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IMPROVEMENT OF GRAIN AND VEGETABLE AMARANTH IN MALAWI

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Table of Contents

Executive Summary

Section 1

A.- Research Objectives

B.- Research Accomplishments

1.- Research in Malawi

2.- Research in Israel

C.- Scientific impact of collaboration

Section 2

A.- Managerial Issues and Budget

B.- Collaboration

Executive Summary

During the third year of the project, research in Malawi continued in the agronomic evaluation of grain amaranth yield in three contrasting agroecological regions. Ten cultivars, including locally grown cultivars, short cycle cultivars and cultivars sent from Israel, that showed good baking characteristics were studied in field experiments.
Most cultivars performed well under rain-fed conditions in terms of grain yield. Grain amaranth seems to be a promising grain crop for certain areas of Malawi. Under rain fed conditions several cultivars reached yields characteristics to cultivation areas of amaranth in USA. This was particularly true at the Bvumwe Research Station that probably represents the best agroclimatic conditions for grain amaranth cultivation in Malawi. Short-cycle amaranth types (e.g. the *A. hyp x A. hyb* (14) hybrid cultivar) can be selected for earliness if terminal stress in the region is frequent. Differences in temperature between regions were a main factor limiting production.

In Israel, field work was centered in production of grain for baking experiments and for leaf production for nutrition experiments. Main efforts were concentrated in the use of air-dried amaranth leaves as a source of iron. The availability of Iron from air-dried amaranth leaves was determined by an *in vitro* technique. Wide variations in the total iron, dialyzable iron and percentage of total iron bioavailability were observed within and between varieties and species. The effects of air dehydration to about 15% residual moisture and storage at ambient temperature (20-25^0 C) in airtight plastic bags for 3 months, on iron and vitamin C in particular, were studied. The implications involved in rating amaranth leaves as a source of iron on the basis of these three indices are discussed. Bioavailable iron, rather than total iron, is probably the appropriate index for ranking the leaves as a source for iron. Our preliminary data suggest that the practice of storing air-dried amaranth leaves is detrimental to iron bioavailability and to vitamin C content. Consequently, despite the high total iron and vitamin C in fresh air-dried leaves, stored air-dried leaves might not be considered as good source for these two nutrients as the fresh leaves.
Studies on flour mixtures, including amaranth, for bread baking were continued and results are currently under evaluation. The will be presented the next annual report.

Section I

A.- Research objectives

The general aims of this 4 year project are to diversify food supply by improving cultivation of amaranth as a dual source for:

a) high quality grain, rich in proteins and lysine,

b) leafy vegetable, rich in vitamins and minerals,

which will be available throughout the year to improve the population diet.

Specific research objectives are:

1.- To select superior amaranth cultivars for leafy vegetable and grain production in different agroecological regions of Malawi.

2.- To study the adaptability of grain amaranth to drought

3.- To develop methods and protocols for the utilization of leaf and grain amaranth in the preparation of local foods.

4.- To develop optimal wheat-amaranth flour mixtures and procedures for incorporation in bread making.

5.- To develop appropriate agronomic practice packages for the production of selected leafy and grain amaranth varieties for smallholder use.

B.- Research accomplishments

1- Research in Malawi

During the 1998/9 crop season, the performance of 10 different cultivars of grain amaranth was evaluated in three contrasting agroecological environments, under rain fed conditions, at the Bunda, Bvumbwe and Kasinthula Research Stations.

Experiment design

Direct sowing was performed shortly after the first reliable rains started, during December 1998 at Bunda, late January at Bvumbwe and February 1999 in Kasinthula. The differences in sowing date were due to the patterns of rains at the three sites.

Experiment had a randomized complete block design in the three sites. The 10 amaranth cultivars were the treatments and were randomly allocated to the experimental plots in each block, with 4 replicates. The experimental plot comprised 6 ridges 6 m long, spaced 60 cm apart. The net plot (area sampled) was 4m long taking the 4 middle ridges.

Plots were heavily seeded to avoid transplanting in case of seedling death that may cause lack of uniformity in plant growth. Spacing between stations was 30 cm and seeds were sown at 1 cm depth. Fertilizer (23:21:20-4S) was applied once at a rate of 25 kg/ ha of N, by splitting the ridge at first thinning. Thinning in Bunda and Bvumwe was done several times to keep three seedlings per station. Plant density was assumed to be 166.667 plants/ha. At Kasinthula, however, high rainfall intensity damaged the young seedlings and plant density was lower. No transplanting was performed due to lack of seedlings.

