HIGHER PLANTS IN BIOREGENERATING SYSTEMS

Chairman

Dr. Alton E. Prince
6570th Aerospace Medical Research Laboratories
Wright-Patterson Air Force Base, Ohio

INVESTIGATIONS OF SELECTED HIGHER PLANTS AS GAS EXCHANGE MECHANISMS FOR CLOSED ECOLOGICAL SYSTEMS*

Bioastronautics Section
THE BOEING COMPANY

INTRODUCTION

During recent years, considerable attention has focused on the use of photosynthetic plants for atmosphere regeneration and food supplements during extended space flight. In most studies, emphasis has been placed on the utilization of microscopic algae. However, the use of higher plants for these purposes offers several potential advantages: they may be grown in light weight substrates; their requirements for environmental control are less critical; and many of the problems of gas exchange that may occur in the zero gravity state with cultures of algae would not be anticipated with the leafed plants. Finally the use of higher plants as a food is more appealing and probably will present fewer psychological problems than the use of algae pastes or powders. The objectives of this program were to:

1. Conduct a survey of the literature with special attention devoted to photosynthetic and nutritional potential of Angiosperms grown under low intensity artificial light.

2. Select a group of plants for experimental studies based on the available information.

3. Evaluate selected plants by measuring photosynthetic activity under carefully controlled conditions of temperature, humidity, and light.

4. Select the three most promising species and carry out detailed studies of photosynthetic activity, water utilization, ease of reproduction, resistance to disease, and response to varying conditions of light, temperature, humidity, and atmospheric composition. Examine these plants for pharmacologically active substances.

5. Establish the food value of the selected plants by chemical analysis and preliminary animal feeding experiments.

---

* This work was accomplished under Air Force Contract 33(616)-7945. This paper is a summary of the more comprehensive final report of the contract. The technical documentary report (by the same title) is AMRL-TDR-62-127, Aerospace Medical Research Laboratories, Wright-Patterson AFB, Ohio, in press.

There is little in the way of pertinent literature to support the selection of Angiosperms that have desirable characteristics for use in an extraterrestrial environment, since the need-to-know concerning specific response under modified conditions is of recent origin. Hence, the role of the literature review is relegated to one of establishing guide lines within which a group of plants may be selected which will, with a reasonable degree of proficiency, fulfill a role in respiratory support and as a food source for man in a space situation.

The principle points developed by a review of the literature led to the establishment of the following criteria for selection:

1. High photosynthesis efficiency under low artificial light conditions.

2. Production of edible parts or plants under low artificial light conditions.

3. Resistance to increasing osmotic pressures.

4. Absence of pharmacologically active substances.

In addition to the criteria above, certain other factors were taken into consideration in the final selection of plants. These were:

1. Compactness of plant. Since space and weight penalties are critical considerations in the selection of any part of system for a space vehicle or base, the volume occupied by the plant system must be kept at a minimum. Therefore, plants which have a maximum leaf area and a minimum of stem would be desirable.

2. Since respiratory support is of paramount interest, plants which tend to flower readily should be avoided since oxygen production declines rapidly after flowering.

Prior to selecting groups of plants which might serve in gas exchange systems, a survey of the plant kingdom as a whole was initiated and complete orders of plants were eliminated in the initial screening because of structure, function, edibility, or availability. The plant kingdom contains over 300,000 different kinds of species of plants which have been observed and described. The present investigation limits selection of plants to the Angiosperms.

SELECTION OF PLANTS

On the basis of the above taxonomic survey, the designated criteria, and personal knowledge of the senior investigators concerning the horticultural programs of the USDA, the State Experiment Stations and commercial firms the following plants were selected for study in this program:

