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Second Annual Report: 1997-1998

IMPROVEMENT OF GRAIN AND VEGETABLE AMARANTH IN MALAWI

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Executive Summary

During the second year of the project, work in Malawi continued the agronomic evaluation in different regions of grain and vegetable cultivars of amaranth. Cultivars included early flowering, short cycle types sent from Israel, that showed good baking characteristics in the first year studies. Of the Malawi grain types, two cultivars showed consistently promising high yields in two consecutive years of the research.

Work in Israel was carried out in two main subjects:

1. Adaptability of grain amaranth to drought. Strategy and physiological mechanisms of adaptation to low water availability were studied in field experiments, under different irrigation treatments during the years 1996-7 and 1997-8, and results are presented together in this report. Work concentrated in the early flowering, short cycle types that are phenologically better adapted to end of season (terminal) drought. Grain amaranth posses an avoidance strategy when coping with drought, that is based on stomata closure at relatively high leaf water potential probably induced by root signals, deep roots and ability to perform photosynthesis at high temperature and light intensity at a relatively low stomatal conductance. These characteristics will enable short cycle types of grain amaranth to achieve good yields if initially provided with moderate amounts of water (rainfall, irrigation) for establishment and terminal drought after the onset of flowering.

2. Assessment of grain amaranth for replacing flour for bread making. Amaranth concentration of 30% in the flour was found as adequate for bread baking. Higher concentrations resulted in lower bread quality. Studies were performed to optimize bread quality by changing other ingredients such as dough extenders, gluten, cystein, vitamin C, hemicellulose, commercial enzymes and timing of salt addition. Different formulations were elaborated to obtain better quality bread. This information will be transferred to Malawi to initiate at Bunda experiments using locally produced grain by the promising cultivars.

Section I

A.- Research Objectives

The overall aims of the project are to diversify food supply by improving the cultivation of amaranth as a dual source for: a) high quality grain, rich in protein and lysine, and B) leafy vegetable, rich in vitamins and minerals, which will be available throughout the year to improve the population diet.

Specific research objectives are:

1. To select superior amaranth cultivars for leafy vegetable and grain production in different agroecological zones 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

The major objective of the research in Malawi was to select superior amaranth cultivars for leafy vegetable and grain production in different agroecological zones.

Field experiments during the 1997/98 season were performed at three sites: Bunda, Bvumbwe and Kasinthula. Fields were ploughed in November 1997. Planting was done with the rains in December 1997. Soil fertilization, and control of weeds, pests and diseased were performed during the crop season, as described in the First Annual Report. Leaves from vegetable cultivars were harvested during the vegetative period and grain from the grain cultivars was harvested at plant maturation. The work at Kasinthula (the farthest site) was discontinued due to financial problems. Therefore, only data for Bunda and Bvumbwe are presented.

Seedling emergence was observed 4 to 5 days after sowing, in all cultivars in both sites. Cultivars differed greatly in time to flowering (Table 1). The two hybrids of *A. hyp* x *A. hyb* (K-432 and K-433) were very early, short cycle types, that flowered 3 to 4 weeks after sowing. They produced short plants (ca. 80-100 cm tall) that, nevertheless, had a relative large inflorescence (Table 2). In contrast to the small size of the hybrid cultivars, their grain yield was about 1000kg/ha, representing 50 to 75 % of the highest yields reached by the taller cultivars. Yield per unit area of these hybrids can be increased by higher plant density in the field. *A. hyp* from the Bunda collection was also an early flowering cultivar (33 days after sowing) with relatively high yields (ca. 1800 kg/ha).

Yield variability was higher at Bvumbwe compared to Bunda. Nevertheless, yield rank of the different cultivars was similar in both sites, with *A. hyp, A.tri* and *A. cru* R-158 showing the highest yield

The fact that these early flowering cultivars had relatively large yields is very promising, since even in dry seasons they probably can provide a reasonable grain production by escaping end of season drought.

Table 1: Grain yield of different cultivars of Amaranth (kg/ha).

Cultivar	Days to flowering	Grain yield (kg/ha)	
	Bunda	Bunda	Bvumbwe
A. hypochondriacus (Bunda collect.) S1	33	1733	1833
A. tricolor (Bunda collection) S2	41	2377	1667
A. cruentus R-158 (2) S3	51	1929	1315
A. cruentus R-283 (11) S4	41	1188	926
A. cruentus R-109 (7) S5	46	1096	963
A. cruentus R-736 (12) S6	42	664	1112
A. cruentus (Bunda collection) S10	54	1358	1185
A. hypochondriacus X *A. hybridus* S8 (14)	27	819	813
A. hypochondriacus X *A. hybridus* S9 (15)	20	990	1041
A. montegazinus (Argentina) S7	46	926	1352
CV (%)	12.3	23.8	44.6
Significance	*	***	n.s.

Table 2: Plant height at flowering and inflorescence dimensions in different Amaranth cultivars.

Cultivar	Plant height(cm)		Inflorescence length (cm)		Inflorescence diameter(cm)	
	Bunda	Bvumbwe	Bunda	Bvumbwe	Bunda	Bvumbwe
A. hyp.	98	79	58	42	6.5	5.0
A. tric.	91	99	33	47	5.5	6.5
A.cru. R-158	83	78	51	56	5.7	3.5
A.cru. R-283	80	81	46	48	6.3	6.0
A.cru. R-109	85	89	51	50	6.2	7.8
A.cru. R-736	83	117	51	56	6.7	6.0
A.cru.	36	100	45	52	5.5	3.5
A.hyp.xA.hyb.	36	22	49	29	2.7	2.0
A.hyp.xA.hyb.	25	24	45	30	2.5	1.7
A.mont.	100	109	48	47	6.5	4.7
CV (%)	9.5	15.5	14.4	15.5	18.1	40.0
Significance	*	***	**	***	***	**

2- Research in Israel

Main goals of the research in Israel were:

a.- to study the adaptability of grain amaranth to drought.

b.- 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).

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* S8 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. Time-table for the experiments is presented in Table1.

In the 1996 experiment (Exp.1) the two cultivars (K-432 and K-433) were planted. 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).

Table 1- Time table, phenology and irrigation treatments in field experiments in Israel.

	Exp. 1 (1996)	Exp.2 (1997)
Time table		
Sowing	14/7	12/7
Emergence	18/7	16/7
Onset of flowering	10/8	7/8
Full flowering	23/8	20/8
Harvest	19/9	12/9
Growth cycle (days)	68	63
Irrigation treatments: amount of water (m^3 /ha)		
100%E	276	205
50%E	218	--
25%E	190	63
Arrested irrigation 1	203 (10/8)	112 (7/8)
Arrested irrigation 2	236 (25/8)	--

Leaf physiological parameters were measured in young, fully developed leaves. Stomata conductance (abaxial side) was measured with a steady state porometer (Licor –1600), rate of photosynthesis (CO_2 uptake) with a gas exchange unit (Licor-6200), osmotic potential of leaf sap with an osmometer (Wescor-5000) and leaf water potential with a pressure chamber (ARI). Soil water potential was measured at 08:00 AM at three depths (30, 60, and 90 cm) with tensiometers (Irrometer Co).

Results

Climatic conditions and soil water potential

Climatic conditions (max and min temperature, % relative humidity -RH) during the two crop seasons are presented in Fig.1. 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 resulted in different patterns of change in soil water availability, according to climatic conditions. In Exp1 (1996), irrigation treatments started at onset of flowering (23/8) and measurements were performed three times during a two week period (25/8, 31/8 and 8/9). Climatic conditions during this period were stable, with 35-38 °C max temperature and 23-28 % min RH (Fig.2). 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.

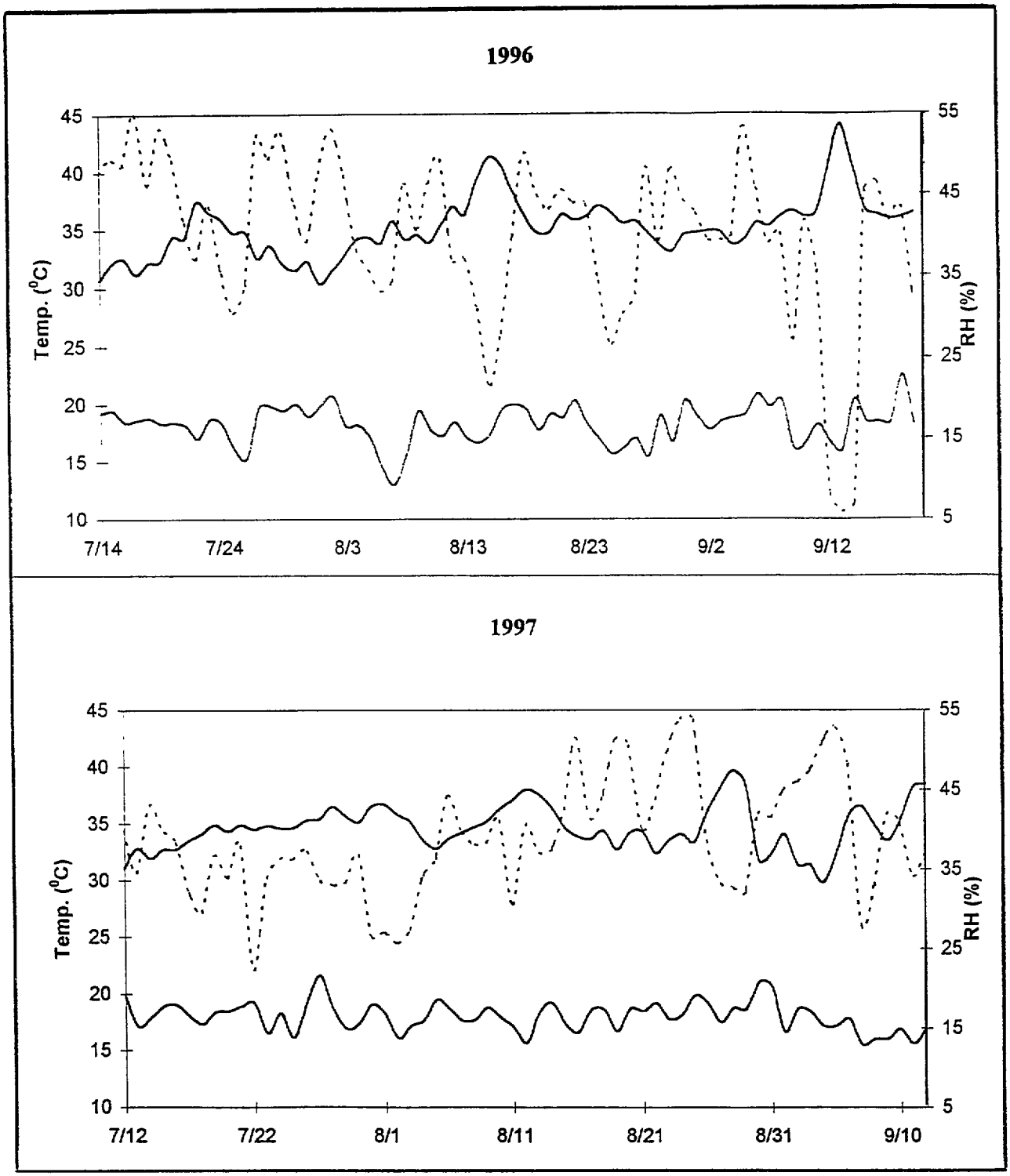

Fig. 1.- Seasonal pattern of changes in min and max temperature (full line) and %RH (broken line) at the Hula Valley during field experiments in 1996 and 1997.