Data collection and analysis:

Ten plants were collected randomly in each experimental plot. At harvest stems, leaves, chaff and seed were separated from each plant. In addition samples of 200 seeds and 10 leaf disks were taken. Fresh weight was recorded, and dry weight was determined after oven drying at 85-90°C for 24 hs. Values were used to calculate total

weight for each experimental plot. Grain yield was adjusted to 14% moisture content. Data was subjected to analysis of variance (ANOVA) using the Matac package. Mean separation was by Duncan's Multiple Range Test where ANOVA indicated significant differences among cultivars. Harvest Index and Leaf Stem Ratio indexes were calculated.

Results

Phenological stages

Rate of plant development in all cultivars, as expressed by days to 50% flowering, days to maturity and duration of grain filling, differed among Research Stations (Table 1). Development was faster at Kasinthula (200 m, hot and dry conditions), followed by Bunda (1100 m, moderate conditions) and Bvumbwe (cooler conditions). Differences among stations in average-days to 50% flowering were relatively small (38 to 49 days) compared to differences in average-days to maturity (93-124 days). Particularly large were the differences among sites in the length of the grain filling period (19-64 days) that can affect grain yield. Plant growth was slower at Bvumbwe, the site with the lowest temperature during the crop season. At Bvumbwe all cultivars had the latest date of flowering and harvest, as well as the longest grain filling period. Between Bunda and Kasinthula differences were smaller. Nevertheless, phenological stages tend to be earlier at Kasinthule (the warmest site).

Clear differences were observed among cultivars in the timing of phenological stages and these differences recurred in the three Research Stations (Table 1). The *A. hyp x A. hyb* hybrids (14 & 15) were sort cycle types, with early flowering (23, 23 and 34 days from sowing vs. 36-42, 39-52 and 47-56 days in the other types at Bunda, Kasinthula and Bvumbwe, respectively. Their earliness is also reflected in the shorter duration of grain filling period: 10, 13 and 31 days vs. 19-25, 24-36 and 70-73 days in the late types, at Kasinthula, Bunda and Bvumbwe, repectively. Grain harvest was also earlier in the short cycle types: 75, 87 and 109 day from sowing vs. 93-101, 102-109 and 116-133 days in the late types, at Kasinthula, Bunda and Bvumbwe, repectively. *A. cruentus* (Bunda) and *A. tricolor* (Bunda) were consistently the latest cultivars in all stations.

Table 1: Phenological characteristics of different amaranth cultivars at Bunda, Bvumbwe and Kasinthula Research Stations in Malawi under rainfed conditions.

Cultivar	Days to 50% flowering			Days to maturity			Duration grain filling		
	Bund	**Bvum**	**Kasi**	**Bund**	**Bvum**	**Kasi**	**Bund**	**Bvum**	**Kasi**
A. hypochondriacus (Bunda)	36	47	39	109	116	97	25	70	11
A. tricolor (Bunda)	42	53	52	107	134	100	36	73	23
A. cruentus R-158 (2)	43	48	44	107	132	98	24	72	19
A. cruentus R-283 (11)	39	53	44	102	130	97	34	72	25
A. cruentus R-109 (7)	43	56	48	106	119	93	24	70	22
A. cruentus R-736 (12)	42	48	44	109	133	101	36	73	24
A. cruentus(Bunda)	42	56	52	102	133	100	36	75	22
A. hyp x A. hybridus (14)	23	34	23	87	109	75	13	31	10
A. hyp x A. hybridus (15	23	34	23	87	109	75	13	31	10
A. montegazinus (10)	42	56	48	105	125	95	28	73	23
Average	**38**	**49**	**42**	**102**	**124**	**93**	**27**	**64**	**19**

Plant size at harvest

Plant size at harvest was measured by biomass (total dry weight/ha), plant height and stem diameter at the base of the plant (Table 2).

Average plant height was quite similar in the three sites (108-116 cm). Height of the short-cycle cultivars 14 and 15 was much lower than the in the other cultivars (ca. 60 vs. 92-151 cm in Bunda and Bvumbwe). This difference between cultivars was larger at Kasinthula, where plants of the short-cycle cultivars were very small (20-24 cm vs. 94-143 cm).

