C16-025
PN-ACM-363

CDR Program – MOU – 96 C16-025

Final Report: 1996-2000

IMPROVEMENT OF GRAIN AND VEGETABLE AMARANTH IN MALAWI

Submitted by:

Principal Investigator in Israel:	**Collaborating Investigator in Malawi:**
Prof. Jaime Kigel Department of Agricultural Botany Faculty of Agriculture Hebrew University of Jerusalem P.O.Box 12, Rehovot 76100, Israel Tel: 972-8-9489916 Fax: 972-8-9467763 e-mail: kigel@agri.huji.ac.il	Dr. M. Kwapata Bunda College of Agriculture University of Malawi Lilongwe, P.O. Box 219 Malawi Tel: 265-277-222 Fax: 265-277-364
Co-Investigators in Israel:	**Co-Investigator in Malawi:**
Dr. Noemi Trostler Prof. Sam Saguy Dep. Biochemistry and Human Nutrition Faculty of Agriculture Hebrew University of Jerusalem P.O.Box 12, Rehovot 76100, Israel	Dr. M. Ngwira Bunda College of Agriculture Lilongwe, P.O. Box 219 Malawi

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Executive Summary

In this Final Report summarizing 4 years of research, we present the main accomplishments achieved by the four research groups involved in the Amaranth project: 1- Agrobiology and grain production (Malawi); 2- Ecophysiology of the crop and adaptation to drought (Israel); 3- Use of amaranth leaves as a source of vitamins and minerals in human nutrition (Israel); 4- Assessment of amaranth for replacing flour for bread baking (Israel). Results obtained during the 4th year of the project, as well as technologies developed in relation to nutrition value and baking procedures using vegetable and grain amaranth are presented in detail. This approach and the fact that results of 4 research working on different subjects is presented in the Final Report explains its length. **A summary of achievements and of their possible impact in Malawi is presented in the Section 1C- Scientific impact of collaboration.**

During the **4th year of the project** (year 2000), **research in Malawi** continued in two main directions: a) the agronomic evaluation of grain amaranth yield in three contrasting agroecological regions, and b) evaluation of yield responses to irrigation during the dry season. Ten cultivars, including locally grown cultivars, short cycle cultivars and cultivars sent from Israel, that showed good baking characteristics were studied in these field experiments. Several cultivars, including two cultivars from Malawi, performed well under rain-fed conditions in terms of grain yield, and consistently showed good yields in two consecutive, contrasting years. Drought during the flowering or the grain filling period reduced yield. However, in some cultivars this reduction was relatively small, expressing a higher yield stability under drought. Grain amaranth seems to be a promising grain crop for certain areas of Malawi. Under rain fed conditions the promising cultivars reached yields characteristics to cultivation areas of amaranth in USA. This was particularly true at the Bunda and Bvumbwe Research Stations that probably represent 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 Israel, fieldwork was centered in production of grain for baking experiments and for leaf production for nutrition experiments. Studies on flour mixtures, including amaranth, for bread baking were continued. Experiments to increase the level of amaranth beyond 30% were not successful. Thus, the work during the year 2000 was focused on preparing other products containing amaranth at levels higher than 30%, such as waffles and crackers, since they are not based on a leavened structure and gluten. Such products are wafers (or biscuits), that may contain up to 100% amaranth and could also be enriched with amaranth leaves. It was shown that wafers could be baked with amaranth as sole source of flour, or in addition to wheat flour. The wafers have high protein content and could be stored with a relatively long shelf life when water activity is below 0.7. These products no not need special and expensive equipment, thus allowing direct technology transfer. Commercial production will require optimization for the local pallet and market needs.

Section I

A.- Research objectives

The general aims of this 4 year project were 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 were:

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

Experimental sites: Field experiments were performed at three sites in Malawi: at Bunda College and at the Bvumbwe and Kasinthula Agricultural Research Stations, that represent the main agroecological areas of production in Malawi. Data on location, altitude and climate during crop season are presented in Table 1. Bunda and Bvumbwe sites are located on the high plateau, at about 1100-1200 m a.s.l. They have a tropical seasonal rainfall. In Bvumbwe there are frequent mists (Chiperoni winds), occasional frosts and light rains.

Table 1.- Geographic and climatological data for Bunda, Bvumbwe and Kasinthula Research Stations. Mean monthly temperature and monthly rainfall. Crop seasons 1998/1999 and 1999/2000.

	Bunda			**Bvumbwe**			**Kasinthula**		
Altitude (a.s.l.)	1100 m			1250 m			70 m		
Latitude	14° 11' S			15° 55' S			16° 00' S		
Longitude	33° 46' E			35° 04' E			34° 05' E		
Month	**Temp. (°C)**		**Rain**	**Temp. (°C)**		**Rain**	**Temp. (°C)**		**Rain**
	Max.	**Min.**	**(mm)**	**Max.**	**Min.**	**(mm)**	**Max.**	**Min.**	**(mm)**
1998/1999									
December	33.0	17.6	126						
January	27.9	17.3	229	25.4	17.8	588			
February	26.0	17.6	144	25.9	17.0	359	32.2	17.7	204
March	25.4	16.6	378	24.7	17.0	274	31.9	17.3	150
April	26.5	15.6	29	23.0	15.6	96	30.4	16.1	61
May				19.8	13.4	16	29.5	12.8	2
Total rainfall			905			1333			417
1999/2000									
December	32.0	17.5	99						
January	30.0	17.4	228	27.8	17.9	68			
February	29.4	16.3	133	25.8	17.9	346	29.2	22.1	146
March	27.6	14.5	51	26.9	18.4	236	26.5	22.0	102
April	24.8	14.8	0	25.8	17.9	104	23.9	21.0	23
May							23.3	19.6	9
Total rainfall			510			753			281

Field Experiments

The soil at Bunda is sandy clay loam and at Bvumbwe dominant soils are red latosols and dark red sandy clay or clay loam. Kasinthula is located at the lowland at the lower Shire Valley. The site is usually hot and dry. Soils are moderately coarse fine textured alfisols, with a sandy clay loam in surface soil, moderately drained and with a water table at 6.5 m from ground surface.

Rain-Field Trials

During the 1997 and 1998 crop seasons preliminary experiments were carried out in the different sites, to calibrate agrotechnology aspects of cultivation (fertilization, plant spacing, harvesting methods, first variety screening, etc), as presented in first two annual reports. Main rain-fed trials to study promising cultivars obtained in the preliminary screening were performed during the 1999 (3rd annual report) and 2000 (this report) crop seasons. For comparative purposes and to show variation in interannual stability of yield, results from both seasons are presented in this Final report. The performance of 10 different cultivars of grain amaranth was evaluated in the above described 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, usually during December at Bunda, late January at Bvumbwe and February in Kasinthula. The differences in sowing date were due to the patterns of rains at the three sites.
Experiments 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: For analysis of yield components, 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 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 2). 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 Kasinthula (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 2). 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, respectively. 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, respectively. *A. cruentus* (Bunda) and *A. tricolor* (Bunda) were consistently the latest cultivars in all stations.

Table 2: 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**		
	Bun	**Bvu**	**Kas**	**Bun**	**Bvu**	**Kas**	**Bun**	**Bvu**	**Kas**
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: Average plant height was quite similar in the three sites (108-116 cm) (Data in 3rd Report). 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).

Grain yield and HI: Significant differences in grain yield were found among sites: 1.42, 1.36 and 0.46 Ton/ha at Bvumbwe, Bunda and Kasinthula, respectively (averages for 1999 and 2000 crop seasons, Table 3). Overall yields were similar at Bunda and Bvumbwe, but particularly lower (by ca. 64%) at Kasinthula (the warmer and driest site). ANOVA showed that cultivar, location and cultivar x location interaction had significant effect on grain yield ($p<0.05$)(Table 4). Cultivars differed in their yield response to agroclimatic conditions in the different regions. At Bunda, *A. cruentus* R-109 had the highest yield (2.21 Ton/ha), followed by *A. hypochondriacus* (Bunda) (1.87 Ton/ha) and *A. cruentus* (Bunda) (1.81 Ton/ha). At Bvumbwe, *A. hypochondriacus* (Bunda) and *A. montegazinus* showed the highest yields (1.83 and 1.60 Ton/ha). At Kasinthula, *A. cruentus* (Bunda) and *A. cruentus* K-736 were the highest yielding cultivar (ca 0.71 Ton/ha). Thus, the local cultivars of *A. hypochondriacus* and *A. cruentus* from Bunda were the best adapted ones for the different agroecological regions in Malawi. The lower yields in the short cycle hybrid cultivars 14 and 15 compared to the taller accessions ($p<0.05$) were probably due to relatively low plant density, since these are smaller plants.

The average harvest index (HI) also varied between sites (data in 3rd Report): 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 and. 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.

Seed weight: Seed weight at Kasinthula was 15-25% higher than in the other sites, despite the lower grain yield at this site (Table 5). This suggests either an 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 heaviest 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 3.- Grain yield of different amaranth cultivars at Bunda, Bvumbwe and Kasinthula Research Stations in Malawi under rainfed conditions. Crop seasons 1998/9 and 1999/2000.

Cultivar	Yield (Ton/ha)								
	1998/9			1999/2000			Average		
	Bun	Bvu	Kas	Bun	Bvu	Kas	Bun	Bvu	Kas
A. hypochondriacus (Bunda)	1.32	2.02	0.35	2.42	1.63	0.35	**1.87**	**1.83**	**0.35**
A. tricolor (Bunda)	1.43	1.32	0.54	1.17	1.16	0.54	**1.30**	**1.24**	**0.58**
A. cruentus R-158 (2)	1.36	1.12	0.43	1.60	1.38	0.43	**1.49**	**1.25**	**0.54**
A. cruentus R-283 (11)	0.90	1.30	0.65	0.89	1.25	0.31	**0.90**	**1.27**	**0.48**
A. cruentus R-109 (7)	1.62	1.36	0.48	2.80	1.06	0.35	**2.21**	**1.21**	**0.42**
A. cruentus R-736 (12)	1.34	1.58	0.82	0.94	1.29	0.58	**1.14**	**1.43**	**0.70**
A. cruentus (Bunda)	1.16	1.50	0.68	2.47	1.50	0.76	**1.81**	**1.50**	**0.72**
A. hyp x A. hyb (14)	1.14	1.83	0.30	1.20	0.51	0.04	**1.17**	**1.17**	**0.17**
A. hyp x A. hyb (15)	0.92	1.52	0.36	1.22	0.76	0.05	**1.07**	**1.14**	**0.20**
A. montegazzinus (10)	1.34	1.99	0.34	1.25	1.20	0.57	**1.29**	**1.60**	**0.45**
Average	**1.25**	**1.55**	**0.52**	**1.60**	**1.18**	**0.40**	**1.42**	**1.36**	**0.46**
CV (%)	29.7	18.8	29.9	30.5	24.3	28.4			
LSD 5%	0.57	0.51	0.21	0.45	0.36	0.15			

Table 4.- Two way Anova for cultivar and season effects (* $p<0.05$; ** $p<0.01$).

Factor	Bunda	Bvumbwe	Kasinthula
Cultivar	*	*	**
Crop season	*	*	**
Cultivar x Season	*	*	*

Table 5.- Seed dry weight (g/1000 seeds) of different amaranth cultivars at Bunda, Bvumbwe and Kasinthula Research Stations in Malawi under rainfed conditions. Crop season 1998/9.

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**	**-**

Irrigated field trials

The possibility that grain amaranth can be grown during the dry season under irrigation was studied at Bunda College (August-November 2000). Mean monthly Max temperature varied in the range 28.9-32.8 °C and Min temperature 14.2-15.8 °C . Seeds of the ten amaranth cultivars cultivars were sowed on 26 August. Cultivation methodology was similar to the rainfed experiments, except irrigation treatments. Plots were irrigated before sowing. Sprinkler irrigation was applied up to seedling emergence and thereafter furrow irrigation was used to control irrigation to each plot-treatment. Fertilization (23:21:0:+4) was applied by the banding method at a rate of 25 kg N/ha.

Experiment design: split-plot in a randomized complete block design with three replicates. The main plots were irrigation regimes while the 10 cultivars formed sub-plots. The main plot was manipulated to create three different moisture regimes: T1- irrigation throughout the growing period; T2- not irrigated at flowering period, T3- not irrigated at grain filling period. The purpose of these treatments was to evaluate the ability to recover from drought at flowering (mid season drought) and grain filling period (end of season drought). Irrigation frequency was twice a week. A drought of 2 weeks was applied to both the short and long cycle cultivars. Estimated total amounts of water applied through the season were 1788, 1169 and 1375 mm for the tall cultivars, and 1714, 964 and 1190 mm for the in the short cultivars in the T1, T2 and T3 treatments, respectively. % reduction in yield by drought at flowering and grain filling stage were calculated.

Cultivar differed in their responses to drought according to growth habit and to the phenological stage at which drought was applied. In the tall cultivars drought reduced grain yield while in the short cultivars drought increased yield, compared to the continuously irrigated control. The unexpected increase in the short cultivars was probably related to excessive watering in the control. Similar trends were observed in Israel with these cultivars. Excessive watering may cause diseases. On the other hand, a two week interruption in irrigation was probably not enough to cause a significant stress, since the smaller plant size in these cultivars probably resulted in a slower rate of evapotranspiration and water loss from the soil. The relative reduction in yield observed in the tall cultivars when drought was induced at the flowering and grain filling stages was similar in *A. hypochondriacus, A. cruentus* R-109, *A. cruentus* (Bunda) and A. *montegazzinus.* In contrast, the effect of drought was larger at the -736. The local cultivar *A. cruentus* (Bunda) showed a relatively small reduction in grain yield during both stages of growth. Drought treatments did not affect Days to maturity (89-91 days) nor seed weight (data not shown).