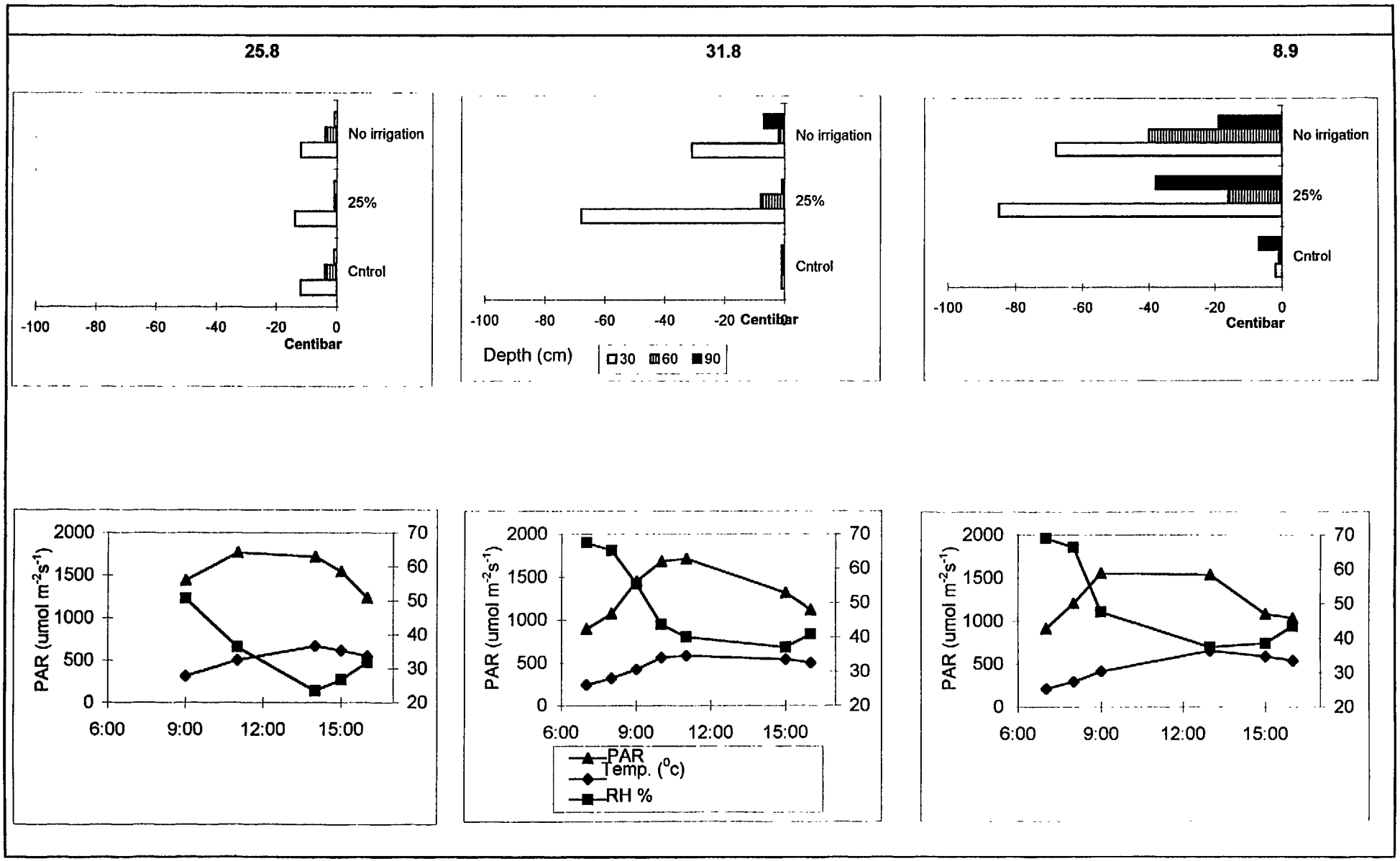

Fig. 2.- Soil water potential (at 8 AM), solar irradiation (PAR), air temperature and %RH during measurement days in 1996. Water potential was measured at 30, 60 and 90 cm depth.

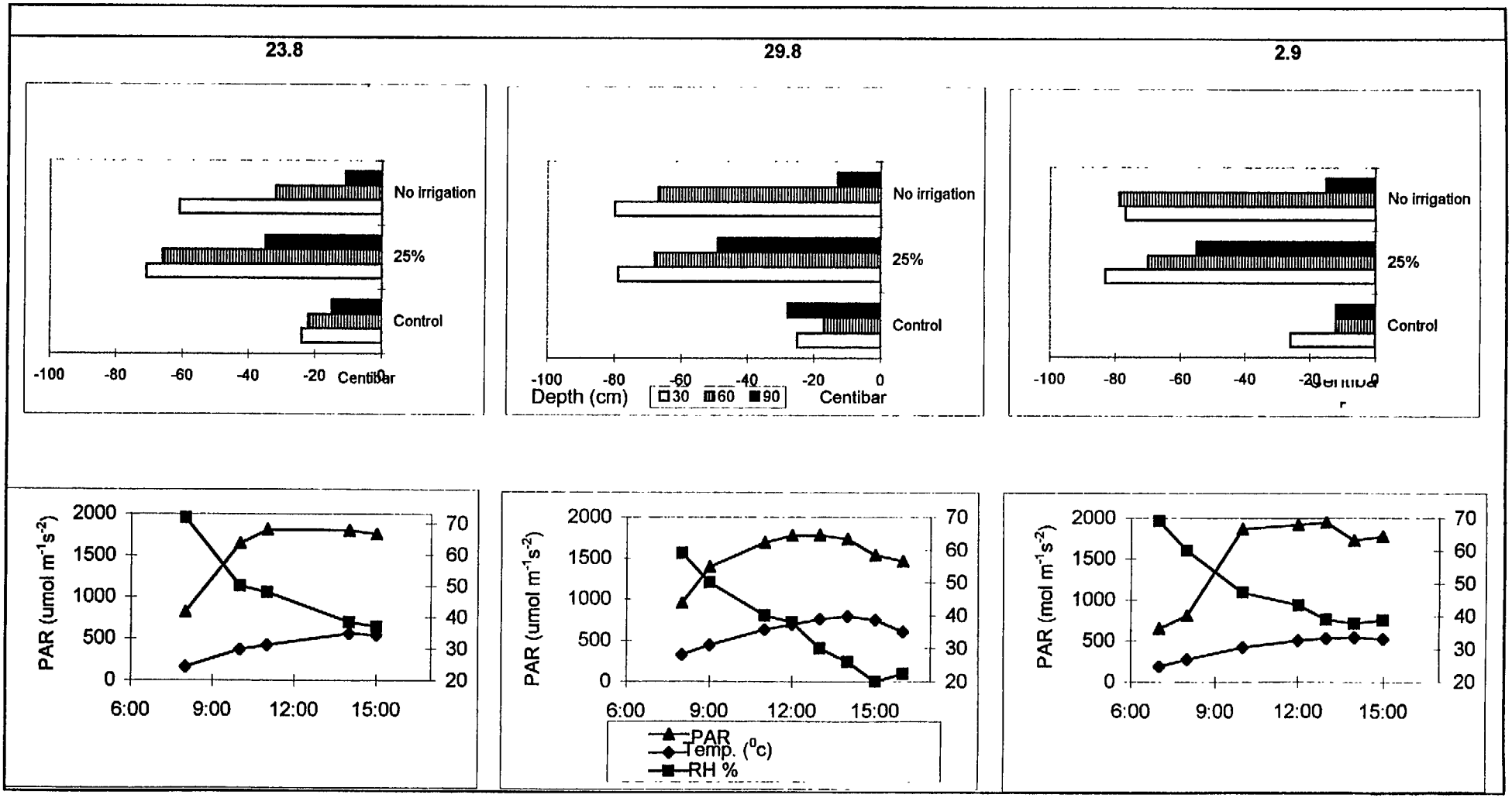

Fig. 3.- Soil water potential (at 8 AM), solar irradiation (PAR), air temperature and %RH during measurement days in 1997. Water potential was measured at 30, 60 and 90 cm depth.

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 (Table 1). Measurements were performed three times (23/8, 29/8 and 2/9). Climatic conditions were more severe during 29/8, with 40^0C max temperature and 20% min RH. 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 (Fig. 4). 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. It seems that 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 (Fig.5). 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 (Table 2). 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.