Stem diameter at the base of the plant showed similar trends to plant height (Table 2). Differences between sites were relatively small (14-17 mm) and short-cycle cultivars had a smaller stem diameter than the taller cultivars (6-10 vs. 15-22 mm).

Average total plant biomass per unit area was similar in Bunda and Kasinthula, but ca. 44% smaller at Kasinthula (Table 2). The short-cycle cultivars had the smallest biomass and *A. cruentus* R-109 reached the largest biomass. Differences among cultivars were not significant at Bvumbwe.

Grain yield and HI

Significant differences in grain yield were found among sites: 1.88, 1.31 and 0.45 Ton/ha at Bvumbwe, Bunda and Kasinthula, respectively (Table 3). Highest yields were consistently observed in all cultivars at Bvumbwe (the coolest site). On the other hand, yield was particularly low at Kasinthula (the warmer and driest site), 24-34% compared to the other sites.

Cultivars differed in their yield response to agroclimatic conditions in the different regions were the Research Stations are located. At Bunda, *A. cruentus* R-109 had the highest yield (1.76 Ton/ha) that was significantly greater than in *A. cruentus* R-283 and *A. hyp x A. hyb* (14) (0.94 Ton/ha). At Bvumbwe, *A. hypochondriacus, A. montegazinus* and *A. hyp x A. hyb* (14) showed the highest yields (2.27-2.11 Ton/ha) and *A. cruentus* R-158 and *A. cruentus* R-283 the lowest yields (ca. 1.54 Ton/ha). At Kasinthula, *A. cruentus* R-736 was the highest yielding cultivar (0.74 Ton/ha).

Table 2: Plant characteristics of different amaranth cultivars at Bunda, Bvumbwe and Kasinthula Research Stations in Malawi under rainfed conditions.

Cultivar	Plant biomass (kg/ha)			Plant height (cm)			Stem diameter (mm)		
	Bund	**Bvu**	**Kasi**	**Bund**	**Bvum**	**Kasi**	**Bund**	**Bvum**	**Kasi**
A. hypochondriacus (Bunda)	6116bc	6578	3970 a	144	92	94	14	13	16
A. tricolor (Bunda)	7311 b	5448	4037 a	127	116	140	16	16	19
A. cruentus R-158 (2)	6778 b	4815	4066 a	126	118	139	15	14	20
A. cruentus R-283 (11)	4919cd	4977	4181 a	115	106	131	15	16	22
A. cruentus R-109 (7)	9039 a	6070	4422 a	151	123	130	20	16	18
A. cruentus R-736 (12)	7364 b	5766	3766 a	131	135	127	16	18	22
A. cruentus (Bunda)	5856bc	5703	3469 ab	147	132	128	16	15	20
A. hyp x A. hybridus (14)	3580dc	4464	1589 bc	68	57	20	10	9	6
A. hyp x A. hybridus (15)	3018 c	4094	2151 bc	57	57	24	10	9	10
A. montegazinus (10)	6660 b	6982	3845 ab	122	136	143	16	18	20
Average	**6064**	**5490**	**3550**	**116**	**107**	**108**	**15**	**14**	**17**
CV %	**28.9**	**10.7**	**33.1**	**12.8**	**10.0**	**4.8**	**11.1**	**16.3**	**10.3**
LSD 5%	**2544**	**805**	**1537**	**21.6**	**21.6**	**7.6**	**2**	**4**	**2**

Table 3: Grain yield and Harvest Index of different amaranth cultivars at Bunda, Bvumbwe and Kasinthula Research Stations in Malawi under rainfed conditions.

Cultivar	Yield (kg/ha)			HI (%)		
	Bunda	Bvumwe	Kasinth	Bunda	Bvumwe	Kasinth
A. hypochondriacus (Bunda)	1280 ab	2266 a	298 c	21	34	8
A. tricolor (Bunda)	1483 ab	1875 ab	557 ab	21	34	14
A. cruentus R-158 (2)	1486 ab	1510 b	562 ab	22	31	14
A. cruentus R-283 (11)	940 b	1589 b	500 abc	19	32	12
A. cruentus R-109 (7)	1763 a	1745 ab	448 abc	19	29	12
A. cruentus R-736 (12)	1357 ab	1823 ab	674 a	18	32	16
A. cruentus (Bunda)	1213 ab	1849 ab	576 a	21	32	17
A. hyp x A. hyb (14)	1159 ab	2109 a	273 c	32	47	17
A. hyp x A. hyb (15)	937 b	1823 ab	339 bc	31	45	16
A. montegazinus (10)	1506 ab	2240 a	287 c	23	32	7
Average	**1312**	**1883**	**451**	**22.7**	**34.8**	**13.3**
CV (%)	**29.7**	**18.8**	**29.9**			
LSD 5%	**566**	**513**	**206**			