<u>Lactuca sativa</u>

1. Lettuce - variety Slobolt. Broadleaf, round head. Leaves edible.

2. Lettuce - variety Early Great Lakes. Broad leaf, round head.

3. Lettuce - variety Great Lakes. Broadleaf, round head. Leaves edible.

4. Romaine - variety Paris Dark Green. Long, narrow leaf. Leaves edible.

5. Celtuce - variety Celtuce. Non-heading, large leaf. Edible, large, fleshy stem.

Brassica chinensis

6. Chinese cabbage - variety Wong Bok Poatung. Round head; large, savoyed leaves. Leaves and stem edible.

B. oleracea

7. Cabbage - variety Savoy Iron Head. Head flat, large leaf. Leaves and stem edible.

8. Cabbage, celery - variety Special Selections. Head elongate, large leaf. Leaves and stem edible.

9. Cauliflower - variety Snowball, non-heading, very large leaf. Leaves edible.

10. Kale - variety Blue Green Curled. Non-heading, very large leaf. Leaves edible.

11. Kale - variety Georgia, non-heading, very large leaf. Leaves edible.

12. Collards - variety Georgia. Non-heading, large leaf. Leaves edible.

B. rapa

13. Turnip - variety Seven Top

Beta vulgaris cicla

14. Swiss chard - variety Fordhook Giant. Broad succulent leaf. Leaves edible.

Cichorium endiva

15. Endive - variety Salad King. Finely curled, edible leaf.

Taraxacum officinale

16. Dandelion - variety Thick Leaf. Elongate, edible leaf.

Raphanus sativas

17. Radish - variety Cherry Belle. Elongate leaf. Edible root.

Tetragonia expansa

18. New Zealand spinach - no varietal name. Small, thick-leaf; prostrate habit of growth with young stem tips. Leaves edible.

Amaranthus gangeticus

19. Tampala - variety Regular

Ipomoea batatus

20. Sweet potato - variety Yellow Gem. Vine-like; large leaf. Edible tuberous roots.

EXPERIMENTAL STUDIES

Methods and Characteristics of Growth in Low Intensity Artificial Light.

For these studies, the seeds were germinated under blue fluorescent lights in plywood cabinets painted a flat white and equipped with twelve fluorescent lamps each. Temperatures were held at 83°±2°F. during the day and 68°±2°F. at night. The length of the light period was 14 hours and the intensity, as measured by a Weston light meter was 700 f.c. at plant height.

The growth medium was a mixture of equal parts of Perlite and peat moss moistened with a solution composed of 30 gm KNO_3, 30 gm $Ca(NO_3)_2$, and 30 gm H_2SO_4 in 7 liters of water. The wet mix was limed so that random samples had a pH of 6.5±0.2. Prior to planting, the seeds were soaked in warm water for one hour.

After two weeks under blue light, the seedlings were thinned to five per pot and placed under green fluorescent light, assuming an increase in dry weight was probable. Following two weeks of exposure to green light, the plants were transferred to cabinets containing white light for an additional two weeks. The six-week seedlings were then ready for experimental use. During the entire period, the plants were watered daily with tap water and weekly with dilute Shives Solution (Table I).

Growth Response to Low Intensity Artificial Light

Observations were made as to general growth characteristics under these conditions. Members of the genus Lactuca exhibited extreme etiolation (blanching) and elongation of the hypocotyl and epicotyl. Cotyledons and true leaves were strap-shaped and leaf margins were entire whereas the typical morphology is one of crinkled or curled broad leaves (2). The leaves were a very pale green color instead of light green. No change was noticed when the seedlings were placed in the green light or later under the white light. A similar situation existed when several varieties of lettuce were grown in pink light in an earlier study.

Similar observations were recorded for members of the genus Brassica with the exception that leaf margins were the normal crinkled or curled leaf. However, the stems and petioles of kale, varieties Blue Green Curled and Georgia, and the Georgia Collard remained etiolated. Swiss chard exhibited signs of etiolation of the hypocotyl but not the epicotyl. However, the number of leaves and their size was considerably reduced.

TABLE I

CHEMICAL COMPOSITION OF SHIVES SOLUTION*

Macronutrients	Grams per 20 liters
KH_2PO_4	5.9
$Ca(NO_3)_2{}^4H_2)$	20.1
$MgSO_4{}^7H_2)$	10.7
$(NH_4)_2SO_4$	1.8
Micronutrients	**In 500 ml H_2O**
H_3BO_3	0.8 gm
$MnSO_4$	0.8 gm
$Zn\ SO_4$	0.8 gm
$CuSO_4$	0.4 gm

* 10 ml added to each liter of solution.