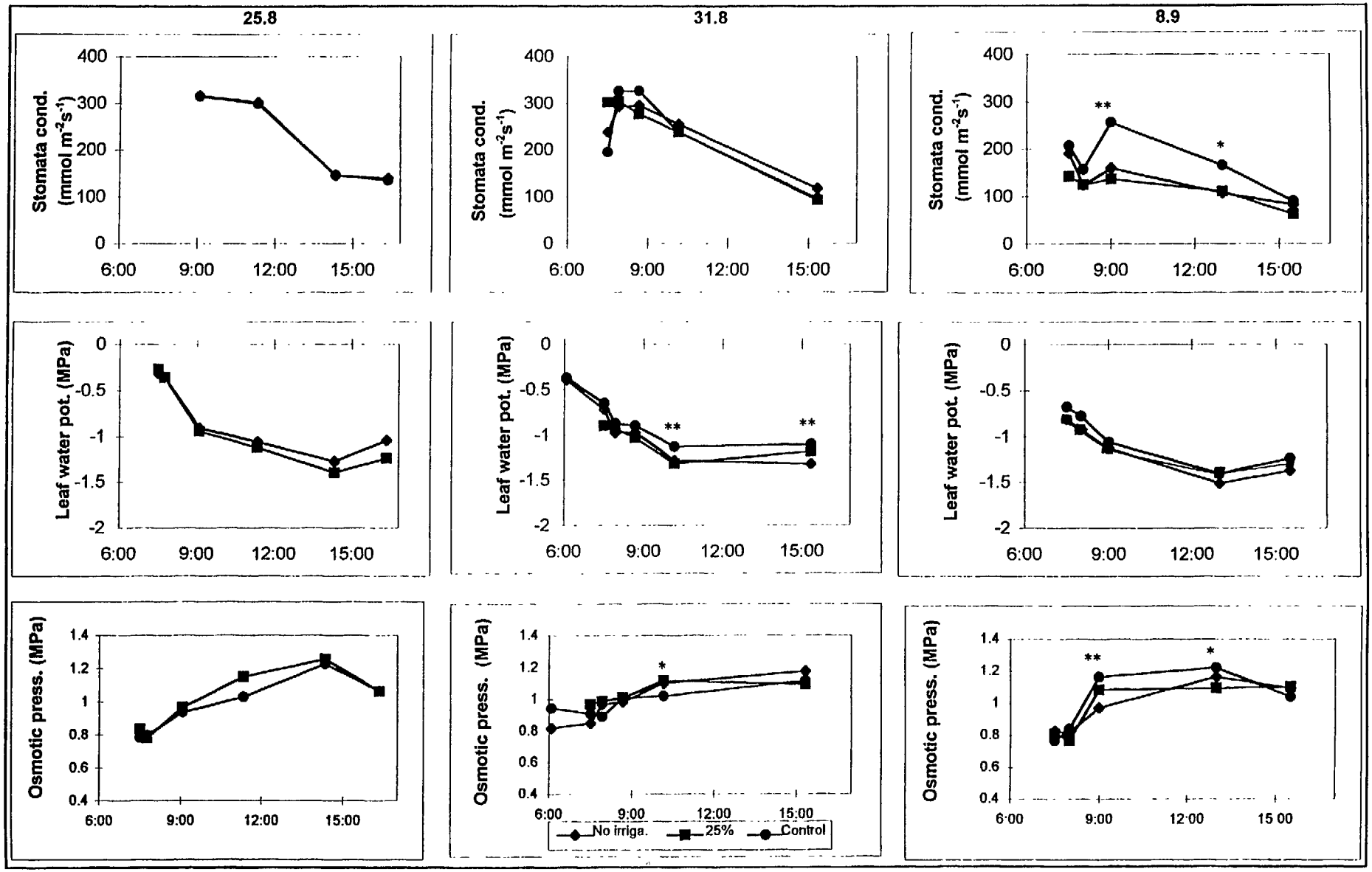

Fig. 4.- Effect of irrigation treatments on stomatal conductance, leaf water potential and osmotic potential in Exp 1 (1996). Treatments were continued irrigation (control), 25%E and cessation of irrigation 30 days after emergence (no irigation). * and ** indicate significant differences at P 0.05 and 0.01.

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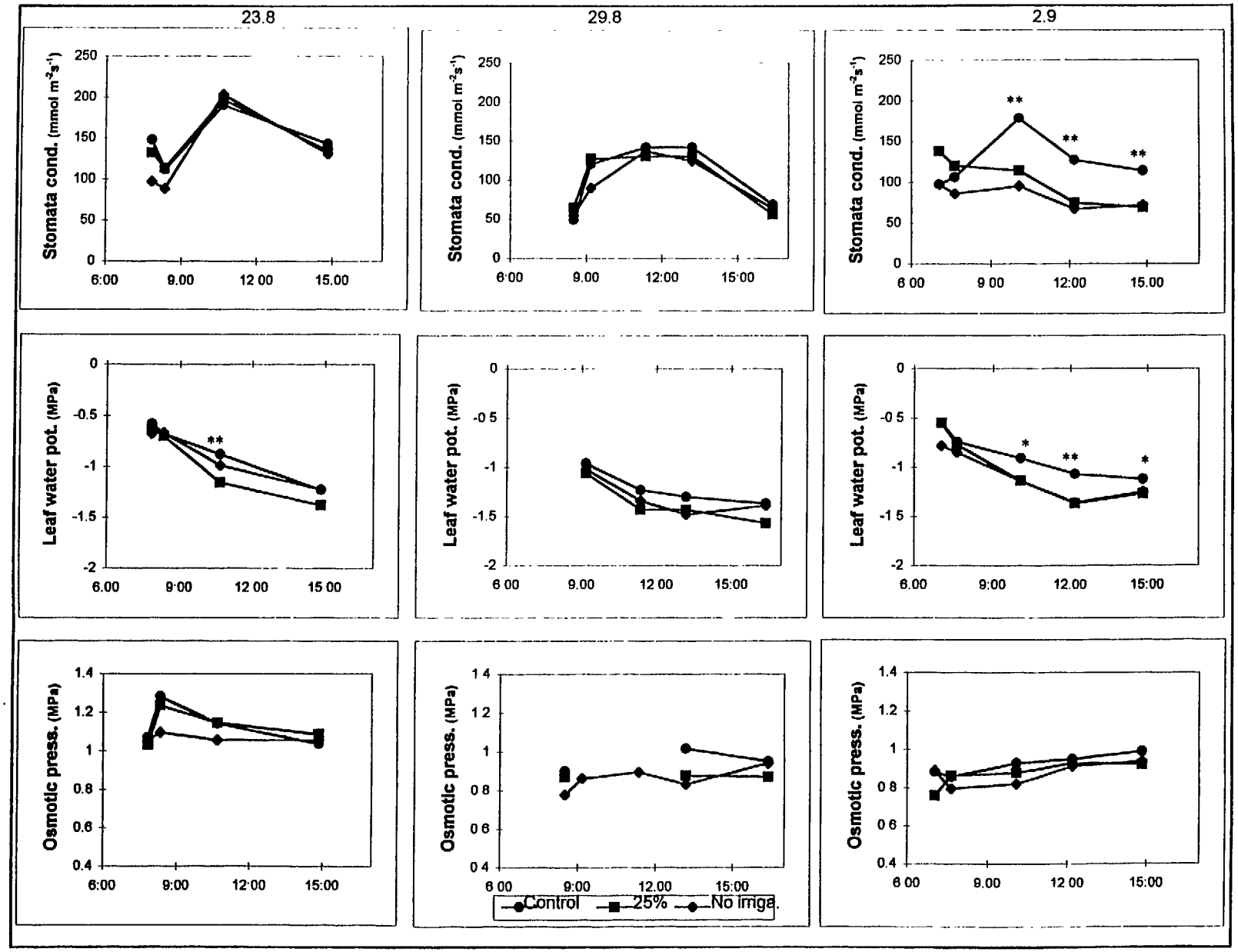

Fig. 5.- Effect of irrigation treatments on stomatal conductance, leaf water potential and osmotic potential in Exp 1 (1997). Treatments were continued irrigation (control), 25%E and cessation of irrigation 30 days after emergence (no irigation). * and ** indicate significant differences at P 0.05 and 0.01.

Table 2.- Effect of irrigation treatments on leaf osmotic potential at full turgor. Similar letters indicate no significant differences at P=0.05.

Exp.2 – 1997				Exp.1 - 1996			
Date	**Treatment**	**Osmotic potential (MPa)**	**Sign**	**Date**	**Treatment**	**Osmotic potential (MPa)**	**Sign.**
31.8	Control	0.91	a	**23.8**	Control	0.88	a
	25%E	1.03	a		25%E	0.83	a
	Int. irrigation	0.96	a		Int. irrigation	0.89	a
8.9	Control	0.88	a	**29.9**	Control	0.88	a
	25%E	0.86	a		25%E	0.83	a
	Int. irrigation	0.96	a		Int. irrigation	0.84	a
				2.9	Control	0.86	a
					25%E	0.81	a
					Int. irrigation	0.83	a

Relationships between stomatal conductance and leaf water potential

No linear relationship was found between leaf water potential and stomatal conductance (Fig. 5 and 6). 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). 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 tensiometers located at 90 cm depth indicated very low water availability (Fig. 3), suggests that the root system of amaranth remains active, pulling water from soil layers deeper than one meter.

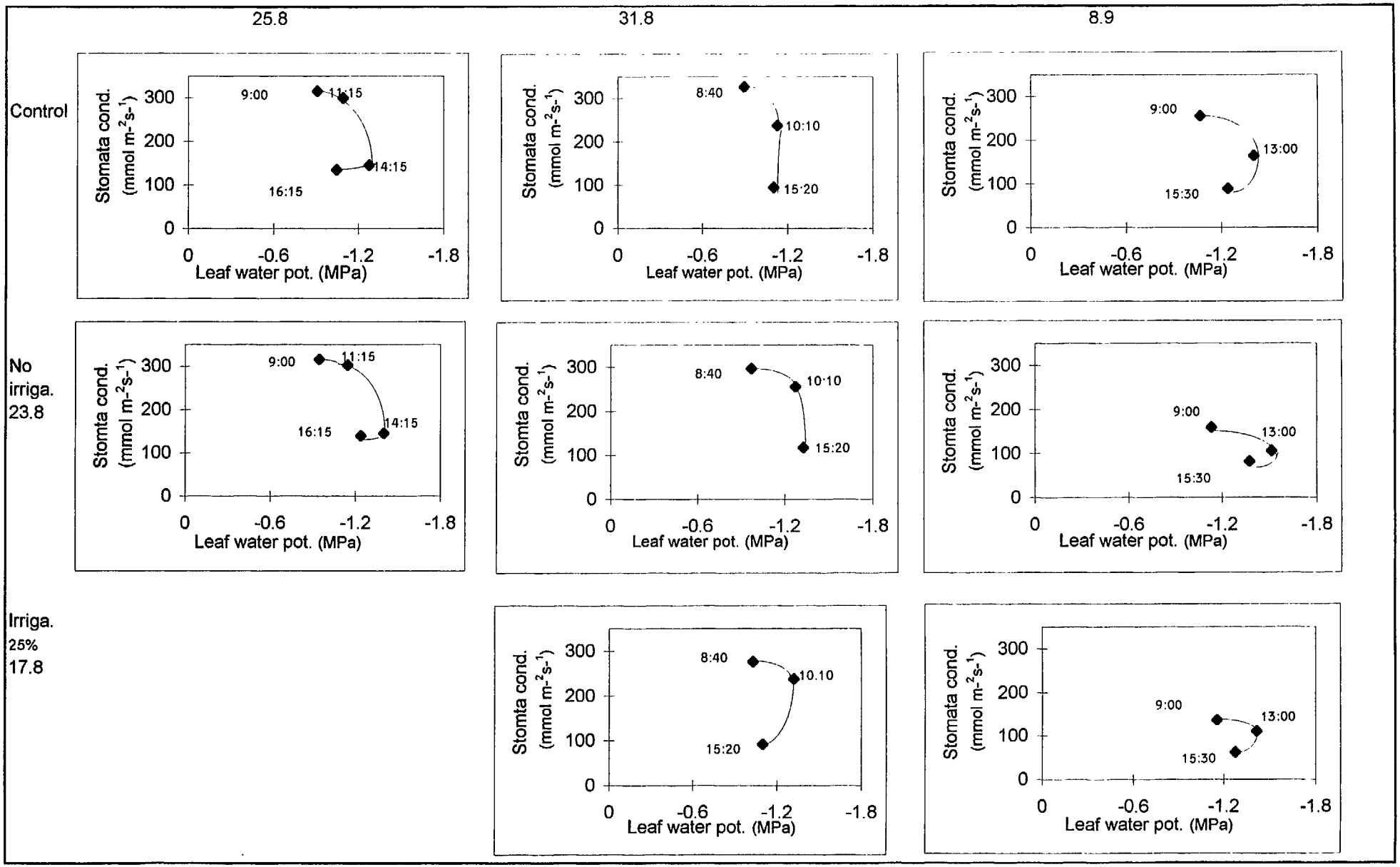

Fig. 6.- Relationship between stomatal conductance and leaf water potential in the different irrigation treatments in Exp 1 (1996).

