 1909; KNUDSON 1922). The establishment of a successful symbiosis in which the orchid survives and grows into a mature plant is less frequent. This would be a distinct disadvantage for the orchid. An infection must be established, even though most are harmful, if the seedling is to mature. On the other hand, since the fungus supplies the needed carbohydrate(s), it is not necessary for these substances to be stored in the seed. Thus, less storage material is required per seed, and more seeds could be produced per parent plant. The number of seeds produced per capsule varies from several hundred thousand to at least 4 million (ARDITTI 1967). It is possible that the advantage of producing numerous seeds outweighs the disadvantage of their dependence on the fungus.

In summary, germinating orchid seeds reach the protocorm stage using their own reserves and the products of limited photosynthesis. Further development is arrested, however, and protocorms persist by a slow utilization of reserves. Upon infection, carbohydrates are supplied, and if the seedlings survive, leaf-bearing plants are produced.

Acknowledgments

We thank Dr. ERNEST A. BALL for his help and encouragement. This study was supported in part by the American Orchid Society Fund for Research and Education and the University of California Industrial Associates.

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