6

BARD

United States - Israel Binational Agricultural Research and Development Fund

FINAL REPORT

PROJECT No. I-127-79

Physiological Disorders in Vegetable Crops: Basic and Practical Aspects

B. Aloni, E. Pressman, M.J. Jaffe

1984

ALO
630.72
BAR

2030 8

BARD

P.O.B. 6

Bet Dagan, ISRAEL

Date Jan. 31.1984

יש למלא את הטופס במכונה כתיבה

BARD TECHNICAL REPORTS

BARD No. of Proposal I-127-79

Title of Proposed Research Physiological disorders in vegetable crops:
Basic and practical aspects

Name(s) of investigator(s):

Principal Investigator Dr. Beny Aloni

Cooperating investigator(s) Dr. Mordechai J. Jaffe, Dr. Etan Pressman

Name and address of Affiliated institution(s):

Principal Institution: Dept. of Vegetable Crops, ARO, The Volcani Center,
Bet Dagan, POB 6, Israel

Grantee institution

Cooperating institution(s) Wake Forst University

Winston-Salem, North Carolina 27109, USA

Project's starting date

Type of Report: initial ☐ Comprehensive ☐ Final ☒

Signature

Principal Investigator

Name

Beny Aloni

Signature

Beny Aloni

Institution's Authorizing Official

Name

Yuval Eshdat

Dr. YUVAL ESHDAT

Head, Div. for Research Projects and Budgets

Signature

Agricultural Research Organization

- 1) Celerys - Diseases and pests
- 2) Tomatoes - Diseases and pests

630.72 : 635.53 : 635.64 : 581.2

BAR

2nd copy

We like to thank the Binational Agricultural Research
and Development Fund (BARD) for supporting this project.

We also like to thank Miss Gila Rosenstein and
Miss Sofia Levanon for their skillful assistance.

Many thanks to the staff members of the Besor
Experimental Station and of Ramat Hanegev
Experimental Station for their help in carrying
out the field experiments.

We also like to thank Dr. Beny Bar Yosef and
Ms. Bijan Sagiv for their fruitful cooperation.

<u>Table of contents</u>	<u>Page</u>
Introduction	1
Abstract	3
Petiole pithiness in celery leaves: Induction by environmental stresses and the involvement of abscisic acid	5
Interaction with salinity of GA ₃ -induced leaf elongation petiole pithiness and bolting in celery	10
The effect of nutrient solution composition and transient nutrient starvation on the yield and petiole pithiness of celery plants	18
The effect of irrigation with brackish water on yield and quality of three leafy crops	24
Effects of salts on NADA-Malate dehydrogenase activity in celery leaves during their maturation and senescence	31
Tomato	43
Stem pithiness in tomato plants: The effect of water stress and the role of abscisic acid	44
Effect of flooding on tomato cultivars: The relationship between proline accumulation and other morphological and physiological changes	50
Proline accumulation: A parameter for evaluation of sensitivity of tomato varieties to drought stress ?	55
Stem pithiness and collapse of tomato plants - field experiment	72
Work done in the U.S.A.	82
Thigmomorphogenesis: the effect of mechanical perturbation and ethrel on stem pithiness in tomato [<i>Lycopersicon esculentum</i> (Mill.) plants .	83
Benefit to agriculture	91
Description of cooperation	92

Physiological disorder in Vegetable Crops:

Basic and practical aspects

Introduction

A large part of the vegetable growing areas in Israel and the U.S.A. are located in semi-arid regions in open fields, where the plants are often exposed to environmental stresses (osmotic and mechanical) and subsequently exhibit some physiological disorders. Often the stress affected crops exhibit degradation of various plant tissues, collapse of the vegetative part of the plant, and reduction in the quantity and quality of the yield.

In this research we proposed to study two of the most severe and economically important stress-induced disorders: stem pithiness in tomato plants, and petiole pithiness in celery leaves. Pithiness of celery petioles reduces the yield's quality and therefore also its eligibility for export. Pithiness of the stem of tomato plants is associated with collapse of the plants in the late growth stages, when they are heavily burdened with unripened fruits.

We proposed to focus our study on the following points:

1. Definition of the relationships between several types of environmental stresses and the development of stem and petiole pithienns in both controlled environments and the open field.
2. Evaluation of the physiological and biochemical mechanisms which bring about the degradative procedss.
3. Study of cultivation techniques which prevent the induction of these disorders.

The following types of methodology were proposed to be employed:

1. Measurement of the plant's growth by conventional growth parameters.
2. Measurement of the water status in the plants (water potential, osmotic potential and turgor).
3. Membrane structure and function-related techniques.
4. Plant growth regulators, application and measurements of plant hormones endogenous levels.
5. Development of cultivation techniques for attenuation of these disorders in the open field, and in particular, methods which will endow the plants with osmotic adaptation properties.

The results of this study were expected to contribute to the better understanding of the behavior of celery and tomato plants under stress conditions, especially in terms of the relationships between the water status in the plant, changes in the hormonal balance in the vegetative tissues, and the induction of degradative processes in stem and petiole cells.

In this final report we present the data, and discuss them mainly in papers which were published in the due course. The unpublished results are presented separately.

Abstract

Following the three year research in this project the following major conclusions emerged.

1. Celery is a crop extremely sensitive to fluctuation in water and nutrient supply. We have obtained a nutritional formula which minimizes the risk of petiole pithiness in winter outdoor celery crop.
2. In greenhouse and field experiments we have established the beneficial effect of saline water (E.C. = 4.5 mmhos/cm) on petiole pithiness in celery and on the quality of other leafy crop like lettuce.
3. The study on tomato plants indicated that tomato varieties differ significantly in their sensitivity to stem pithiness. Following characterization of some major features of this disorder in several varieties it is evident that the more drought and flooding sensitive variety are more prone to pithiness and collapse. We have defined the minimal drought conditions which cause stem degradation and concluded that temporary wilting especially during the late developmental stages may cause stem pithiness and plant collapse.
4. Consequently we have shown in field experiment that under reduced irrigation frequency (for establishment of larger and deeper root system) tomato plants were less susceptible to periodic wilting, grew faster, produced greater tomato yields and were significantly less affected with pithiness and collapse.
5. Investigation of phytohormones involvement in the degradation of tomato stem and celery petiole revealed that while both ABA and kinetin enhances this process while given in combination with drought, ethylene at least in tomato endows the plant with partial hardening, making it less susceptible to pithiness. It is also shown that mechanical

perturbation of the sensitive tissues has a similar effect as ethylene has, suggesting that ethylene is the agent of hardening in our system. Agrotechnical practises were applied in the field according to our recommendations in both crops. In addition, many other aspects of the research are now being investigated in further research programs.

The effect of irrigation with brackish water on yield and quality of three leafy crops.

This part has been carried out in collaboration with Dr. D. Pasternak

M s. Y. De Malach of the Ramat Hanegev Experimental Station and H. Aviram from Shaham, Ministry of Agriculture.

Following the results obtained in the greenhouse experients we carried out a field experiment for testing the resposne of celery plants and other leafy crops to irrigation with brackish water.

Materials and Methods

The experiment was conducted in the experimental station of Ramat Hanegev - Kibbutz Revivim. The field was flushed with 150 m³/dunam tap water and then was fertilized with super phosphate 90 kg/dunam, ammonium sulfate 15 kg/dunam, potassium sulfate 35 kg/dunam.

Seedlings of the following crops were transplanted between October 22-25, 1979: Celery var. Earlybell and tendercrisp, Lettuce. var. Vaugard and Chinese cabbage var. Michilli and spring A. Each variety was planted in 12 plots (72 m² each) arranged in random blocks. The transplanted seedlings were irrigated with tap water during the first 5 days to ensure proper adsorbtion. The differential irrigation began thereafter. In each variety six plots were irrigated with brakcish water (F C. 4 mmho/cm, SAR=10), and six were irrigated with tap water (E.C. 1.0 mmho/cm). Mineral composition of both water types are seen in table 1. The amount of water irrigated was calculated according to 80% of the total evaporation determined by a class A evaporation pan. Due to plenty of precipitation

during the experiment a significant leaching of salts from the root zone was observed and it was necessary to resalinate the treated plots with 20 mm of brackish water after each rain storm. Data of the electrical conductivity in the root zone layers are presented in table 2.