Ipomoea batatus

Sweet potato, grew normally for a short period of time, then the stems of succeeding flushes of growth produced abnormally small leaves.

Hypocotyl and epicotyl etiolation were also noted in the cabbage, variety Savoy Iron Head, although stem elongation was reduced as the plant aged. Epicotyl elongation was minor in the cabbage, variety celery. This variety, however, could not be distinguished from Chinese cabbage at maturity.

The plants eliminated from further testing for reasons cited above were:

Lactuca sativa

Lettuce - variety Slobolt

Lettuce - variety Early Great Lakes

Lettuce - variety Great Lakes

Romaine - variety Paris Dark Green

Celtuce - no varietal name

Brassica Oleracia

Cabbage, celery - variety Special Selections

Kale - variety Blue Green Curled

Kale - variety Georgia

Collards - variety Georgia

Beta vulgaris cicla

Swiss chard - variety Fordhook Giant

Ipomoea batatus

Sweet potato - variety Yellow Gem

Preliminary Screening for Photosynthetic Activity

Two methods were employed for the measurement of photosynthetic activity. In the first, a well-watered plant was placed under a 5-liter glass battery jar which was sealed with vacuum grease to an aluminum base plate. The base plate contained inlet and outlet ports through which the atmosphere was recirculated by means of a small diaphragm pump (Dyna-pump). Two 10-lamp fluorescent light banks were placed on each side of the battery jar and a 500 watt photoflood lamp was placed directly above. The total incident light intensity, as measured by a Weston light meter, Model 756, was approximately 2000 f.c. from above and 1400 f.c. from the sides. The atmosphere was circulated in series through a Beckman E2 oxygen analyzer for measurements of oxygen concentration and an Aerograph gas chromatograph for carbon dioxide estimation. The system was cooled by an evaporative blower which maintained a temperature of 85 - 90°F inside the battery jar. A volume of carbon dioxide equivalent to two percent of the volume of the system was injected into the battery jar and measurements of carbon dioxide utilization and oxygen production were started. After the initial period required for mixing the atmospheric components, data were recorded at fifteen minute intervals. During these measurements, humidity and temperature could not be adequately controlled and the condensate on the sides of the battery jar varied from plant to plant. Measurement of the effect of the condensate on light intensity showed a decrease of up to eleven percent. The alternate method was similar to that described above except that a lucite box (1 ft^3) was used in place of the battery jar. The data obtained are recorded in Table II.

TABLE II

PRELIMINARY SCREENING FOR PHOTOSYNTHETIC ACTIVITY

Plant	Light Intensity (fc)	$+O_2$ ml/dm^2/hr.	$-CO_2$ ml/dm^2/hr.
Chinese Cabbage	1200	3.8	6.8
	2000	3.6	3.0
	8000	6.2	5.1
	8000	7.0	5.6
Cabbage, Savoy	1500	4.4	11.6
	2000	15.1	9.8
	8000	11.2	13.5
Endive	1500	3.6	8.7
	2000	6.5	5.2
	2000	4.2	3.7
Tampala	1200	3.8	8.0
	1500	3.2	7.4
	2000	3.2	4.7
	8000	6.6	4.2
N. Z. Spinach	1500	6.6	11.3
	8000	13.2	13.4
Turnip	1500	6.8	17.5
	2000	16.4	13.7

Following the oxygen and carbon dioxide measurements described, further evaluation of the usable photosynthetic potential was made by measurement of the leaf area versus the area occupied by the whole plant. The plants were approximately forty-five days old. These data are shown in Table IV. For comparative purposes the plants species are listed in order from highest to lowest parameters in Table III.

As may be seen, Tampala, Chinese cabbage, turnip, the Savoy variety of cabbage, cauliflower, endive and New Zealand spinach appeared to be the most likely candidates for further study. Plants eliminated from this group were: cabbage, cauliflower, turnip and New Zealand spinach. Cabbage and cauliflower were eliminated because of the gas they produce upon ingestion which would result in extreme discomfort at high altitude. Turnip and radish exhibit an unfavorable mode of growth for maximum photosynthesis.