The average harvest index (HI) also varied between sites (Table 3): 34.8, 22.7 and 13.3 at Bvumbwe, Bunda and Kasinthula, respectively. The short cycle cultivars showed the highest HI at Bvumbwe and Bunda (46 and 32 % vs 18-23% and 31-34%). However their HI at Kasinthula were in the same range with the other cultivars (16-17% vs. 7-17%).

The relatively similar values of HI within each group of cultivars (short-cycle vs. taller cultivars) in each site, indicate that differences in yield among cultivars within each group are related to total plant growth. Therefore, differences among cultivars in their growth (accumulated biomass) due to different agroclimatic conditions dictate the differences in yield. Yield is much less affected by variation in HI within each group of cultivars. On the other hand, allocation to seed production is much larger in the short-cycle cultivars, as shown by their greater HI. It is worthwhile to note that at Bvumbwe, the short-cycle *A. hyp x A. hyb* (14) reached yields in the range of the taller cultivars with larger biomass, due to its greater relative allocation to seed production (HI).

Seed weight

Seed weight at Kasinthula was 15-25% higher than in the other sites (Table 4) despite the lower grain yield at this site (Table 3). This suggest either a n enhancement of individual seed growth by the warmer conditions at Kasinthula or a negative correlation between amount of developing seeds and seed size.

A. montegazinus was the cultivar with the heavier seeds in the three sites. On the other hand, variation among cultivars in seed weight was relatively small (if the two cultivars with the heaviest seeds at Kasinthula are not compared), with ca. 20 % range variation around a general average of 780 g/1000 seeds.

Table 4: Seed dry weight (g/1000 seeds) of different amaranth cultivars at Bunda, Bvumbwe and Kasinthula Research Stations in Malawi under rainfed conditions.

Cultivar	Seed dry weight (g/1000 seeds)			
	Bunda	**Bvumbwe**	**Kasinthula**	**Average**
A. hypochondriacus (Bunda)	765 b	745 a	925 b	810
A. tricolor (Bunda)	830 a	725 a	815 b	790
A. *cruentus* R-158 (2)	740 b	675 a	1240 a	885
A. cruentus R-283 (11)	790 b	690 a	950 b	810
A. cruentus R-109 (7)	800 b	700 a	765 b	755
A. cruentus R-736 (12)	950 a	625 b	875 b	755
A. cruentus (Bunda)	815 a	740 a	675 a	925
A. hyp x A. hybridus (14)	725 b	765 a	800 b	765
A. hyp x A. hybridus (15)	740 b	715 a	915 b	790
A. montegazinus (10)	950 a	765 a	1065 b	745
Average	**790**	**715**	**905**	**803**
CV(%)	**12**	**12**	**21**	**-**
LSD 5%	**27**	**25**	**55**	**-**

Conclusions

Grain amaranth seems to be a promising grain crop for certain areas of Malawi. Under rain fed conditions several cultivars reached yields characteristics to cultivation areas in USA (1.8-3.0 Ton/ha). This was particularly true at the Bvumwe Research Station that probably represents the best agroclimatic conditions for grain amaranth cultivation in Malawi. Short-cycle amaranth types (e.g. the *A. hyp x A. hyb* (14) hybrid cultivar) can be selected for earliness if terminal stress in the region is frequent. In addition, increasing planting density of the short-cycle cultivars can also result in increased yield. However, if mid season stress is frequent, short –cycle cultivars will be at a disadvantage, since their recover after drought is poorer. This probably explains their poor performance at Kasinthula. Late maturing cultivars usually exhibit compensatory growth at a later stage of development if exposed to mid season stress.

2- Research in Israel

Main goals of the research in Israel during this year were:

One- To study air dried amaranth leaves as a source of iron in nutrition.

Two- To grow plants in the field to provide grain for the bread making studies.

Three- To grow plants during the crop season in the field and under controlled environmental conditions all year around to provide fresh leaves for the nutrition studies.