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 Bunda and Bvumbwe Research Stations that probably represent the best agroclimatic conditions for grain amaranth cultivation in Malawi. Local accessions from Bunda *A. hypochondriacus* and *A. cruentus* were the best-adapted cultivars. They showed relatively high yields in the two seasons across the three agroecological regions. *A. cruentus* also had large leaves and hence canopy cover on the ground was good. The shade reduced water evaporation from the soil and also suppressed weeds. Short-cycle amaranth types (e.g. the *A. hyp x A. hyb* hybrid cultivars) 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 poor. This probably explains their low performance at Kasinthula. Late maturing cultivars usually exhibit compensatory growth at a later stage of development if exposed to mid season stress.

Amaranth cultivars showed a good recovery after short drought periods during flowering or the grain filling stage. Seed weight was not affected. Cultivars differed in their drought tolerance, with local Malawian accessions showing good levels of tolerance. These results support the idea that amaranth is a promising crop even in areas with short spells of dry weather.

Table 6.- Effects of drought at different phenological stages on grain yield. T1- continuous irrigation, T2- no irrigation during flowering, T3- no irrigation during grain filling. Irrigation was interrupted during two weeks.

Cultivar	**Grain Yield (Ton/ha)**				**% change**	
	T 1	**T 2**	**T 3**	**Average**	**T 2/T1**	**T 3/T1**
B. hypochondriacus (Bunda)	0.81	0.45	0.48	0.58	-44	-41
B. tricolor (Bunda)	0.95	0.79	0.56	0.77	-17	-41
A. *cruentus* R-158 (2)	0.55	0.44	0.33	0.44	-20	-40
B. cruentus R-283 (11)	0.80	0.49	0.30	0.53	-39	-63
B. cruentus R-109 (7)	0.86	0.53	0.54	0.64	-38	-37
B. cruentus R-736 (12)	0.52	0.28	0.38	0.39	-46	-27
B. cruentus (Bunda)	0.98	0.67	0.64	0.76	-32	-35
B. hyp x A. hybridus (14)	0.46	0.59	0.57	0.54	+28	+24
B. hyp x A. hybridus (15)	0.55	0.88	0.64	0.69	+60	+16
B. montegazinus (10)	1.02	0.59	0.65	0.75	-42	-36
Average	**0.75**	**0.57**	**0.51**	**0.61**		
Two way Anova						
Irrigation	P<0.05					
Accession	P< 0.01					
Irrigation X Accession	n.s.					

2- Research in Israel

Main goals of the research in Israel were:

a- to study physiological aspects of amaranth adaptation to drought

b- to study amaranth leaves as a source of vitamins and iron in human nutrition.

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

2.1- Adaptability of grain amaranth to drought

The aim of these experiments were to study the adaptation of Amaranth to drought by examining the changes in parameters related to the water balance of the plant and to photosynthesis, as influenced by a decrease in water availability (irrigation). Full details and results were presented in the 2nd report.

Field experiments and methodology: Experiments were carried out during the summer, at the Hula Valley, during 1996 and 1997. Two hybrid cultivars of amaranth were used: *A. hypochondriacus* X *A. hybridus* K-432 (14) and K-433 (15). These are early flowering, short-cycle cultivars. Under the summer conditions in Israel they reach plant maturation and seed harvest about two months after sowing. Therefore, they are well adapted to short growing seasons with terminal drought. Seed source from Rodale Press (Minnesota, USA) and reproduced for two seasons in Israel.

Soil characteristics at experiment site were: 68% clay, 24% silt, 8% sand and 1.7% organic matter, with pH 7.9, and 45% field soil water capacity. Top fertilization of ammonium sulfate, super phosphate and KCl was applied before sowing. Drip irrigation was used to get a better control of water availability to the plants. Additional fertilization was applied by mineral solution (20:20:20 N,P,K) through the irrigation system (fertigation). Experiments were performed with a complete randomized block design, with four replicates (5 x 2 m each). Seeds were sown in rows 40 cm apart (along the drippers), and seedlings were thinned to 10 plants/m along the row. In the 1996 experiment (Exp.1) the two cultivars (K-432 and K-433) were planted. Range of max and min temperature was similar in the two seasons: 30-45 °C and 15-20 °C, respectively. Minimal RH was 5 to 54%. In the 1996 season, several hot and dry days (hamsin) occurred, with temperature reaching 44°C and 6-7%RH.

Irrigation treatments started ca.15 days after emergence and were of two types: a-continuos irrigation with different water levels, and b- cessation of irrigation: 35 (onset of flowering) and 50 days after emergence (end of flowering). Levels of irrigation were 100, 50 and 25% of Pan A evaporation (E). In treatments with interrupted irrigation a 100%E irrigation was applied. In the 1997 experiment (Exp.2) only K-433 was grown under three irrigation treatments: a-continuous 100%E, b- continuous 25%E, and c- 100%E until 30 days from emergence (onset of flowering).

Irrigation treatments resulted in different patterns of change in soil water availability, according to climatic conditions. In Exp. 1 (1996), irrigation treatments started at onset of flowering (23/8). Differences in soil water availability between irrigation treatments appeared 8 or 14 days after onset of the treatments, first in the upper soil layer (30 cm). During this period, soil water potential in the 25%E and no irrigation treatments decreased to –70 to –90

centibar (cbar) at 30 cm depth, and –20 to –40 cbar at 60 and 90 cm depth, compared to -5 to –10 cbar in the irrigated control. In Exp. 2 (1997), amount of irrigation was reduced compared to Exp. 1 and the 25%E started earlier (after emergence), to obtain a more severe stress. Ten days after cessation of irrigation soil water potential was much lower compared to the irrigated control, e.g. ca. –60 cbar at 30 cm and –30 to –65 cbar at 60 cm depth, compared to –20 cbar in the control. Differences were much larger at the last measurement day, with –70 to –85 cbar at 30-60 cm and –55 cbar at 90 cm depth in the stressed treatments, compared to –10 to –30 cbar in the control.

Variation in stomatal conductance, water potential and osmotic potential of leaves during plant development: In Exp. 1 (1996), no significant differences were found between the two amaranth cultivars (K- 432 and K- 433) and results were pooled together. Stomatal conductance was not affected by irrigation treatments until the third measurement, 53 days after sowing. At this stage conductance was significantly lower in the 25%E and interrupted irrigation ($p<0.01$ and $P<0.05$, respectively). Nevertheless, even in the drier treatments, average stomatal conductance did not drop below 80 mmol $m^{-2}s^{-1}$.
Leaf osmotic potential and leaf water potential were little affected by decreased and interrupted irrigation. Leaves succeeded in maintaining a minimal potential higher than –1.5 MPa. It is probable that in amaranth leaves do not tolerate desiccation and, therefore, in the dryer treatments amaranth prevents leaf dehydration mainly by stomata closure.

In Exp. 2 (1997) only the cultivar K– 433 was planted. Similar response trends were observed even though the drought treatments started earlier. Again, significant differences in stomatal conductance and leaf water potential were recorded at the third measurement, 50 days after sowing ($P<0.01$ and $P<0.05$, respectively), while leaf osmotic potential was unaffected.

The possibility of leaf osmotic adjustment as a mechanism of adaptation to drought in amaranth was examined by measuring leaf osmotic potential at full turgor in leaves collected in the early morning. Leaves were brought to full turgor by keeping their petioles in water in a 100%RH chamber. No significant effects of irrigation treatments on osmotic potential at full turgor were found in the 1996 and 1997 field Experiments. Therefore, it seems that osmotic adjustment is absent in amaranth leaves, and prevention of leaf dehydration at low soil water availability is mainly due to stomata closure.

Relationships between stomatal conductance and leaf water potential: No linear relationship was found between leaf water potential and stomatal conductance. In Exp1 (1996) and Exp. 2 (1997), stomatal conductance reached 200 to 300 mmol $m^{-2}s^{-1}$ in the absence of drought stress. During the early hours of the morning conductance increased in parallel to a reduction in leaf water potential (Exp. 2)(Fig. 1). Later on, when leaf water potential falls below a threshold of about –1.0 to –1.2 MPa, stomatal conductance is reduced with lowest levels reaching 50-100 mmol $m^{-2}s^{-1}$. It is important to note that leaf water potential did not fell below ca. –1.5 MPa. Thus, it is conceivable that a controlling mechanism is operating, preventing dehydration of the leaves by closing the stomata, but still enabling same gas exchange to take place since stomata did not close completely. The fact that amaranth is a C4 plant contributes to the ability to perform photosynthesis at a low stomatal conductance.

The facts that: a) under drought conditions a threshold leaf water potential was maintained even though stomata were not completely closed, and b) at late stages of plant growth the

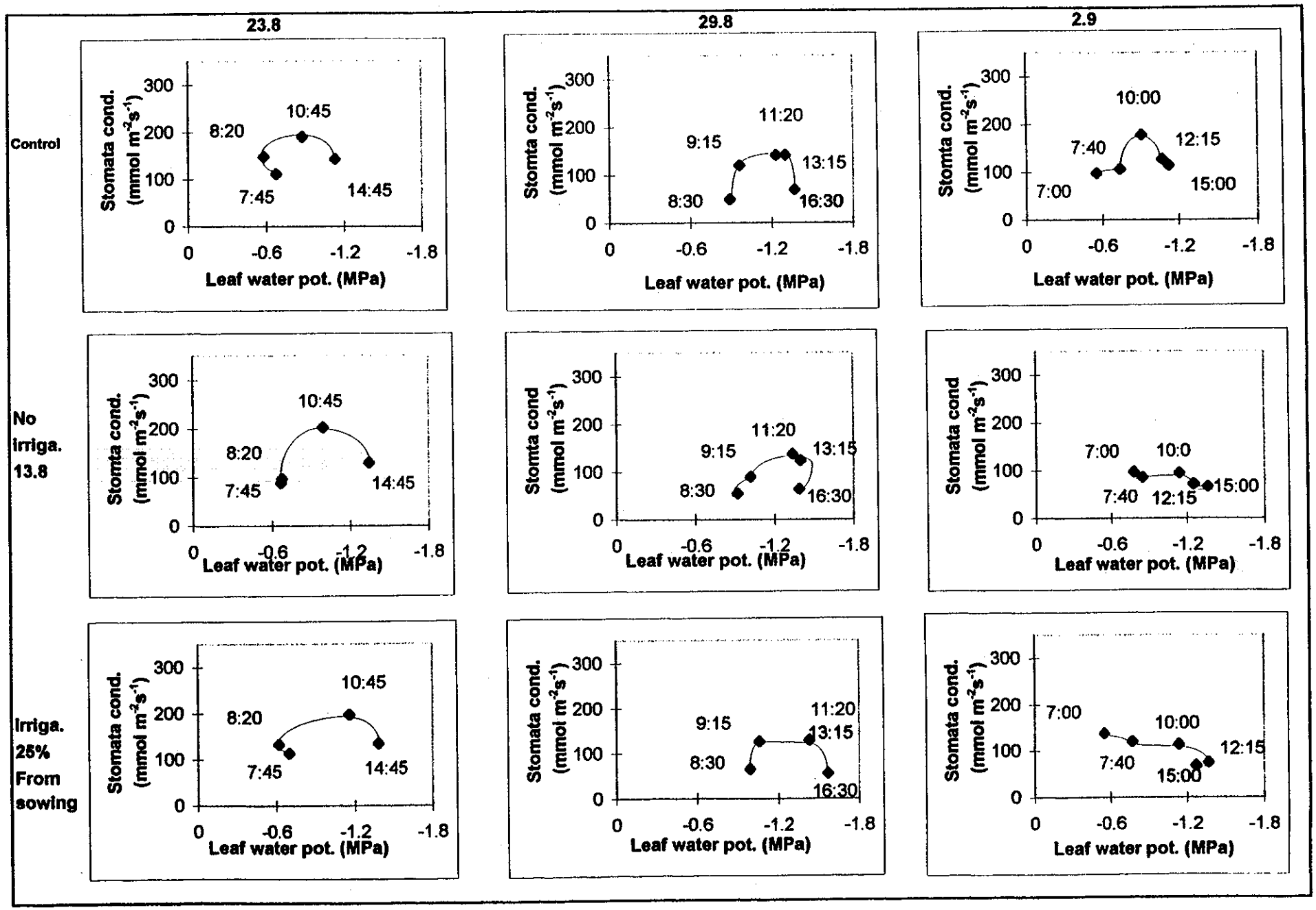


Fig. 1.- Relationship between stomatal conductance and leaf water potential in the different irrigation treatments in Exp 2 (1997).

14A

tensiometers located at 90 cm depth indicated very low water availability, suggests that the root system of amaranth remains active, pulling water from soil layers deeper than one meter.

Relationships between soil water availability, stomatal conductance and leaf water potential: The relationships between soil water availability and leaf water parameters was examined by pooling together the data from both experiments. For the analysis, values of minimal leaf water potential and maximal stomatal conductance were plotted against soil water potential measured in the morning of the same day. No relationship was found between minimal leaf water potential and soil water potential (Fig. 2). Minimal leaf potential was –1.5 MPa in the range of 0 to 90 cbar at the three depths were water potential was measured. In contrast, the maximal stomatal conductance decreased the lower the soil water potential, until it reached ca 100 mmol $m^{-2}s^{-1}$ (Fig. 3).

Results suggest that stomata closure occurs at relatively high leaf water potential, in response to root signals produced with water depletion in the soil, thus preventing leaf dehydration. Root signals, such as abscissic acid, have been proposed as an essential component of mechanisms of drought avoidance by stomata closure.

Photosynthesis and stomatal conductance: Variation in rate of photosynthesis was measured at noon hours (11:00-13:00), during the 8.9 and 13.9 in the 1996 experiment. These two days differed greatly in max temperature and min RH conditions, e.g.: 36 °C and 40% RH vs. 44°C and 6% RH. At noon minimal leaf potential was reached: -1.3 and –1.6MPa, respectively, and stomata were quite closed. In both days, a significant ($P<0.001$) linear relationship was found between rate of photosynthesis and stomatal conductance in the irrigated and stressed treatments pooled together (Fig. 4). This relationship was not affected by the irrigation treatments. In contrast, no relationship was found between rate of photosynthesis and internal concentration of CO_2 (Ci) (Fig.5).