It appears that we have succeeded in maintaining an average E.C. of about 3-4.5 mmho/cm in the root zone throughout the experiment. Table 3 shows that irrigation with brackish water reduced yield of lettuce and chinese cabbage varieties. However, no reduction in yield was observed in celery varieties. Salinity also affected quality parameters (table 4). Chinese cabbage was found to be a highly sensitive crop. Significant deterioration of the heads in both varieties was detected. On the other hand lettuce and celery quality was promoted in all respects. As expected, petiole pithiness in celery was significantly attenuated under salinity conditions. It is worth emphasizing that in both chinese cabbage and celery, bolting was inhibited under salinity which fits well the observations in the greenhouse experimentation (see publication No. 2). Irrigation with brackish water also improved durability of celery head in storage under conditions which simulated exportation of celery (table 5). Consequently cultivation of lettuce and celery in the Arava and the Jordan Valley which use brackish water and saline soils is now being tested under more commercial scale.

Table 1.
The quality of the tap and brackish water in Revivim Experimental Station.

Type of water	Salt conc. (ppm)	E.C. (mmhos/cm)	SAR	concentration of principal ions (meq./lit.)				
				Na	Ca	Mg	K	Cl HCO ₃ SO ₄
Tap	600	1.3	3.3	4.7	1.8	2.0	0.2	6.1 2.6 1.1
Brackish	2300	4.4	8.9	20.7	6.0	5.9	0.4	28.4 4.7 6.3

Table 3.

Effect of irrigation with brackish water on the yield components of lettuce, chinese cabbage and celery

Crop and Variety	Total yield (kg/m ²)		Net yield (kg/m ²)		Trimmed head weight (g)	
	tap water	brackish water	tap water	brackish water	tap water	brackish water
Lettuce, Vangard	8.4	7.4	5.4	4.8	800	600
Chinese cabbage,						
Michili	13.5	10.9	7.4	6.3	1300	1100
Spring A	12.0	8.8	5.4	3.8	1000	1100
Celery, Earlybell	15.5	17.1	8.8	8.8	800	800
Tendercrisp	14.8	16.8	7.9	9.1	700	700

Table 4.

Effect of irrigation with brackish water on variety yield quality parameters of lettuce, chinese cabbage and celery at harvest.

Crop and variety	Rotten heads (%)		Firm heads (%)		Bolting plants (%)		Pithy pitioles (%)	
	t.w.	b.w.	t.w.	b.w.	t.w.	b.w.	t.w.	b.w.
Lettuce, Vanguard	24.9	4.3	48.1	68.2	0	0	-	-
Chinese cabbage, Michili	52.5	90.0	28.7	19.6	53.3	34.2	-	-
Spring A	12.8	58.6	-	-	-	-	-	-
Celery, Earlybell	0	0	-	-	64.7	43.4	38.3	22.9
Tendercrisp	0	0	-	-	1.9	1.8	48.7	29.1

Table 5.

Celery quality after 2 weeks cold storage (at 10c) and 4 days shelf life (at 200c)

Variety	Irrigation treatment	Q u a l i t y			p a r a m e t e r s	
		Black heart (%)	Petiole rot (%)	Pithy heads (%)	Pithy petioles (%)	
Earlybell	tap water	36.1 a	13.5 a	64.2 a	9.4 a	
	brackish	21.3 b	9.8 b	35.0 b	0.7 b	
Tendercrisp	tap water	35.2 a	12.6a	81.8 b	2.5 a	
	brackish	27.0 b	6.3 b	36.7 b	0.8 b	

EFFECTS OF SALTS ON NADH-MALATE DEHYDROGENASE ACTIVITY IN CELERY LEAVES DURING THEIR MATURATION AND SENESCENCE

BENY ALONI, ETAN PRESSMAN and RACHEL SHAKED

Institute of Field and Garden Crops, Agricultural Research Organization, The Volcani Center, Bet Dagan (Israel)

(Received August 8th, 1980)

(Accepted September 25th, 1980)

SUMMARY

The effect of several inorganic salts on the activity of NADH-malate dehydrogenase (MDH) from the petiole and the blade of celery leaves of different age, was investigated. KCl and NaCl, in a concentration range of 100–180 mM, activated the enzyme similarly when given in the assay medium. At higher concentrations, both salts became inhibitory. CaCl_2 and MgCl_2 activated the enzyme at a much lower concentration, with MgCl_2 being the weaker activator. The activation of celery leaf MDH by KCl was found to vary with the leaf age. As the leaf matured, the extracted enzyme exhibited increased activation by KCl. However, the transition from maturity to senescence was accompanied by a decline in the activation of the enzyme by the salt. During senescence of detached leave blades in the dark, the MDH activation by KCl undergoes the same changes found in the intact plant. The changes in MDH activity due to either age or KCl, were a result of changes in the $V_{\max}$ and not of the K_m -value for oxaloacetate. Acceleration of senescence by transient water stress or flooding induced a marked increase in the MDH activation by KCl in vitro.

It is suggested that the change in the enzyme response to ions is part of a regulation mechanism by which MDH activity is maintained during various developmental stages of the leaf. However, in accelerated senescence situations, this regulation mechanism is disrupted.

INTRODUCTION

Various aspects of the senescence process in plant tissue were studied intensively. In general, senescence of plant leaves is characterized by loss of total nitrogen, protein, chlorophyll, RNA, DNA and dry weight [1,17]. Accordingly, changes in enzymatic activities associated with the degrada-

tion of these constituents, have been reported by many workers. While most of the studies on enzyme changes are concerned with the level of total or specific enzyme activities, a few studies dealt with changes in the intrinsic (structural as well as kinetic) properties of the enzyme molecule in the course of senescence.

Among the various enzymes investigated, NADH-related malate dehydrogenase (MDH) activity was shown to be stimulated at the onset of senescence [12]. In apple leaves this rise in activity was followed by a decrease to the basal level. Similar results were obtained for *Cucurbita maxima* leaves [9]. However, when tested in detached leaves during dark starvation, MDH activity hardly changed. In a study on malate dehydrogenase extracted from banana fruits during maturation, an increase in activity was measured, which did not absolutely correspond to an increase in the amount of the MDH antigen. It was postulated that increase in activity was probably due to an accumulation of enzymatic proteins and also to an activating system at the enzymatic protein level [10].

MDH was shown to be highly sensitive to ions [4,5]. Blackwood and Mifflin [2] reported that monovalent as well as divalent cations stimulated MDH activity from barley and corn leaves in vitro. Furthermore, they showed that the activation was accompanied by changes in the aggregative properties of the enzyme. In the present study we examined the ionic sensitivity of MDH in the course of celery leaf development from a very young age until the stage at which the leaf undergoes marked yellowing. The effects of water deprivation and flooding on this enzyme property were also investigated.

It is shown that during the processes of leaf maturation and senescence, MDH undergoes modifications in its ionic sensitivity. These modifications may be of significance in the regulation of the enzyme activity during these processes.

MATERIALS AND METHODS

Plant material

Celery (*Apium graveolens* L.) seeds, var. Earlybell, were germinated on peat-vermiculite mixture in Speedling trays. After 2 months the seedlings were transferred to 10-liter pots filled with inert volcanic ash, and irrigated and fertilized daily with an N:P:K 20 : 20 : 20 fertilizer dissolved in the irrigation water. Growth of the plant continued for another 2 months until the average leaf number was 33 and the average total canopy weight was 600 g. At this stage leaves were sampled for the enzymatic activity measurements and stress treatments were started. The experiments were carried out during autumn and winter, in a greenhouse with average day and night temperatures of 24°C and 15°C, respectively. For labeling of leaf age, leaves of five celery plants were marked (every third leaf), with number 1 being

the youngest leaf (at the center of the canopy) and number 11 being the oldest leaf (at its outer circle).