2.1- AIR DRIED AMARANTH LEAVES AS A SOURCE OF IRON

Of the numerous species consumed as leaf vegetable in east Africa, the Amaranth is widely used. Its leaves are common as cooked vegetable in the drier and warmer regions. The leaves are frequently dried for use during seasons when vegetables are scarce. The inclusion of dried leaves in the local diet is an important cultural adaptation, supplementing nutritional needs.

The leaves have been noted to be exceptionally high in carotenoids, protein, iron, calcium and vitamin C. The latter is an important water soluble antioxidant and plays significant role in maintaining the preferred oxidation-reduction potential in tissues (1). The presence of vitamin C and Fe in the leaves has an advantage, the former provides protective conditions for the latter, a synergism which promotes Fe absorbability (2).

In Malawi Amaranth leaves are sun- and shade- dried on mats and stored in sacks or clay pots. In case of the latter, water is added, the content boiled for about half an hour, the resulting mixture is than spread to dry in the sun, packed in leaves and hung for storage. The dry product is added to soup, to other vegetable and mixed dishes (3).

Wilting, vapor and storage temperature, as well as the effects during traditional drying, all have a negative affect on the nutritional qualities of amaranth leaves.

The purpose of this study was to learn the dynamics of the change in vitamin C content and iron bioavailability from dried leaves over 3 and 6 months storage.

Methods

The species used for these studies were *A. tricolor* (a leafy cv. from Germany source) and *A. hypochondriacus x A. hybridus* (cv. 15). The leaves for this study were produced during the crop season in the Faculty farm at Rehovot, and out of season in a Phytotron (28/22°C). *In vitro* studies were conducted on air-dried leaves.

Leaf preparation: the freshly cut leaves were rinsed with double distilled water, lightly dried between paper towels, placed in single layer on a plastic sheet, outdoor (Ave. day tem. 25-30^0 C, night Ave. tem 20-25^0 C) under a black net. Drying was terminated when the leaves maintained a constant weight, between 72 and 86 hours. The dry leaves were stored in airtight plastic bags, protected from light, at ambient temperature (20-25^{0}C). Samples were ground in an electric coffee grinder immediately preceding each analysis.

Sample analysis: The samples were analyzed in duplicates for protein by the Micro-Keldjhal method; vitamin C was analyzed, in triplicates, by the reduction of molybdophosphoric acid by ascorbic acid. Concentration of the resulting compound, phosphomolybdenum, was determined reflectrometrically (Reflectoquant ascorbic acid test, Merck); total iron, dialyzable and bioavailable iron was estimated using the *in vitro* assay described by Kapsokefalou (4) adapted by Rangarjan (5), with one additional change, we have measured the iron in each assay by ICP rather than colorimetrically; minerals were analyzed by ICP.

Results

Protein concentration, 20-35% in dry leaves (3 – 5.2% wet weight) in the two amaranth varieties is in agreement with values reported in the literature for amaranth leaves (6). At the same time, the about 50% higher protein concentration in the *A. tricolor than A. hypochondriacus* is of much interest and deserves further study of this cultivar (Table 1).

Table 1: Nutritional Composition: Protein, Minerals (Ca, Zn, Cu) and NO_3, of fresh Air-dried Amaranth (*A. hypochondriacs* and *A. tricolor*). Leaves, grown in the field and the Phytotron.

Species	Dry Weight %	Protein % dry wt	Ca mg/g dry wt	Zn ug/g dry wt	Cu ug/g dry wt	NO3 mg/g dry wt
A. hypochondriacus	**14 - 16**	**20 - 24**	**19 - 44**	**62 - 73**	**46 – 78**	**1.1 - 3**
Mean	[15]	[21]	[26]	[65]	[57]	[2]
A. tricolor	**14 - 15**	**33 - 36**	**20 - 35**	**60 - 100**	**55 – 144**	**8.1 - 19**
Mean	[14]	[35]	[28]	[73]	[90]	[14]

N = 6; to convert to 100g fresh weight multiply the value by 15.

