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CHEMICAL ADAPTATION OF ROOTS TO
PHYSIOLOGICAL DROUGHT.

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CHEMICAL ADAPTATION OF ROOTS TO
PHYSIOLOGICAL DROUGHT

by
Mohamed N. El-Shourbagy

A Dissertation Submitted to the Graduate Council
In Partial Fulfillment of Requirements for
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THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my
direction by Mohamed Nabih El-Shourbagy
entitled Chemical Adaptation of Roots to
Physiological Drought
be accepted as fulfilling the dissertation requirement of the
degree of Doctor of Philosophy

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ABSTRACT

CHEMICAL ADAPTATION OF ROOTS TO
PHYSIOLOGICAL DROUGHT

by

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A biochemical basis for the adaptation of plant roots to conditions of physiological drought was investigated. This study tested the hypothesis that salt inhibits metabolic step essential to growth and that salt tolerance may be induced by supplying the salt-inhibited tissue with the product of the inhibited reaction.

Clone material from a single root of a salt-intolerant plant, tomato (Lycopersicum esculentum L.), was grown in vitro culture media containing inhibitory concentrations of sucrose or NaCl and with or without various concentrations of metabolites. These metabolites included amino acids, vitamins, purines,

pyrimidines, organic acids, and plant alkaloids. Root length, number of side roots, fresh weight, and dry weight were measured and results were analysed statistically.

In preliminary studies, the roots showed a trend towards increased growth only when a mixture of eighteen amino acids was added to the medium containing inhibitory concentrations of salt. Threonine, phenylalanine, tyrosine, tryptophan, arginine and cystine supplied individually or in a mixture, restored a significant proportion of growth of roots inhibited by salt. The effect of a mixture of these six amino acids was most pronounced when roots were subcultured twice in salt media.

Because it was possible to induce partial salt tolerance of roots from the salt-intolerant tomato plant by supplying them with certain amino acids, it seemed evident that the mechanism of salt tolerance or intolerance is in part metabolic. Based on the results provided, it was suggested that either the enzyme systems involved in the synthesis of these amino acids, or other enzymes containing these amino acids, or both, were inhibited under saline conditions. The mechanism of this inhibition may involve some physical change in the enzyme protein or a competitive inhibition.

INTRODUCTION

Physiological drought is defined as the condition of a plant limited by insufficient water although the habitat contains ample water. In physiological drought, the most common response of plants is a suppression of growth. Sodium chloride is among the salts found in excess in saline soils. Some plants can tolerate high levels of salinity and develop high osmotic pressure in their tissue. The growth of these plants is not inhibited by the same concentration of salts in the root environment that inhibits other plants. The mechanism by which saline-tolerant plants are able to grow under saline conditions is unknown.

Pettersson and Gray (1958) stated that the drier-living species have inherently drier cells and cells which have a smaller volume per chromosome in their lam-
inas.

Langridge (1958) isolated an osmotic mutant of Arabidopsis thaliana which appeared to have a lower osmotic pressure than the normal type. He attributed the lower osmotic pressure to at least four possible reasons, one of which was a lowered rate of metabolism and alterations in the physical properties of the cytoplasm.

West (1962) reported a quantitative reduction of protein and nucleotides and an accumulation of ribonucleic acid (RNA) in seedlings grown in water stress where ribonucleic acid contained higher ratios of guanosine monophosphate and uridine monophosphate to cytosine monophosphate and adenosine monophosphate.

Kessler and Frank-Tishel (1962) postulated that the extent of resistance of plants to unfavorable conditions such as drought and low temperatures is related to either a high guanine-cytosine content of ribonucleic acid or their capacity to synthesize guanine-cytosine-rich ribonucleic acid molecules.

Wadleigh and Ayers (1945) noticed that the increase in soil moisture tension or salt concentration tended to cause an increase in the percentage of nitrate nitrogen and proteins in the leaves of kidney beans. They concluded that whether water stress was due to osmotic forces or to moisture tension, reduced growth of a plant is to a great extent brought about by reduced hydration of protoplasmic proteins.

Laties (1954) exposed cauliflower buds to an environment of low osmotic pressure and observed an injury to the mitochondrial respiratory systems. He attributed this to the action of certain soluble protoplast components.

Nieman (1962) studied some effects of NaCl on growth, photosynthesis, and respiration of twelve crop

plants grown in gravel cultures. In most species, the water content of the leaves was increased and the size of palisade cells in tomato leaves was increased. For the salt-induced increase in leaf respiration, the author proposed that salinity affected the mitochondrial and non-mitochondrial enzymes. He also suggested a stimulation of enzymatic activity rather than an increase in the amount of respiratory substrate since NaCl decreases the amount of organic acids in some plants.

Ter-Karapetyan (1957) noticed that lysine, threonine, and glycine were apparently missing in leaves of Goebelia alopecuroides grown under saline conditions, but were present under non-saline conditions. Akopian (1957) reported a decrease in the monosaccharide and an increase in the disaccharide concentration in the leaves of that plant under saline conditions (1.3 % total salt).

Prusakova (1960) found more tryptophan, alanine, and tyrosine and less proline in the leaves of wheat plants when grown under sufficient water supply than if the water supply was insufficient.

Kurtz (1958) proposed the concept that it should be possible to control the growth of an organism, inhibited by temperature inactivation of one of its essential metabolites, by supplying the known metabolite. Later, the same author (1960) presented the concept of chemical

adaptation as applied to environmental factors other than temperature, including osmotic effects and salinity problems.

The studies reported in this dissertation represent an attempt to provide a biochemical basis for the adaptation of plant roots to conditions of physiological drought. In this work, several experiments were made to provide information about the mechanism of the inhibition of root growth under such conditions.

Clone material of the roots of a salt-intolerant plant, tomato (Lycopersicum esculentum L.), from one seedling was maintained for two years. These roots were grown in vitro in culture solutions containing inhibiting concentrations of sugar (sucrose) or salt (NaCl) and with or without various concentrations of metabolites. The importance of using tomato roots resides in the fact that it has been demonstrated by several workers that it is possible to grow excised roots of tomato in vitro indefinitely (White, 1963). Thus, by using a clone of roots from one seedling, there is the advantage that all members of a clone have theoretically identical genetic constitutions and can be expected to behave similarly under given environmental conditions. This principle is sound and important although there is a tendency of some cells to undergo somatic mutations.

METHODS AND MATERIALS

Methods of cleaning glassware and sterilization of nutrients and supplies similar to those used for tissue culture techniques (White, 1954, 1963), have been applied throughout this study. A transfer room of an inside-room-type, equipped with ultraviolet sterilizing lamps has been used to protect tissues, implements, and culture flasks from contamination during the transfer operations. After the transfer, the culture vessels were incubated at a constant temperature of 28°C.

Root Propagation:

Roots of tomato (Lycopersicum esculentum L.), from one seedling were propagated in multiple flask cultures. A number of sound seedlings were selected from 4-day germinated tomato seeds (Early Pak. No. 7, Ferry Morse Seed Co.), grown aseptically in a Petri dish containing 10 ml of pH 6.8 culture solution. This culture solution was modified from White (1943) and consisted of: 3×10^{-3} M MgSO_4 , 1.4×10^{-3} M Na_2SO_4 , 8.5×10^{-4} M $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 8.7×10^{-4} M KCl , 7.9×10^{-4} M KNO_3 , 1.2×10^{-4} M $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 2.4×10^{-5} M H_3BO_3 , 2.4×10^{-5} M $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$, 6.3×10^{-6} M $\text{Fe}_2(\text{SO}_4)_3$, 5.2×10^{-6} M ZnSO_4 ,

4.5 x 10⁻⁶ M KI, 4.1 x 10⁻⁵ M nicotinic acid, 4.9 x 10⁻⁶ M pyridoxine.HCl, 3 x 10⁻⁶ M thiamine.HCl, 4 x 10⁻⁴ M glycine, and 5.8 x 10⁻² M sucrose. Root tips about one cm. long were excised with a scissors from each of the selected seedlings, each being transferred into a 250-ml. Erlenmeyer flask containing 50 ml. of the above culture solution. The flasks were closed with cotton plugs, covered with 100-ml. beakers, and then incubated in a constant temperature chamber. After eleven days, one healthy root with well-established branches was selected for subsequent subcultures; the rest were discarded. In the transfer room, tips of some branches about one cm. long were excised inside the culture flask by means of a nose-and-throat type scissors. Each of these excised root tips was transferred to a flask containing fresh culture solution. The original clone of roots was split into two sets, A and B, and each was cut back and subcultured every eleven days. A period was allowed between the subculture of each set, so that tissue material was available from the same seedling every 5 to 6 days for a study. Root tips approximately one cm. in length were excised at random from either set A or B for each experiment. Each set, A and B, of the clone has been grown in vitro and maintained for two years.