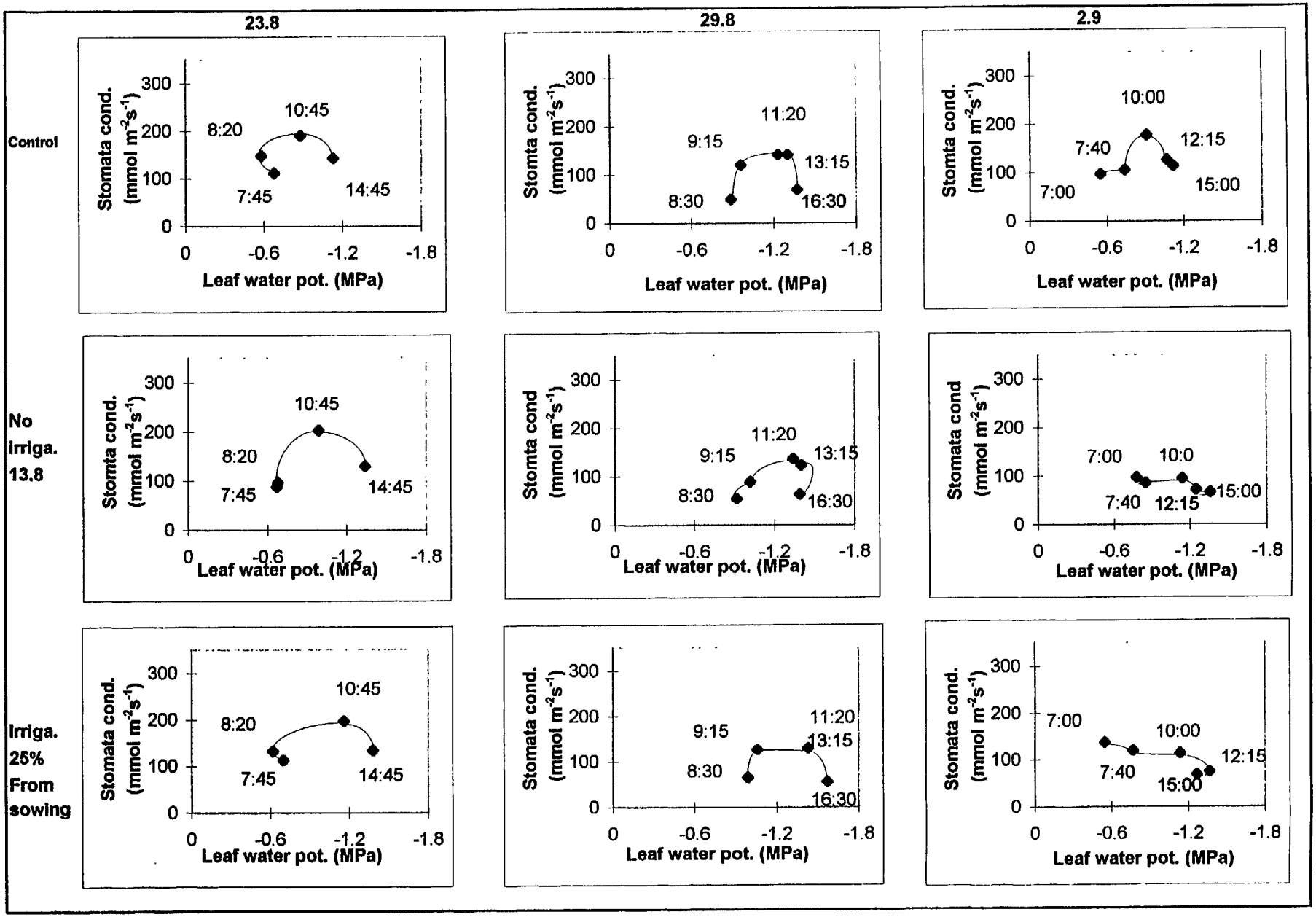

Fig. 7.- Relationship between stomatal conductance and leaf water potential in the different irrigation treatments in Exp 2 (1997).

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. 8). 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. 9).

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. 10). 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.11).

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 3.

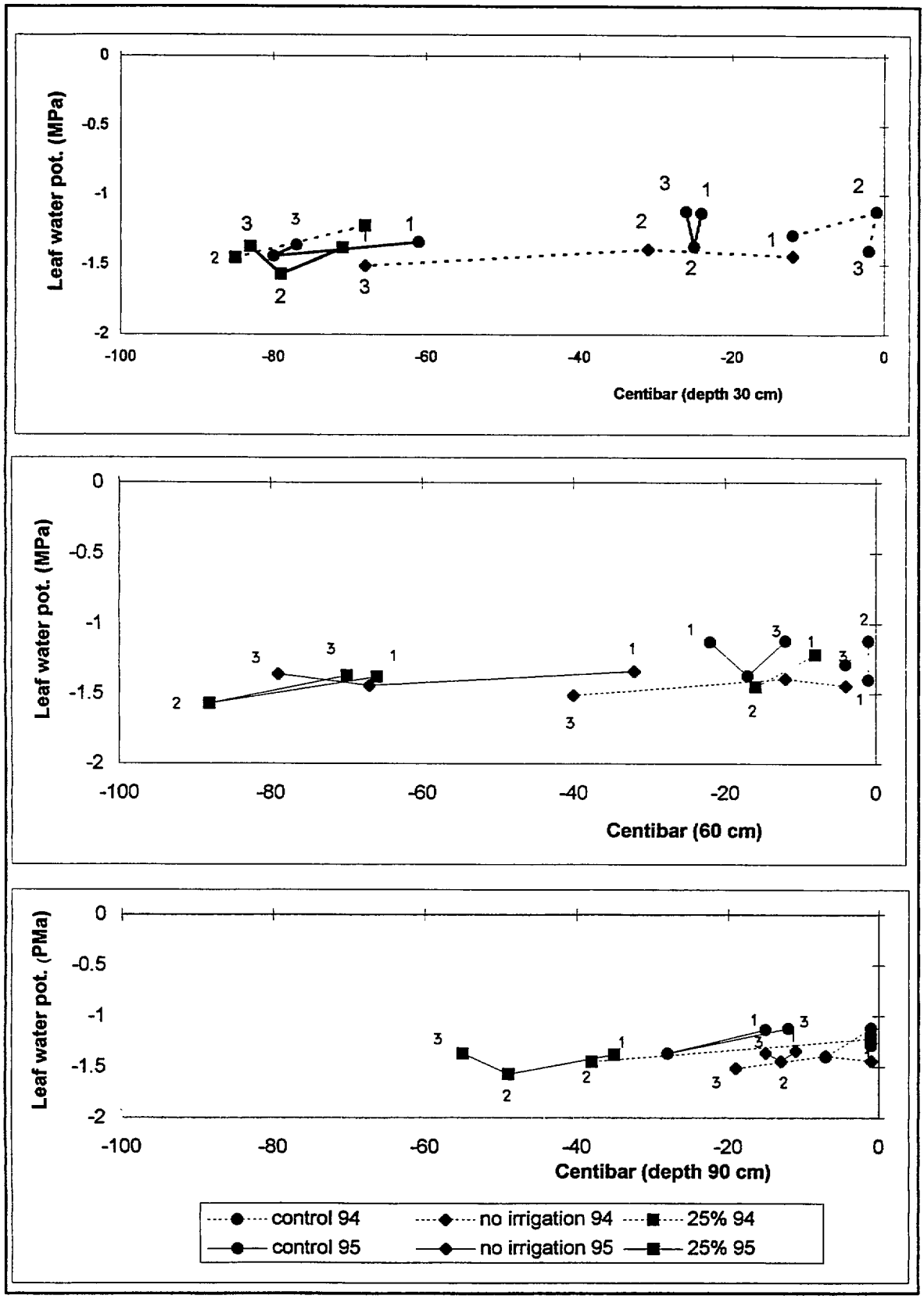

Fig. 8.- Relationships between min leaf water potential and soil water potential at 30 (top), 60 (middle) and 90 (bottom) cm depth. Data from 1996 (broken lines) and 1997 (full lines) were pooled together. Numbers (1,2 and 3) indicate measurement days. (•) Irrigated controls; (■) 25%E; (◆) cessation of irrigation.

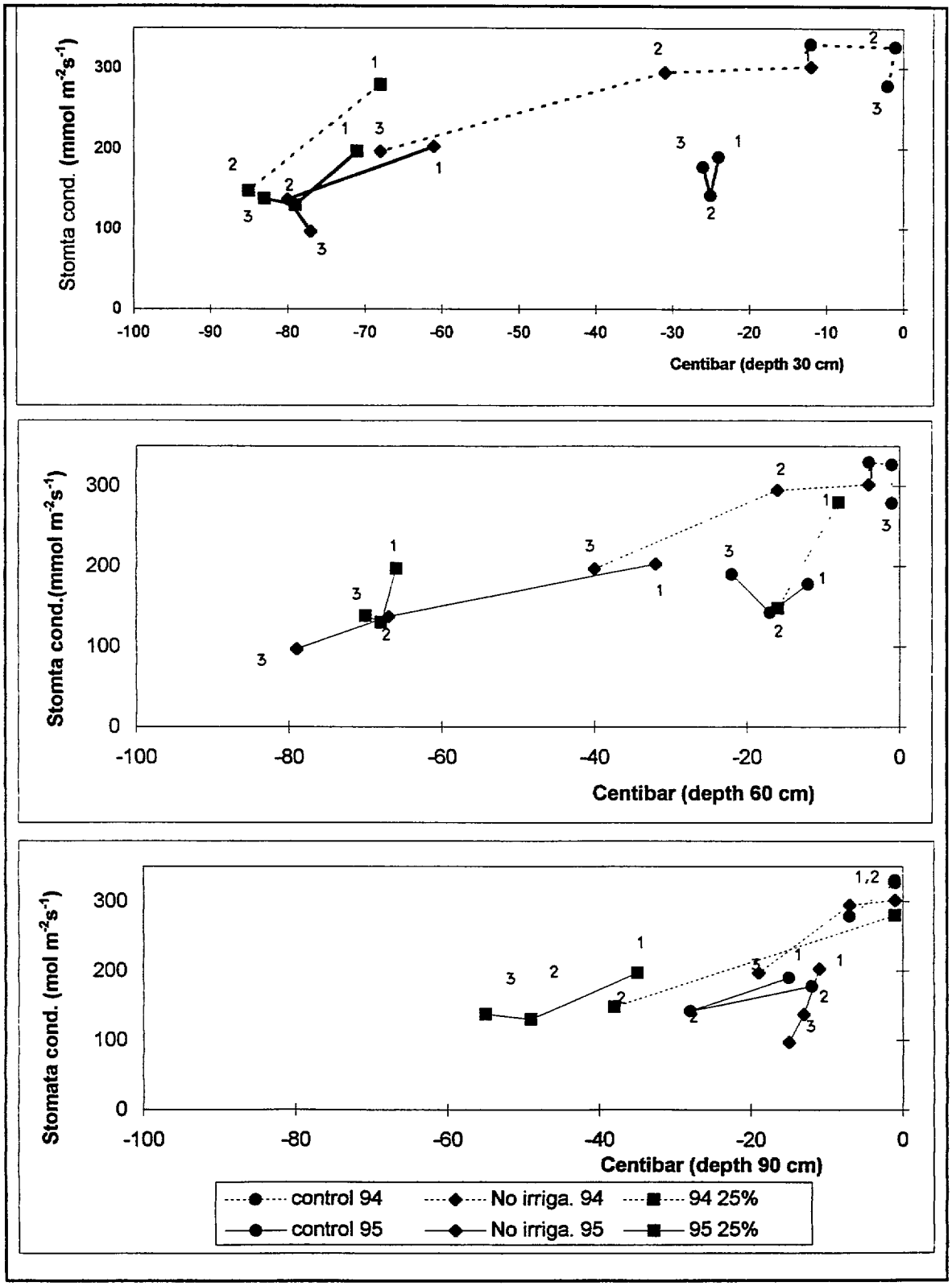

Fig. 9.- Relationships between max stomatal conductance and soil water potential at 30 (top), 60 (middle) and 90 (bottom) cm depth. Data from 1996 (broken lines) and 1997 (full lines) were pooled together. Numbers (1,2 and 3) indicate measurement days. (●) Irrigated controls; (■) 25%E; (◆) cessation of irrigation.