During the period of oxygen and carbon dioxide measurements the New Zealand spinach plants flowered and were eliminated from further consideration.

TABLE III

Weight in Grams	Total Leaf Area	Area Covered By Plant	Total Leaf Area/ Area Covered By Plant
Chinese Cabbage	Chinese Cabbage	Cabbage, Savoy	Turnip
Turnip	Cabbage, Savoy	Chinese Cabbage	Chinese Cabbage
Cabbage, Savoy	Endive	Endive	Dandelion
Endive	Turnip	Cauliflower	N. Z. Spinach
Cauliflower	Tampala	Tampala	Endive
N.Z. Spinach	Cauliflower	N. Z. Spinach	Tampala
Tampala	N. Z. Spinach	Dandelion	Cauliflower
Dandelion	Dandelion	Turnip	Cabbage, Savoy

The dandelion, although it maintained a rosette mode of growth, produced an insufficient number of leaves and was dropped from further consideration. The remaining three species, chinese cabbage, endive, and Tampala, were retained for additional study.

Tolerance to Salts

In closed ecological systems, the nutrient substrate for plant growth will be supplied by human waste products. One of the problems involved in using these waste products is the high ratio of sodium, to other ions. Table V shows the ion content, i.e., calcium, magnesium, potassium and sodium, of human waste as opposed to a standard plant nutrient solution. Because of this high ratio the plants must have considerable tolerance to sodium chloride in the nutrient solution, if concentrations of calcium, magnesium and potass m are high enough to support adequate plant growth.

For preliminary screening purposes, a medium composed of 1/10 Shives solution (Table I) plus 0.01, 0.02, 0.03 or 0.04 molar sodium chloride were prepared. These solutions were equivalent to 585, 1170, 1755, and 2340 Mg/L NaCl respectively. Approximately 800 ml of each solution was placed in wide mouth one-quart mason jars leaving about one centimeter for airspace. Holes were drilled in a cork stopper so that it would hold 5 six week old seedling plants and an outlet and inlet glass tube for aeration. The seedlings were placed in holes in the stopper and sealed in with cotton and warm wax. Jars, in duplicate, containing the various solutions were connected to a humidified aeration line.

A preliminary evaluation of water loss indicated that approximately one ml of water was lost per day per mason jar. Water loss due to transpiration

TABLE IV

Weight and Area Measurements of Leaves of
Eight of the Selected Plant Species

An Average of Three Plants

Variety	Leaf Weight/Plant in grams	Area/1 gm of Leaf Tissue	Total Area in Sq. Cm.	Total Shadow Area Covered by Plant	Ratio: Total Area/ Area of Plant
Cabbage (Savoy Iron Head)	18.3	26.8	478.0	379.5	1.26
Cauliflower	10.3	23.0	237.1	188.10	1.26
Chinese Cabbage	22.8	23.9	533.0	261.4	2.04
Dandelion	3.8	40.8	155.2	72.0	2.16
Endive	15.4	28.9	444.6	254.9	1.74
New Zealand Spinach	7.3	23.6	172.0	85.1	2.02
Tampala	6.1	39.2	239.0	171.8	1.39
Turnip	19.4	22.2	431.3	8.0	53.9

TABLE V

Comparison of a Plant Medium with the Average Composition of Human Excretion Products

	Plant Media * Mg/L	Excretion Products ** mg per Kg Body Weight/24 Hrs.
Ca	168	10
Mg	50	4
K	241	34
Na	Trace	46

* Table 192 in Handbook of Biological Data, Wright Air Development Center Technical Report 56-273, Wright-Patterson Air Force Base, Ohio, October 1956.

** Table 222, ibid.

and evaporation was replaced on Mondays, Wednesdays, and Fridays during the two week run. Data were obtained on water loss and the death rate of the plants.

Tampala and cabbage showed a greater loss of water at high concentration of sodium chloride than was exhibited by the controls (standard Shives Solution). The reverse was true for Swiss chard and New Zealand spinach.