Treatments:

Tomato roots cultivated in vitro were grown in solutions containing inhibiting concentrations of sugar (sucrose) or sodium chloride and with or without various concentrations of metabolites. Metabolites used included amino acids, vitamins, purines, pyrimidines, plant alkaloids, and organic acids. The metabolites and source of material were as follows:

<u>Metabolites</u>	<u>Source of Material</u>
<u>Amino acids:</u>	
L-Alanine	Calif. Corp. for Biochem. Research
L-Arginine. HCl	Calif. Corp. for Biochem. Research
L-Aspartic acid	Calif. Corp. for Biochem. Research
L-Cystine	Calif. Corp. for Biochem. Research
L-Glutamic acid	Calif. Corp. for Biochem. Research
L-Glycine	Calif. Corp. for Biochem. Research
L-Histidine. HCl, H ₂ O	Calif. Corp. for Biochem. Research
L-Isoleucine	Calif. Corp. for Biochem. Research
L-Leucine	Calif. Corp. for Biochem. Research
L-Lysine. HCl	Calif. Corp. for Biochem. Research
L-Methionine	Calif. Corp. for Biochem. Research
L-Phenylalanine	Calif. Corp. for Biochem. Research
L-Proline	Calif. Corp. for Biochem. Research
L-Serine	Calif. Corp. for Biochem. Research
L-Threonine	Calif. Corp. for Biochem. Research

<u>Metabolites</u>	<u>Source of Material</u>
<u>Amino acids:</u> (Contd.)	
L-Tryptophan	Calif. Corp. for Biochem. Research
L-Tyrosine	Calif. Corp. for Biochem. Research
L-Valine	Calif. Corp. for Biochem. Research
<u>Vitamins:</u>	
L-Ascorbic acid	General Biochem., Inc.
Biotin	General Biochem., Inc.
Ca Pantothenate	Nutritional Biochem. Corp.
Inositol	General Biochem., Inc.
Riboflavin	Calif. Corp. for Biochem. Research
<u>Purines:</u>	
Guanine. HCl	General Biochem., Inc.
Adenine	Calif. Corp. for Biochem. Research
Xanthine	General Biochem., Inc.
Hypoxanthine	Nutritional Biochem. Corp.
<u>Pyrimidines:</u>	
Cytosine	Nutritional Biochem. Corp.
Thymine	General Biochem., Inc.
Uracil	General Biochem., Inc.
<u>Plant alkaloids:</u>	
Caffeine	Calif. Corp. for Biochem. Research
Hyoscyamine	Calif. Corp. for Biochem. Research
Theophylline	Calif. Corp. for Biochem. Research
Trigonelline. HCl	Calif. Corp. for Biochem. Research

<u>Metabolites</u>	<u>Source of Material</u>
<u>Plant alkaloids: (Contd.)</u>	
Nicotine	Calif. Corp. for Biochem. Research
Yohymbine. HCl	Calif. Corp. for Biochem. Research
<u>Organic acids:</u>	
Citric acid	Mallinckrodt Chem. Works
a-Ketoglutaric acid	General Biochem., Inc.
Succinic acid	Calif. Corp. for Biochem. Research

These metabolites were added to the culture solution and autoclaved at 230°F, except the vitamins which were sterilized by dissolving in 50% ethanol and then were added to the culture solution. Water used throughout the experiments was deionized (0.1 to 0.5 p.m.m.).

Measurements:

At the end of eleven days, roots were removed from the culture medium and laid out in a wide Petri dish containing distilled water to a depth of about 3 mm. The total length of each root, including the main axis and the side branches, was measured with a flexible plastic ruler to the nearest mm. in experiments 15 through 19; they were measured to the nearest 0.5 mm. in experiments 20 through 43. For each root, the increase in length was obtained by subtracting the original length from the total length and all values were rounded off to the nearest integer. At the same time, the number of side roots was

recorded for each root. After recording the total length and the number of side roots, the roots from each treatment were pooled, washed with deionized water to remove excess ions, blotted with filter paper to remove excess moisture, and weighed to the nearest mg. For measuring the dry weight, roots from each treatment were transferred to an open Petri dish and dried to constant weight at 60°C. Growth in length and number of side roots was recorded in all experiments, while fresh weight and dry weight were recorded from experiment 21 to experiment 43. In each treatment and control within a given experiment, three to five culture flasks were used, each containing two root tips. When contamination occurred in a flask or a root tip died (brown and not floating) during the experimental period, growth measurements were not recorded for these roots. This is represented in the tables by a (-). Statistical methods used (Steel and Torrie, 1960) involved comparison of all means with the salt control to test the effects of individual amino acids by applying Dunnett's procedure for 0.05 level. However, group comparison of two sample means was applied to test the effects of amino acid mixture (Tables 24 and 25) and the hypothesis was tested by the two tailed "t" test. The standard deviations of the mean increase in length were also applied in all tables.

RESULTS

Inhibition of Root Growth by Sucrose and Sodium Chloride:

The growing root as a highly organized and actively metabolizing system is directly affected by the surrounding physical and chemical environment. Response to drought differs among different species. When environmental conditions are varied a given species is able to change its osmotic value in large amount. The osmotic value is one of the means of defense against drought that is inherent in each species to a different degree.

Bernstein and Pearson (1954) have shown that growth of peppers was reduced to almost zero if osmotic pressure is maintained continuously at 6 atm. This corresponds to the osmotic pressure of the root of the pepper plant rather than to that of the leaves. Many workers support the view that water deficit induced by high osmotic pressure of the root medium is the factor restricting growth and that absorption of water by roots is greater from dilute than from concentrated media. Smirnov et al. (1960), using isolated lucerne roots grown on media with gradually increasing NaCl concentration, have shown that the growth was impaired with 0.1% NaCl and the roots died at 1.1%.

The data in Tables 1 to 4 indicate that the growth of tomato roots was greatly inhibited as the concentration of sucrose or NaCl was increased in the culture media. The roots ceased to form side branches when the concentration of sucrose was increased above 0.20 M. At the same concentration of NaCl, the increase in length and the number of side branches were more inhibited at 0.20 M sucrose than at 0.06 M. It seems likely that increasing the osmotic pressure of the culture medium causes a reduced growth rate. The effects on root growth of excessive concentrations of sugar in the culture media was mediated by osmotic inhibition of water absorption, while the effects of NaCl may involve osmotic inhibition of water absorption as well as direct toxicity of the constituent ions. The mechanism of NaCl toxicity remains unknown. The similarity of NaCl accumulation levels at which injury occurs in NaCl-sensitive species suggests that there is a common mechanism.

Salt tolerance is the degree to which osmotic adjustment can be made without significant sacrifice of growth. The inhibition of growth of tomato roots by salt may have involved biochemical processes, and this suggests that it may be possible to restore growth at high concentrations of salt by supplying the roots with the metabolites involved in the inhibited biochemical processes.