Iron: Table 2a (leaves grown in the greenhouse) and 2b (leaves grown in the field) show that significant differences ($p < 0.01$) were detected among the two species tested for total, dialyzable and percent bioavailable Fe. Total Fe content of leaves ranged from 200 to 269 ppm, 250 to 430 ppm and 45.5 to 56.6 ppm, 84 to 110 ppm for *A. hypocondriacus* and *A. tricolor*, grown in the greenhouse or the field, respectively. *A. tricolor* had in all cases almost twice the total Fe content than *A. hypochondriacus.*

In vitro digestion provided estimated bioavailable Fe (dialyzable). The values of fresh air-dried leaves, from both species, is in close proximity (6.5 and 5.5 ppm), but expressed as percent bio-available Fe, *A. hypochondriacus* provides significantly more Fe ($p<0.01$), twice the amount than *A. tricolor*. There was no correlation between total and dialyzable Fe in greenhouse grown leaves

The Fe values for three-month old air-dried leaves (stored in airtight dark boxed in room temperature) are shown in Tab. 2a. Total Fe was not significantly different from the fresh values, but both dialyzable and bioavailable Fe were significantly ($p<0.01$) reduced. For *A. hypochondriacus*, dialyzable Fe decreased from an average value of 6.5 to 1.7 ppm, and the bioavailable value decreased from 12.7 to 4.1 ppm; for *A. tricolor* the decrease was from 5.5 to 3 and from 5.7 to 3.2 ppm, respectively.

Table 2: Total Iron, Dialyzable Iron and Bioavailable Iron in fresh air-dried and 3 months stored air-dried *A. hypochondriacus* and *A. tricolor* leaves grown in the Phytotron (2a), and air-dried one year old leaves, grown in the field (2b).

2a

	AIR-DRIED LEAVES					
	FRESH			THREE MONTHS STORAGE		
Species	Fe Total ug/	Fe Dialyzable g dwt	Fe Bioavailable %	Fe Total ug/	Fe Dialyzable g dwt	Fe Bioavailable %
A. hypochondriacus	45.5 - 56.6	4.7 - 8.3	10.3 - 14.7	35.5 - 46.6	0.9 - 2.5	2.5 - 5.4
Mean	[51]	[6.5]	[12.7]	[41]	[1.7]	[4.1]
A. tricolor	84 - 110	4 - 6.9	4.8 - 6.3	71 - 116	1.2 - 4.8	1.7 - 4.1
Mean	[97]	[5.7]	[5.7]	[3.5]	[3]	[3.2]

N=6;

2b

	AIR DRIED LEAVES		
	ONE YEAR STORAGE		
	Fe Total ug/	Fe Dialyzable g dry wt	Fe Bioavailable %
Species			
A. hypochondriacus	200 - 269	0.8 - 1.9	0.4 - 0.7
Mean	[234]	[1.4]	[0.5]
A. tricolor	250 - 430	0.9 - 2.5	0.4 - 0.6
Mean	[340]	[1.7]	[0.5]

N=3; to convert to 100g wet weight multiply the value by 15.

The very low values for dialyzable and bioavailable Fe found in the one-year-old samples of the field grown leaves (Tab. 2b), strongly suggest continuous decrease in Fe bioavailability over time.

Vitamin C content showed some variation (Tab. 3), but was not different between or within species and variety of amaranth. Fresh air-dried leaves content of ascorbic acid was 78 – 105 mg%, wet weight. A substantial decrease was found in vitamin C levels following 3-month storage (21–38% wet weight). Further deterioration during one-year storage was detected, as values were below 20 mg% in the one sample on hand (data not shown). Data reported in the literature also shows very large variation in vitamin C content both within and between species (62 to 288 mg% for leaves from grain varieties and 62 to 209 mg% for leaves from vegetable variety (7).

Table 3: Ascorbic Acid levels in fresh air-dried and 3 months stored air-dried *A. hypochondriacus* and *A. tricolor* leaves grown in the Phytotron.

	VITAMIN C (mg/g dwt) air- dried	
	FRESH	**3 MONTHS STORAGE**
Species		
A. hypochondriacs	**6.3 - 7**	**1.5 - 2.1**
Mean	[6.7]	[1.8]
A. tricolor	**5.2 - 7.3**	**1.4 - 2.5**
Mean	[6.2]	[2]

N=6; to express for 100g multiply value by 15.

Discussion

This study represents the first attempt to determine the effect of air-drying and long term storage of amaranth leaves on iron bioavailability. The species selected for the study were those popular in home-gardens and which are consumed by both rural and urban population in Malawi.

In general it was observed, both by others (7) and us that vegetable type amaranth had lower amounts of vitamin C than grain varieties. The content of vitamin C decreased significantly due to air drying and further due to storage (95 to 20 mg/100g wet weight, respectively). Since the daily requirement for vitamin C for an adult is 60 mg per day, 25 – 100 g of fresh leaf (depending on variety and cultivar)

would supply this amount. Unfortunately this will not be the case once the leaves have been air-dried.