In C4 plants grown under good water availability, high light intensity and average temperature, C_i ranges between 100-150 ul $^{-1}$ CO_2 . In our experiments Ci ranged between 150-250 ul^{-1} and in this range it was not correlated with rate of photosynthesis. Thus, under drought conditions photosynthesis was constrained by stomatal closure but not by internal CO_2 concentration in the leaves.

Effect of irrigation treatments on min leaf water potential and leaf temperature during measurement days is presented in Table 7. As in previous measurements leaf water potential reached a low threshold that, even in a very warm and dry day, was not lower than ca. –1.7 MPa. Leaf temperature at noon was relatively high, ca 38 °C and 44 °C with little transpirational cooling due to stomata closure. Interestingly, high rates of photosynthesis were reached at these high temperatures as shown in Fig. 10. Thus, it seems that the photosynthetic system in amaranth is very tolerant to high temperature and drought, suggesting a well-developed mechanism to avoid photoinhibition under these extreme conditions.

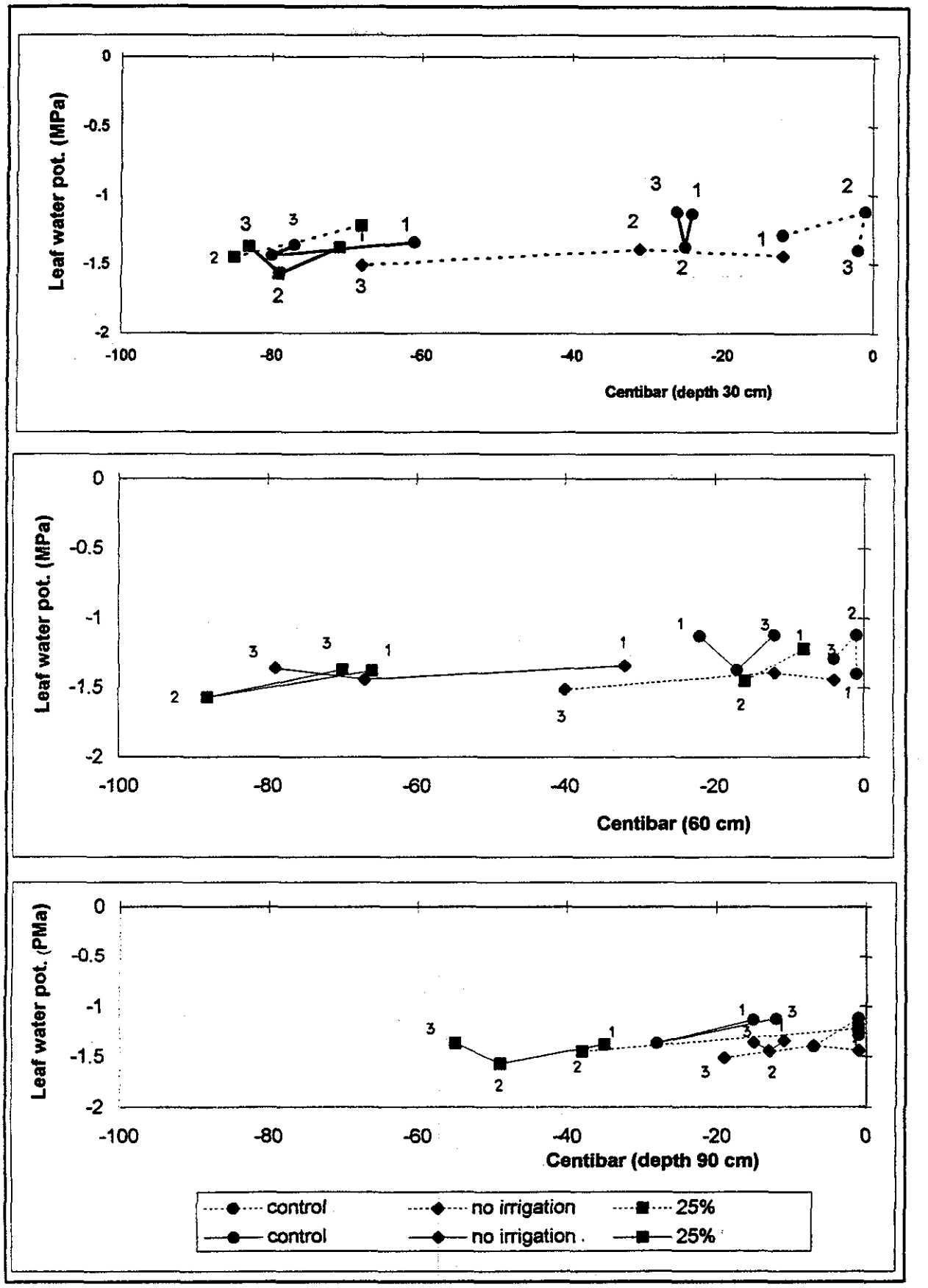


Fig. 2.- Relationships between min leaf water potential and soil water potential at 30 (top), 60 (middle) and 90 (bottom) cm depth. Data from 1996 (broken lines) and 1997 (full lines) were pooled together. Numbers (1,2 and 3) indicate measurement days. (•) Irrigated controls; (■) 25%E; (◆) cessation of irrigation.

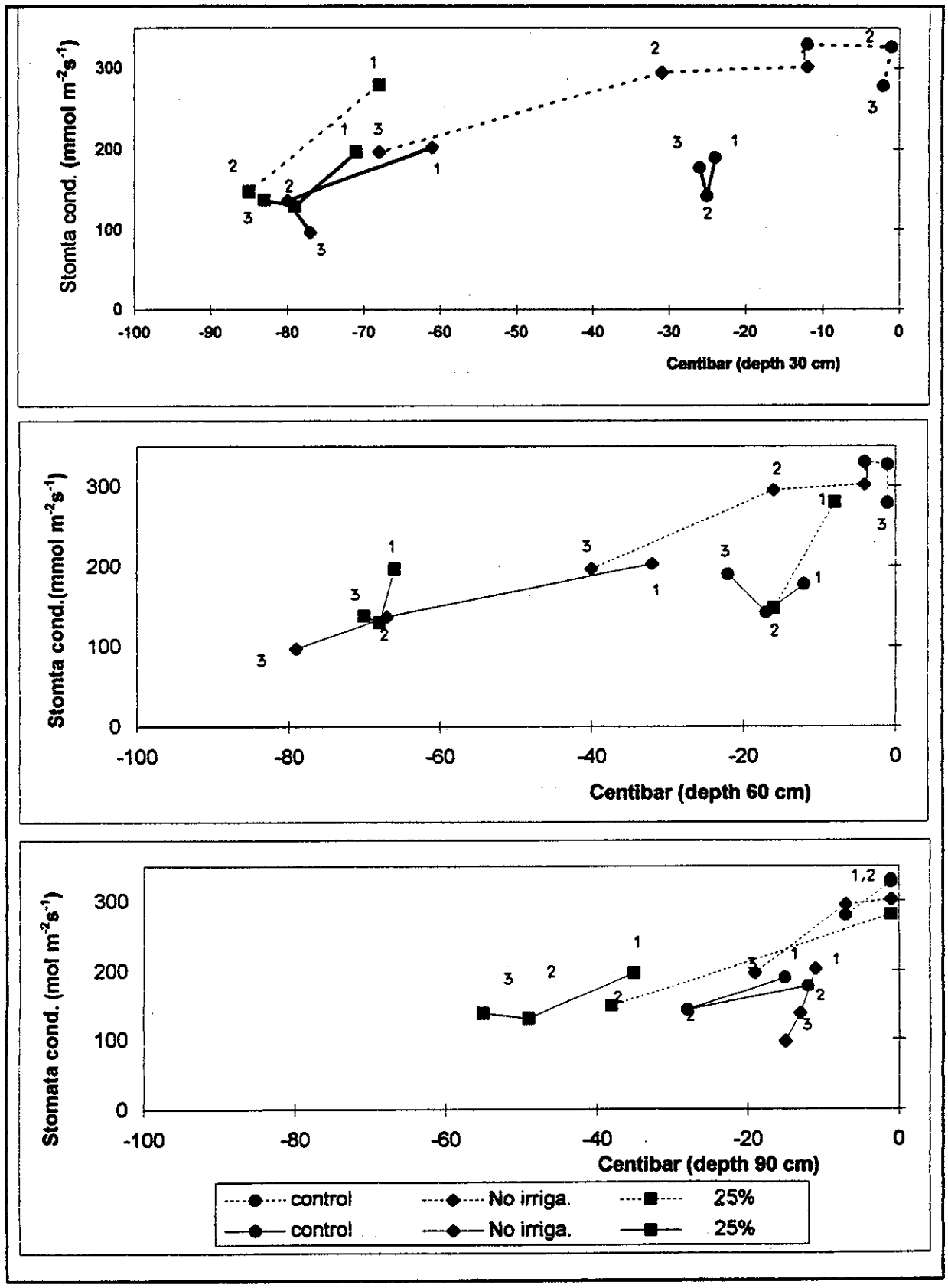


Fig. 3.- Relationships between max stomatal conductance and soil water potential at 30 (top), 60 (middle) and 90 (bottom) cm depth. Data from 1996 (broken lines) and 1997 (full lines) were pooled together. Numbers (1,2 and 3) indicate measurement days. (●) Irrigated controls; (■) 25%E; (◆) cessation of irrigation.

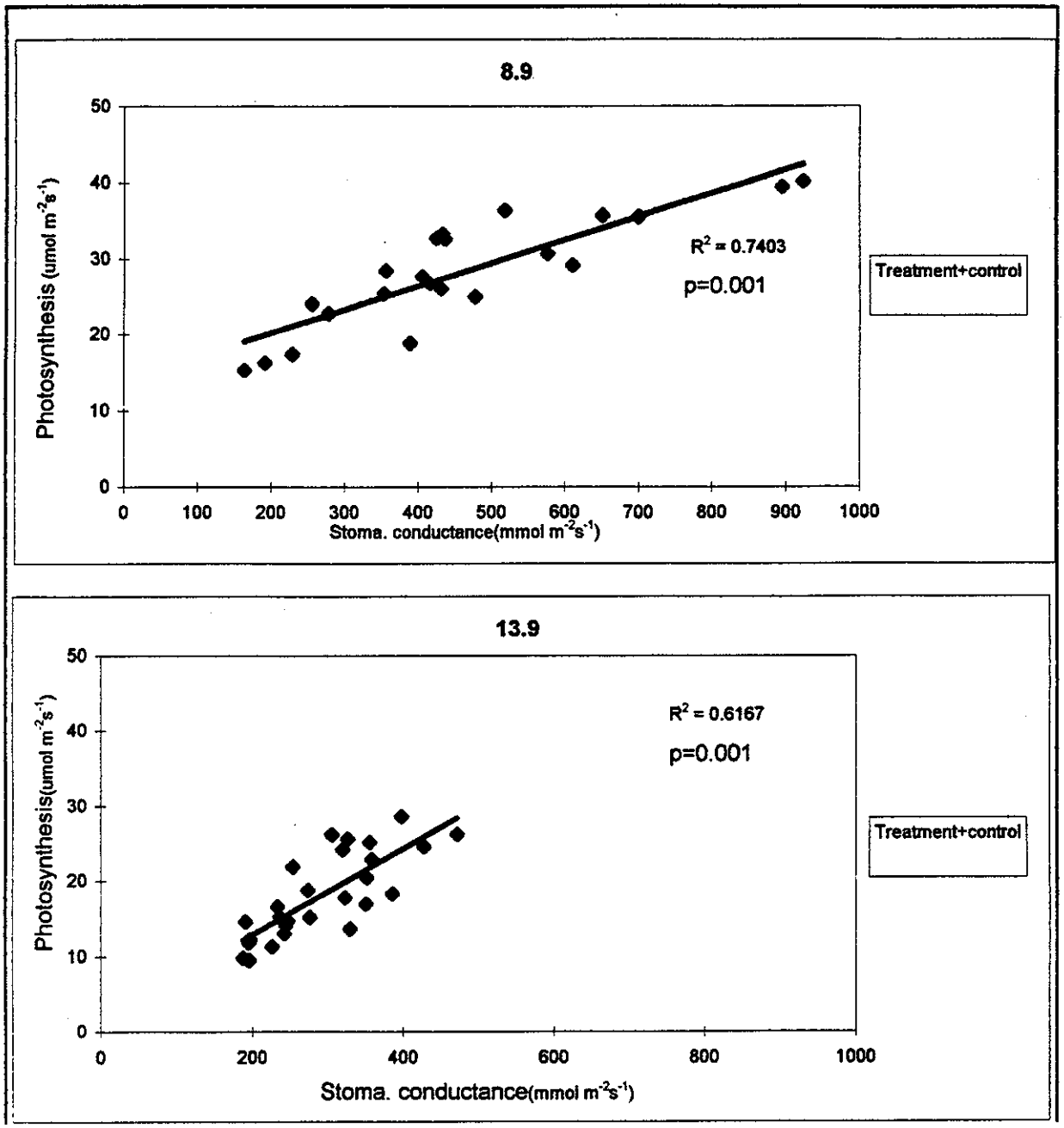


Fig. 4 .- Relationship between rate of photosynthesis and stomatal conductance. Data from all treatments were pooled together. Exp 1 (1996).