Table 1. Effect of sucrose concentration on root growth.
Experiment No. 15.

sucrose concentration M							
	0.06	0.16	0.18	0.20	0.22	0.24	0.26
<u>Increase in length, mm.</u>							
	96	20	17	24	23	14	14
	229	-	18	-	22	-	3
	230	17	-	5	28	20	17
	235	24	21	41	22	9	11
	152	30	24	3	0	0	10
	-	28	-	-	13	18	14
Mean:	188	24	20	18	18	12	12
$\pm s$	± 62	± 5	± 3	± 18	± 10	± 8	± 5
<u>Number of side roots</u>							
	7	3	0	1	0	0	0
	27	-	0	-	0	-	0
	30	2	-	0	0	0	0
	39	2	2	3	0	0	0
	26	2	3	0	0	0	0
	-	3	-	-	0	0	0
Mean:	26	2	1	1	0	0	0

s = standard deviation.

Table 2. Effect of sucrose concentration on root growth.
Experiment No. 16.

	sucrose concentration M					
	0.06	0.26	0.28	0.30	0.32	0.34
	<u>Increase in length, mm.</u>					
	78	20	5	15	10	17
	162	11	11	18	6	16
	172	25	9	14	9	6
	124	10	11	-	9	15
	209	16	17	12	-	-
	131	13	19	16	20	11
Mean:	146	16	11	15	11	13
$\pm s$	± 45	± 6	± 5	± 2	± 5	± 5
	<u>Number of side roots</u>					
	7	0	0	0	0	0
	21	0	0	0	0	0
	19	3	0	0	0	0
	12	0	0	-	0	0
	31	0	0	0	-	-
	18	0	0	0	0	0
Mean:	18	0.5	0	0	0	0

Table 3. Effect of different concentrations of NaCl on root growth in culture media containing 0.06 M sucrose. Experiment No. 17a

NaCl concentration M							
	0.00	0.02	0.04	0.06	0.08	0.10	0.12
<u>Increase in length, mm.</u>							
	215	353	245	209	129	48	50
	353	126	89	48	-	47	23
	178	231	129	89	93	68	18
	391	-	46	96	50	50	-
	-	156	39	63	92	26	26
	214	-	-	36	89	45	-
	141	169	-	-	38	27	42
	172	-	-	-	32	-	32
Mean:	238	207	110	90	75	44	32
± s	± 96	± 90	± 84	± 63	± 36	± 15	± 12
<u>Number of side roots</u>							
	27	43	22	28	12	5	3
	45	15	8	3	-	5	1
	20	23	14	4	6	7	0
	44	-	0	4	2	6	-
	-	16	3	1	9	3	2
	23	-	-	2	8	2	-
	16	15	-	-	2	1	4
	15	-	-	-	1	-	1
Mean:	27	22	9	7	6	4	2

Table 4. Effect of different concentrations of NaCl on root growth in culture media containing 0.20 M sucrose. Experiment No. 17b.

		NaCl concentration M						
		0.00	0.02	0.04	0.06	0.08	0.10	0.12
		<u>Increase in length, mm.</u>						
		32	37	22	0	4	1	0
		29	34	10	10	4	0	1
		28	15	16	0	0	0	1
		55	19	12	5	6	1	0
		31	21	30	4	17	3	1
		49	-	-	7	0	1	0
		21	12	0	1	0	0	0
		20	-	8	14	3	1	2
Mean:		33	23	14	5	4	1	1
$\pm s$		± 12	± 10	± 10	± 5	± 6	± 1	± 1
		<u>Number of side roots</u>						
		1	1	0	0	0	0	0
		1	2	0	0	0	0	0
		2	0	0	0	0	0	0
		3	2	0	0	0	0	0
		1	2	2	0	0	0	0
		2	-	-	0	0	0	0
		0	0	0	0	0	0	0
		0	-	0	1	0	0	0
Mean:		1	1	0.3	0.1	0	0	0

In the following studies we have tested the effects of a number of compounds on root growth and by these in vitro culture methods we have tried to determine the mechanism of the adaptation of roots under conditions of physiological drought. This method has the advantage that growth of isolated roots depends completely on the culture environment and complex root-shoot relationships are not involved. The studies reported here include two parts. The first part contains the results of screening of a number of compounds to test the extent of their effects on roots inhibited by 0.20 M sucrose and by 0.12 M NaCl; the second part centers on the role of amino acids in roots inhibited by NaCl. It was reasonable to select 0.12 M NaCl at 0.06 M sucrose and 0.00 NaCl at 0.20 M sucrose because root growth at these two concentrations exhibited greater inhibition but not a complete one and the growth measurements showed approximately similar values, although the effects were different.

I. Screening of Different Compounds:

Compounds applied in culture media containing inhibitory concentrations of either sucrose (0.20 M) or NaCl (0.12 M), included amino acids, vitamins, purines, pyrimidines, organic acids, and plant alkaloids.

1. Amino acids

Six treatments of amino acids were used, each treatment included eighteen amino acids (see page 7), and each amino acid was tested over a concentration range of 4×10^{-8} M to 4×10^{-4} M. In Table 5, 4×10^{-6} M, 4×10^{-5} M, and 4×10^{-4} M amino acid mixtures were used, and in Table 6, concentrations of 4×10^{-8} M, 4×10^{-7} M, and 4×10^{-6} M were used. Data presented in Table 5 show that the average increase in length was larger and the roots formed more side branches when treated with amino acids at a concentration of 4×10^{-6} M in either medium containing inhibitory concentrations of sucrose or NaCl. The other two concentrations of amino acids were ineffective or inhibitory. In both of these experiments, however, results were so variable that effects were not statistically significant.

2. Vitamins

Two mixtures of vitamins were used; each mixture contained ascorbic acid, biotin, Ca-pantothenate, inositol, and riboflavin. In one mixture, each vitamin was present in a concentration ten times greater than the other mixture. The results (Table 7) show that vitamins at these two concentrations did not relieve the inhibitory effects of NaCl or sucrose. On the contrary, the growth of roots in these two treatments was inhibited.

Table 5. Effect of amino acids (AA) on root growth in two culture media, one containing 0.20 M sucrose and the other containing 0.06 M sucrose + 0.12 M NaCl. Eighteen amino acids were used in the mixture, and each amino acid was at the concentration indicated. Experiment No. 18.

0.06 M sucrose 0.00 NaCl	0.20 M sucrose 0.00 NaCl	0.20 M sucrose 4×10^{-6}	0.20 M sucrose 4×10^{-5}	0.20 M sucrose 4×10^{-4}	0.06 M sucrose + 0.12 M NaCl	0.06 M sucrose + 0.12 M NaCl + AA mixture (M)	0.06 M sucrose + 0.12 M NaCl + AA mixture (M)	0.06 M sucrose + 0.12 M NaCl + AA mixture (M)
<u>Increase in length, mm.</u>								
66	28	25	33	11	34	31	18	3
135	20	23	9	1	9	21	15	3
97	9	22	16	5	23	22	25	3
210	15	25	17	3	24	37	7	6
137	25	18	16	12	22	29	18	0
148	12	26	11	0	18	18	17	4
56	-	20	-	10	-	-	-	0
-	-	21	-	0	-	-	-	1
Mean: 121	18	23	17	5	22	26	17	3
\pm s \pm 53	\pm 8	\pm 3	\pm 8	\pm 5	\pm 8	\pm 7	\pm 6	\pm 2
<u>Number of side roots</u>								
10	0	2	2	2	4	2	1	0
18	0	3	0	0	0	1	0	0
12	0	0	0	0	0	2	0	0
18	0	0	0	0	0	4	0	0
17	2	0	0	1	2	3	1	0
14	1	0	0	0	0	1	0	0
3	-	1	-	0	-	-	-	0
-	-	0	-	0	-	-	-	0
Mean: 13	0.5	1	0.3	0.4	1	2	0.3	0

Table 6. Effect of amino acids (AA) on root growth in two culture media, one containing 0.20 M sucrose and the other containing 0.06 M sucrose + 0.12 M NaCl. Eighteen amino acids were used in the mixture, and each amino acid was at the concentration indicated. Experiment No. 19.