Of the six plants tested only Chinese cabbage and endive were affected by the sodium chloride solutions. Endive would not tolerate 0.03 M NaCl while Chinese cabbage showed a loss of fifty percent of the plants after six days at a concentration of 0.04 molar. Browning of the root tips was the first evidence of injury followed by a general darkening of the entire root system with an accompanying permanent wilt. Necrotic areas in the leaves also appeared at the onset of root system darkening.

A second series of tests were run with 0.01, 0.02, 0.03 and 0.04 molar calcium chloride and Shives solution. The results of this test were similar to the results obtained with sodium chloride.

Photosynthetic Studies

Carbon dioxide utilization and oxygen production data were obtained on two species, endive and Chinese cabbage. The method used for photosynthetic measurement was quite similar to the methods described previously. Twelve each of the endive and Chinese cabbage plants were placed in the chamber for a conditioning period of ten days prior to start of the tests. Each group of twelve plants was divided into three lots. The endive plants were (a) the control, (b) 50 mg/l added sodium chloride and (c) 100 mg/l added sodium chloride. The

Chinese cabbage were (a) the control, (b) 250 mg/l added sodium chloride, and (c) 750 mg/l added sodium chloride.

In the Chinese cabbage Table VI, a general increase in utilization of CO_2 and O_2 production were noted as NaCl concentration was increased.

Although endive was the most susceptible species to salt damage in the preliminary screening tests, increases of salt concentration of up to 200 mg/l in the three plants used for the 100 mg/l experiment above were without visible effect. At 2200 mg/l some leaf curl appeared. The 750 mg/l Chinese cabbage plants were increased to 2500 ppm sodium chloride without visible signs of damage.

TABLE VI

AVERAGE OXYGEN PRODUCTION AND CARBON DIOXIDE UTILIZATION DURING LIGHT PERIOD BY ENDIVE AND CHINESE CABBAGE DURING LIGHT PERIOD

	Ml/dm^2/hr *		Photosynthetic Quotient
	$+O_2$	$-CO_2$	O_2/CO_2
Endive, Control	3.38	4.63	0.73
Endive, +50 ppm NaCl	2.98	2.67	1.12
Endive, +100 ppm NaCl	2.85	2.80	1.02
Chinese Cabbage, Control	0.74	0.94	0.79
Chinese Cabbage, +250 ppm NaCl	1.10	1.32	0.83
Chinese Cabbage, +750 ppm NaCl	1.31	1.25	1.04

* Volume of gas utilized or produced per square decimeter of cross sectional area of plant leaf per unit time.

The data obtained on the endive plants was similar to that obtained in preliminary screening tests (Table II). Both carbon dioxide and oxygen measurements were essentially linear with time. No differences in oxygen production could be attributed to differences in carbon dioxide content of the atmosphere. Endive plants proved superior in oxygen production and carbon dioxide utilization. However, the differences may be due to experimental techniques rather than photosynthetic activity (Tables VI and VII).

Differences occurred between the response of endive to sodium chloride treatments. As the salt content of the medium was increased, oxygen production and carbon dioxide utilization decreased (Table VI).

TABLE VII

HOURLY OXYGEN PRODUCTION AND CARBON DIOXIDE UTILIZATION DURING LIGHT PERIOD BY ENDIVE AND CHINESE CABBAGE AS EXPRESSED IN MILLILITERS PER GRAM OF WET WEIGHT OF TISSUE PER HOUR

	ml/gm/hr	
	$+O_2$	$-CO_2$
Endive, Control	0.67	0.92
Endive, +50 ppm NaCl	0.62	0.55
Endive, +100 ppm NaCl	0.62	0.59
Chinese Cabbage, Control	0.12	0.15
Chinese Cabbage, +250 ppm NaCl	0.14	0.17
Chinese Cabbage, +750 ppm NaCl	0.19	0.19

Nutritional Studies

This study was conducted in two parts. The first consisted of chemical analyses designed to reveal information pertaining to the nutritional value of the selected species and the second part to determine if the plants were toxic during short-term animal feeding tests.