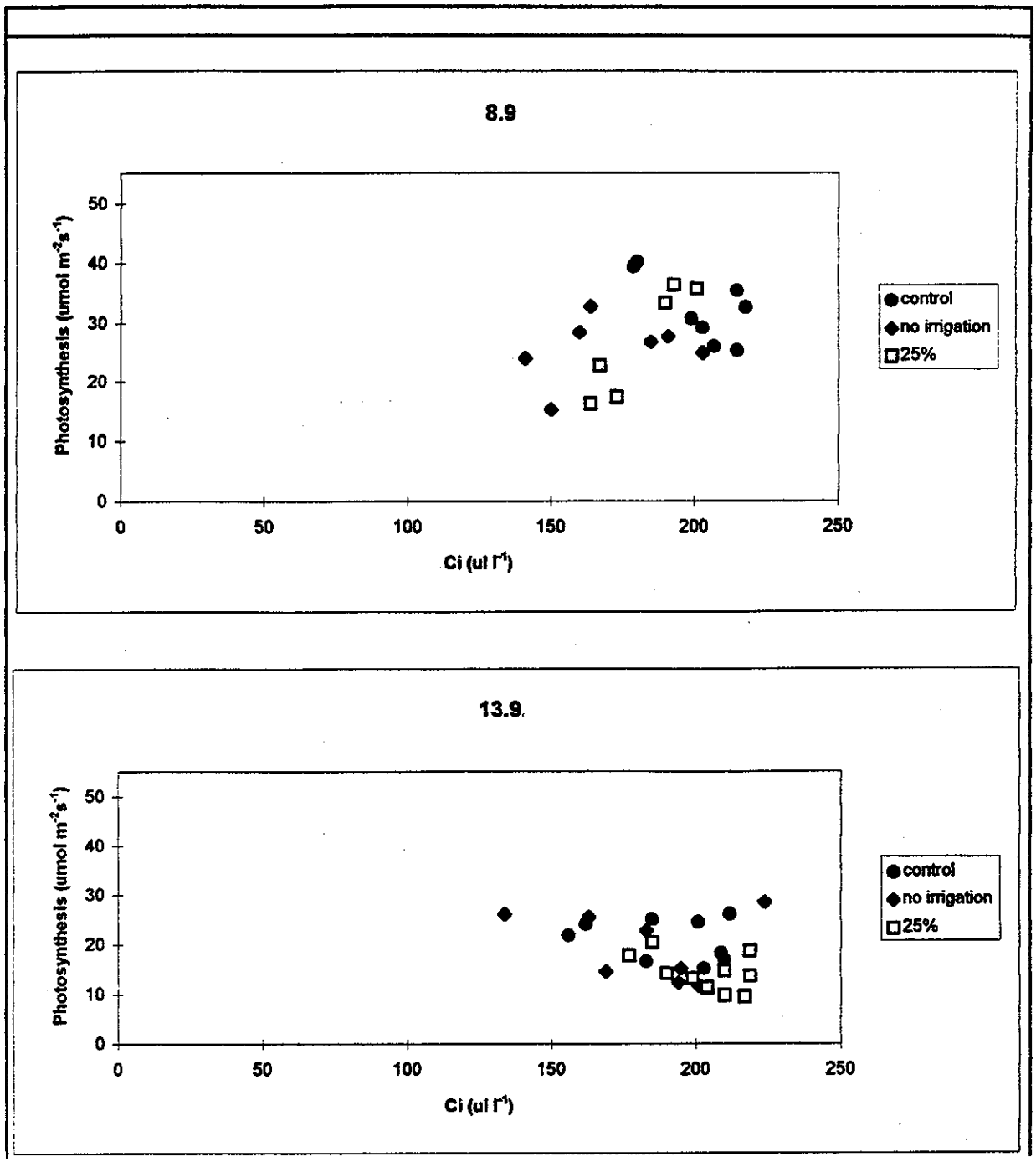


Fig. 5 .- Relationship between rate of photosynthesis and leaf internal CO_2 concentration (C_i). Data from all treatments were pooled together. Exp 1 (1996).

Table 7.- Effects of irrigation treatments on leaf water potential and leaf temperature.

Treatments	8.9.96		13.9.96	
	Water potential (MPa)	Leaf temp. (°C)	Water potential (MPa)	Leaf temp. (°C)
Irrigated control	-1.35	37.5	-1.63	41.9
25%E	-1.34	38.9	-1.35	44.9
Interrupted Irrigation	-1.31	38.3	-1.67	42.3
Significance	n.s.	<0.05	n.s.	<0.05
Air temperature	36.1 °C		44.1 °C	
RH	40.2 %		5.8 %	

Drought effects on grain yield: Even though the main purpose of these experiments was to study physiological responses to drought, preliminary results on grain yield were also obtained. Treatments of reduced irrigation and cessation of irrigation did not result in a reduction in yield compared to irrigated control. They rather caused a decrease in the total plant biomass. The applied levels of drought did not affect grain production. It is conceivable that during seed development at low water availability assimilates were transferred to them at the expense of vegetative growth. As a result, seed weight was not affected by drought but harvest index was increased.

Conclusions

According to the results of this study, grain amaranth belongs to the type of plants whose response to drought is based on the avoidance strategy: stomata closure at relatively high leaf water potential, ability to perform photosynthesis at high temperature and low water availability, reduction of leaf expansion, and deep roots. No evidence of osmotic adjustment was found in response to drought. Thus grain amaranth is apparently well adapted for cultivation in hot regions, with rains or irrigation during early stages of growth and with end of season (terminal) drought. Short cycle cultivars, planted at high density are recommended.

2.2- Amaranth leaves as a source of vitamins and minerals in human nutrition

Of the numerous species consumed as leaf vegetable in east Africa, 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. 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.

The main purpose of our studies was to examine the potential of amaranth leaves as a source of vitamins and minerals (including iron) and to develop simple methods for drying and conserving the leaves for consumption during seasons when fresh leaves are not available for human nutrition. Information presented here is based mainly on results from the 1st and 3rd Reports. The species used for these studies were *A. tricolor* (a leafy cv. from Germany source) and *A. hypochondriacus x A. hybridus* (cv. 15) (a short cycle grain type). Total protein and fat content in amaranth leaves were within the range of values reported in the literature: 25.4 % protein, 4.3% fat (in lyophilized samples). These levels were not affected by drying methods. Mineral content is shown in Table 8.

Table 9: 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 content of leaves ranged from 200 to 269 ppm, 250 to 430 ppm and 46 to 57 ppm, 84 to 110 ppm for *A. hypocondriacus* and *A. tricolor*, grown in the greenhouse or the field, respectively (Table 9). *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. 9a. 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 9: 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).

9a

	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;

9b

	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. 9b), strongly suggest continuous decrease in Fe bioavailability over time.

Vitamin C content showed some variation (Tab. 10), but was not different between or within species. 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.

Table 10: 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.

In general it was observed, 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.

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 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.9a) 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. 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.

Summary: Main efforts were concentrated in the use of air-dried amaranth leaves as a source of iron an Vitamin C. 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. Supplementing the leaves during the dehydration process with a source of vitamin C might be advantageous during storage.

2.3.- Assessment of amaranth for replacing flour for bread baking

The blend of amaranth and wheat flour determines the baking quality, nutritional values and product acceptance. It also has a market effect on the price and the economic feasibility of the approach. In our experiments, the level of amaranth was raised gradually to 30% replacement. This level was found as a technological hurdle. Thus, during the second and third year of the project, efforts were made to increase the level of replacement. Following results are based on the 2nd Report. During the 4yh year work was oriented to the production of other baking products.

Use of amaranth flour for bread baking

A. **INGREDIENTS**

1. **Amaranth/Flour** - Baking experiments were carried out on amaranth/wheat blend flour replacement of 30% (all quantities and percents are based on initial flour weight defined as 100%). To compensate for the expected low volume, both enzymes and emulsifiers were utilized as indicated in Table 1. Amaranth was purchased from the US (NuWorld Amaranth Inc. Earlville, IW), as no alternative seeds with adequate baking quality were available either from Malawi or Israel. White wheat flour was purchased at a local baking industry (Maadanei Bar, Kibbutz Einat, Israel) and was characterized by these values: 13.9% moisture, falling number (385 s) and 12% protein.
2. **Enzymes** – The selection included commercial -amylase from fungal source, hemicellulose and Vernon 2000 (mainly α-amylase, Rohm Enzyme, Darmstadt, Germany).
3. **Emulsifiers** – DATEM, 0 to 0.2%; CSL - calcium stearoyl-2-lactylate 0 to 0.4%; GMS distilled mono and di-glyceride of fatty acids 0 to 0.5%;
4. **Dough Extender** - Two commercial formulation were utilized: Adomix (Adomim Chemicals, Maleh Adomim, Israel), 0 to 1.0%, and Green (Sharon Laboratories, Ashdod, Israel) 0 to 2.0%.
5. **Dough Improver** - Ascorbic acid (0 to 750 ppm), L-cysteine (0 to 200 ppm) and potassium carbonate (0 to 200 ppm) were utilized.
6. **Salt** – The level of salt was fixed at 1.7% (5 g) to reduce the number of variables. This level was defined by taste. The salt was added at several stages of the baking.
7. **Yeast** – Dry baking yeast (Paca, Bat Yaam, Israel) at 3 g (1%). Dried yeast was used to ensure complete repeatability of the experiments and possible application at Malawi.
8. **Gluten** - Vital gluten (Adomin Chemicals, Israel) was used at levels ranging from 0 to 2%.
9. **Sugar** - Sucrose level of 1% was added for taste, gas formation and color.
10. **Oil** – Commercial margarine 1% was added during all baking tests.
11. **Water** – A constant amount of 200 g (66.6%) per baking experiment was utilized

B. **BAKING PROCESS**

1. **Control** – Basic baking formula was: 300 g flour, 3 g sugar, 5 g salt, margarine 3 g, dry yeast 3 g, commercial dough extender and 200 mL tap water.
2. **Amaranth** – The seeds were cleaned manually before milling on a coffee grinder (Moulinex Supper Junior S) for ca. 60 s. The flour was sieved through a 60/100 standard sieves (Retsch D-42752 Haan, Germany) on a vibrating unit (Retsch AS200 basic). Sieving conditions were full amplitude (100%) for 15 min. Only the fraction that passed 60 Mesh and remained on 100 Mesh was collected for further usage. The larger particles were re-milled once, and the whole cycle was repeated. The flour was utilised on the same day to avoid any possible enzymatic activity and possible deterioration.
3. **Baking instruments** – In addition to the automatic breadmaker machine utilised previously (Novac AV, BM-152ASL, China), we looked for a different unit that would allow us to control the process and the length of its various stages. For this purpose, three Zojirushi (Home Bakery, Zojirushi America Corp. Commerce, CA Model BBCC-S15A) were purchased. Only after calibrating the units and ensuring repeatability they were utilised for the baking experiments. Four different baking programs were applied:

a. Baking Program A (total time: 219 min)

1) Heating, 5 min, 2) Kneading "1," 8 min, 3) Rest 4 min, 4) Kneading "2," 22 min, 5) 1st Rising (proofing), 60 min, 6) 2nd and final rising, 60 min, 7) Baking 60 min and 8) Cooling.

b. Baking Program B (total time: 216 min)

1) Heating, 5 min, 2) Kneading "1," 6 min, 3) Rest 5 min, 4) Kneading "2," 20 min, 5) 1st Rising, 60 min, 6) 2nd and final rising, 60 min, 7) Baking 60 min, and 8) Cooling.

c. Baking Program C (total time: 211 min)

1) Heating, 5 min, 2) Kneading "1," 6 min, 3) Rest 5 min, 4) Kneading "2," 15 min, 5) 1st Rising, 60 min, 6) 2nd and final rising, 60 min, 7) Baking 60 min, and 8) Cooling

d. Baking Program D (total time: 196 min)

1) Heating, 5 min, 2) Kneading "1," 6 min, 3) Rest 5 min, 4) Kneading "2," 10 min, 5) 1st Rising, 60 min, 6) 2nd and final rising, 60 min, 7) Baking 60 min and 8) Cooling.

C. **MEASUREMENTS**

1. **Volume** – Loaf volume was used as an index for quality comparison. The volume was determined using a seed displacement method utilising unpuffed popcorn. The bulk density of the corn was calculated as 0.87 g/mL. The baking pan volume was measured with water, and the volume of the loaf was derived from the weight of the corn that was required to fill the baking pan.
2. **Fractal number** – The loaf was cut in the middle and one slice (ca. 1 cm) thick was cut with an electric knife. The slice was scan (RGB 300 DPI) on a UMAX Scanner (1200 Astra) using Presto! PageManager software (Ver. 2.3, NewSoft Technology Corp.). The image (TIFF file or BMP file) was converted into a 257 x 257 x 256 pixels format with a Paint Shop Pro Program for Windows (Ver. 4.12 Shareware, JASC Inc.). The BMP image generated was converted to a file that can be read by the Russ program (Russ, 1994), and both Minkowski and Kolmogorov values were derived with their appropriate standard deviations (S.D). The higher the number derived, the rougher the surface is.
3. **Visual Assessment** – The bread loaf was also visually assessed and its overall appearance was documented using the scanner described previously.
4. **Taste** – At this point, the organoleptic quality was assessed for general appearance and taste by the small research team only. As no expert panel was utilised these attributes were not quantified and were utilised for general direction.

5. D. RESULTS

A list of the pertinent experiments carried out is listed in Table 1. The data obtained showed that volume and the ingredients, and the order of addition affected quality of the bread loaf. The following **main conclusions** could be drawn:

a. **Commercial dough extenders** – Two different commercial dough extenders were utilized. These improvers were chosen based on the specific recommendations made by two leading baking laboratories in Israel. Their recommendations were based on previous experience with amaranth. Baking experiments with both Adomix and Green were very effective to produce high quality bread with even distributed small pores. The loaf was filling the width of the baking pan, and the volume was significantly higher than the other experiments (Fig. 1; baking experiments 1 through 6 and 27). When comparing the apparent fractal dimension, which describes the ruggedness of the surface, it revealed a constant value. The difference between Minkowski and Kolmogorv method (Russ, 1994) yielded an average 2.478 and 2.79 respectively. Similar results (Figs. 1 and 2; Baking experiment #9 and #11) were obtained when the following were added: (0.6 g CSL, 0.3 g DATEM, 0.465 GMS, 0.075 vitamin C, 0 g gluten, 0.03 g α-amylase).