Table 3.- 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 %	

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 temperetaures 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.

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 based on: 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.

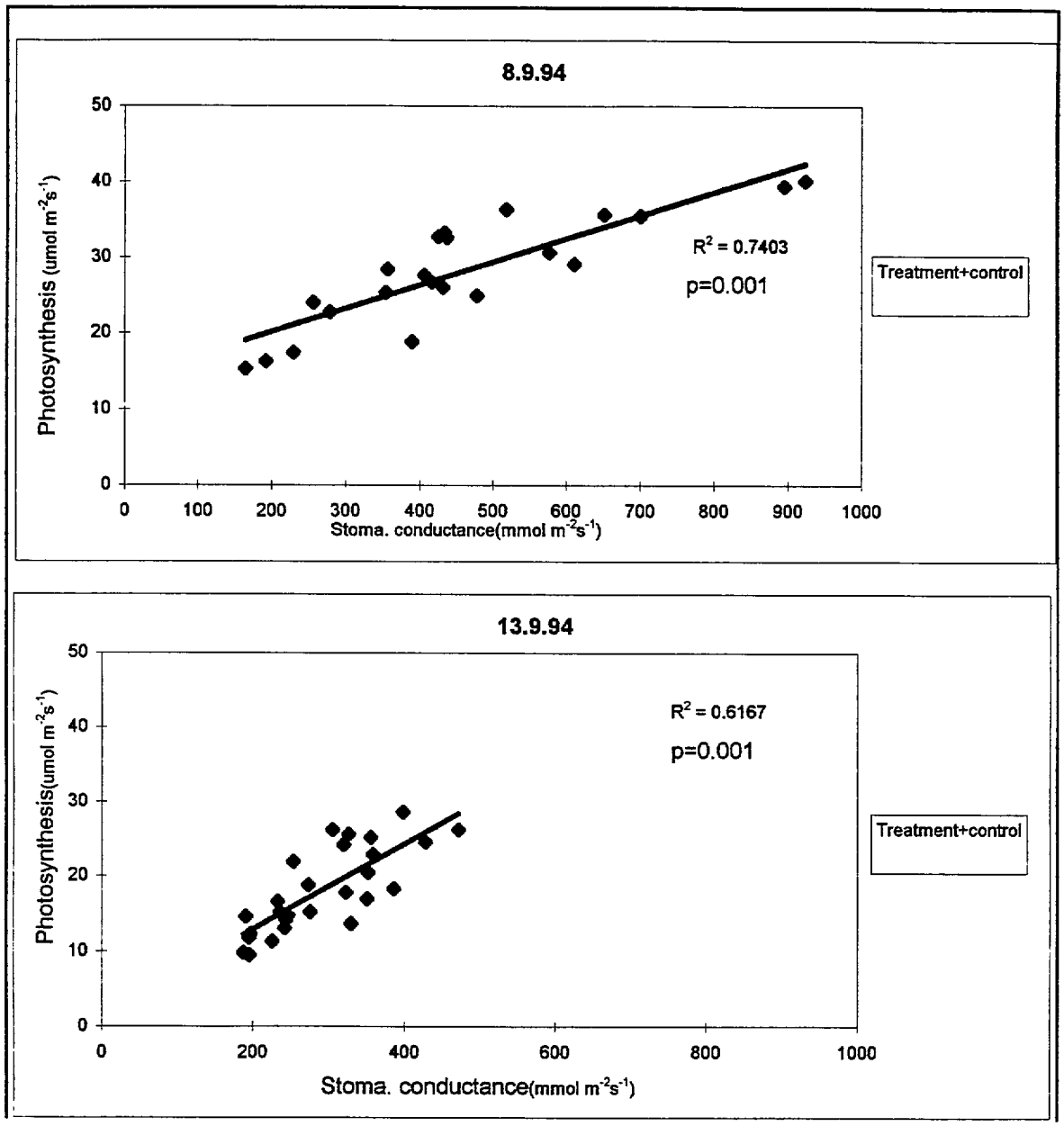

Fig. 10.- Relationship between rate of photosynthesis and stomatal conductance. Data from all treatments were pooled together. Exp 1 (1996).

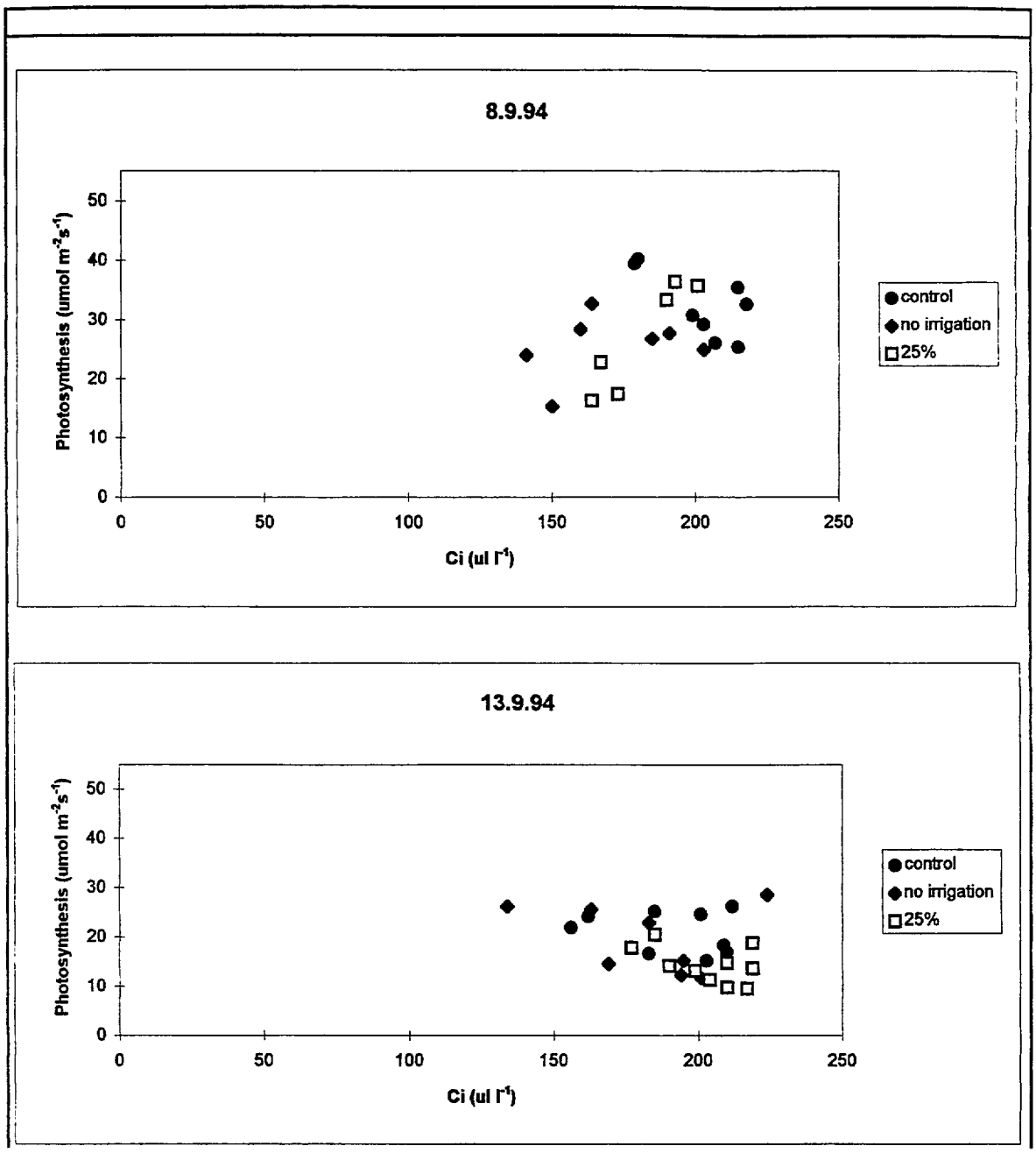

Fig. 11.- Relationship between rate of photosynthesis and leaf internal CO_2 concentration (C_i). Data from all treatments were pooled together. Exp 1 (1996).

2.2- Assessment of amaranth for replacing flour for bread baking

S. Saguy and P. Weinberg

SHORT LITERATURE REVIEW

Utilisation of amaranth in bread making is widely documented (e.g., Bressani et al., 1992; Bressani, 1988; Bressani et al., 1987; Moreira-Sales and Vitti, 1987; Schnetzler and Breene, 1994; Stone and Lorenz, 1984). These studies focused on both composition and technological properties of amaranth seeds with a view to their use and utilisation in mixtures with wheat, soybeans, cassava or other foods. Nevertheless, amaranth practical utilisation is quite limited due to numerous deficiencies (Schnetzler and Breene, 1994). Other applications such as snacks containing amaranth were recently reported (e.g., Annapure et al., 1998a, b).

The granules of starch isolated from seeds of Amaranthus cruentus were found very small, angular and polygonal in shape. Compared to corn starch, the amaranth starches have a higher swelling power, lower solubility, a greater water binding capacity, a lower susceptibility to alpha-amylase, a higher amylograph viscosity and much lower amylose content These properties showed that ***utilisation of amaranth starches for breadmaking produced very poor quality products***. Nevertheless, they have a very promising effect of freeze-thaw relationships maintaining the original shape (Baker and Rayas, 1998a, b) and moisture retention (Pollio et al., 1998). The bread containing amaranth was low in volume, lacked symmetry, and had inferior grain, texture and crumb colour (Stone and Lorenz, 1984). Hence, it was concluded that utilisation of amaranth, as a sole ingredient for breadmaking is not very promising. Similar results were recently reported showing that the optimal range of amaranth for extrusion of rice/amaranth puffed product was 20 to 40% (Ilo et al., 1999).