MDH activity: extraction and measurement

One gram of freshly sampled leaf petioles or leaf blades was homogenized in a chilled mortar and pestle with 4 ml of 25 mM Tris-HCl buffer (pH 8.3), containing 1 mM EDTA and 2 mM dithiothreitol (DTT). This was followed by vigorous homogenization of the same homogenate in a Polytron homogenizer. The homogenate was centrifuged at $20\,000 \times g$ for 30 min and the supernatant was assayed, without additional purification, for enzyme activity and protein [7]. The temperature of the homogenate did not exceed 8°C throughout the extraction procedure. Supernatants of $20\,000 \times g$ and $100\,000 \times g$ exhibited the same enzymatic activities and protein levels.

Malate dehydrogenase was assayed spectrophotometrically by recording the disappearance of NADH in the course of oxaloacetate reduction to malate at 340 nm [13]. The reaction mixture contained 20 ml of 25 mM Tris-HCl buffer (pH 8.0), 0.5 ml of 3 mM oxaloacetic acid dissolved in 0.6% EDTA, 50 μ l of 6 mM NADH, and 0.5 ml of H₂O. The reaction was initiated by addition of up to 50 μ l of enzyme solution. When the effect of salts was examined, the H₂O portion in the reaction mixture was replaced by concentrated salt solutions of the appropriate concentration. Enzyme activity was expressed as units per mg protein per minute, where one unit was considered the amount of enzyme causing a change of 1.0 O.D. at 340 nm.

Chlorophyll determination

For determination of chlorophyll content, discs of about 200 mg of fresh leaf blades were extracted for 48 h by 8.0 ml *N,N*-dimethylformamide at 4°C in the dark. The absorption of the clear extract was determined at 660 nm.

Dark starvation experiments

Blades from leaves of intermediate age (leaf No. 4) were rinsed in tap water, surface sterilized and put in petri dishes with 4 ml of distilled water. The senescing blades were incubated in the dark at 25°C. At various intervals these petri dishes, each containing three blades, were sampled for chlorophyll and enzyme activity determinations. Each blade was cut in half, one half being for chlorophyll determination and the other for MDH analysis.

All measurements were carried out in triplicate. The experiments were repeated at least three times and the most representative are described in this paper.

RESULTS

MDH activity, extracted from petioles of celery leaves, is highly sensitive

to the ionic environment in the assay medium. Figure 1 shows that a minute concentration of 20 mM KCl increased the enzyme activity and that an activation peak was reached at a salt concentration of 100 mM. Subsequent experiments, in which the concentration range of 0–0.3 M KCl was examined in detail, showed that maximum activation of the enzyme was reached in the range of 100–180 mM KCl. Concentrations above 400 mM were inhibitory. The activation effect of KCl was not due to the osmotic potential of the medium, since sucrose at concentrations giving osmotic potentials similar to those of the KCl solution, did not affect the enzyme activity at all. NaCl was found to be as effective as KCl and showed a similar concentration-dependent activation curve (Fig. 1).

The chloride salts of the divalent cations Mg^{2+} and Ca^{2+} increased the MDH activity to the same extent as found for KCl and NaCl. However, both $CaCl_2$ and $MgCl_2$ activated the enzyme at a concentration range of 20–40 mM, which is much lower than the concentrations found for KCl and NaCl. $CaCl_2$ was twice as effective as $MgCl_2$. At concentrations above 100 mM, $CaCl_2$ totally inhibited the enzyme activity, whereas $MgCl_2$ started to be inhibitory only when its concentration exceeded 160 mM (Fig. 2).

MDH exhibited different responses to KCl when extracted from petioles of celery leaves at different stages of development. Figure 3 shows that KCl had practically no effect on MDH from the youngest leaf (leaf No. 1), whereas the activation by KCl was much more apparent in MDH from the mature

Fig. 1. The effect of different KCl and NaCl concentrations on the activity of malate dehydrogenase extracted from petioles of mature celery leaves.

Fig. 2. Comparison between the effects of monovalent (KCl and NaCl) and divalent cations (CaCl₂ and MgCl₂) on the activity of malate dehydrogenase extracted from petioles of a mature celery leaf.

Fig. 3. The effect of different KCl concentrations on the activity of malate dehydrogenase of celery leaves at different stages of development. The leaf age is marked on the right side by Nos. 1-8, where 1 is the youngest leaf and 8 is a mature leaf in which yellowing has not been observed. The intermediate numbers indicate advancing stages of development.

TABLE I

THE EFFECT OF POTASSIUM CHLORIDE (0.16 M) ON THE $V_{\max}$ - AND K_m -VALUES OF MALATE DEHYDROGENASE, EXTRACTED FROM THE PETIOLES OF YOUNG AND MATURE CELERY LEAVES

Leaf age	KCl	$V_{\max}$ (units/mg protein/min)	K_m (oxalacetate (mM))
Young	—	2.864	0.049
Mature	—	0.648	0.052
Young	+	3.522	0.053
Mature	+	1.887	0.047

leaf (leaf No. 8). While KCl activated the youngest leaf MDH by about 20% it increased the enzyme activity from the mature leaf 2.5-fold. MDH from leaves of intermediate stages of development, were activated by KCl to a moderate extent, depending on the leaf position in the plant. The optimum KCl concentration for MDH activation was found to be in the range of 80–180 mM and similar in leaves from different stages. The activation of the MDH from both young (leaf No. 1) and older (leaf No. 8) leaves by KCl was a result of the potassium effect on the enzyme $V_{\max}$ and not on the K_m -value for oxaloacetate (Table I). When examined in the absence of KCl, the enzymes from both young and mature leaves showed similar K_m -values, with the former having $V_{\max}$ -values nine times higher than that of the latter. In the presence of KCl, the difference between the $V_{\max}$ -values narrowed significantly, due to the greater activation effect of KCl on MDH from the mature leaf than on MDH from the young leaf.

Different responses of MDH from the young and mature leaves were apparent also when examined with CaCl_2 and MgCl_2 . Both divalent cations were more effective in activating MDH from the mature leaf, with CaCl_2 being more effective than MgCl_2 (Table II). The chlorophyll content in leaves Nos. 1–8 (Table III) indicates that these leaves represent stages in the maturation process and do not include senescing leaves, since a decline

TABLE II

THE EFFECT OF CaCl_2 AND MgCl_2 (40 mM) ON MALATE DEHYDROGENASE ACTIVITY, EXTRACTED FROM PETIOLE OF YOUNG AND MATURE CELERY LEAVES

The numbers in brackets represent the percentage of activation.

Leaf age	MDH activity (units/mg protein/min)		
	Control	+ CaCl_2	+ MgCl_2
Young	2.53	5.38 (212)	4.01 (158)
Mature	0.57	1.98 (347)	1.22 (214)

TABLE III

THE EFFECT OF LEAF AGE ON ITS CHLOROPHYLL CONTENT, MALATE DEHYDROGENASE SPECIFIC ACTIVITY AND THE ACTIVATION OF MALATE DEHYDROGENASE BY 0.16 M KCl

The enzymatic activity was extracted from the petioles of the indicated leaves

Leaf age/No.	Chlorophyll O.D. ₆₈₀ /g fresh wt.	MDH spec. act. (units/mg protein/min)	MDH activation by 0.16 KCl (%)
Young	1 0.63	2.86	123
	2 1.85	1.97	141
	3 5.44	1.98	151
	4 10.68	1.66	155
	5 19.01	0.94	190
	6 20.31	0.72	182
	7 22.24	0.65	230
Mature	8 21.42	0.34	255
	9 15.18	0.70	152
	10 8.66	1.24	139
Old	11 2.33	1.98	104

in chlorophyll was not observed within this set of leaves. The response of MDH to potassium appeared to be different when the leaf entered the process of senescence. Table III shows that with the decline in chlorophyll content in leaves No. 9, 10 and 11, the MDH-specific activity, measured with absence of KCl, starts to increase after being reduced during the process of maturation. Concomitantly, this MDH exhibits less activation by KCl in vitro.