0.06 M sucrose + 0.00 NaCl	0.20 M sucrose + 0.00 NaCl	0.20 M sucrose + 0.00 NaCl + <u>AA mixture (M)</u> 4×10^{-8}	0.20 M sucrose + 0.00 NaCl + <u>AA mixture (M)</u> 4×10^{-7}	0.06 M sucrose + 0.12 M NaCl	0.06 M sucrose + 0.12 M NaCl + <u>AA mixture (M)</u> 4×10^{-8}	0.06 M sucrose + 0.12 M NaCl + <u>AA mixture (M)</u> 4×10^{-7}	0.06 M sucrose + 0.12 M NaCl + <u>AA mixture (M)</u> 4×10^{-6}	
<u>Increase in length, mm.</u>								
396	57	47	27	52	77	47	29	103
138	34	52	22	28	47	109	19	35
351	34	53	74	50	63	41	89	19
289	12	49	42	48	48	102	14	33
158	65	23	33	63	41	54	39	44
269	37	59	23	38	19	45	27	-
241	25	41	17	28	47	57	56	-
219	23	37	15	80	42	-	42	-
Mean: 258	36	45	32	48	48	65	39	47
\pm s \pm 89	\pm 18	\pm 11	\pm 19	\pm 18	\pm 17	\pm 28	\pm 24	\pm 33
<u>Number of side roots</u>								
37	2	0	1	1	6	4	2	8
24	0	0	1	0	4	11	1	1
34	0	2	2	1	5	5	7	0
33	0	1	2	1	3	14	0	1
27	2	1	0	1	1	4	1	2
32	1	1	0	0	0	2	1	-
27	0	1	0	0	3	7	3	-
28	0	1	0	3	3	-	2	-
Mean: 30	1	1	1	1	3	7	2	2

Table 7. Effect of vitamins on root growth in two culture media, one containing 0.20 M sucrose and the other containing 0.06 M sucrose + 0.12 M NaCl. Ascorbic acid, biotin, Ca-pantothenate, inositol, and riboflavin were used in two mixtures; in the high concentration mixture each vitamin was at a concentration ten times that in the low concentration mixture.* Experiment No. 20.

	0.06 M sucrose +	0.20 M sucrose +	0.20 M sucrose + 0.00 NaCl + vitamin mixt.		0.06 M sucrose +	0.06 M sucrose + 0.12 M NaCl + vitamin mixt.	
	0.00 NaCl	0.00 NaCl	low conc.	high conc.	0.12 M NaCl	low conc.	high conc.
	<u>Increase in length, mm.</u>						
	200	26	17	16	13	30	18
	174	-	-	5	26	19	9
	367	28	24	-	46	31	33
	113	34	25	-	31	16	14
	266	-	24	18	49	-	17
	-	-	-	14	-	-	13
	216	23	23	12	40	26	12
	-	33	14	10	-	19	-
	-	35	27	-	-	30	24
	-	24	56	-	-	-	10
Mean:	223	29	26	13	34	24	17
$\pm s$	± 87	± 5	± 13	± 5	± 14	± 7	± 8
	<u>Number of side roots</u>						
	23	0	0	3	0	0	0
	32	-	-	1	0	0	0
	30	0	0	-	3	0	2
	7	0	0	-	0	0	0
	20	-	0	0	4	-	0
	-	-	-	1	-	-	1
	16	0	0	0	1	1	1
	-	0	0	0	-	0	-
	-	0	0	-	-	1	0
	-	0	3	-	-	-	2
Mean:	21	0	0.4	1	1	0.3	1

* Lower concentration contained 4×10^{-8} M ascorbic acid, 4×10^{-10} M biotin, 4×10^{-8} M Ca-pantothenate, 4×10^{-7} M inositol, and 4×10^{-9} M riboflavin. Higher concentration contained 4×10^{-7} M ascorbic acid, 4×10^{-9} M biotin, 4×10^{-7} M Ca-pantothenate, 4×10^{-6} M inositol, and 4×10^{-8} M riboflavin.

3. Purines

A mixture of purines composed of adenine, guanine-HCl, xanthine, and hypoxanthine was applied. Each was at a concentration of 4×10^{-5} M, as indicated in Table 8. From the data, it is clear that purines inhibited roots subcultured in all treatments. When the same mixture was applied to culture media containing either 0.06 M or 0.20 M sucrose, the same effects were noticed (Table 9).

It is possible that purines, because they profoundly inhibited root growth, acted as partial structural analogues of certain enzyme systems where inhibition was competitive in nature. Similar evidence has been reported by Boll (1954) with excised tomato roots who found that 1.5×10^{-5} M desoxypyridoxin caused 50% inhibition of growth which could be relieved by the addition of pyridoxin. Walaas and Walaas (1956) found that the partial structural analogues of flavin adenine-dinucleotide (FAD) inhibit the activity of D-amino acid oxidase.

4. Pyrimidines

Two mixtures of pyrimidines were applied; each mixture contained cytosine, thymine, and uracil. In one mixture, each of these pyrimidines was 4×10^{-5} M and in another mixture, each was 4×10^{-4} M. It can be seen (Table 10) that 4×10^{-4} M pyrimidines showed certain effects on the formation of side roots. At this concentration of pyrimidines more side roots were formed by these

Table 8. Effect of purines on root growth in two culture media, one containing 0.20 M sucrose and the other containing 0.06 M sucrose + 0.12 M NaCl. Guanine-HCl, adenine, Xanthine, and hypoxanthine were used in the purine mixture, and each was 4×10^{-5} M. Experiment No. 21.

	0.06 M sucrose + 0.00 NaCl	0.20 M sucrose + 0.00 NaCl	0.20 M sucrose + 0.00 NaCl + purines	0.06 M sucrose + 0.12 M NaCl	0.06 M sucrose + 0.12 M NaCl + purines
<u>Increase in length, mm.</u>					
	157	45	7	21	0
	73	67	0	-	0
	152	27	4	27	4
	-	58	0	2	0
	219	13	0	7	2
	158	17	3	0	2
	281	35	0	18	0
	-	49	0	27	3
	69	33	2	0	5
	182	20	0	0	4
Mean:	161	36	2	11	2
± s	± 70	± 18	± 2	± 12	± 2
<u>Number of side roots</u>					
	17	2	0	1	0
	5	3	0	-	0
	18	0	0	1	0
	-	3	0	0	0
	30	0	0	0	0
	22	1	0	0	0
	39	2	0	0	0
	-	3	0	0	0
	12	0	0	0	0
	22	0	0	0	0
Mean:	21	1	0	0.2	0

Table 9. Effect of purines on root growth in two culture media, one containing 0.06 M sucrose and the other containing 0.20 M sucrose. Guanine-HCl, adenine, xanthine, and hypoxanthine were used in the purine mixture, and each was 4×10^{-5} M. Experiment No. 23.

0.06 M sucrose + 0.00 NaCl	0.06 M sucrose + 0.00 NaCl + purines	0.20 M sucrose + 0.00 NaCl	0.20 M sucrose + 0.00 NaCl + purines
<u>Increase in length, mm.</u>			
262	1	33	1
138	0	32	2
209	0	36	2
272	1	87	0
221	0	38	0
235	0	83	0
228	0	56	0
251	1	90	0
288	0	48	0
263	0	37	1
Mean: 237	0.3	54	0.4
$\pm s \pm 43$	± 0.5	± 24	± 0.8
<u>Fresh weight, mg.</u>			
Mean: 34	0.3	10	1
<u>Dry weight, mg.</u>			
Mean: 2.3	0.05	1.2	0.05
<u>Number of side roots</u>			
33	0	0	0
26	0	0	0
26	0	2	0
28	0	3	0
20	0	1	0
20	0	2	0
27	0	2	0
31	0	4	0
25	0	1	0
31	0	1	0
Mean: 27	0	2	0

Table 10. Effect of pyrimidines on root growth in two culture media, one containing 0.20 M sucrose and the other containing 0.06 M sucrose + 0.12 M NaCl. Cytosine, thymine, and uracil were in the mixture, and each pyrimidine was at the concentration indicated. Experiment No. 22.