Table 1: Description of the experiments

#	Description	Amaranths (%)	Emulsifier (%)	CSL (g)	DATTEM (g)	GMS (gr)	Vitamin C (g)	Gluten (%)	Hemicellulose (g)	α-amylase (g)	Others	Salt (g)	Baking Program	Loaf length (cm)	Loaf width (cm)	Loaf volume (cm3)	Kolmogorov	S.D (Kolmogorov)	Minkowski	S.D. (Minkowski)	Comments
1	Control	0	0.5% Adomix									5	A	153.2	126.9		2.781	0.081	2.469	0.038	
2	Control	0	0.5% Adomix									5	A	162.0	124.1		2.769	0.078	2.468	0.038	
3	Control	0	0.5% Adomix									5	A	157.7	122.5		2.769	0.074	2.382	0.034	
4	Control	0	0.5% Adomix									5	B	150.3	122.3		2.749	0.072	2.480	0.034	
5	Control	0	0.5% Adomix									5	B	147.5	127.0		2.761	0.077	2.430	0.037	
6	Control	0	0.5% Adomix									5	B	144.3	125.0		2.715	0.084	2.420	0.035	
7		30	1.0% Adomix					1.0				5	A	101.8	124.7		2.782	0.070	2.486	0.037	
8		30	1.0% Adomix					1.0				5	B	104.7	124.2		2.798	0.077	2.488	0.038	
9	Control	0		0.6	0.3	0.465	0.075		0	0.03		5	B	170.7	121.2		2.773	0.060	2.446	0.035	
10		30		1.2	0.6	0.930	0.150	1.0	0.1	0.06		5	B	124.2	125.5		2.823	0.068	2.518	0.038	
11	Control	0										5	B	131.6	123.8		2.723	0.071	2.431	0.035	
12		30		1.2	0.6	0.870	0.150	1.5	0.1	0.06		5	B	133.6	124.1		2.822	0.069	2.516	0.037	
13		30		1.2	0.6	0.750	0.150	1.5	0.2	0.06		5	B	133.4	124.9		2.796	0.064	2.478	0.036	
14		30		1.2	0.6	0.870	0.150	1.5	0.1	0.12		5	B	117.6	121.4	1,641.5	2.824	0.072	2.517	0.037	
15		30		1.2	0.6	0.810	0.150	1.5	0.1	0.12		5	B	119.8	124.1	1,613.8	2.797	0.065	2.503	0.037	
16		30		1.2	0.6	0.810	0.150	1.5	0.1	0.12		5	A	109.9	124.2	1,514.0	2.837	0.076	2.523	0.038	
17		30		1.2	0.6	0.870	0.150	1.5	0.1	0.06		5	C	112.5	125.0	1,600.1	2.860	0.062	2.644	0.028	
18		30		1.2	0.6	0.870	0.150	1.5	0.1	0.06		5	D	114.6	125.5	1,535.8	2.750	0.068	2.477	0.034	
19		30		1.2	0.6	0.795	0.225	1.5	0.1	0.06		5	B	124.2	125.6	1,484.1	2.818	0.074	2.487	0.035	

Table 1 (con..): Description of the experiments

#	Description	Amaranths (%)	Emulsifier (%)	CSL (g)	DATTEM (g)	GMS (g)	Vitamin C (g)	Gluten (%)	cellulose (g)	α-amylase (g)	Others	Salt (g)	Baking Program	Loaf length (cm)	Loaf width (cm)	Loaf volume (cm3)	Kolmogorov	S.D (Kolmogorov)	Minkowski	S.D. (Minkowski)	Comments
20		30		1.2	0.6	0.720	0.300	1.5	0.1	0.06		5	B	117.2	124.7	1,511.6	2.820	0.071	2.483	0.035	
21		30		1.2	1.0	0.530	0.150	1.5	0.1	0.06			B	107.1	125.0	1,492.4	2.789	0.077	2.478	0.038	
22	Salt after mix 1	30		0.6	0.6	1.530	0.150	1.5	0.1	0.06		5	B	107.4	125.4	1,410.9	2.808	0.071	2.499	0.033	
23		30		1.2	0.6	0.930	0.150	1.5	0.1	0.06			B	87.2	126.4	1,243.0	2.835	0.079	2.475	0.037	
24	Salt at beg. mi:	30		1.2	0.6	0.930	0.150	1.5	0.1	0.06		5	B	111.8	125.1	1,540.0	2.817	0.073	2.510	0.037	
25	Salt at 10 min	30		1.2	0.6	0.930	0.150	1.5	0.1	0.06		5	B	104.3	125.0	1,375.4	2.816	0.068	2.504	0.034	
26	Salt at beg. mix	30		1.2	0.6	0.870	0.150	1.5	0.1	0.06		5	B	105.6	126.4	1,384.4	2.829	0.074	2.493	0.036	
27	Control	0	0.5% Green									5	B	164.1	125.2	2,267.2	2.703	0.066	2.416	0.033	Holes
28		30	1.0% Green									5	B	91.0	125.5	1,315.1	2.841	0.073	2.423	0.041	Sticky
29	Salt after mix 1	30		1.2	0.6	0.330	0.200	1.5	0.1	0.06	0.6 g Cystein	5	B	70.9	125.8	1,094.2	2.808	0.075	2.408	0.041	
30	Salt before mix	30		1.2	0.6	0.330	0.200	1.5	0.1	0.06	0.6 g Potassiu	5	B	98.3	125.9	1,351.6	2.836	0.070	2.484	0.036	
31		30		1.2	0.6	1.065	0.200	1.5	0	0.06	0.045 g Rohm	5	B	103.3	126.0	1,403.5	2.832	0.072	2.512	0.036	
32	Salt before mix	30		1.2	0.6	1.065	0.200	1.5		0.06	0.045 g Rohm	5	B	105.6	125.0	1,390.7	2.829	0.073	2.486	0.034	
33		30		1.2	0.6	1.005	0.200	1.5	0.1	0.06	150 ppm 0.07	5	B	108.6	125.4	1,432.7	2.800	0.077	2.465	0.037	Uneven loaf
34	Salt before mix	30		1.2	0.6	1.005	0.200	1.0		0.06	0.075 g Rohm	5	B	98.7	125.1	1,373.4	2.851	0.074	2.499	0.033	
35		30		1.2	0.6	1.095	0.200	2.0			0.075 g Rohm	5	B	103.6	126.3	1,383.0	2.807	0.072	2.491	0.035	
36	Salt before mix	30		1.2	0.6	1.095	0.200	2.0			0.075 g Rohm	5	B	112.0	126.5	1,390.1	2.792	0.072	2.423	0.032	
												Overall Average:		119.7	124.9	1467.3	2.799	0.072	2.478	0.036	

b. **Amaranth** - The addition of amaranth has a negative effect on the length and the volume of the loaf. The two commercial dough extenders that were used increased the volume of the bread and improved somewhat the quality of the 30% amaranth bread loaf (Table 1 and Figs. 1 and 2). However, even at levels of 0.5 and 1.0% the improvement obtained was not sufficient, and the quality of the baked bread loaf was very low. Thus, clearly indicating that optimization of the bread containing 30% amaranth was necessary. Picture 1 clearly indicates that the optimization process finally yielded good quality bread, where volume and length were comparable to the non amaranth bread. The various formulations utilized are listed in Table 1.

c. **Gluten** - The addition of gluten at level of at least 1.0% to 1.5% was paramount in order to maintain a volume that was comparable with the regular bread baked without amaranth. On the other hand, the addition of 2.0 % was not efficient and proved not to be cost-effective and beneficial.

d. **Amaranth concentration** - Experiments to increase the level of amaranth beyond 30% were not successful even when the gluten level was increased above 1.5% to 2.0%. Hence, it was concluded that increasing the level of amaranth above 30% replacement suffers from two main obstacles: 1. the volume of the loaf is reduced significantly. The bread loaf was very dense and its quality inferior. Secondly, the taste became very grainy and was defined as taste like "earth." We have originally assumed that this organoleptic inferiority could be due to the origin of the amaranth seeds. However, several other amaranth seed sources have not improved this perceived low quality attribute.

e. **Salt** - The timing of salt addition was found to have a significant effect on the volume. This finding is not surprising as the effect of salt on gluten and baking characteristics is very well known. In 30% amaranth bread slices without salt (Baking Experiment #30) the texture was very dense, and the volume very small (i.e. 1243 mL). Additional experiments suggested that salt should be added after the first kneading, allowing the development of the continuous phase (gluten), which is cumbersome in the presence of amaranth. The continuous phase and sustaining the volume of the loaf during baking is affected by salt presence and therefore salt is an important ingredient.

f. **Cysteine** – Addition of cysteine (0.06 g) was not promising as the structure was very porous and large holes were observed and the volume was very low (1,094 mL).

g. **Enzyme** – The addition of a commercial enzyme Rohm Vernon 2000 at levels of 150 to 250 ppm as recommended by Rohm's expert (Dr. N. Burkhardt, private communication). However, the data showed that was not sufficient to increase the volume of the bread containing 30% amaranth.

h. **Vitamin** C - Addition of vitamin C was found to be crucial for the development of the loaf and its structure. The level found as optimal is 100 to 200 ppm.

I. The addition of hemicellulose (0.06 to 0.12 g / 300 g flour) was beneficial to increase loaf volume. Yet, its efficiency is not clear, as it may be possible that the α-amylase utilized contained already some hemicellulose.

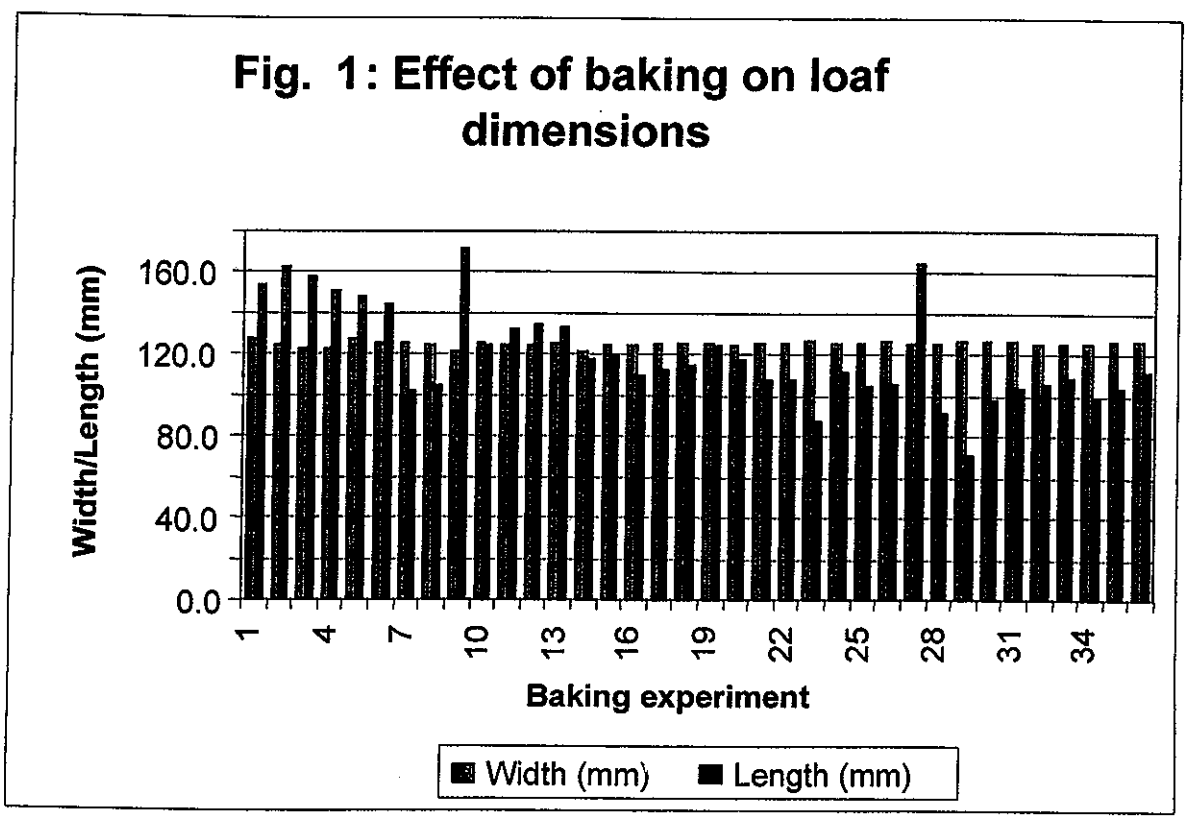


Fig. 1.- Effect of baking conditions on loaf dimensions.