Chemical Analyses

The analyses which were performed on the plants were chosen because of the relatively complete picture of dietary essentials which could be assembled from the data. All major groups of essential nutrients were studied and, although a finer definition within some groups may be desirable, it is felt that the results reported leave little to be desired, at least for a preliminary screening.

The environmental conditions and the analytical procedures used were controlled as closely as possible. All plants used for analysis were brought to maturity under identical conditions of temperature, light, and humidity, and all plants of a given species were 12 weeks old when taken for analysis. The tissues of ten plants of each species were pooled, and samples for analysis were drawn from this pool. All procedures were carried out as rapidly as possible to minimize the effect of autolytic changes in labile components.

A group of plants was harvested, weighed, and rinsed in cold running tap water. The roots, stems, and leaves were separated, and the tissues frozen between blocks of solid carbon dioxide. After freezing, the tissues were pooled and ground to a fine powder in a mortar and pestle with powdered dry ice. Following thorough mixing, the ground tissues were stored at -20°C. in polyethylene bags until used. The wet weights of the plants are shown in Table VIII.

TABLE VIII

Wet Weight, of Leaf, Stem and Root Tissues

of Endive, Chinese Cabbage and Tampala (One Plant)*

	Leaf	Stem	Root	Total Weight
Endive	212.4	5.6	54.4	272.4
Chinese Cabbage	151.9	4.2	11.4	167.5
Tampala	37.3	11.1	17.1	65.5

* Each value represents the average of data from ten plants.

Methods and Results

Moisture and Ash

The water and ash content of the plant materials were estimated by standard methods and reported in Table IX. Moisture was determined as weight loss after drying to constant weight at 100°C. and ash is reported as residue remaining after ignition for four hours at 600°C.

TABLE IX

Water and Ash Content*

	Endive			Tampala			Chinese Cabbage		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Moisture %	92.8	85.7	95.8	82.3	86.6	92.6	91.7	87.7	93.2
Ash %	16.39	6.71	20.48	10.6	8.06	15.0	13.25	9.76	20.0

*Data are expressed as percent of wet weight.

Total Nitrogen

The nitrogen content of the three tissues of each plant was estimated by the addition of Nessler's reagent to a Kjeldahl digest of the frozen tissue. The results are recorded on Table X.

Protein:

A weighed amount of frozen tissue was homogenized in a Waring Blender with 25 ml of Tris* buffer, 0.2 M, pH 7.6. After centrifugation the residue was extracted twice more with 25 ml portions of the buffer (preliminary experiments showed that this procedure resulted in essentially quantitative recovery of the plant protein). Sufficient cold 50% trichloroacetic acid was added to the combined extracts to stand at 3° for one hour. The precipitate was collected by centrifugation, extracted twice with cold 5% TCA and washed twice with a cold acetone-ether mixture (1:1) to remove adsorbed pigments and residual TCA. The precipitate was dissolved in dilute NaOH and an aliquot taken for the estimation of protein by the method of Lowrey, et al (3). The results are expressed as gm of protein per 100 gm of dry tissue in Table X.

---

*Tris (hydroxymethyl) amino methane.

TABLE X

Total Nitrogen and Protein*

	Endive			Tampala			Chinese Cabbage		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Nitrogen %	4.65	2.52	6.20	4.08	2.46	3.85	3.70	3.68	6.93
Protein %	8.83	3.78	2.70	6.90	1.79	2.97	6.56	0.90	1.74

*Data are expressed as percent of dry weight.

Ascorbic Acid

Ascorbic acid in the plant tissues was measured by the method of Roe and Oesterling (4). Because of the labile nature of this substance, the determinations were performed with minimum delay. Tissues taken for ascorbic acid analysis were extracted within 24 hours after harvest. The results are expressed as mg. per 100 gm. dry tissue in Table XI.

Carotenes

The method used for the estimation of carotenes in the plant tissues is a modification of the methods reported by Wall and Kelly (6). The results of the carotene estimation are reported as International Units per 100 gm. plant tissue in Table XI.