In order to characterize the maturation-senescence-related MDH changes further, we followed the activity of the enzyme during a period of dark starvation of detached leaf blades. In preliminary experiments, we observed that MDH activities extracted from leaf blades and petioles were similar in their response to KCl solution. The dark-starvation experiments were therefore performed on detached blades from almost fully mature leaves, which at zero time exhibited a high degree of KCl activation. Figure 4 shows that in the course of incubation in the dark, two phases of chlorophyll changes could be distinguished: an early phase during which chlorophyll content in the blades remained practically unchanged and a late phase, from the sixth day on, during which there was rapid chlorophyll degradation. In the course of the early phase, a gradual but constant decrease in MDH-specific activity was observed. Concomitantly, the enzyme exhibited an increase in KCl activation. The elevation in the degree of KCl activation continued until rapid chlorophyll degradation was initiated. At this time, a transition occurred in the MDH activity. While the enzyme-specific activity started to increase, there was a sharp decline in its KCl sensitivity. When the same experiment was performed on younger leaves with an etiolated appearance, this transition was delayed by 4 days.

Fig. 4. Changes in chlorophyll content, malate dehydrogenase-specific activity (in the absence of KCl, and malate dehydrogenase activation by 0.16 M KCl during dark starvation of blades from celery leaves. The detached blades were incubated on moist filter paper in petri dishes at 25°C in the dark.

Water stress and flooding effect

If the changes in MDH activity are associated with the maturation-senescence process in the leaves, the water stress or flooding conditions, through their accelerating effect on this process, should also influence the enzyme activity and its sensitivity to salt solution. In order to examine this possibility, groups of five celery plants were either watered daily or deprived of water for 5 days until there was severe wilting. The stressed plants were then rewatered for an additional 5 days, regained full turgor, but their mature leaves showed marked yellowing. Another group of five plants was flooded by keeping their root zone in an excess of water for 10 days without aeration. Under these conditions, no wilting was observed, but there was a marked reduction in chlorophyll in the plants' peripheral leaves.

Following the water deprivation and the flooding treatments, identical leaves — representing three stages of development — were sampled from the control and the stressed plants and their petioles were extracted for MDH assay. Table IV shows that in the control plants the MDH from the youngest leaf (leaf No. 1) was only slightly affected by KCl, whereas the enzyme activities from the older leaves (No. 2 and 3) were greatly increased in the presence of the salt. Activation of the enzyme by KCl was much more pronounced in the water-stressed plants. While in the young etiolated leaves of these plants MDH was only slightly activated by the KCl solutions, the enzyme from the more mature leaf (No. 2) was activated 10-fold, in com-

TABLE IV

ACCELERATION OF SENESCENCE BY FLOODING AND TRANSIENT WATER STRESS, AND ITS EFFECT ON THE ACTIVITY OF MDH FROM PETIOLE OF CELERY LEAF AND ITS ACTIVATION BY KCl (0.16 M)

The measurements were made on leaves of identical chronological age. Leaf 1: young; leaf 2: intermediate; leaf 3: mature.

Treatment	Leaf No.	Chlorophyll O.D. ₆₆₀ /g fresh wt.	MDH activity (units/mg protein/min)		MDH activation (%)
			- KCl	+ KCl	
Control	1	0.63	1.86	2.04	109
	2	25.64	0.91	2.35	258
	3	24.11	0.40	1.45	362
Water stress	1	1.92	1.93	2.54	121
	2	5.38	0.31	3.44	1109
	3	2.71	1.45	1.91	131
Flooding	1	4.83	1.76	1.84	104
	2	20.31	0.94	3.24	344
	3	11.98	1.31	2.50	190

parison with 3-fold activation of MDH from identical control leaves. In the oldest leaf (No. 3), which showed marked yellowing following the stress, the KCl activation was greatly reduced, down to the value of the young leaf (No. 1). The assumed relationship between maturation-senescence processes (as manifested by the leaf chlorophyll content) and changes in the MDH, seems to be interrupted under the stress conditions. Table IV shows that in the case of water stress treatment, the highest activation degree was reached in leaves of an intermediate stage (No. 2), which also showed marked decrease in chlorophyll content. Only after severe yellowing could a decline in the activation degree be observed. The same interruption was apparent, although to a lesser extent, in the flooded plants.

DISCUSSION

Malate-dehydrogenase is known to occur in multiple forms [8,14,17]. Its isoenzymes were found in mitochondria, microbodies and the cytosol [12]. Therefore, it is difficult to attribute the changes in the enzyme ionic sensitivity to any particular form. In some experiments (not reported here) we observed that the cytosolic MDH activity, which was found to constitute more than 80% of the total activity, exhibited the same ionic sensitivity changes as were found for the bulk of the MDH activity. Since it has been reported that the soluble form and the organelles-associated forms do not differ much in their response ions [16], it is conceivable that the enzymatic changes found in the present study are characteristic of the cellular MDH activity as a whole.

Flowers et al. [3] showed MDH to respond similarly to monovalent and divalent cations. Blackwood and Miflin [2] showed CaCl_2 , NaCl and KCl to have almost identical effects on MDH extracted from *Zea mays* leaves; however, when tested on barley leaves, CaCl_2 exerted strong inhibition at its high concentration, whereas NaCl and KCl were much less inhibitory. The present study indicates that the activation could not be due merely to ionic strength effect for the following reasons: (a) Both CaCl_2 and MgCl_2 produced the maximum activation at a concentration at least four times lower than that of KCl or NaCl; (b) CaCl_2 was two times more activating than MgCl_2 ; (c) CaCl_2 was totally inhibitory at concentrations at which both KCl and NaCl show their maximum activation effect. Based on the same reasons, it is obvious that the activating species is the cationic portion of the salt rather than the chloride ion. It is conceivable that each ionic species has a different interaction with the enzyme molecule, thus producing different effects. Jefferies et al. [6] reported that divalent ions promote MDH activity by stabilization of a high molecular weight MDH aggregate from *Lemna minor*. Blackwood and Miflin [2] concluded that the presence of the ions causes a progressive disaggregation, resulting in an increase and then a decrease in total activity at high salt concentration. The difference between monovalent and divalent cations in our case can be interpreted as a difference in their affinities to the aggregating sites on the enzyme molecule.

The changes in MDH ionic sensitivity in the course of leaf development imply that the maturation-senescence processes are associated with an alteration in enzyme conformation. It is shown here that with respect to MDH alteration, leaf development can be divided into two distinct stages: (i) The first stage, at which the MDH-specific activity gradually declines with the maturation of the leaf; at this stage, the KCl sensitivity increases. (ii) The second stage, which is characterized by an increase in the enzyme activity with a parallel decline in its activation by KCl (Table III). The activity changes, due to either age or ionic environment, were found to be a result of changes in V_{max} , with almost no change in the enzyme affinity (K_m) to the oxaloacetate (Table I). These findings are in contradiction with those of Blackwood and Miflin [2], who found that in the presence of 100 mM NH_4Cl the K_m for OAA was greatly reduced. However, these authors did not indicate whether the V_{max} -values were altered by the salt and did not present any data on the effect of KCl on these kinetics parameters.

The transition from maturation stage to senescence seems to be associated with both an increase in MDH-specific activity and a decrease in its KCl sensitivity. In the whole plant as well as in the detached leaf experiments, this transition coincided with the initiation of the rapid decline in chlorophyll content.

The reasons for the age-related MDH changes are not understood. However, it can be speculated that the changes in ionic sensitivity of the enzyme

might be associated with the 'need' of the plant tissue to maintain a high MDH activity. This 'need' is materialized when a decline in the enzyme activity (in the course of the maturation process) is compensated for by elevation of its ionic sensitivity and when the activity increases (during senescence), the enzyme ionic sensitivity drops. If this scheme is correct, the site of regulation is expected to reside at the enzyme synthesis level, where a change in MDH activity would trigger the synthesis of a modified MDH with a different ionic sensitivity. This would re-establish the 'needed' level of activity. Simon [11] suggested that acclimation for energy of activation of MDH in *Lathyrus japonicus* is associated with de novo synthesis and degradation of MDH isoenzymes. During developmental studies of MDH in corn, Yang and Scandalios [18] found that de novo synthesis of MDH isoenzymes was completed in about 3–5 days, indicating that this enzyme has a rapid turnover.