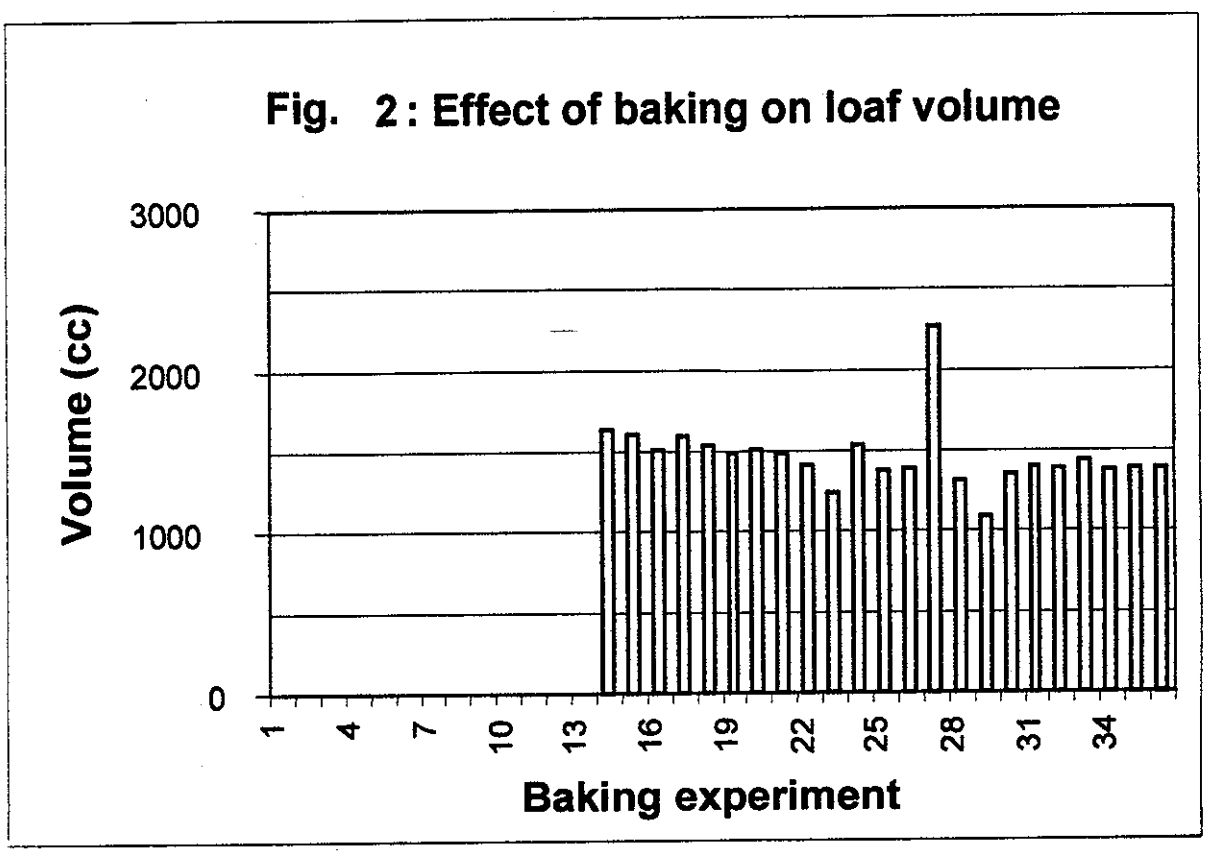


Fig. 2.- Effect of baking conditions on loaf volume.

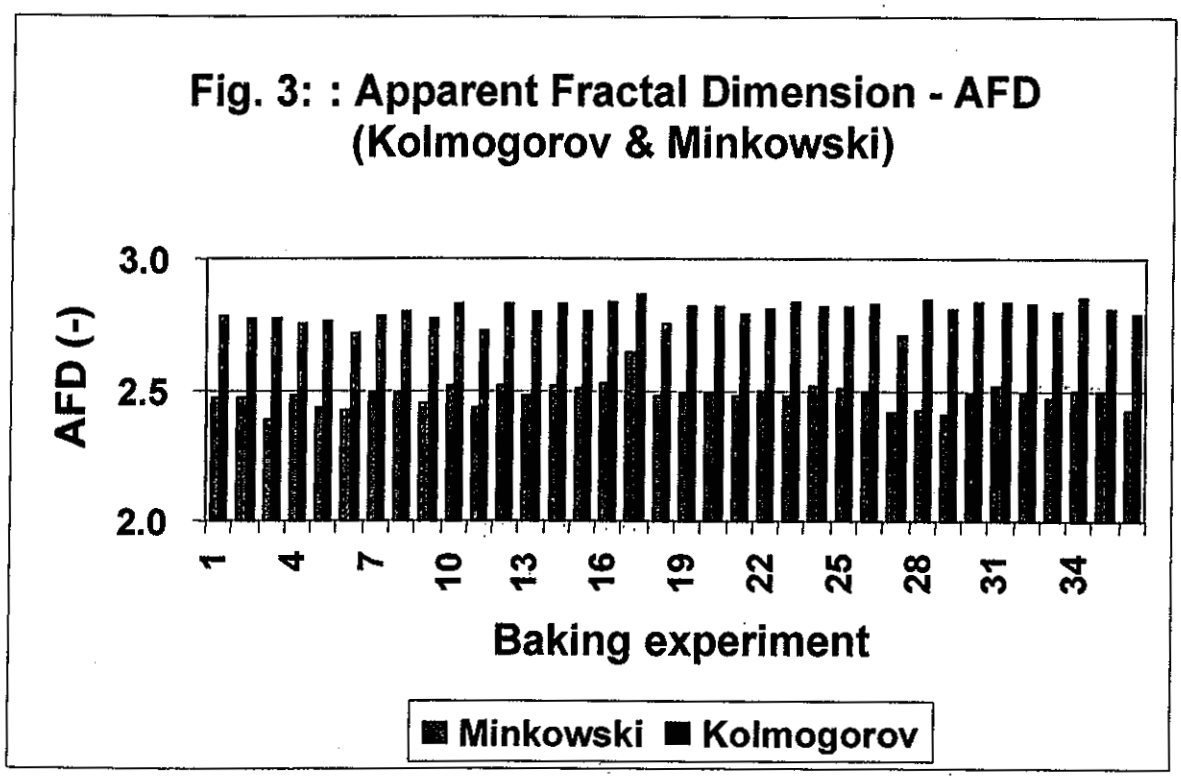
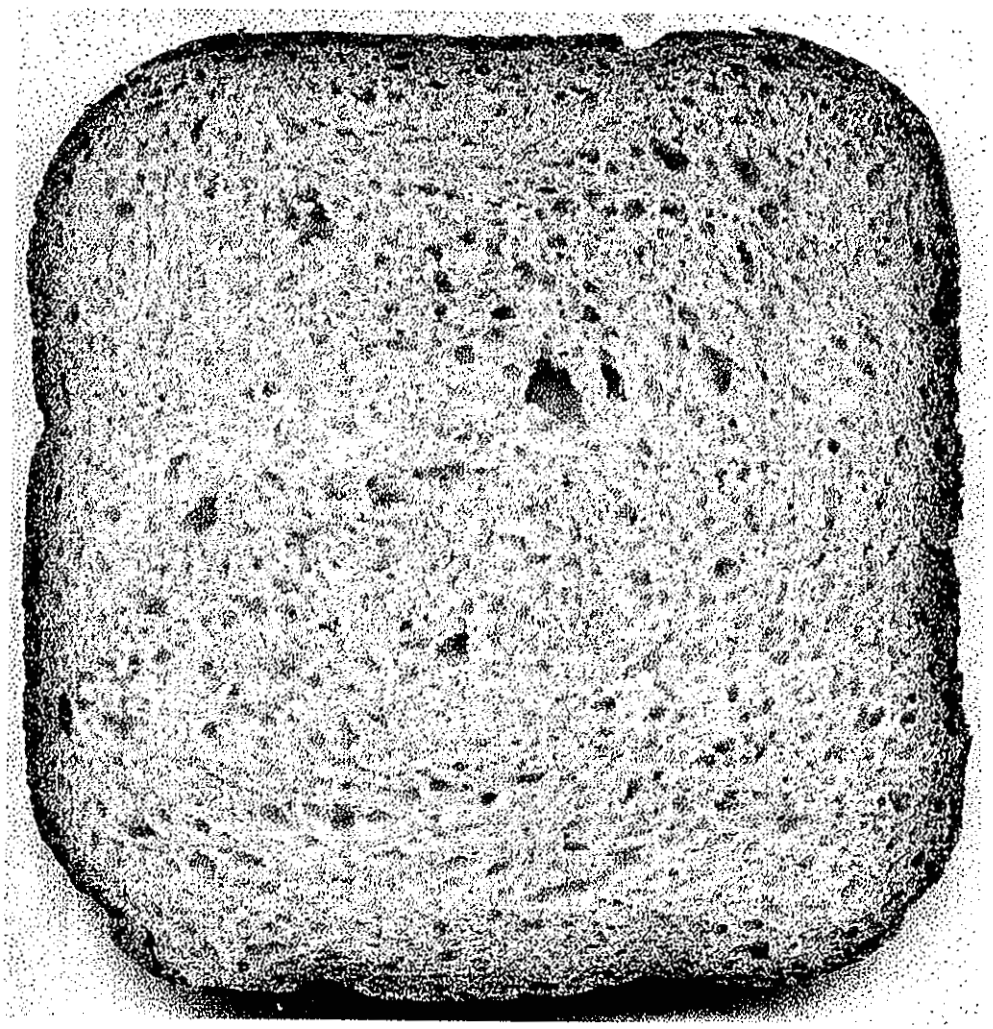


Fig. 3.- Apparent fractal dimension – AFD (Komogorov and Minkowski)

Picture : Effect of 30% amaranth replacement on baked bread (additive: 0.06 g α-amylase; 0.1 g hemicellulase, 1.0% gluten, 0.15 g Vitamin C, 0.93 GMS, 0.6 g DATEM, 1.2 g CSL)



Use of amaranth flour for baking wafers (biscuits)

Consumption of amaranth is not necessarily limited to bread baking. In the previous experiments the level of amaranth was raised bread gradually to 30% replacement. This level was found as a technological hurdle during bread baking. Thus, the work during the year 2000 as focused on preparing other products containing amaranth at levels higher than 30%. Products such as waffles and crackers are not limited to the 30% barrier encountered in our research, as they are not based on a leavened structure and they are not depending on the formation of the structure by gluten. Such products are wafers (or biscuits), that may contain up to 100% amaranth and could also be enriched with amaranth leaves. Moreover, these products no not need special and expensive equipment, thus allowing direct technology transfer.

MATERIALS AND METHODS

Ingredients

a. **Amaranth flour** – Wafers were prepared using various concentration of amaranth purchased locally (Alser, Or Yehuda, Israel). The Amaranth was biologically grown. These seeds were used mainly due to convenience.

b. **Wheat flour** - White wheat flour was purchased at a local supermarket.

c. **Salt** – The level of salt was determined mainly on flavor and taste.

d. **Oil** – Commercial soy oil (Milumor, Haifa, Israel) was purchased locally.

e. **Egg protein** - We have incorporated two different possibilities, either a fresh egg protein to simulate conditions where other sources are not available, and also a commercial source (i.e., Sigma Catalog #A55253). Obviously, the source of egg could affect cost and also the microbial status of the product.

f. **Water** – Tap water was added at specific concentrations that are listed.

g. **Emulsifiers** – Glycerine monostearate, GMS 41% (Catalog #030089; Sharon Labs. Industrial Zone Ad Halom, Ashdod, Israel) containing 41% distilled 1-monoglycerine was used. Lecithin – Lecimuthin 100 (Lucas Meyer, Hamburg, Germany) was also used as a releasing agent.

h. **Sucrose** – White commercial sucrose was added for taste and color.

a. **Amaranth leaves** – Green young leaves of Amaranthus cruentus R-736 grown locally were used. The leaves were harvested, cleaned and let to dry at ambient temperature.

The dry leaves were grinded and stored at –20°C. The frozen leaves were thawed before mixing with the amaranth flour according to the various formulations.

2. Production process

a. **Amaranth flour** – The seeds were cleaned manually before milling on Grindomix (Retsch, Haan, Germany) operating at 10,000 RPM for 30 s. The flour was sieved through a 60/100 standard sieves (Retsch) on a vibrating unit (AS200 basic, Retsch). Sieving conditions were full amplitude (100%) for 15 min. Only the fraction that passed 60 Mesh and remained on 100 Mesh was collected for further usage. The larger particles were re-milled once, and the whole cycle was repeated. The flour was utilized on the same day to avoid any possible enzymatic activity and possible deterioration. Throughout the experiments, the 100% basis was set as the flour initial concentration (before mixing). This system is widely and frequently used in the baking industry.

b. **Wafer production** – Baking was carried out on a heated home type grill pressing plates (Multi Grill, SOL, K36, Italy). The procedure was as follows:

   1). Mixing of all dry ingredients in a pan.

   2). Addition of oil, egg protein, and water, and gentle mixing to avoid any lumps, until a uniform batter is produced.

   3). Preheating the hot plates to ca. 200°C (processing temperature on the surface of the wafer was 180 to 185°C).

   4). A small amount (5 to 6 g) was poured on the lower plate, and the upper plate was closed slightly leaving ca. 1 cm gap between it and the wafer, for 6 min. The upper plate was then closed touching the wafer and baking continued for additional 6 min.

   5). The wafer was replaced from the plates with a spatula, cooled down and stored for further analyses and assessments.