Tocopherols

The procedure used for the estimation of tocopherol content of the plant tissues is a modification of the method of Wall and Kelly (6). This procedure consists of removal of interfering substances with concentrated H_2SO_4 and measuring the tocopherols colorimetrically after reaction with α-α-dipyridyl-$FeCl_3$. The standard used in this procedure was a solution of α-d-tocopherol in cottonseed oil. Replicate determinations agreed within 5%. The results are expressed as mg. per 100 gm. dry tissue in Table XI.

A survey of the literature has failed to reveal any compositional studies of these particular plants which might be used for comparative purposes. Some of the values reported here for certain constituents are outside the range which has been reported for many related plants, but it is not possible to say whether this is an inherent characteristic of these species or a function of the techniques and conditions used in their cultivation.

TABLE XI (Vitamins)

	Endive			Tampala			Chinese Cabbage		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
A-Ascorbic Acid (mg/100 gm)	168.5	52.0	105.5	242.4	53.7	5.9	135.5	28.5	86.0
C-Carotene I.U./100 gm	96,931	3,727	41,785	69,265	12,269	15,310	69,880	9,211	7,838
E-Tocopherol (mg/100 gm)	22.5	2.59	12.62	2.88	3.21	5.54	24.8	3.01	10.00

* Data given are per 100 gm dry weight.

Amino Acids

The amino acid content was estimated by the procedures of Spackman, Stein, and Moore (5). The results are presented in Table XII, expressing amino acid concentration as gm per 16 gm N.

TABLE XII

AMINO ACID RECOVERY - GRAMS PER 16 GRAMS OF NITROGEN

Amino Acid	Chinese Cabbage Leaves	Chinese Cabbage Roots	Tampala Leaves	Tampala Roots	Tampala Stems	Endive Leaves	Endive Roots	Endive Stems
Cystic Acid	1.874	0.518	0.151	0.858	1.318	0.973		5.924
Aspartic Acid	7.784	14.927	12.393	12.517	10.007	10.078	10.409	5.441
Theonine	3.995	6.031	5.477	3.077	5.660	5.595	5.342	2.522
Serine	2.099		5.154	2.192	4.248	5.062	4.918	3.759
Glutamic Acid	10.598	11.091	12.796	7.873	12.417	9.919	10.563	7.732
Proline	3.095	5.004	5.193	5.452	8.084	3.089	3.394	1.009
Glycine	4.785	6.275	5.033	7.761	5.909	5.204	6.208	4.493
Alanine	4.840	5.849	6.315	7.257	6.256	6.205	6.149	3.512
Valine	0.260	0.319			2.742			1.881
Methionine	3.308	9.978	6.860	9.140	9.523	8.676	11.860	6.861
Cystine		0.199	0.743					0.885
Isoleucine	3.636	3.389	5.528	3.779	4.744	4.903	4.625	2.872
Leucine	6.617	5.382	9.599	7.080	7.721	7.753	11.135	5.074
Tyrosine	0.552	0.440	1.804	0.421	4.755			
Phenylalanine	0.549	0.402	3.164		6.443			
Lysine	8.509	10.575	8.962	10.358	7.050	9.284	10.215	6.616
Histidine	3.182	3.490	3.805	3.288	3.851	5.511	3.680	3.737
Arginine	7.592	8.710	9.849	9.907	4.184	16.329	5.583	4.613
Ammonia	2.075	3.064	1.959	3.111	1.928	2.693	3.323	2.404

Water Soluble Carbohydrates

Water soluble carbohydrates, including the soluble polysaccharides, in the plant tissues were estimated by application of the anthrone reagent to water extracts. Samples of plant tissues were dropped into boiling water and allowed to boil for 30 minutes. The entire contents of the flask were then transferred to a Waring Blender, ground for 2 minutes, and transferred to an alundum or pyrex extraction thimble. Extraction in a Sohxlet apparatus was continued for no less than eight hours. The results are reported as gm. glucose per 100 gm. dry plant tissue in Table XIII.

Alcohol Soluble Carbohydrate

The procedure used for determination of alcohol soluble carbohydrate is the same as that reported above for water soluble compounds except that 80% ethanol was used as the solvent. The results are expressed as gm. glucose per 100 gm. dry plant tissue in Table XIII.