The assumed coupling between the development of the leaf and the alteration in its MDH properties, seems to be disrupted under stress conditions. Accelerated senescence due to water stress induces a rapid decline in the leaf chlorophyll content accompanied by pronounced elevation in the enzyme ionic sensitivity (Table IV), while, under normal senescence conditions, yellowing was always associated with a decrease in KCl activation. We do not know what role this 'uncoupling' phenomenon plays in the response of the plant to the applied stress. Malate dehydrogenase is a key enzyme in carbon metabolism. It is involved in the citric and glyoxylate cycles [15]. This enzyme plays an ionic and osmotic role and causes redox regulation through the production of malate from oxaloacetate and the regeneration of NADH. The relation of the enzymatic changes discovered in this study to any known function of the MDH is still to be resolved.

ACKNOWLEDGEMENT

This work was supported by the United States–Israel Agricultural Research and Development Fund (BARD).

REFERENCES

- 1 L. Beevers, Growth regulator control of senescence in leaf discs of nasturtium (*Tropaeolum majus*), in: F. Wightman and G. Setterfield (Eds.), *Biochemistry and Physiology of Plant Growth Substances*, Runge Press, Ottawa, 1968, p. 1417.
- 2 G.C. Blackwood and B.J. Miflin, *Plant Sci. Lett.*, 7 (1976) 435.
- 3 T.J. Flowers, J.L. Hall and M.E. Ward, *Phytochemistry*, 15 (1976) 1231.
- 4 H. Greenway and C.B. Osmond, *Plant Physiol.*, 49 (1972) 256.
- 5 E. Hason-Porath and A. Poljakoff-Mayber, *Plant Physiol.*, 44 (1969) 1031.
- 6 R.L. Jefferies, D. Laycock, G.R. Stewart and A.P. Sims, The properties of mechanisms involved in the uptake and utilization of Ca^{++} and K^{++} by plants in relation to an understanding of plant distribution, in: I.H. Rorison (Ed.), *Ecological Aspects of Mineral Nutrition of Plants*, Blackwell, Oxford, 1968.
- 7 O.H. Lowry, J. Rosenbrough, A.L. Farr and A.R. Randall, *J. Biol. Chem.*, 193 (1951) 265.

- 8 S.A. O'Sullivan and R.T. Wedding, *Plant Physiol.*, 49 (1972) 117.
- 9 C. Postius, B. Klemme and G. Jacobi, *Z. Pflanzenphysiol. Bd.*, 78 (1976) 122.
- 10 V. Rocha and I.P. Ting, *Arch. Biochem. Biophys.*, 147 (1971) 114.
- 11 J.P. Simon, *Plant, Cell Environ.*, 2 (1979) 35.
- 12 A. Skakonn and J. Dausant, in: *Facteurs de la Regulation et de la Maturation des Fruits*, Vol. 238, Coll. Int. CNRS, Paris, 1975, p. 281.
- 13 R.W. Spencer and J.S. Titus, *Plant Physiol.*, 49 (1972) 746.
- 14 I.P. Ting, *Arch. Biochem. Biophys.*, 126 (1968) 1.
- 15 I.P. Ting, I. Fuhr, R. Curry and W.C. Zschoche, *Malate dehydrogenase isoenzymes in plants: Preparation, properties and biological significance*, in: C.L. Markert (Ed.), *Isoenzymes*, Vol. 2, Academic Press, New York, 1975, p. 369.
- 16 R. Weinberg, *Plant Physiol.*, 43 (1968) 622.
- 17 R. Wollgiehn, *Symp. Soc. Exp. Biol.*, 2 (1967) 231.
- 18 N.S. Yang and J.G. Scandalios, *Biochim. Biophys. Acta*, 384 (1975) 293.

TITLE OF ARTICLE

Proline accumulation: A parameter for evaluation of sensitivity of tomato varieties to drought stress?

by

Dr. Ben y Aloni and Gila Rosenshtein

ABSTRACT

The pattern of proline accumulation and the growth response were followed in several tomato (Lycopersicum esculentum) varieties which were exposed to 7 days of drought stress followed by a 15-day period of rewatering. During dehydration, water potential and leaf elongation rates decreased more in var. 'Hosen' and 'S-5', than in 'LX-11', '1970', 'Pakmor', 'Faculty-16', 'Alcobaca' and '475'. Proline accumulation during stress was greatest in the first two varieties. In 'Hosen' and 'S-5' rewatering resulted in a decrease of proline to control levels upon rewatering, whereas in the other varieties accumulation of proline continued long after turgor had been regained. The extent of this continued accumulation was not correlated with the degree to which each variety was dehydrated. Upon rewatering of the plants the rate of leaf elongation was increased, but the final leaf size as well as whole shoot and root fresh weight of the recovered plants were not correlated with the degree of "suffering" that each variety experienced during the drought period. Incubation of detached young tomato leaves in polyethylene glycol solution for 48 h resulted in a substantial accumulation of proline. The varietal differences observed under these conditions were reminiscent

of the differential responses in proline accumulation obtained in the intact plants. It is concluded that proline accumulation at the time of dehydration signals drought stress in tomato plants, but does not correlate with the overall varietal sensitivity to transient dehydration in recovered plants.

Additional key words: Diffusive resistance, leaf elongation, water potential.

Ben y Aloni and Gila Rosenshtien: Agricultural Research Organization,
Institute of Field & Garden Crops, Department of Vegetable Crops,
POB 6, Bet Dagan, 50250, ISRAEL.

INTRODUCTION

Accumulation of free proline in tissues of plants grown under stress is evident in many studies (Stewart and Laher 1980). However, the significance of high proline levels to the plant is still a matter of dispute. Several possible roles have been attributed to supraoptimal levels of proline: osmoregulation under drought and salinity conditions (Wyn Jones and Storey 1978), stabilization of proteins (Schobert and Tschesche 1978), prevention of heat denaturation of enzymes (Paleg et al. 1981) and conservation of nitrogen and energy for a post-stress period (Barnett and Naylor 1966). On the other hand, Hanson et al. (1979)

suggested that accumulation of proline is a mere symptom of the deleterious response of the plant to the stress and does not have any adaptive value. In several studies attempts were made to use proline accumulation for evaluation of tolerance or sensitivity of plants to stresses.

Ta et al. (1979) have shown that in tomato plants less proline accumulated in Solanum pinelli (tolerant to salinity) than in the more sensitive L. esculentum. They also found that under both salinity and water stress conditions the wild species L. peruvianum accumulated less proline than cultivated tomato known to be less tolerant. Kuo and Chen (1980) have shown that proline accumulation was highest in tomato cultivars which revealed the greatest sensitivity to flooding. Aloni and Rosenshtein (1982) showed that under flooding conditions more proline accumulated in tomato cultivars which were more dehydrated during flooding stress. In the present study we examined whether proline accumulation can be used as a measure of drought sensitivity in several tomato varieties.

Abbreviations: Abscissic acid - ABA, polyethylene glycol - PEG.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of eight tomato (Lycopersicum esculentum) varieties were sown in vermiculite on Dec. 1, 1981. After germination, the seedlings were planted in 10 liter pots with volcanic ash (tuff) as the growth medium.

The plants were irrigated daily with a commercial nutrient solution (N:P:K, 20:20:20, Chemicals Corp. Haifa, Israel). A concentration of 7.3 mM nitrogen was maintained in the irrigation solution throughout the experiment. The plants were grown in a greenhouse, with average day and night temperatures of 24 and 18°C, respectively, and natural day length and light intensity. After one month under these conditions, five plants out of ten of each variety were subjected to drought stress by withholding irrigation for a period of 7 days, during which time wilting was observed in plants of several varieties. Following that period, irrigation with nutrient solution was resumed and continued until the termination of the experiment 15 days later. The control (non-stressed) plants were irrigated daily throughout the experiment.

Growth parameters

Leaf elongation rates were determined by measuring leaf length at 2-day intervals. The leaves measured (one per plant) were the youngest leaves which were 1 cm long at the beginning of the experiment. At the termination of the experiment the fresh weights of shoot and roots were determined.