Utilisation of amaranth as a flour blend for bread is more promising. Research originated in Brazil (Moreira-Sales and Vitti, 1987) tested 3 flour blends (I) Amaranth 50%, cassava 40%, soybean 10%; (ii) wheat 80%, amaranth 20%; and (iii) wheat 50%, amaranth 25%, cassava 20%, soybean 5%, were used for breadmaking trials. Blends (I) and (iii) showed poor breadmaking properties, although the blend (iii) showed promise in the biscuit making trial, with good organoleptic properties. Evaluations of specific volume symmetry, colour and texture of bread produced from blend (ii) showed satisfactory results, being similar to pure wheat flour bread. Similar results were presented for selected Amaranthus cruentus ecotypes from different places of origin used to prepare baking products from mixtures of white wheat flour with whole amaranth grain flour from raw, toasted and popped amaranth seeds, as well as with higher protein fractions obtained by air classification, presented clear differences with respect to wheat flour (Sanchez-Marroquin et al., 1985). The data displayed different behaviours according to amaranth origin and the seeds' previous treatment.

The opportunity of employing wheat-amaranth mixtures in programs developed to improve the diet of the population's marginal sectors was one of the main conclusions (Sanchez-Marroquin et al., 1985). For instance, it was found that amaranth enriched cookies and "bolillos" (French bread) with net protein retention values of 3.63 and 4.35, respectively, are slightly higher than casein (3.0 and 4.0), and are especially recommended.

BAKING EXPERIMENTS WITH AMARANTH BLENDS WITH FLOUR

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 we have concentrated our efforts searching for the possibilities to increase the level of replacement.

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. These main conclusions could be drawn:

a. **Commercial dough extenders** – Two different commercial dough extenders were utilised. 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. Pictures 1 and 2 clearly show that 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 (Picture 3 and 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).

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 optimisation of the bread containing 30% amaranth was necessary. Picture 3 clearly indicates that the optimisation process finally yielded good quality bread, where volume and length were comparable to the non amaranth bread. The various formulations utilised 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. Picture 5 shows a typical image of the 30% amaranth bread slice without salt (Baking Experiment #30). The texture was very dense, and the volume very small (i.e. 1243 mL). Additional experiments suggested that the addition of salt should be added after the first kneading. Thus, 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 utilised contained already some hemicellulose.

D. NEXT STEPS

Our next steps will focus on preparing other products containing amaranth at higher level than 30%. The products such as waffles and crackers are not limited to the 30% barrier we have 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. Also, these products also no not need special and expensive equipment, thus allowing direct technology transfer.

We will also continue with the baking experiments looking for new and advanced enzyme from commercial sources leading the market in this domain.
In addition, we will also focus on shelf-life studies (staling prevention), nutritional assessment consumer acceptance (and economic analysis). Also, technology transfer will be reviewed.

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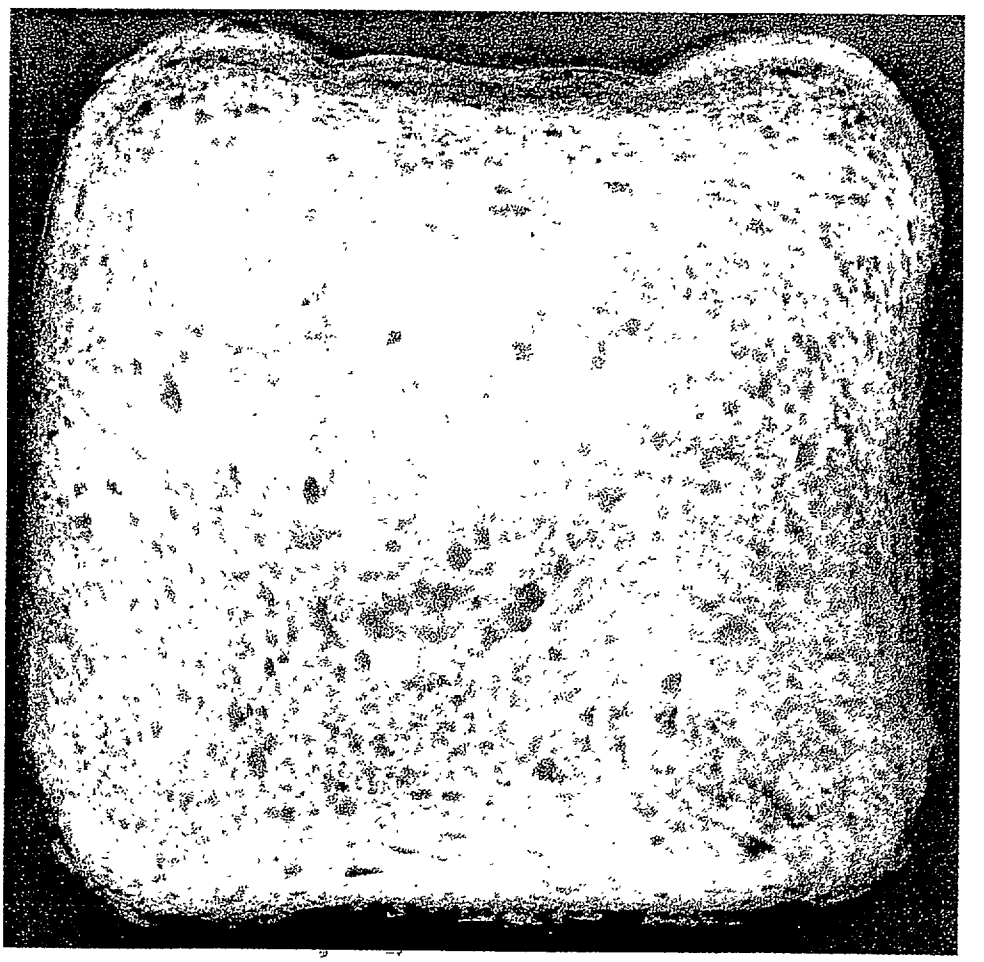
Table 1: Description of the experiments

#	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	

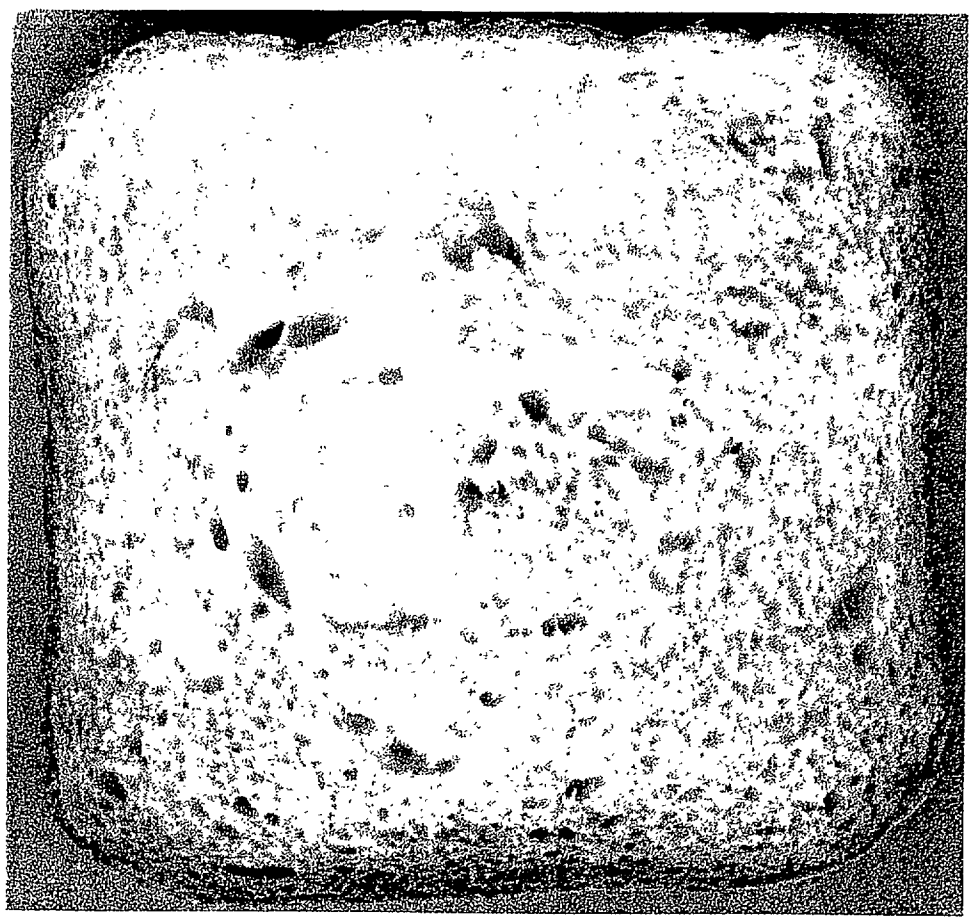
Table 1 (con..): Description of the experiments

#	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	

Picture 1: Effect of 0.5% Adomix (Adomim Chemicals) on white bread.



Picture 2: Effect of 0.5% Green improver (Sharon Chemicals) on while bread.