3. Measurements

a. **Sorption isotherm** – Constant water activities measurements were carried out using the following saturated salt solutions: KCl, KI, K_2CO_3, KCH_3COO yielding at 25°C these water activities: 0.85, 0.68, 0.43, 0.2, respectively.

b. **Visual Assessment** – Following the baking process, the wafer was assessed for its integrity, stickiness, overall appearance, number of air bubbles, overall quality, and its

possible resemblance to a commercial wafer. The following visual score scale (from 0 to 10) was utilized: 0 – the wafer disintegrated to small pieces; 3- sticky wafer containing a large number of air bubbles; 5 – small holes were obvious, but the wafer integrity and quality was low and barely acceptable; 7 – improved quality, not too rough surface, tiny holes, acceptable overall appearance; 10 – excellent appearance and color, no holes, smooth and shiny surface. This subjective scale and was used for appearance judgment. However, the overall acceptability of the wafer should be also based on organoleptic taste, which needs to be evaluated at the local site where the product will be sold. The consumer tests are one of the most crucial for judging the product acceptability and therefore remain to be carried out. No flavors were added.

c. **Chemical Analyses** – Ca, Mg, Fe, Zn, Na and P were determined using an ICP apparatus (Spectroflame Module E, Kleve, Germany). Data is listed in PPM.

d. **Protein concentration** – Standard Kjeldhal (Model 1031, Tecator, Sweden) procedure were applied

RESULTS

All the pertinent experiments carried out are given in Appendix A (Tables 1 to 5). The data obtained showed that wafer produced with 100% amaranth flour and containing emulsifiers such as lecithin or distilled mono and di-glyceride of fatty acids provided a product that was judged acceptable and could be developed for commercial utilization. These main conclusions could be drawn:

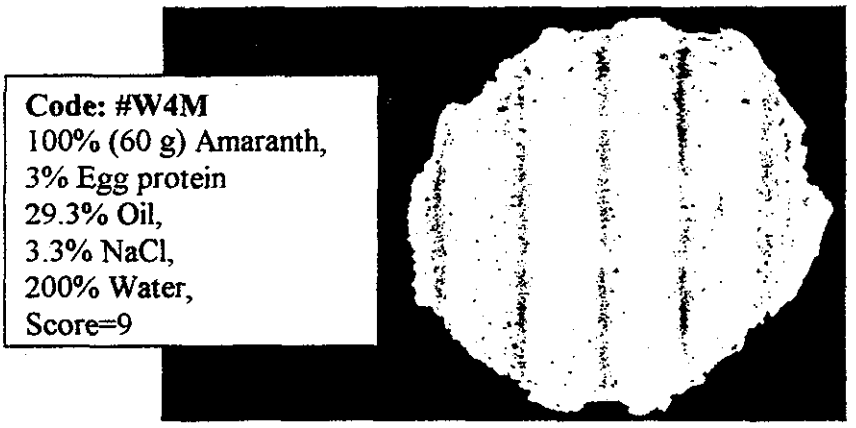
1. **No additives** – When no emulsifiers were added, and egg protein was used as the binder, both 60 and 100% amaranth flour formulations yielded satisfactory results as could be judged from the picture of the typical formulation highlighted in Appendix B. Initial egg protein concentration was fixed to 3% as below this level the wafer disintegrated and could not maintain its desired form and shape. An optimal wafer was produced using the following concentration:

Table 1: Optimal formulation for wafer (no additives; See appendix A for details)

Code:	**W18**	
Ingredient	**g**	**%**
Amaranth	40.0	66.7
Flour	20.0	33.3
Egg protein	1.8	3.0
Oil	17.6	29.3
Salt	2.0	3.3
Water	117.6	196.0
Score (1-10)	**10**	

This combination yielded a wafer that was judged superior and was visually scored as 10, which describes an excellent appearance, no holes, smooth and shiny surface. Typical appearance is give in the following picture:

Picture 1: Typical appearance of a wafer containing no added emulsifiers (picture codes are and complete formulation are listed in Appendix A):

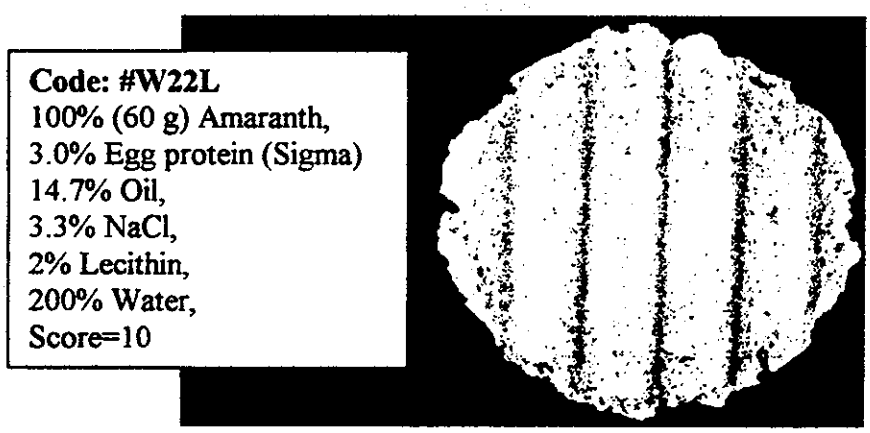


2. **Oil Concentration** – We have tested several formulations that included from 14.7 to 44% oil. This wide range could provide a completely different product aiming to a large spectrum of consumers. Those with specific health oriented requirements that on one hand would like to benefit from Amaranth renown nutritional benefits of reducing cholesterol and other health positive aspects (e.g., fibers), to those who need an intensive energy source. Obviously a product with an initial oil content of 44% becomes a very intensive health snack.

3. **Protein source** – Data supported the assumption that protein source for baking of the wafer could be the most convenient and economically feasible. Obviously, utilizing fresh egg as a protein source needs to comply with the strict health and microbial requirements especially concerning salmonella. The high temperature utilized for the baking could be sufficient to destroy most health related microorganisms, yet this assumption needs to be verified and was not tested herewith.
4. **Emulsifiers** – Utilization of emulsifiers in baking procedure are required for several purposes, namely, improving the dough consistency, enhancing the distribution of the oil into the matrix and improving the release mechanism between the wafer and the baking plates. The stickiness problem could become a severe problem as it directly affects the wafer surface roughness and appearance. Moreover the batter that sticks to the surface is exposed for relatively long time and high temperature, and could lead to the creation of burned speckles affecting the overall appearance of the yielded wafer. It is worth noting that fresh egg protein itself could act as emulsifiers due to its phospholipids. Lecithin is used frequently for baking as a release agent. However, our data (Table 2, Appendix A) showed that Lecthin at concentration of up to 2% was not beneficial at all and the quality of the wafer was inferior (the overall score decreased to 5 due mainly to stickiness). These results indicate that probably the added lecithin resulted in a negative impact affecting the quality of the final product.

When a commercial protein source was used, lecithin had a very positive impact as could be seen from Picture 2.

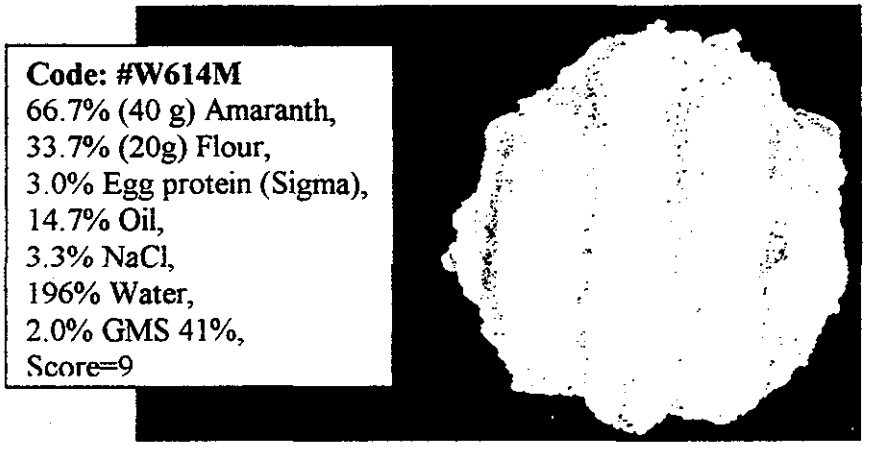
Picture 2: Typical appearance of a wafer containing lecithin and a commercial egg protein (picture codes are and complete formulation are listed in Appendix A):



These results clearly indicate that lecithin has a positive affect on the release mechanism and should be used with commercial sources of protein.

Other emulsifiers were also quite effective. For instance GMS 41% had a similar effect and could be used to replace lecithin (Picture 3).

Picture 3: Typical appearance of a wafer containing GMS 41% and a commercial egg protein (picture codes and complete formulation are listed in Appendix A).



Thus, GMS 41% could offer another option, which could be more economical, and the final decision which emulsifier should be used depends, mainly on the effectiveness, as well as functionality and cost. There are many additional emulsifiers that could be used, and the final choice should be made locally.

5. **Added Leaves** – In order to fully utilize the amaranth (both the leaves and the seeds) several baking experiments were carried out. Up to 10% dry leaves were used. The leaves had only a minor impact on the overall appearance when both emulsifiers were used (lecithin and GMS 41%). The color however was affected to be greener. This could have a negative consumer impact on its marketability and therefore needs careful consideration.

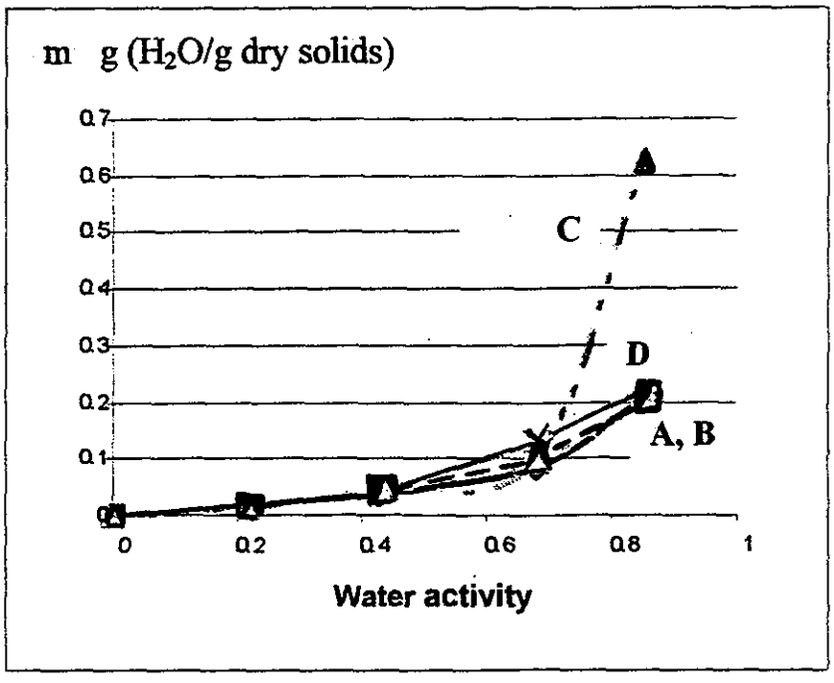
Table 2: Optimal formulation for wafer containing leaves and emulsifiers (Details in Appendix A)

CODE:	**W8LM**	
Ingredient	**g**	**%**
Amaranth (natural grown)	37.0	61.7
Flour	20	33.3
Protein (Sigma)	1.8	3.0
Oil	8.8	14.7
Salt	2.0	3.3
Water	117.6	196.0
Lecithin	0.6	1.0
GMS 41%	0.6	1.0
Leaves	3.0	5.0
Score (1-10)	**9**	

6. ***Added Sucrose*** – Wafer could be providing not only as a nutritious snack, it could also be produced with added sucrose and could serve completely other market needs. Not only that this added sugar could change the consumer appeal, it could also control the texture and color. It could also cover up some of the off flavors that could be attributed to the leaves that may be included in the formulation. Our tests (Table 4, Appendix A) showed clearly that excellent visual appearance could be obtained.
7. **Water activity** – The wafer is a dry product that should provide adequate shelf life to allow its commercialization. In our experiments, the final water content of the baked wafers was: 19 to 27% (wet basis). This water content although is a significant reduction from the initial water content of ca. 60%, nevertheless, resulted in a water activity of ca. 0.75 to 0.80. This is quite high water activity that indicates that for a commercial product, the wafer needs further water removal, or formulation change in order to guarantee a microbial stable product. We have not further optimized this water content, as it should be carried out in combination with texture and flavor that appeals to the local market. In order to enhance water removal, there are many approaches. For instance, higher baking temperature and/or longer time, reduced water in the formulation, etc.

As indicated, water activity is an important quality attribute. Hence, we have utilized several formulations for determining their sorption isotherms. Typical data is listed in Fig. 1.

Figure 1: Amaranth wafer isotherm at 25°C (A= Amaranth 30 g; Egg protein, 0.9 g; Oil, 8.8 g; Salt, 1g; Water 60 g; B- Amaranth 30 g; Egg protein, 0.9 g; Oil, 4.4 g; Salt, 1g; GMS 41%, 0.6 g; Water 60; C- Amaranth 30 g; Egg protein, 0.9 g; Oil, 4.4 g; Salt, 1g; GMS 41%, 0.6 g; Sucrose, 3 g; Water 60g; D- Amaranth 28.5 g; Amaranth leaves, 1.g g; Egg protein, 0.9 g; Oil, 8.8 g; Salt, 1g; GMS 41%, 0.6 g; Water 60).



These data shows that only when sucrose was added the moisture content increased significantly. Hence, for most wafers, reducing slightly the water content reduces significantly the water activity and the product should be shelf stable. Product containing sucrose on the other hand could absorb much more water. The exact shelf life should be determined based on the local conditions (temperature and relative humilities) taking also in consideration the final package.

8. **Mineral content and protein** – Table 5 (Appendix lists several chemical analyses carried out on the wafer. It could be seen that the wafer is an excellent source of protein containing up to 15%. It also shows that the wafer could provide only

marginal calcium, and lacks iron. Thus, clearly indicates that for nutritional reason if this wafer is to be utilized for children or to improve the diet of the consumers, it should be supplemented with nutrients to meet the recommended RDA. It is worth noting that these wafers could be used to address special nutritional requirements such as individuals with celiac disease. Due to the special structure of this wafer it could provide for these people a bread substitute, which is easy to prepare and requires no special preparations.

In conclusion, it has been demonstrated that Amaranth could be used as either a sole flour source, or in addition to wheat flour, and/or with amaranth leaves to produce wafers. The wafers have high protein content and could be stored for a significant shelf life (when their water activity is below 0.7). Both egg protein and commercial egg protein were used. The wafer could be sweetened with sucrose to produce a sweet product appealing to various market needs. The wafer however needs to be enriched if it should be used to provide the RDA required for children or young adults. The ability to transfer this technology to developing country is almost guaranteed, as it requires a low investment in equipment. However, the wafer needs to be optimized for the local pallet and market needs. The possibility to incorporate up to 10% dry leaves in the formula is very promising as it could enhance the utilization of the amaranth plant and reduce the cost of the wafer. It should however be pointed out that the final formulation needs to be tested locally and address the local taste, flavors, customs and habits as well as occasions where wafers are utilization.