Leaf diffusive resistance (r) and water potential

Measurements of r were carried out with a LI-65 autoporometer (Lambda Instruments Corp., Lincoln, Nebraska, U.S.A.) precalibrated at 25°C according the procedure described by Kanemasu *et al.* (1969). The measurements were taken on the youngest fully expanded leaf (third from the apex) from 11:00 to 14:00 h. In preliminary experiments, the

diffusive resistance was found to level off at that time of the day. Leaf water potentials were determined using a pressure bomb (Scholander et al. 1964).

Proline determination

At various intervals during the drought stress and after recovery, samples of leaf tissue were taken from prelabeled leaves at the youngest part of the plant canopy. Proline was determined on a sample of about 200 mg fresh weight. The extraction procedure and colorimetric determination with acidic ninhydrin were carried out according to Singh et al. (1973).

Incubation with polyethylene glycol (PEG)

Intact young leaves, about 300 mg fresh weight, were floated on 15 ml 25% PEG 6000 made up in distilled water. Incubation took place in a 10 cm petri dish at 25°C under conditions of continuous fluorescent light ($200 \mu\text{E m}^{-2}\text{s}^{-1}$, Sylvania fluorescent lamps, New Jersey, USA) for 48 h. At the termination of the incubation period the leaves were rapidly rinsed free of residual PEG and processed for proline assay as described above.

RESULTS

The pattern of elongation and proline accumulation in young leaves of tomato varieties which were exposed to transient water deprivation is illustrated in Fig. 1. In all varieties except 'LX-11', inhibition of leaf elongation started only 4 days after the onset of the water stress

treatment. Therefore we chose to use leaf elongation which occurred between day 4 and 7 as a measure of leaf elongation rate under the applied stress. Leaf elongation rates in 'Hosen' and 'S-5' were only 17 and 35% of the controls, respectively, whereas in the least inhibited varieties '475' and '1970' the corresponding values were 85 and 70%. Resumption of irrigation resulted in enhancement of leaf elongation, although at different rates in each variety. After 15 days of recovery the rehydrated leaves reached the control leaf size in 'F-16', '366' and 'Hosen'. The transient stress caused a significant reduction in the final leaf size in 'S-5', 'Pakmor' and 'LX-11". In the varieties '475' and '1970', which were the ones least affected by the stress, final leaf size exceeded the size of the control leaves.

Accumulation of proline in all the varieties was not initiated until the fourth day of water stress, at which time the reduction in leaf elongation rate started. At the end of the stress period proline level in the leaves was in 'Hosen' and 'S-5' 4.5 and 4.1 μmol per g fresh weight respectively, whereas in all other varieties it did not exceed the level of 1.7 μmol per g fresh weight. Resumption of irrigation caused a rapid disappearance of the accumulated proline in 'Hosen' and cessation of accumulation in 'S-5'. On the other hand, in all other varieties elevation of proline content continued during the recovery period, (Fig. 1), even though diffusive resistance and water potential declined (Table 1) and full turgor was regained within 24 h in all varieties.

These results raise the question of whether proline accumulation is a mere reflection of the water potential that each variety had reached during the stress, or whether it expresses the varietal variability to accumulate proline regardless of the differences in leaf water potential. Our approach was to measure proline in detached leaves which were evenly stressed by dehydrating them in non permeable polyethylene glycol. Detached, fully expanded young leaves from non-stressed plants accumulated free proline when incubated for 48 h in 25% (-1.6 MPa) polyethylene glycol solution. Distinct differences were found between varieties under these conditions. 'Hosen' and 'S-5' accumulated more proline than all the other varieties. Proline levels in both detached and the corresponding attached leaves were inversely correlated with the relative leaf elongation rates and water potentials that were determined during the stress in the intact plants (Figs. 2 and 3). In both proline assays the distinction between the more affected varieties ('Hosen', 'S-5') and the less affected ones (all the rest) was apparent. However, proline accumulation did not correlate with the differential sensitivity within the less dehydrated varieties.

The transient water deprivation had a profound effect on the subsequent vegetative growth of the plants. Following the stress, shoot growth of '366', 'Faculty-16' and 'LX-11' was greater than that of the control (Tab. 2). Enhancement of growth was observed in root development of all varieties except in roots of 'Hosen' and '475'.

DISCUSSION

On the basis of leaf response to transient drought, one can distinguish two groups of varieties: (i) A wilted type (vars. 'Hosen' and 'S-5') which wilts rapidly upon dehydration, and the leaves of which stop elongating. In this group rewatering results in an immediate resumption of leaf elongation when turgor is regained. (ii) The other group, which is best represented by LX-11, wilted less and does not seem drastically affected by the dehydration-rehydration regime.

This distinction is also apparent in the pattern of proline accumulation. The wilted type accumulated and then rapidly metabolized proline when transferred from the dehydrated to the rehydrated state. In the less wilted type proline accumulation continued long after turgor had been regained until a transition point, at which proline declined back to the initial level. The biochemical basis for this continued proline accumulation was not resolved. Nevertheless, it does not relate to the changes in water potential during the dehydration period, nor does it correlate with any of the growth parameters that were examined. On the other hand, the levels of proline which were accumulated within the dehydration period did correlate positively with the inhibition of leaf elongation (Fig. 2 A) and with the decrease in water potential (Fig. 3 A). A distinction between wilted and the less wilted varieties can also be made on the basis of proline accumulation in detached leaves, dehydrated in PEG solution (Figs. 2B and 3B). This implies that the difference between the two groups is not dependent solely on the decrease in water supply to the whole plant, but might be associated with a metabolic response to the stress, typical for each variety. However, determination of proline in

both attached and detached leaves (floated on PEG) cannot be used for evaluation of the overall sensitivity or tolerance to transient drought. Following rehydration, leaf elongation and shoot fresh weight recover in a specific pattern for each variety and, independent of the previous proline accumulation or the extent of dehydration intensity.

If proline does play a role in the adaptive response of tomato plants to water deficit by serving as substrate for post-stress recovery, it is probably masked by other factors or processes which are concomitantly active during rehydration. For instance, accumulation of ABA during the stress may affect both leaf wilting and elongation (Milborrow 1978). ABA produced or translocated to the root system may alter root development and permeability, which in turn may affect the recovery rates of the shoot. Indeed several studies have indicated that ABA has a strong effect on root exudation rates and mineral uptake (Tal and Imber, 1971; Karmoker and van Steveninck, 1978). We have observed that in most of these tomato varieties transient drought stress greatly enhanced root exudation after plant recovery, in comparison with non-stressed plants (unpublished data). This fact raises the possibility that these varieties might differ in their ABA production capacity during the stress. Presently we are investigating the role of ABA in the drought-recovery process in tomato plants and its relation to differences in varietal sensitivity.

Acknowledgments. We wish to thank Drs. E. Kopilevich and N. Kadar of the Hebrew Univ. of Jerusalem, Faculty of Agriculture in Rehovot, and Dr. M. Pilovsky of the ARO, The Volcani Center, Bet Dagan, for supplying us with seeds of the different cultivars and for useful advice.

This work was supported by a grant from the United States - Israel Binational Agricultural Research and Development Fund (BARD).

Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel. No. 22-E, 1983 series.

REFERENCES

- Aloni, B. & Rosenshtein, G. 1982. Effect of flooding on tomato cultivars: The relationship between proline accumulation and other morphological and physiological changes. - *Physiol. Plant.* 56: 513-517.
- Barnett, N.M. & Naylor, A.W. 1966. Amino acids and protein metabolism in Bermuda grass during water stress. - *Plant Physiol.* 41: 1222-1230.
- Hanson, A.D., Nelson, C.E., Pedersen, A.R. and Everson, E.H. 1979. Capacity for proline accumulation during water stress in barley and its implications for breeding for drought resistance. - *Crop Sci.* 19: 489-493.
- Kanemasu, E.T., Thurtell, G.W. and Tanner, C.G. 1969. Design, calibration and field use of a stomatal diffusive prometer. - *Plant Physiol.* 44: 881-885.
- Karmoker, J.L. and van Steveninck, R.F.M. 1978. Stimulation of volume flow and ion flux by abscissic acid in excised root systems of Phaseolus vulgaris L. cv. Redland Pioneer. - *Planta* 141: 37-43.
- Kuo, C.G. and Chen, B.W. 1980. Physiological responses of tomato cultivars to flooding. *J. Am. Soc. Hortic. Sci.* 105: 751-755.
- Milborrow, B.V. 1978. Abscissic acid. In: *Phytohormones and Related Compounds - A Comprehensive Treatise*, (D.S. Letham, P.B. Goodwin and T.G. Higgins, eds.), Vol. 1, pp. 295-347, Elsevier/North Holland Biochemical Press, Amsterdam. ISBN 0-444-80053-0.
- Paleg, L.G., Douglas, T.J., Van Dail, A. and Keech, D.B. 1981. Proline, betaine and other organic solutes protect enzymes against heat inactivation. - *Aust. J. Plant Physiol.* 8: 107-114.