0.06 M sucrose + 0.00 NaCl	0.20 M sucrose + 0.00 NaCl	0.20 M sucrose + 0.00 NaCl + pyrimidines(M)		0.06 M sucrose + 0.12 M NaCl	0.06 M sucrose + 0.12 M NaCl + pyrimidines(M)	
0.00 NaCl	0.00 NaCl	4×10^{-5}	4×10^{-4}	0.12 M NaCl	4×10^{-5}	4×10^{-4}
<u>Increase in length, mm.</u>						
529	16	33	-	98	11	72
-	28	12	27	97	-	25
431	37	39	36	50	36	22
259	34	-	19	41	42	-
256	89	34	23	65	31	59
201	27	-	62	46	35	-
301	80	36	68	33	39	59
480	28	40	35	30	20	57
312	-	49	26	-	45	82
-	-	112	-	-	32	52
Mean: 346	42	44	37	58	32	53
$\pm s \pm 118$	± 27	± 29	± 18	± 27	± 11	± 21
<u>Fresh weight, mg.</u>						
Mean: 47	7	9	6	8	7	8
<u>Dry weight, mg.</u>						
Mean: 3.8	0.8	1.1	0.9	0.7	0.6	0.7
<u>Number of side roots</u>						
45	0	0	-	4	0	9
-	0	1	2	6	-	1
79	0	0	0	4	1	0
32	0	-	0	2	2	-
39	4	0	1	5	2	3
35	1	-	3	4	1	-
38	3	0	7	1	1	5
74	1	0	6	0	0	6
39	-	2	2	-	4	5
-	-	5	-	-	1	4
Mean: 48	1	1	3	3	1	4

roots than in either sucrose or NaCl treatments. The 4×10^{-5} M pyrimidines were without effect on roots in the NaCl treatment. Because of these results we cannot be sure at present about the influence of pyrimidines.

5. Organic acids

Two mixtures of organic acids (Krebs' cycle type) were used; each mixture contained citric acid, α -ketoglutaric acid, and succinic acid. In one mixture, each of these organic acids was 10^{-4} M and in another mixture each was 10^{-3} M. The results (Table 11) indicate a profound growth inhibition of roots cultured in either 0.12 M NaCl or in 0.20 M sucrose; this effect was more pronounced in the salt treatment than in the sugar treatment. In this regard, Nieman (1962) reported that NaCl stimulates enzymatic activity and decreases the amount of organic acids in bean leaves. Thus, addition of organic acids to NaCl-inhibited roots might cause further inhibition. This was the case in the present study.

6. Plant alkaloids

a. Selecting suitable concentrations

Six plant alkaloids were tried in media containing 0.06 M sucrose to determine a suitable concentration for roots cultured in this medium. Each alkaloid was used individually in either one of two experiments. In experiment No. 24 (Table 12), theophylline, caffeine, and

Table 11. Effect of organic acids (OA) on root growth in two culture media, one containing 0.20 M sucrose and the other containing 0.06 M sucrose + 0.12 M NaCl. Citric acid, -ketoglutaric acid, and succinic acid were used in the mixture, and each organic acid was at the concentration indicated. Experiment No. 25.

	0.06 M sucrose + 0.00 NaCl	0.20 M sucrose + 0.00 NaCl	0.20 M sucrose + 0.00 NaCl + OA mixture (M) 10^{-4}	0.20 M sucrose + 0.00 NaCl + OA mixture (M) 10^{-3}	0.06 M sucrose + 0.12 M NaCl	0.06 M sucrose + 0.12 M NaCl + OA mixture (M) 10^{-4}	0.06 M sucrose + 0.12 M NaCl + OA mixture (M) 10^{-3}
<u>Increase in length, mm.</u>							
	281	22	3	0	60	0	0
	165	-	0	0	26	0	0
	245	16	13	1	52	0	1
	164	17	17	0	31	1	2
	167	20	0	0	10	0	0
	203	-	1	0	-	1	-
	219	48	3	2	22	0	1
	178	16	11	2	6	0	0
	-	16	13	0	6	0	1
	-	21	3	0	-	0	0
Mean:	203	22	6	0.5	27	0.2	0.6
$\pm s$	± 43	± 11	± 6	± 0.8	± 20	± 0.4	± 0.7
<u>Fresh weight, mg.</u>							
Mean:	21	5	2	1	4	1	1
<u>Dry weight, mg.</u>							
Mean:	1.4	0.5	0.2	0.1	0.4	0.1	0.1
<u>Number of side roots</u>							
	33	3	0	0	4	0	0
	25	-	0	0	0	0	0
	26	0	1	0	4	0	1
	22	0	2	0	0	0	1
	20	1	0	0	1	0	0
	21	-	0	0	-	0	-
	29	2	0	0	1	0	0
	26	1	2	0	0	0	0
	-	0	2	0	0	0	0
	-	0	0	0	-	0	0
Mean:	25	1	1	0	1	0	0.2

Table 12. Effect of theophylline, caffeine, and nicotine on root growth in culture media containing 0.06 M sucrose. Each alkaloid was at the concentration indicated. Experiment No. 24.

	0.06 M sucrose + 0.00 NaCl + theophylline(M)			0.06 M sucrose + 0.00 NaCl + caffeine(M)			0.06 M sucrose + 0.00 NaCl + nicotine(M)			
	10 ⁻⁵	10 ⁻⁴	10 ⁻³	10 ⁻⁵	10 ⁻⁴	10 ⁻³	10 ⁻⁵	10 ⁻⁴	10 ⁻³	
	<u>Increase in length, mm.</u>									
	334	134	145	122	333	200	39	73	3	0
	258	-	208	49	99	0	0	-	4	0
	494	471	308	138	-	0	0	85	1	0
	335	242	217	-	286	0	0	244	3	1
	352	310	77	50	207	51	38	174	0	0
	457	252	247	-	-	0	0	-	0	0
	-	310	302	-	87	0	0	172	-	1
	-	-	-	-	-	-	-	189	-	0
Mean:	372	287	215	90	202	36	11	155	2	0.3
<u>± s</u>	<u>± 88</u>	<u>± 111</u>	<u>± 83</u>	<u>± 47</u>	<u>± 110</u>	<u>± 75</u>	<u>± 19</u>	<u>± 68</u>	<u>± 2</u>	<u>± 0.4</u>
	<u>Fresh weight, mg.</u>									
Mean:	37	35	35	13	23	2	1	29	1	1
	<u>Dry weight, mg.</u>									
Mean:	2.8	2.4	2.4	1.1	1.4	0.2	0.1	2.6	0.1	0.1
	<u>Number of side roots</u>									
	28	12	10	8	24	16	0	4	2	0
	22	-	18	3	15	0	0	-	1	0
	79	59	32	13	-	0	0	5	0	0
	49	36	33	-	38	0	0	26	0	0
	41	42	12	5	16	6	1	13	0	0
	55	35	36	-	-	0	0	-	0	0
	-	30	27	-	10	0	0	35	-	0
	-	-	-	-	-	-	-	30	-	0
Mean:	46	36	24	7	21	3	0.1	19	0.5	0

nicotine were used. Each was 10^{-5} M, 10^{-4} M, and 10^{-3} M. It is shown that as the concentration of each of these alkaloids was increased, the root length, number of side roots, fresh weight, and dry weight decreased. In experiment No. 27 (Table 13), trigonelline-HCl, hyoscyamine, and yohimbine-HCl were used; each was 10^{-6} M, 10^{-5} M, and 10^{-4} M. Again, as the concentration of each was increased, the growth of roots decreased.

b. Addition of alkaloids to culture media containing 0.12 M NaCl and 0.20 M sucrose.