APPENDIX A

Table 1: Formulation for experimenting in production of Amaranth wafer

Code:	W1		W2		W3		W4		W15		W16		W17		W18		W19	
Ingredient	**g**	**%**	**g**	**%**	**g**	**%**	**g**	**%**	**g**	**%**	**g**	**%**	**g**	**%**	**g**	**%**	**g**	**%**
Amaranth	60.0	100.0	60.0	100.0	60.0	100.0	60.0	100.0	40.0	66.7	40.0	66.7	40.0	66.7	40.0	66.7	40.0	66.7
Flour	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	33.3	20.0	33.3	20.0	33.3	20.0	33.3	20.0	33.3
Egg protein	1.8	3.0	1.8	3.0	3.0	5.0	4.2	7.0	1.8	3.0	1.8	3.0	3.0	5.0	1.8	3.0	1.8	3.0
Oil	17.6	29.3	26.4	44.0	8.8	14.7	8.8	14.7	17.6	29.3	8.8	14.7	8.8	14.7	17.6	29.3	26.4	44.0
Salt	2.0	3.3	2.0	3.3	2.0	3.3	2.0	3.3	2.0	3.3	2.0	3.3	2.0	3.3	2.0	3.3	2.0	3.3
Water	120.0	200.0	120.0	200.0	120.0	200.0	120.0	200.0	120.0	200.0	120.0	200.0	117.6	196.0	117.6	196.0	117.6	196.0
Score (1-10)	**0**		**7**		**8**		**2**		**6**		**5**		**8**		**10**		**4**	

Table 2: Formulation for wafer experiments containing emulsifiers

Code:	**W611L**		**W612L**		**W613M**		**W614M**	
Ingredient	**g**	**%**	**g**	**%**	**g**	**%**	**g**	**%**
Amaranth	40.0	66.7	40.0	66.7	40.0	66.7	40.0	66.7
Flour	20.0	33.3	20.0	33.3	20.0	33.3	20.0	33.3
Egg protein	1.8	3.0	1.8	3.0	1.8	3.0	1.8	3.0
Oil	8.8	14.7	8.8	14.7	8.8	14.7	8.8	14.7
Salt	2.0	3.3	2.0	3.3	2.0	3.3	2.0	3.3
Water	117.6	196.0	117.6	196.0	117.6	196.0	117.6	196.0
Lecithin	0.6	1.0	1.2	2.0				
GMS 41%					0.6	1.0	1.2	2.0
Score (1-10)	**5**		**5**		**5**		**9**	

Table 3 Formulation for wafer experiments containing emulsifiers and industrial protein source

Code:	W211LM		W22L		**W23M**	
Ingredient	**g**	**%**	**G**	**%**	**g**	**%**
Amaranth	40.0	66.7	60.0	100.0	60.0	100.0
Flour	20.0	33.3	0.0	0.0	0.0	0.0
Egg protein (sigma)	1.8	3.0	1.8	3.0	1.8	3.0
Oil	8.8	14.7	8.8	14.7	8.8	14.7
Salt	2.0	3.3	2.0	3.3	2.0	3.3
Water	117.6	196.0	120.0	200.0	120.0	200.0
Lecithin	0.6	1.0	1.2	2.0		
GMS 41%	0.6	1.0			1.2	2.0
Score (1-10)	**9**		**10**		**8**	

Table 3 Formulation for wafer experiments containing emulsifiers and industrial protein source (cont.)

CODE	W4M		W613M		W614M	
Ingredient	g	%	g	%	g	%
Amaranth	60.0	100.0	40	66.7	40	66.7
Flour		0.0	20	33.3	20	33.3
Egg protein (Sigma)	1.8	3.0	1.8	3.0	1.8	3.0
Oil	17.6	29.3	8.8	14.7	8.8	14.7
Salt	2.0	3.3	2	3.3	2	3.3
Water	120.0	200.0	117.6	196.0	117.6	196.0
Lecithin		0.0		0.0		0.0
GMS 41%		0.0	0.6	1.0	1.2	2.0
Score (1-10)	**9**		**5**		**9**	

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Table 4 Formulation for wafer experiments containing natural grown Amaranth and leaves

CODE:	W7		W7M		W7SM		W7VLM		W7V2M		W7V3M		W7V4M		W7V5M	
Ingredient	g	%	g	%	g	%	G	%	g	%	g	%	g	%	g	%
Amaranth	60	100.0	40	66.7	40	66.7	40	66.7	34	56.7	57	95.0	54	90.0	54	90.0
Flour		0.0	20	33.3	20	33.3	20	33.3	20	33.3		0.0		0.0		0.0
Egg protein	1.8	3.0	1.8	3.0	1.8	3.0	1.8	3.0	1.8	3.0	1.8	3.0	1.8	3.0	1.8	3.0
Oil	17.6	29.3	8.8	14.7	8.8	14.7	8.8	14.7	8.8	14.7	8.8	14.7	8.8	14.7	17.6	29.3
Salt	2	3.3	2	3.3	2	3.3	2	3.3	2	3.3	2	3.3	2	3.3	2	3.3
Water	120	200.0	117.6	196.0	117.6	196.0	120	200.0	120	200.0	120	200.0	120	200.0	120	200.0
Lecithin		0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0
GMS 41%		0.0	1.2	2.0	1.2	2.0	1.2	2.0	1.2	2.0	1.2	2.0	1.2	2.0	1.2	2.0
Sugar					6	10.0							6	10.0	6	10.0
Leaves*							6	10.0	6	10.0	3	5.0	6	10.0	6	10.0
Score (1-10)	**9**		**10**		**9**		**9**		**7**		**8**		**9**		**8**	

Table 5: Formulation for wafer experiments containing natural grown Amaranth and leaves and chemical analyses

CODES:	W7		W7M		W7VM		W7V3M		W7V5M		F-1	
Ingredient	**g**	**%**	**g**	**%**	**g**	**%**	**g**	**%**	**g**	**%**	**g**	**%**
Amaranth*	60	100.0	40	66.7	40	66.7	57	95.0	54	90.0	40	66.7
Flour		0.0	20	33.3	20	33.3		0.0		0.0	20	33.3
Protein*	1.8	3.0	1.8	3.0	1.8	3.0	1.8	3.0	1.8	3.0	1.8	3.0
Oil	17.6	29.3	8.8	14.7	8.8	14.7	8.8	14.7	17.6	29.3	8.8	14.7
Salt	2	3.3	2	3.3	2	3.3	2	3.3	2	3.3	2	3.3
Water	120	200.0	117.6	196.0	117.6	196.0	120	200.0	120	200.0	117.6	196.0
Lecithin		0.0		0.0		0.0		0.0		0.0	0.6	1.0
GMS 41%		0.0	1.2	2.0	1.2	2.0	1.2	2.0	1.2	2.0	0.6	1.0
Sugar									6	10.0		
Leaves*		0.0		0.0	6	10.0	3	5.0	6	10.0		0.0
Chemical Analyses (PPM, Except protein, %)												
Ca	1.21		1.67		2.4		1.86		2.52		0.76	
Mg	1.96		1.73		2.52		2.58		2.59		1.44	
Fe	0.08		0.06		0.07		0.09		0.07		0.07	
Zn	0.13		0.1		0.09		0.1		0.11		0.14	
K	4.03		3.48		4.64		5.57		4.79		3.29	
Na	9.55		10.59		8.11		8.98		0.43		8.51	
P	3.63		2.99		3.12		3.85		3.09		2.78	
Protein (%)	13.86		14.17		13.46		14.70		12.79		12.94	

Table 5: Formulation for wafer experiments containing natural grown Amaranth and leaves and chemical analyses (cont..)

CODE:	W8M		W8VM		W8V3M		W8V5M		W8LM		W5SM		W5SV1M		W5SV2M	
Ingredient	g	%	g	%	g	%	g	%	g	%	g	%	g	%	g	%
Amaranth*	40	66.7	40	66.7	57	95.0	54	90.0	37	61.7	40	40.0	57	57.0	57	57.0
Flour	20	33.3	20	33.3		0.0		0.0	20	33.3	20	20.0		0.0		0.0
Protein*	1.8	3.0	1.8	3.0	1.8	3.0	1.8	3.0	1.8	3.0	1.8	1.8	1.8	1.8	1.8	1.8
Oil	8.8	14.7	8.8	14.7	8.8	14.7	17.6	29.3	8.8	14.7	8.8	8.8	8.8	8.8	8.8	8.8
Salt	2	3.3	2	3.3	2	3.3	2	3.3	2	3.3	2	2.0	2	2.0	2	2.0
Water	117.6	196.0	117.6	196.0	120	200.0	120	200.0	117.6	196.0	117.6	117.6	120	120.0	120	120.0
Lecithin	1.2	2.0	1.2	2.0	1.2	2.0	1.2	2.0	0.6	1.0		0.0		0.0		0.0
GMS 41%									0.6	1.0	1.2	2.0	1.2	2.0	1.2	2.0
Sugar		0.0		0.0		0.0	6	10.0		0.0	12	12.0	6	6.0	12	12.0
Leaves*		0.0	6	10.0	3	5.0	6	10.0	3	5.0		0.0	3	3.0	3	3.0
Score (1-10)	10		8		8		6		9		7		6		7	
Chemical Analyses (PPM; Except protein, %)																
Ca	1.19		3.38		2.76		2.69		1.95		1.67		6.63		1.96	
Mg	1.85		3.27		3.1		2.92		2.03		1.53		2.8		2.55	
Fe	0.1		0.11		0.11		0.09		0.08		0.07		0.16		0.08	
Zn	0.1		0.08		0.09		0.07		0.07		0.23		0.33		0.12	
K	4.24		6.29		5.41		5.56		4.06		3.11		5.17		5.16	
Na	10.15		10.5		10.7		7.8		8.05		7.6		11.42		8.24	
P	3.81		4.49		5.1		3.98		2.89		2.76		4.16		3.88	
Protein (%)	14.35		13.8		15.14		12.4		13.06		12.74		13.16		14.56	

C.- Scientific impact of collaboration

This research showed the high potential of vegetable and grain amaranth for cultivation in Malawi and its use for human nutrition.

1- 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), particularly at the Bunda and Bvumbwe Research Stations that probably represent the best agroclimatic conditions for grain amaranth cultivation in Malawi. Adapted germplasm is available. Local accessions from Bunda *A. hypochondriacus* and *A. cruentus* were the best cultivars, showing relatively high yields in the two seasons across the two agroecological regions. *A. cruentus* also had large leaves and hence canopy cover on the ground was good. The shade reduced water evaporation from the soil and also suppressed weeds. Short-cycle amaranth types (e.g. the *A. hyp x A. hyb* hybrid cultivars) 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 was poor. Late maturing cultivars exhibited compensatory growth at later stages of development if exposed to mid season stress. Amaranth cultivars showed a good recovery after short drought periods during flowering or the grain filling stage. Seed weight was not affected. Cultivars differed in their drought tolerance, with local Malawian accessions showing good levels of tolerance. These results support the idea that amaranth is a promising crop even in areas with short spells of dry weather.

2- According to the results of this study, grain amaranth belongs to the type of plants whose response to drought is based on the avoidance strategy: stomata closure at relatively high leaf water potential, ability to perform photosynthesis at high temperature and low water availability, reduction of leaf expansion, and deep roots. No evidence of osmotic adjustment was found in response to drought. Thus grain amaranth is apparently well adapted for cultivation in hot regions, with rains or irrigation during early stages of growth and with end of season (terminal) drought.

3- The use of air-dried amaranth leaves as a source of iron and Vitamin C was examined. 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 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. Bioavailable iron, rather than total iron, is probably the appropriate index for ranking the leaves as a source for iron. Our 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. Supplementing the leaves during the dehydration process with a source of vitamin C might be advantageous during storage.

4- The use of amaranth flour for bread baking was studied. "Amaranth bread", with an amaranth concentration up to 30% in the flour and richer in protein compared to regular bread, was produced. Experiments to increase the level of amaranth beyond 30% were not successful, even when the gluten level was increased above 1.5% to 2.0%. Increasing the level of amaranth above 30% replacement suffers from two main obstacles: 1- the volume of the loaf is reduced significantly and the bread loaf was very dense and its quality inferior; 2-.the taste became very grainy ("like earth"). This organoleptic inferiority was not due to the origin of the amaranth seeds, since, several other amaranth seed sources did not improved this perceived low quality attribute. Thus, work was also focused on preparing other products containing amaranth at levels higher than 30%, such as waffles and crackers, since they are not based on a leavened structure and gluten. Such products are wafers (or biscuits), that may contain up to 100% amaranth and could also be enriched with amaranth leaves. It was shown that wafers could be baked with amaranth as sole source of flour, or in addition to wheat flour. The wafers have high protein content and could be stored with a relatively long shelf life when water activity is below 0.7. These products no not need special and expensive equipment, thus allowing direct technology transfer. Commercial production will require optimization for the local pallet and market needs.

In summary: Potential areas of amaranth cultivation were defined in Malawi according to the crop requirements. Seed of promising cultivars was exchanged for grain and leaf production in Malawi. Formulations for bread and wafer baking and use of leaves for human nutrition were elaborated and transferred to Malawi, for use with locally produced grain and leaves. A better understanding of physiological requirements and mechanisms of drought tolerance of the amaranth crop was achieved, and will improve its cultivation in areas prone to drought in Malawi.

Section II

A.- Managerial and Budget Items

During the 4th year of the project experiments were performed as planned. See attached letter about budget changes.

B.- Collaboration

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