Vitamin C is known to act as an anti-oxidant, which among its other functions protects iron in the leaves from oxidizing. It also results in chelation with iron, which increases the absorbability of the iron from the complex. The presence of vitamin C to iron bioavailability is therefore very important, and is expected to contribute to the percent of Fe absorbed.

The significant differences observed for total and bioavailable (dialyzable) Fe between the two species studied (A. hypochondriacus and A. tricolor) indicates the importance of the species consumed, should amaranth leaves be considered a significant contributor to Fe status in the body. It also may indicate potential genetic variability that may be utilized to improve the Fe nutritional quality and enhance its bioavailability. A. tricolor which accumulated high levels of Fe is used predominantly for its leaves, while the A. hypochondriacus, which accumulated lower levels of total Fe, is predominantly cultivated for grain. We have interest in the latter since the idea of a dual use of the plant (for seeds and leaves) could be attractive in cases of decreased land and/or water availability.

Field grown amaranth plants accumulated 2 to 5 times the amount of total iron than the greenhouse grown plants (Tab. 2a & b). This has been previously reported (5), and to-date no clear explanation has been given for this phenomena. The relative differences in total Fe among the amaranth species tested were maintained between the two environmental modalities. The effects of secondary compounds or differences in the Fe form in the field-grown leaves may affect the total and available Fe. The difference in soil pH, the light intensity and light quality of the controlled environment may be more important than soil Fe concentration in explaining the differences observed in total Fe accumulation from plants grown in field environment (8).

Under optimal conditions the availability of iron in plant foods is quite poor. It ranges from less than one percent to 10 %. The term "bioavailability" describes the percentage of iron in the food that is absorbed and used for physiological purposes.

This report provides for the first time data on the bioavailability of Fe from air-dried stored amaranth leaves. The percent bioavailable Fe (dialyzable, 6-13% in the fresh air-dried samples) was in both growth environments, field and greenhouse, in our study, within the range reported in the literature, but differed significantly

between species. The one other study to test bioavailability of Fe from amaranth leaves (5) reported a ten fold difference between field and greenhouse values, but we can not compare our data to those results since the leaves in the latter study were lyophilized while those in our study were air dried.

The decrease in bioavailability of Fe after three-month storage, from 13 to 4% in A. hypochondriacus, and from 6 to 3% for A. tricolor (Tab.2a) might be due to difference in dry leaf structure and nutrient survival (mainly protective anti oxidants like ascorbic acid), change in pH, and other yet unknown causes which could contribute to the reduction in Fe solubility. Chelation of Fe to oxalic or phytic acids could not be responsible for decreased bioavailability since their levels in amaranth leaves were reported to be negligible (9). The almost negligible bioavailability of Fe in year-old dry-leaf samples confirms the deterioration of the sample, which probably results in increase of non-soluble Fe compounds. No reported values for Fe bioavailability from air-dried stored leaves have been found in the literature.

Any assay of Fe bioavailability only provides a relative measure of nutritional quality among the tested foods. Actual bioavailability of Fe will vary based upon the diverse interactions of total diet components and the iron status of the subject tested. In view of the fact that traditional practice of seasonal storage of leafy vegetable is by air-drying, in Africa in general and Malawi in particular, and considering the centrality of this product in the diet during the dry season, the actual level of bioavailability of nutrients is of importance. The use of the total Fe analysis for screening for high bioavailability is emerging to be incorrect and misleading. The traditional data of total iron will have to be combined with *in vitro* dialyzable and soluble Fe analysis, for more accurate rating of foods as sources for iron.

In conclusion, from the very preliminary data presented here we would suggest that total iron should not be the sole indicator for grading the quality of air-dried amaranth leaves as a source for iron. Supplementing the leaves during the dehydration process with a source of vitamin C might be advantageous during storage.

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C.- Scientific impact of collaboration

Scientists from Malawi and Irael collaborated in the planning of field experiments. Seed of promising cultivars was exchanged for grain and leaf production in field experiments. Formulations for bread making and use of leaves for human nutrition are being elaborated and transferred to Malawi, for use with locally produced grain and leaves.

Section II

A.- Managerial and Budget Items

No special managerial items emerged during the third year of the project. Experiments were performed as planned.

B.- Collaboration

Main collaboration during this year was based on exchange of seed material, consultation for nutrition studies and transfer of protocols.