In experiment No. 30, a concentration of each alkaloid was selected for both salt and sugar treatments based upon concentrations reported in biochemical studies in the literature. The data (Table 14) illustrate the fact that none of these alkaloids in its particular concentration can relieve inhibition of root growth in salt media. In addition, when these alkaloids were applied at the same concentrations to media containing 0.20 M sucrose (Table 15), no significant change in root growth was noticed.

It has been reported that alkaloids are physiologically active compounds. They are related structurally to amino acids or their derivatives, but it is difficult to interpret their effects on tomato roots in the present study.

Table 13. Effect of trigonelline-HCl, hyoscyamine, and yohimbine-HCl on root growth in culture media containing 0.06 M sucrose. Each alkaloid was at the concentration indicated. Experiment No. 27.

0.06 M sucrose + 0.00 NaCl + 0.00 NaCl	0.06 M sucrose + 0.00 NaCl + <u>trigonelline(M)</u>			0.06 M sucrose + 0.00 NaCl + <u>hyoscyamine(M)</u>			0.06 M sucrose + 0.00 NaCl + <u>yohimbine(M)</u>		
	4×10^{-6}	10^{-5}	10^{-4}	4×10^{-6}	10^{-5}	10^{-4}	4×10^{-6}	10^{-5}	10^{-4}
<u>Increase in length, mm.</u>									
209	39	24	0	41	5	3	95	14	0
198	127	27	1	87	8	4	71	21	6
149	50	22	5	87	11	2	76	107	9
-	85	16	7	36	3	0	53	15	0
253	83	94	8	92	58	0	93	21	8
171	27	30	2	61	8	2	99	19	0
305	72	72	10	54	12	3	99	81	14
310	107	-	13	26	22	1	93	9	0
Mean: 228	74	40	6	61	16	2	85	36	5
$\pm s \pm 64$	± 34	± 30	± 5	± 26	± 18	± 1	± 16	± 37	± 5
<u>Fresh weight, mg.</u>									
Mean: 24	10	5	1	9	3	1	12	6	1
<u>Dry weight, mg.</u>									
Mean: 1.4	0.8	0.5	0.1	0.8	0.3	0.1	1.0	0.6	0.1
<u>Number of side roots</u>									
23	6	3	0	11	2	0	24	2	0
29	15	2	0	15	2	0	13	3	1
9	12	3	0	12	2	0	8	19	2
-	9	5	0	8	0	0	8	1	0
22	13	20	2	14	8	0	29	1	1
12	5	5	0	11	0	0	17	2	0
36	10	12	0	13	2	0	15	9	0
31	18	-	0	4	3	0	22	1	1
Mean: 23	11	7	0.3	11	2	0	17	5	0.5

Table 14. Effect of theophylline, caffeine, nicotine, trigonelline-HCl, hyoscyamine, and yohimbine-HCl on root growth in culture media containing 0.12 M NaCl. Experiment No. 30.

0.06 M sucrose		0.06 M sucrose + 0.12 M NaCl		0.06 M sucrose + 0.12 M NaCl + alkal. treatment				
+	+	10 ⁻⁵	10 ⁻⁵	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶	
0.00 NaCl	0.12 M NaCl	theo- phyl- line	caf- feine	nico- tine	trig- onel- line	hyo- scya- mine	yohym- bine	
<u>Increase in length, mm.</u>								
152	102	121	81	33	65	95	70	
176	146	43	53	22	73	-	43	
269	105	125	37	32	75	29	32	
161	151	115	63	20	39	31	-	
192	108	39	59	47	56	31	25	
250	65	36	56	53	-	87	26	
131	57	112	54	28	47	35	26	
214	79	33	-	34	100	50	22	
192	-	36	84	28	29	48	30	
135	-	-	33	57	76	45	31	
Mean:	187	102	73	57	35	62	50	34
± s	± 46	± 35	± 43	± 17	± 13	± 22	± 27	± 15
<u>Fresh weight, mg.</u>								
Mean:	30	16	13	8	6	10	8	4
<u>Dry weight, mg.</u>								
Mean:	2.8	1.5	1.3	0.7	0.7	0.8	0.9	0.5
<u>Number of side roots</u>								
22	8	9	6	1	6	9	7	
24	13	1	4	0	5	-	3	
28	11	10	2	1	6	0	1	
26	14	9	5	0	2	1	-	
16	10	2	5	3	6	1	0	
32	5	1	5	4	-	8	0	
15	3	10	7	0	2	2	0	
15	4	0	-	0	10	4	0	
21	-	0	9	0	1	3	1	
14	-	-	2	5	7	3	1	
Mean:	21	9	5	5	1	5	3	1

Table 15. Effect of theophylline, caffeine, nicotine, trigonelline-HCl, hyoscyamine, and yohimbine-HCl on root growth in culture media containing 0.20 M sucrose. Experiment No. 31.

0.06 M sucrose	0.20 M sucrose	0.20 M sucrose + 0.00 NaCl + alkal. treat.					
+	+	10 ⁻⁵	10 ⁻⁵	10 ⁻⁶ ^M	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶
0.00 NaCl	0.00 NaCl	theo- phyl- line	caf- feine	nico- tine	trig- onel- line	hyo- scya- mine	yohy- mbine
<u>Increase in length, mm.</u>							
252	83	31	20	35	63	40	60
202	-	24	23	74	45	17	58
187	59	61	36	23	42	109	77
220	29	21	31	29	57	59	35
379	50	74	51	83	38	-	28
187	99	62	79	74	90	66	15
225	41	37	12	61	26	42	30
285	84	28	26	48	-	21	69
230	94	92	89	69	72	18	32
226	57	27	35	83	69	21	44
Mean: 239	66	46	40	58	56	44	45
± s ± 58	± 25	± 27	± 25	± 23	± 20	± 30	± 20
<u>Fresh weight, mg.</u>							
Mean: 32	12	11	11	13	11	9	13
<u>Dry weight, mg.</u>							
Mean: 2.3	0.9	1.1	1.1	1.4	1.0	1.1	1.2
<u>Number of side roots</u>							
42	3	0	2	0	1	0	2
28	-	1	0	2	0	0	1
23	1	2	0	0	0	3	1
27	0	1	0	1	1	2	2
44	2	2	1	2	1	-	0
23	5	2	2	3	3	1	0
28	1	2	1	1	0	1	0
35	2	1	2	1	-	0	2
20	2	3	4	2	1	0	0
19	1	0	2	3	2	0	0
Mean: 29	2	1	1	2	1	1	1

II. Induction of Salt Tolerance in Roots by Amino

Acids:

In the previous section there was some suggestion of stimulation by amino acids of the growth of excised tomato roots grown in media containing inhibitory concentrations of sucrose or NaCl. When a mixture of eighteen amino acids, each at 4×10^{-6} M, was applied to roots cultured in media contained 0.20 M sucrose, the roots showed a trend toward increased length. On the other hand, when they were cultured in media containing 0.12 M NaCl, and the amino acid mixture (each acid 4×10^{-8} M), they increased in length as well as forming more side roots.

In the present section, effort centered upon inducing tolerance by individual amino acids in roots. Later, a mixture of those amino acids which counteracted the effects of salt on roots was tried in media containing NaCl at concentrations ranging from 0.0 to 0.16 M. Finally the effect of this mixture was demonstrated on roots subcultured twice in media containing 0.12 M NaCl.

1. Selecting a suitable concentration of each amino acid.

Several workers have studied the effects of amino acids on different plant tissues grown in vitro.