Total Carbon:

The method used is based upon the Schöniger technique as modified by Cheng and Smullin (1). The results are reported as percent carbon in Table XIII.

Fiber

Crude fiber in each of the plant tissues was estimated by a slightly modified AOAC method (7) which involved serial extraction with ether, sulfuric acid, and sodium hydroxide. The results are expressed as gm. per 100 gm. dry plant tissue in Table XIII.

TABLE XIII

	Endive			Tampala			Chinese Cabbage		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
CHO % (H_2O Sol)	24.6	63.7	33.1	17.63	42.99	29.54	40.12	43.85	16.71
CHO % (Alc. Sol)	23.51	45.94	34.05	7.10	28.7	21.67	30.2	40.65	14.79
Total C	42.2	41.1	41.7	42.4	41.9	41.5	40.2	41.9	38.0
Fiber %	10.83	11.61	12.38	8.25	18.66	15.14	7.89	21.87	18.5
Lipid %	6.30	2.03	4.52	2.49	5.45	4.59	5.66	2.76	2.65

*Data are expressed as percent of dry weight.

DISCUSSION

The screening of a wide variety of plants for possible use in closed ecological systems is a desirable area of study. Although little or no significant information to assist in the selection of plants was developed from the literature survey, a consideration of ecology, structural characteristics, and environmental requirements of a large number of plants was helpful in limiting the initial study to a few familiar species.

Although the fluorescent lamp is the simplest to use in laboratory studies a large number of plants were unable to develop normally under this type of light, and it would appear that a major effort is needed in the field of lighting technology if wider use is to be made of broadleaf plants. Detailed photosynthetic and nutritional studies were limited to those plants which grew in weak fluorescent light without marked deviation from normal growth and developmental characteristics. In the experimental studies concerned with photosynthetic activity and tolerance to salt a great deal of variability was observed between species and between individual plants of the same species.

Consideration of the analytical data does reveal that the three plants selected may provide a valuable nutritional supplement for man in a closed system. One of the most important considerations in assessing nutritive value in material of this type is accurate values for the content of amino acids, especially those which are indispensible to man. Inspection of the tables reveal that these plants contain adequate quantities of all these compounds. The content of vitamins A and C would also provide valuable supplies of these substances.

The only additional work necessary to completely characterize these plants and define their nutritive value would be estimation of the members of the B group of vitamins.

Preliminary feeding tests served only to demonstrate that the plants did not contain any highly toxic materials. Before they could be used as a food source for man, the fraction of these or any other uncommon plant would have to be thoroughly tested in long term feeding experiments.

The studies which are reported represent a fairly thorough screening of the three species of plants and represents the type of data which will be required to evaluate the potential of any plant species in a closed system. One additional study which is necessary is evaluation of the response of the plant when human waste materials or their degradation products are used as the growth substrate. This evaluation would be of primary interest in any further study, which should also include:

1. The screening of more species of plants, in particular those non-commercial horticultural lines which have a rosette mode of growth or a minimum of stem area such as is found in the double dwarf types.

2. Determine the optimal means of plant reproduction (vegetative vs. seed production) under conditions which will exist in closed ecological systems.

3. Determine photosynthetic activity as a function of the quality and quantity of artificial light.

REFERENCES CITED

1. Cheng and Smullin. Micro. Chem. J. 4:213. 1960

2. Hayward, H. E. The Structure of Economic Plants. The Macmillan Co. New York. 1948.

3. Lowrey, O. H., N. S. Rosebrough, A. L. Fare, and R. S. Randall. J. Biol. Chem. 193:265. 1951.

4. Roe, J. H. and M. S. Oesterling. J. Biol. Chem. 152:511. 1944.

5. Spackman, D. H., W. H. Stein, and S. Moore. Anal. Chem. 30:1185. 1958.

6. Wall, M. E. and E. G. Kelly. Ind. and Eng. Chem. 15:18. 1943.

7. Association of Official Agricultural Chemists, Methods of Analysis, Washington, 8th Edition. 1955.