- Schobert, B. and Tschesche, H. 1978. Unusual solution properties of proline and its interaction with proteins. - Biochim. Biophys. Acta 541: 270-277.
- Scholander, P.F., Hammel, H.T., Hemmingsen, E.A. and Bradstreet, E.D. 1964. Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. - Proc. Natl. Acad. Sci. U.S.A. 52: 119-125.
- Singh, T.N., Paleg, L.G. and Aspinall, D. 1973. Stress metabolism. I. Nitrogen metabolism and growth in barley plants during water stress. - Aust. J. biol. Sci. 26: 5-46.
- Stewart, G.R. and Larher, F. 1980. Accumulation of amino acids and related compounds in relation to environmental stress. In: The Biochemistry of Plants (B.J. Milin, ed.) Vol. 5, pp. 609-635. Academic Press, Inc., New York, N.Y. ISBN 0-12-675405.
- Tal, M. and Imber, D. 1971. Abnormal stomatal behaviour and hormonal imbalance in Flacca, a wilted mutant of tomato. III Hormonal effects on the water status in the plant. - Plant Physiol. 47: 849-850.
- Tal, M., Katz, A., Heikin, H. and Dehan, D. 1979. Salt tolerance in the wild relative of the cultivated tomato: Proline accumulation in Lycopersicon esculentum Mill., L. peruvianum Mill. and Solanum pennellii cor. treated with NaCl and polyethylene glycol. - New Phytol. 82: 349-355.
- Wyn Jones, R.G. and Storey, R. 1978. Salt stress and comparative accumulation in two salt and water-stressed barley cultivars. - Aust. J. Plant Physiol. 5: 817-829.

Tab. 1. Water potential and diffusive resistance values at the peak of the drought stress and 24 h after rehydration.

Mean $\pm$ S.E.

Variety	Water potential (-Mpa)			Diffusive resistance (sec.cm ⁻¹)		
	Control	Peak of stress	24 h recovery	Control	Peak of stress	24 h recovery
'Hosen'	0.52 $\pm$ 0.03	1.15 $\pm$ 0.09	0.65 $\pm$ 0.03	0.5 $\pm$ 0.02	16.4 $\pm$ 1.4	2.6 $\pm$ 0.3
'S-5'	0.57 $\pm$ 0.03	0.88 $\pm$ 0.09	0.61 $\pm$ 0.02	2.7 $\pm$ 0.09	17.3 $\pm$ 2.5	3.7 $\pm$ 0.5
'366'	0.45 $\pm$ 0.04	0.63 $\pm$ 0.03	0.52 $\pm$ 0.05	4.8 $\pm$ 0.20	27.5 $\pm$ 2.5	8.8 $\pm$ 0.5
'Faculty-16'	0.53 $\pm$ 0.03	0.70 $\pm$ 0.05	0.62 $\pm$ 0.05	1.8 $\pm$ 0.17	9.0 $\pm$ 0.5	2.2 $\pm$ 0.3
'Pakmon'	0.48 $\pm$ 0.06	0.65 $\pm$ 0.07	0.46 $\pm$ 0.05	4.6 $\pm$ 0.38	7.0 $\pm$ 1.0	3.2 $\pm$ 0.5
'1970'	0.45 $\pm$ 0.02	0.85 $\pm$ 0.04	0.46 $\pm$ 0.03	2.8 $\pm$ 0.32	11.0 $\pm$ 1.3	7.4 $\pm$ 1.0
'LX-11'	0.52 $\pm$ 0.04	0.56 $\pm$ 0.07	0.55 $\pm$ 0.06	4.0 $\pm$ 0.34	8.0 $\pm$ 0.7	5.0 $\pm$ 0.8
'475'	0.62 $\pm$ 0.03	0.70 $\pm$ 0.07	0.67 $\pm$ 0.08	1.2 $\pm$ 0.08	6.0 $\pm$ 0.5	1.7 $\pm$ 0.3

Table 2

The effect of transient water stress on shoot and root weights

Variety	Tissue fresh weight (g)					
	Shoot			Root		
	control	stressed	%	control	stressed	%
'Hosen'	252±13	252±28	100	43±6	40±3	94
'S-5'	257±20	240±31	93	43±6	51±7	117
'366'	202±18	255±32	126	26±3	35±4	132
'Faculty-16'	156±10	185±15	118	37±4	47±4	127
'Pakmor'	161±17	156±17	96	25±4	34±2	135
'1970'	185±22	177±13	96	46±5	49±6	107
'Lx-11'	156±11	198±22	126	29±2	38±2	130
'475'	315±28	245±30	77	48±6	46±4	96

Legends of figures

Fig. 1. The effect of drought - recovery treatment on elongation and proline content in young leaves of various tomato varieties. Dehydration from day 0 to day 7. The arrows indicate the time of resumption of irrigation. $\bullet$ — $\bullet$ elongation of control leaves; $\circ$ --- $\circ$ elongation of dehydrated leaves; Δ — Δ proline accumulation in dehydrated leaves. Proline levels in the control leaves were in the range of $0.08-0.24 \text{ mol(g FW)}^{-1}$.

Fig. 2. Proline accumulation (A) in attached leaves (dehydration of the whole plant) and (B) in detached leaves (desiccated in PEG) as correlated with the relative elongation of leaves under conditions of dehydration of the whole plant.

Fig. 3. Proline accumulation (A) in attached leaves (dehydration of the whole plant) and (B) in detached leaves (desiccated in PEG) as correlated with the water potential of leaves, determined after 7 days of dehydration of the whole plant.

Fig. 1.

Fig. 2.

Fig. 3.

Stem pithiness and collapse of tomato plants - field experiment

It was previously postulated, by farmers as well as by extension service advisors, that a superficial root system of tomato plants may enhance the risks of plants collapse in the late developmental period under conditions of high evaporative demands. The following experiment was set in order to examine some aspects of tomato cultivation in relation to stem pithiness and collapse in field. Here we examined the effects of irrigation frequency, water stress and other treatments for manipulating the plant's root system. The experiment was carried out on sandy soil in the Besor Experimental Station, on a plot of two dunams which was fumigated with methyle bromide prior to planting. The preparation of the field was conducted as recommended in the region. Irrigation and fertilization were applied with dripping system. The pattern of irrigation was according to the regional practises. Planting of tomato transplants var. Faculty-16 was on August 24, 1980. After the establishment period of two weeks the following treatments were applied:

1. Control - daily irrigation and fertilization;
2. Water stress - plants were irrigated once in four days, namely one fourth of the amount given to the control group. This treatment was begun one month after transplantig.
3. Reduced irrigation frequency - Two weeks after planting this group was irrigated with drippers of 8 liter/hour in comparison to 2 liter/hour used in the rest of the field. Irrigation was applied every fourth day so that the amount of water and fertilizer given were the same as those given to the

control with reduced frequency by a factor of 4.

4. Fruit dilution - In this treatment plants were either devoided of all fruits by removal of the flowers at anthesis (4a) or diluted of fruit number by approximately half at the stage of fruit set (4b).
5. Underground dripping - 30 days after planting the drippers (2 lit/h) were incorporated with attached tubes into a depth of 40 cm and from then on irrigation continued according to the control formulation. The purpose of this treatment was to change the depth in which root system develops with the assumption that at lower depth a more subtle soil temperature changes will be maintained.
6. Physical limitation of root development. - Prior to planting plastic net (0.5 mm) was aligned at a depth of 15 cm under each planting site.