Riker and Gutsche (1948) carried out a number of experiments on the nitrogen nutrition of crown gall tissue of

sunflower and showed that histidine, leucine, valine, and lysine are different in their stimulatory effects at low concentrations, but become toxic as soon as the levels were increased; but alanine, aspartic acid and glutamic acid inhibit growth at medium concentration and become inactive at either lower or higher concentrations. These authors as well as Nickell and Burkholder (1950) indicated no stimulation with tryptophan. Paris and Duhamet (1953) showed that the mixture of eleven amino acids contained in coconut milk stimulates the proliferation of several plant tissues, while Sandstedt and Skoog (1960) found that a mixture of L-amino acids from yeast extract promotes the growth of tobacco callus tissue. Sutton, Scott and Street (1961) presented evidence that the activity of Bacto-casamino acids in promoting the growth of excised wheat roots is partly due to its balanced content of amino acids. These authors showed also that tryptophan enhances lateral initiation and growth of wheat roots in both light and dark cultures. Boszormenyi and Cseh (1962), on the other hand, indicated that L-cystine stimulates the uptake of glycine-C¹⁴ by excised wheat roots, while asparagine stimulates the uptake of tyrosine-C¹⁴ and methionine-S³⁵.

In Tables 16 and 17, six amino acids were selected to test the effect of each at three different

Table 16. Effect of threonine, aspartic acid, and lysine-HCl on roots grown in culture media containing 0.06 M sucrose. Experiment No. 28.

0.06 M sucrose + 0.00 NaCl	0.06 M sucrose + 0.00 NaCl + threonine(M)	0.06 M sucrose + 0.00 NaCl + aspartic acid (M)	0.06 M sucrose + 0.00 NaCl + lysine-HCl(M)								
0.00 NaCl	2×10^{-7}	10^{-6}	10^{-5}	2×10^{-7}	10^{-6}	10^{-5}	2×10^{-7}	10^{-6}	10^{-5}		
<u>Increase in length, mm.</u>											
211	280	220	336	239	171	358	176	311	765		
176	313	410	220	311	434	-	268	208	-		
91	330	239	105	371	320	-	255	203	-		
361	338	343	228	330	338	-	272	283	-		
327	367	208	262	69	316	298	338	163	287		
203	347	269	197	194	304	-	306	395	390		
231	175	173	167	251	331	226	112	138	306		
270	409	406	269	307	157	325	400	170	201		
Mean: 234	320	284	223	259	296	302	266	234	390		
\pm s	\pm 86	\pm 71	\pm 91	\pm 70	\pm 95	\pm 91	\pm 56	\pm 90	\pm 88	\pm 220	
<u>Fresh weight, mg.</u>											
Mean: 38	45	36	44	38	49	46	44	45	56		
<u>Dry weight, mg.</u>											
Mean: 2.4	3.1	2.4	2.8	2.8	3.2	3.2	3.3	3.3	3.8		
<u>Number of side roots</u>											
27	33	29	66	31	23	34	17	35	73		
18	37	53	45	41	57	-	36	28	-		
24	53	24	16	46	44	-	39	38	-		
53	57	44	49	41	38	-	36	39	-		
28	37	22	43	10	32	24	39	16	35		
26	44	38	38	18	29	-	41	40	49		
37	18	26	23	31	37	26	15	32	34		
41	54	52	37	27	31	60	53	21	28		
Mean: 32	42	36	40	31	37	36	35	31	44		

Table 17. Effect of methionine, phenylalanine, and tryptophan on roots grown in culture media containing 0.06 M sucrose. Experiment No. 29.

0.06 M sucrose + 0.00 NaCl	0.06 M sucrose + 0.00 NaCl + methionine(M)	0.06 M sucrose + 0.00 NaCl + phenylalanine (M)	0.06 M sucrose + 0.00 NaCl + tryptophan(M)								
0.00 NaCl	2×10^{-7}	10^{-6}	10^{-5}	2×10^{-7}	10^{-6}	10^{-5}	2×10^{-7}	10^{-6}	10^{-5}		
<u>Increase in length, mm.</u>											
229	169	283	459	332	317	215	468	312	219		
264	419	129	242	105	282	175	-	-	182		
234	332	342	739	257	348	267	296	360	372		
113	301	365	-	330	-	147	268	176	-		
332	198	294	326	144	327	255	326	136	221		
298	169	171	-	206	256	121	262	373	231		
268	140	248	377	142	225	292	211	148	122		
172	230	378	483	309	125	274	318	278	152		
Mean:	239	245	276	438	228	269	218	307	256	214	
$\pm s$	± 70	± 97	± 90	± 172	± 92	± 76	± 64	± 82	± 109	± 80	
<u>Fresh weight, mg.</u>											
Mean:	43	44	51	51	41	44	39	48	42	40	
<u>Dry weight, mg.</u>											
Mean:	3.3	3.3	3.7	3.9	3.1	3.5	3.4	3.8	3.3	2.5	
<u>Number of side roots</u>											
22	28	36	24	34	39	20	48	29	15		
31	47	11	20	12	22	20	-	-	11		
24	45	40	72	25	25	36	30	36	44		
11	35	35	-	32	-	15	40	24	-		
59	27	22	48	15	38	32	41	16	13		
50	24	18	-	15	31	15	34	53	13		
36	21	33	41	12	18	36	40	12	6		
32	39	40	41	24	22	36	54	27	8		
Mean:	33	33	29	41	21	28	26	41	28	16	

concentrations on the growth of tomato roots cultured in media containing 0.06 M sucrose without added NaCl.

The roots exhibited no change in length, fresh weight, dry weight, and number of side roots when cultured in media containing 2×10^{-7} M threonine as compared with the control. The roots showed no change in the dry weight, and the fresh weight was slightly decreased when cultured in media containing 10^{-6} threonine.

The roots showed no change in length, number of side roots, fresh weight and dry weight when treated with 10^{-6} M aspartic acid than with 2×10^{-7} M as compared with the control.

There was no change in the increase in length and in the number of side roots when the roots were cultured in media treated with 10^{-6} M lysine-HCl than those in the control. When treated with 10^{-5} M lysine-HCl, however, the roots exhibited some increase in length as compared with the control.

In Table 17, methionine, phenylalanine, and tryptophan were used. The roots exhibited more increase in length and formed more side branches when cultured in media containing 10^{-5} M methionine. Upon treatment with 10^{-6} M methionine, the roots showed no change in length than those in the control, while the number of side roots slightly decreased. On the other hand, 2×10^{-7} M methionine did not affect root growth.

There was a decrease in length and in the number of side roots when the roots were cultured in media containing 10^{-5} M phenylalanine as compared with those in the control. However, 10^{-6} M phenylalanine brought about a slight increase in length, and a slight decrease in the number of side roots. When the concentration of phenylalanine in the culture media was decreased to 2×10^{-7} M, a decline was noticed in root growth as expressed in the four measurements.

Tryptophan at 2×10^{-7} M brought about better root growth than the other two concentrations as compared with the control. However, 10^{-6} M tryptophan manifested similar growth values as those of the control, while 10^{-5} M tryptophan inhibited growth, especially the dry weight, as well as the number of side roots.

It appears reasonable that any one of the three concentrations of each amino acid tested, except that of 10^{-5} M tryptophan, could be used in subsequent studies with media containing inhibitory concentrations of NaCl or sucrose without exerting any additional growth inhibition. Since each of the amino acids tested represented a specific group of amino acids, it may also be sound to use any member of that group at a similar concentration.

2. Application of individual amino acids to culture media containing 0.12 M NaCl.

In Table 18, threonine, aspartic acid, phenylalanine, and tryptophan were added at 10^{-6} M, while lysine and methionine were 10^{-5} M to culture media containing 0.12 M NaCl. The roots showed greater increase in length, fresh weight, and dry weight and formed more side roots in phenylalanine and tryptophan treatments as compared with the control. Statistical analysis indicated that the difference in the increased length in both phenylalanine and tryptophan treatments were significant (0.05 level) as compared with the NaCl control. Threonine, aspartic acid, lysine, and methionine did not cause any significant increase in root growth.