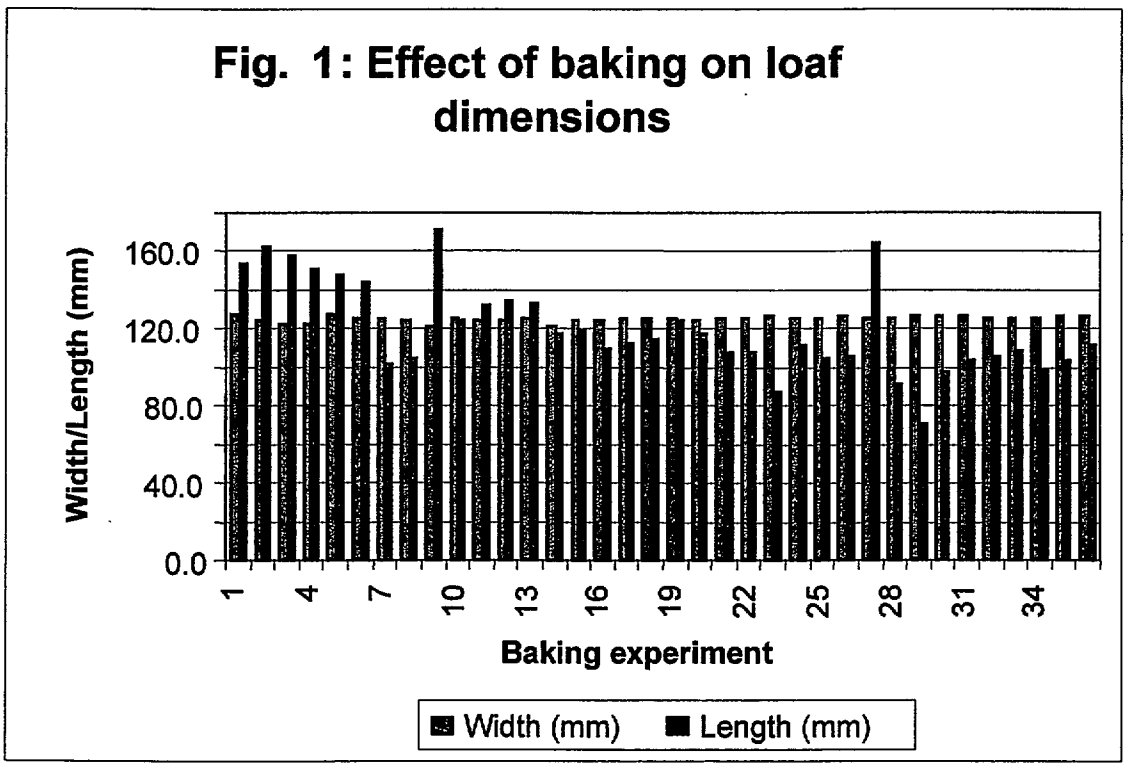
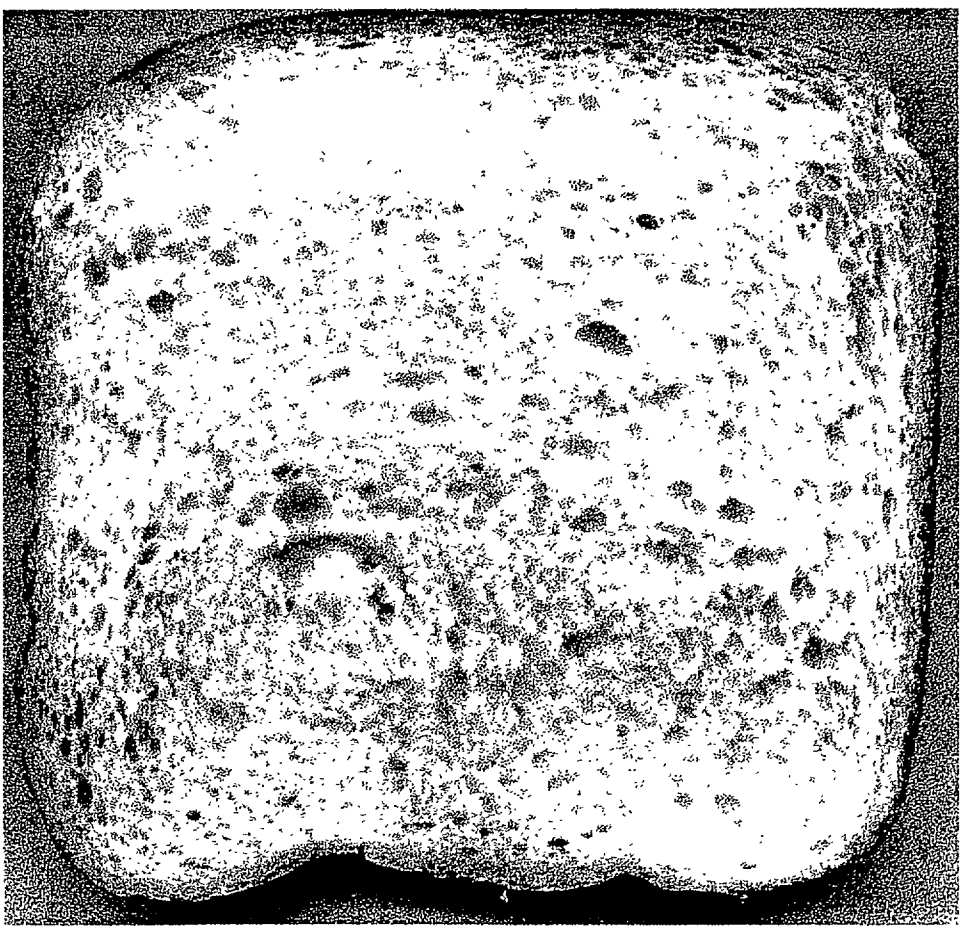


Fig. 1.- Effect of baking conditions on loaf dimensions.

Picture 3: Effect of various additive (0.06 g α-amylase; 0.075 g Vitamin C, 0.465 g GMS, 0.3 g DATEM, 0.6 g CSL) on white bread.



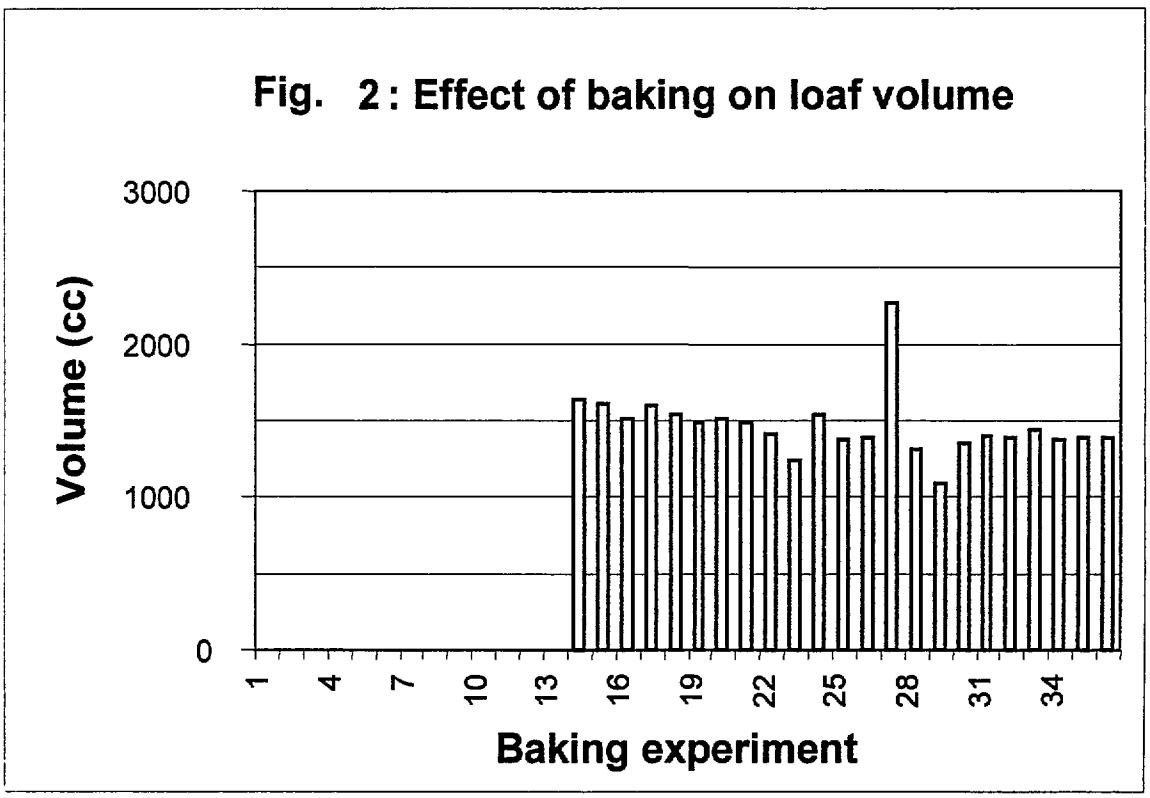


Fig. 2.- Effect of baking conditions on loaf volume.

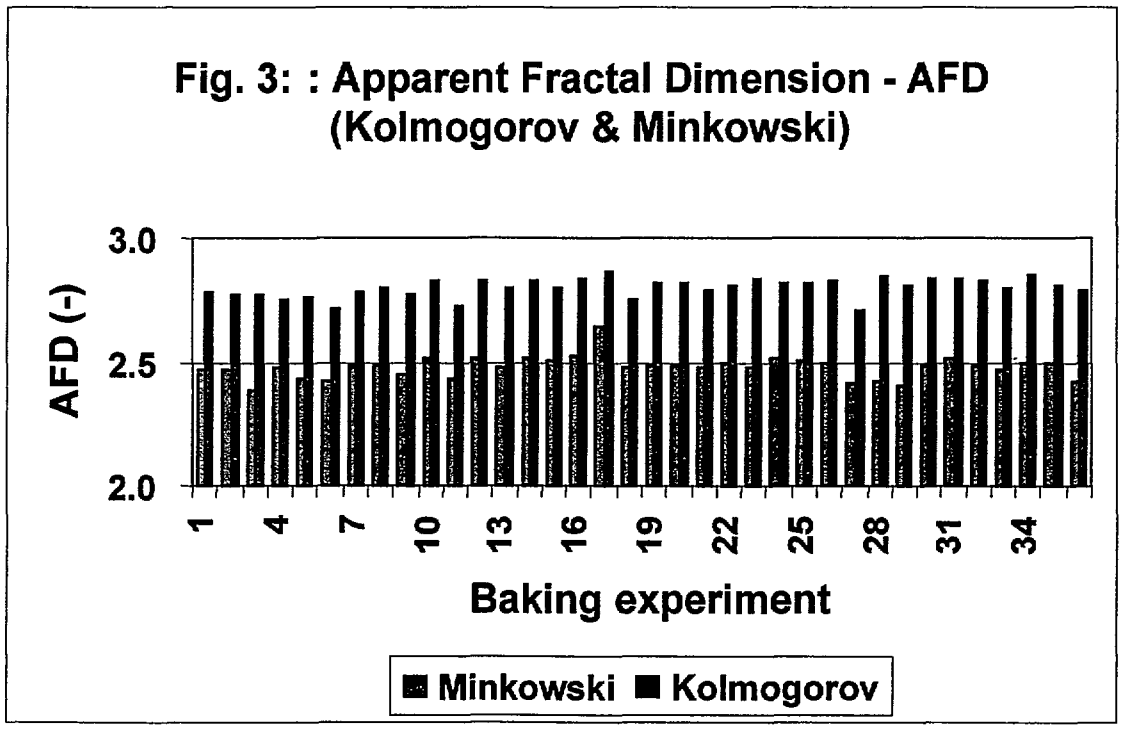
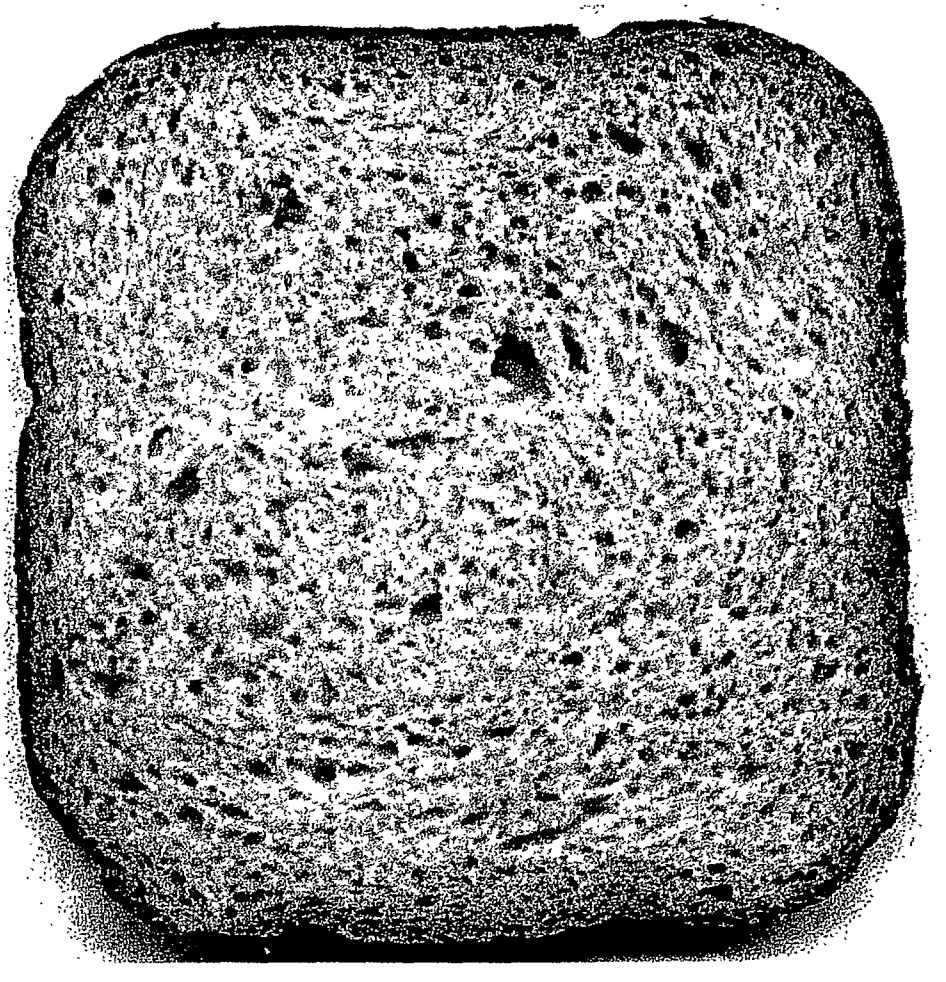
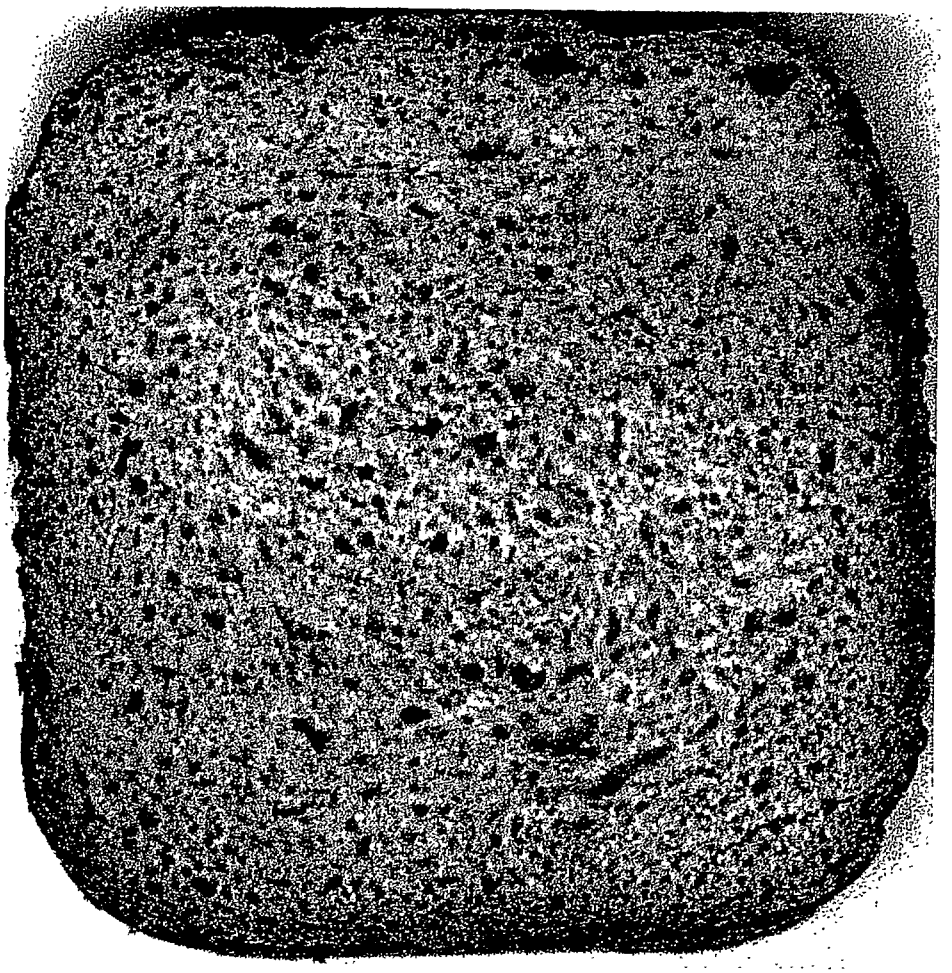