This group was fertilized and irrigated as the control.

Each of the above treatments was applied to four rows of fifty plants. Between the experimental rows a control rows were planted in order to effectively separate the various irrigation treatments. Starting at mid-November fruit yields were measured every two weeks. Stem pithiness and collapse of plants were determined at the same time. Air and soil temperatures at depth of 10-20 cm and 40-50 cm were recorded with THIES Remote thermograph. At November 25 we recorded the diurnal changes in leaf water potential and diffusive resistance in young leaves of 5 plants in each treatment. leaf water potential was measured with a Sholander pressure bomb. Diffusive resistance was determined with Lambda porometer.

Results

Examination of stems 6 weeks from planting, when plants were 60 cm high, revealed that pithiness spots developed along stems of plants from all treatments except treatment 4a. Treatment 4b was also relatively less affected. Fig. 1 shows the advancement of collapse of plants along the growing season. Collapse of few plants was observed in mid-December and from then on the control and the water-stress plants were severely affected. On January 21 40% of the control and 60% of the water stressed plants collapsed. Reduction of irrigation frequency (treatment 3) significantly attenuated the severity of the disorder, being 17% at termination of the experiment. Plants from treatments 5 and 6 were also severely affected with pithiness and collapse being 50 and 55% respectively. We suspect that water supply to these groups of plants was not adequately controlled therefore causing water stress in the former and flooding-like effect in the later treatment. Interestingly, plants which were either devoided or depleted of fruits did not collapse throughout the experiment. Fig. 2 presents the percentage of accumulation of marketable fruits along the season. Water stress greatly reduced yield. On the other hand in treatment 3, persistently greater fruit yield is observed, although this increase was not statistically significant. Nevertheless on January 21, the date of termination of the experiment, plants from treatment 3 had twice as many green fruits than the control plants (Fig. 2) these are potentially fruits which would ripen late in winter.

The recorded changes in temperature are seen in Fig. 3. The fluctuation in soil temperature recorded at depth 10-20 cm were almost abolished at 40-50 cm, however the mean soil temperatures in both depths were the same.

Recording air temperature in the field did not reveal any outstanding conditions at the period at which collapse of plants had begun. Nor did we record any chilling throughout the season. Chilling has been suspected by the farmers to be one of the causes of the collapse of the plants in the field. Measurements of leaf diffusive resistance and leaf water potential at the end of November indicated that the plants from various treatments responded differently to the diurnal changes in water demand. Table 1 shows that plants from treatment 3 had higher values with less closed stomata at 1.00 p.m. than had plants from the control treatment, indicating that these plants were less stressed. Measurement of leaf water potentials in 4 successive days revealed that from the day of irrigation up to the next irrigation in treatment 3 a relatively steady leaf water status was maintained.

Comparison between control and fruits diluted plants indicated that both groups had been similarly diurnally stressed.

At the termination of the experiment root tissue from the depth of 10-20 cm were sampled and screened for possible pathological factors which could be associated with the collapse of the plants. As was assumed in the beginning of the project we did not find such a factor. Visual examination of the roots did not reveal pathological symptoms, although this could not rule out the possibility that pathogen is involved in causing the disorder.

Based on the results of this experiment the following conclusions were drawn:

The existing methodology (which has been changed in the course of carrying out this project) of daily fertigation by dripping, creates an unbalanced plant which has small and superficial root systems with large canopy.

This plant experiences water deficit due to high transpiration rate at mid-day. By enlarging and deepening of the roots with reduction of irrigation frequency one can attenuate the periodic stress at mid-day and thereby presumably avoids the build-up of high abscisic acid concentration in the leaf and stem tissue.

It is also suggested that the presence of high fruit load on the plant reduces its ability to withstand periodic water stress. We presume that high sink activity of the fruit would deplete the vegetative tissue of principle metabolites, mainly soluble sugars and amino acids, which are known to be significant components of plants cells osmotic content. This hypothesis has not been proven in the present study, nevertheless interestingly we observed that both the stems and the leaves of the fruits depleted-plants were thicker and greener than the loaded plants. We also observed that the cold associated characteristic pigmentation of the leaves from the fruit depleted plant was much less than that of the loaded plants. It was therefore conferred by us to the extension service advisors to change the practice of daily fertigation since this methodology encourages pithiness and collapse of the plants.

Until 1979 on initiation of this project the most common variety for winter, out-door grown tomato was "Hosen-Eilon" which as previously shown is most sensitive to environmental stresses, especially water stress and water logging. Since that time the principal variety is Faculty-16 which exhibits much greater tolerance to these conditions. However, as indicated by this experiment, improper fertigation practice may also affect this variety.

Table 1

The effect of various treatments (described on page) on leaf water potential and diffusive resistance (measured at mid-noon) of tomato plants

Treatments	Diffusive resistance		Water potential	
	(sec. cm ⁻¹)		(-ba)	
	November 12	Nov. 12	Nov. 15	
1	14.5	9.8	9.5	
2	23.7*	12.5*	15.4**	
3	10.5*	8.5*	9.8**	
4 a	14.4	9.3	-	
b	14.8	9.6	-	
5	22.1	12.9	-	
6	16.1	10.8	-	

* Measurements done 24 hours following irrigation;

** Measurement done on the fourth day following irrigation.

Fig. 1. Advancement of the collapse of tomato plants during the growing season. Control ●—●, water stress ○—○, reduced irrigation frequency △—△, total fruits avoidance (4a) △, fruit dilution (4b) ⊙.

Fig. 2. (A)-Accumulation of ripe fruits in control ●—●, water stressed ○—○ and plants with reduced irrigation frequency Δ

(b)-Unripe fruits picked at the termination of the experiment.

A-control; B-water stress; C-reduced irrigation frequency.

Fig. 4. Air temperature (maximum and minimum) recorded in the experimental plot.

Fig. 3. Soil temperature, recorded at the vicinity of the control plants.

Work done in the U.S.A.

The research which was carried out in Dr. Jaffe's laboratory is presented here by one paper. Several other papers are in the process of publication. However, until the time of submission of the final report the publication has not been completed.

Benefit to agriculture

The points of contribution to agriculture are presented and discussed in the framework of the report.

Here we outline them in short:

1. Agronomical practises follow our recommendations for elimination of petiole pithiness in celery. It has been recognized that mild fertilization in the early developmental stage together with avoidance of water stress throughout the growing season minimizes the risk for petiole pithiness. On the other hand amendment with rich nitrogen fertilizer towards the stage of maturity guarantees high yields of high quality. In the course of carrying out this project most of our findings were implemented and consequently the petiole pithiness disorder had been almost eliminated.
2. Our findings as well as others on the possibility of using brackish water for irrigation of celery and lettuce, stimulated interest of many agriculture extension advisors. More experiments are presently in progress for further testing cultivation techniques, as well as varietal tolerance to salinity in experimental plots in the Arava and the Jordan Valley regions.
3. Collapse of tomato plants is now avoidable due to introducing changes in irrigation and fertilization schedules. Fortunately tomato varieties with low sensitivity to environmental stresses substituted var. Hosen-Eilon. Our findings in this part of the project reinforced the necessity to incorporate criteria of stressed tolerance to breeding programs of outdoors tomatoes.
4. Probation of the mechanism of hardening tomato and celery plants in association with the effects of mechanical perturbation or ethrel

resulted in interesting findings which are now under further investigation, though not in the same line of research. We are using similar approaches in evaluation of ethrel and other growth substances for hardening vegetable transplants. Although not practically implemented yet we now have preliminary indications that the use of growth substances for controlling transplants quality would be of great value in nursery management.

Description of cooperation

The cooperation between the Israelis (Aloni and Pressman) and the American counterpart (Dr. Jaffe) has been a fruitful one. While being in Israel Dr. Jaffe got acquainted with the methodology used here and with the practical problems in field-grown plants. He also has done some experiments on the mechanism of hardening, using our facilities for measuring kinetics of electrolyte leakage. Dr. Pressman from the Israeli laboratory stayed in Jaffe's lab for a year, where he initiated the experimental work on pithiness there. The research yielded several publications of which two were already submitted.