Fig. 3.- Apparent fractal dimension – AFD (Komogorov and Minkowski)

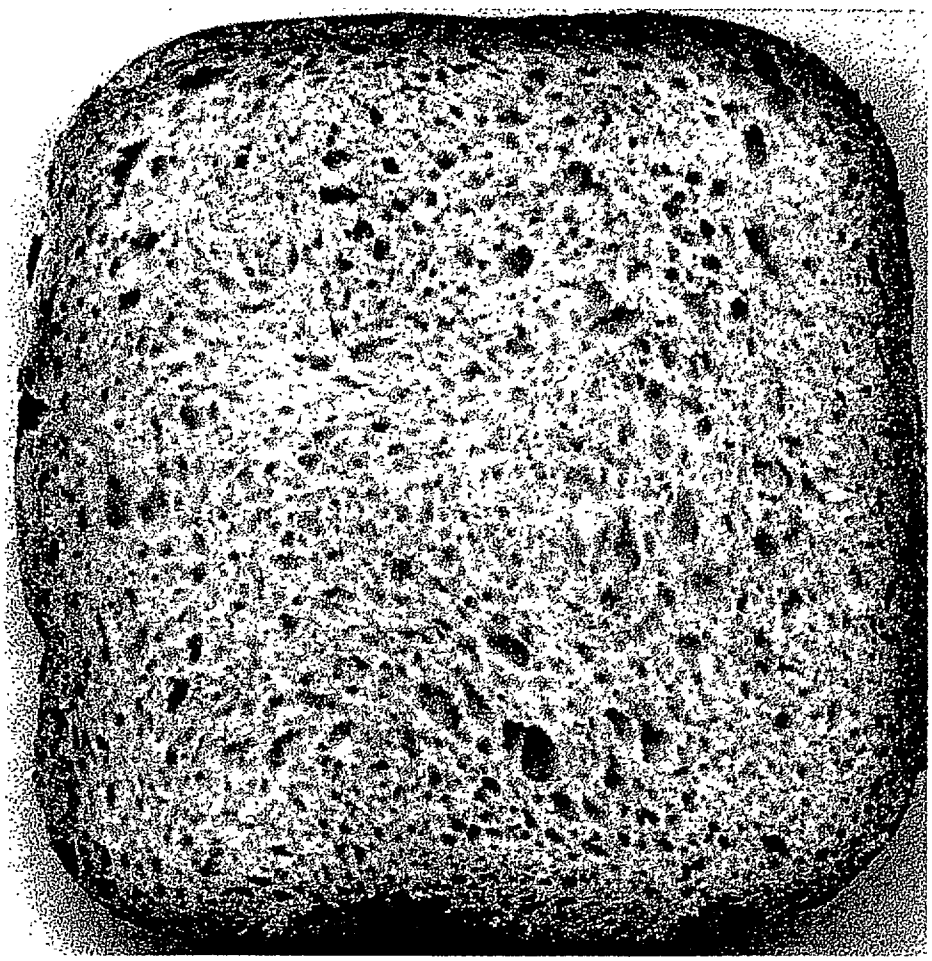
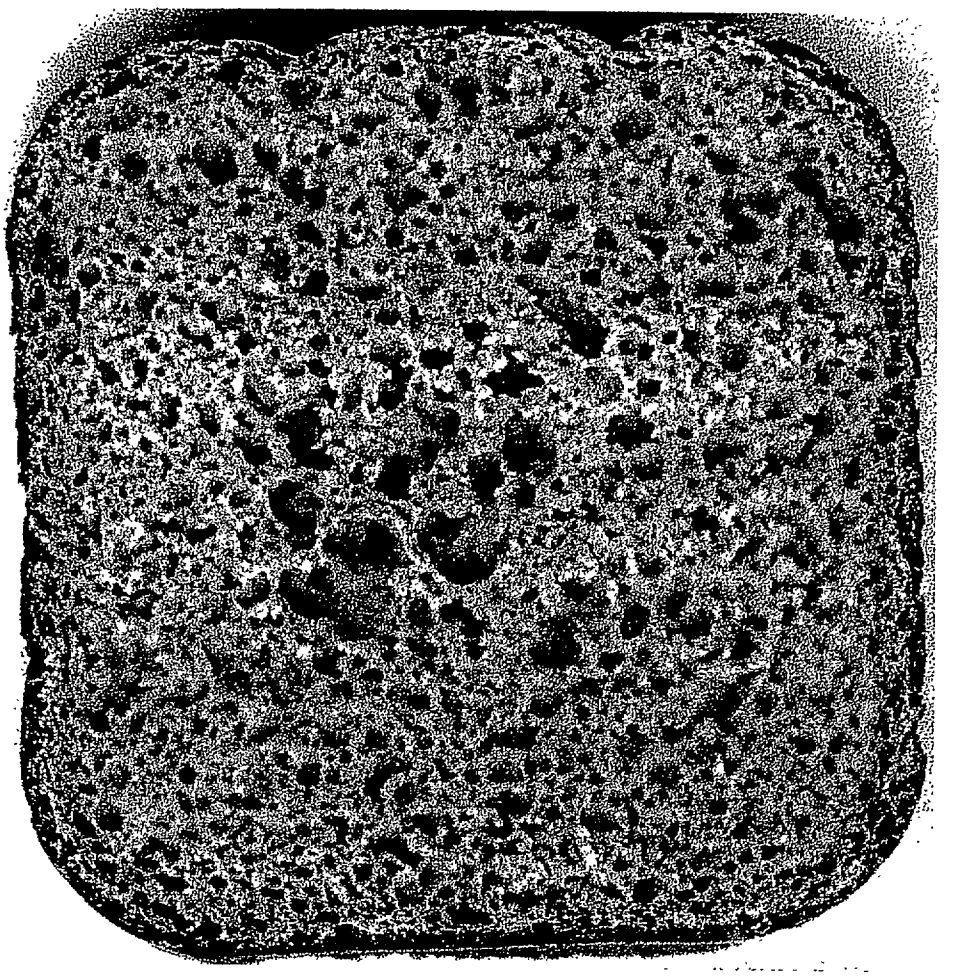
Picture 4: Effect of 30% amaranth replacement on baked bread (additive: 0.06 g α-amylase; 0.1 g hemicellulase, 1.0% gluten, 0.15 g Vitamin C, 0.93 GMS, 0.6 g DATEM, 1.2 g CSL)



Picture 5: Effect of 30% amaranth/ replacement and no salt on baked bread (additives: 0.06 g α-amylase; 0.06 g hemicellulase, 1.5% gluten, 0.15 g Vitamin C, 0.93 g GMS, 0.6 g DATEM, 1.2 g CSL)



Picture 6: Effect of 30% amaranth replacement and salt added prior to mix 2 on baked bread (additives: 0.06 g α-amylase; 0.06 g hemicellulase, 1.5% gluten, 0.15 g Vitamin C, 0.93 g GMS, 0.6 g DATEM, 1.2 g CSL).



Picture 7: Effect of 30% amaranth blend and cysteine (0.06 g).