When media containing 0.20 M sucrose was treated with the same amino acids as in the previous experiment, the phenylalanine and tryptophan treatments still showed greater increase in length, fresh weight, and dry weight and the roots formed more side branches than the sugar control (Table 19).

In the following four experiments (Nos. 34-37), as represented in Tables 20 to 23, detailed study was centered on a number of amino acids (eighteen essential ones), and each was 10^{-6} M in the media containing 0.12 M NaCl. Those treatments which counteracted the inhibitory effects of NaCl were repeated for confirmation.

Table 18. Effect of threonine, aspartic acid, lysine, methionine, phenylalanine, and tryptophan on roots grown in culture media containing 0.12 M NaCl. Experiment No. 32.

0.06 M sucrose + 0.12 M NaCl + AA							
0.00 ⁺	0.06 M sucrose ⁺	0.06 M sucrose treatment.					
	0.12 M	10 ⁻⁶	10 ⁻⁶	10 ⁻⁵	10 ⁻⁵	10 ⁻⁶	10 ⁻⁶
		thre- onine	aspar- tic acid	ly- sine HCl	meth- io- nine	phen- yla- la- nine	tryp- to- phan nine
<u>Increase in length, mm.</u>							
617	14	15	7	19	20	36	21
410	19	13	-	21	24	25	23
435	16	32	10	24	15	39	22
353	12	23	14	20	19	22	20
530	10	21	28	9	12	50	26
425	-	-	12	14	14	26	24
469	24	43	27	19	14	29	23
493	13	29	21	-	7	29	23
755	11	11	7	20	23	25	37
297	-	22	6	21	16	25	28
Mean: 278	15	23	15	19	16	31*	26*
± s ± 132	± 5	± 11	± 9	± 4	± 5	± 9	± 5
<u>Fresh weight, mg.</u>							
Mean: 56.0	2	3	2	3	2	4	3
<u>Dry weight, mg.</u>							
Mean: 3.5	0.2	0.3	0.2	0.2	0.2	0.4	0.3
<u>Number of side roots</u>							
55	3	0	0	0	0	1	0
52	0	0	-	0	0	0	1
28	0	1	0	1	0	2	1
35	0	0	0	0	0	0	0
61	0	0	0	0	0	10	2
49	-	-	0	0	0	3	2
51	1	5	0	0	0	2	0
60	0	0	1	-	0	1	0
82	0	0	0	0	0	2	7
38	-	0	0	0	0	0	3
Mean: 51	0.5	0.9	0.1	0.1	0	2	2

* Significant at 0.05 level with respect to the salt control (Dunnett's two-sided test)

Table 19. Effect of threonine, aspartic acid, lysine, methionine, phenylalanine, and tryptophan on roots grown in culture media containing 0.20 M sucrose. Experiment No. 33.

0.06 M + NaCl		0.20 M + NaCl		0.20 M sucrose + 0.00 NaCl + AA treatment.					
				10 ⁻⁶ thre- onine	10 ⁻⁶ as- partic acid	10 ⁻⁵ ly- sine HCl	10 ⁻⁵ meth- io- nine	10 ⁻⁶ phen- yla- nine	10 ⁻⁶ tryp- to- phan
<u>Increase in length, mm.</u>									
	199	25	35	39	39	27	74	46	
	357	29	43	17	51	26	42	91	
	422	31	28	20	28	26	47	59	
	293	32	25	19	41	31	-	-	
	361	42	24	23	31	32	54	26	
	288	34	-	22	35	31	61	34	
	477	35	54	30	52	32	34	57	
	366	36	41	33	37	28	32	53	
	222	56	41	48	40	50	45	26	
	254	29	33	33	46	28	56	24	
Mean:	324	35	36	28	40	31	49	46	
± s	± 88	± 9	± 10	± 10	± 8	± 7	± 13	± 27	
<u>Fresh weight, mg.</u>									
Mean:	39	7	6	5	7	5	10	7	
<u>Dry weight, mg.</u>									
Mean:	2.6	0.7	0.7	0.6	0.9	0.7	1.0	0.9	
<u>Number of side roots</u>									
	27	0	0	1	0	0	2	1	
	40	1	1	0	1	1	0	3	
	31	0	0	0	0	1	0	3	
	30	0	0	0	1	0	-	-	
	35	1	0	0	0	0	2	0	
	35	0	-	0	0	1	2	0	
	37	1	1	0	1	0	0	1	
	42	1	0	0	0	0	1	1	
	17	2	0	2	0	2	1	0	
	23	0	0	0	0	1	1	0	
Mean:	32	0.6	0.2	0.3	0.3	0.6	1	1	

Table 20. Effect of valine, tyrosine, cystine, proline, glutamic acid, and arginine on roots grown in culture media containing 0.12 M NaCl. Experiment No. 34.

0.06 M sucrose +	0.06 M sucrose +	0.06 M sucrose + 0.12 M NaCl + AA treatment						
0.00 NaCl	0.12 M NaCl	10 ⁻⁶ vali- ne	10 ⁻⁶ tyro- sine	10 ⁻⁶ cys- tine	10 ⁻⁶ pro- line	10 ⁻⁶ glu- tam- ic acid	10 ⁻⁶ argi- nine	
<u>Increase in length, mm.</u>								
235	62	174	132	188	123	18	76	
227	26	106	132	118	92	-	131	
202	92	64	59	46	107	40	173	
260	-	73	93	-	-	31	93	
192	38	34	139	132	41	74	122	
170	32	33	135	-	34	30	109	
243	43	26	71	26	60	123	86	
296	31	29	60	30	-	66	139	
283	56	32	-	35	32	-	115	
212	83	28	-	26	40	-	39	
Mean:	232	51	60	103*	75	66	55	108*
± s	± 40	± 24	± 48	± 36	± 62	± 38	± 36	± 37
<u>Fresh weight, mg.</u>								
Mean:	41	8	10	17	12	10	9	15
<u>Dry weight, mg.</u>								
Mean:	2.6	0.7	0.9	1.6	1.2	1.0	0.7	1.3
<u>Number of side roots</u>								
30	5	8	10	17	13	0	5	
31	1	7	8	13	12	-	13	
22	12	8	2	3	10	0	16	
27	-	9	3	-	-	0	7	
25	0	0	9	17	2	5	11	
18	1	0	9	-	1	0	11	
32	2	0	5	0	2	7	7	
36	0	0	2	0	-	6	14	
30	5	2	-	1	0	-	13	
25	7	0	-	0	1	-	3	
Mean:	28	4	3	6	6	5	3	10

* Significant at 0.05 level with respect to the salt control (Dunnett's two-sided test).

Table 21. Effect of alanine, serine, leucine, isoleucine, histidine, and dihydroxyphenylalanine (Dopa) on roots grown in culture media containing 0.12 M NaCl. Experiment No. 35.

0.06 M sucrose + 0.12 M NaCl		0.06 M sucrose + 0.12 M NaCl + AA		0.06 M sucrose + 0.12 M NaCl + AA				
+ 0.00 NaCl		+ 0.12 M NaCl		treatment.				
				M				
				10 ⁻⁶	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶
				ala-	ser-	leu-	iso-	his-
				nine	ine	cine	leu-	ti-
							cine	dine
								Dopa
								dine
<u>Increase in length, mm.</u>								
	166	65	132	118	66	74	27	61
	294	36	96	93	-	41	24	32
	285	42	45	32	62	79	13	24
	269	46	30	-	46	-	26	-
	415	85	21	33	29	34	32	24
	-	70	-	20	27	39	-	-
	254	33	33	51	46	86	38	21
	-	-	-	39	28	-	25	19
	295	64	28	-	61	-	25	36
	228	-	25	-	94	-	-	31
Mean ¹ :	276	55	51	55	51	59	26	31
± s	± 71	± 19	± 41	± 37	± 22	± 23	± 7	± 13
<u>Fresh weight, mg.</u>								
Mean:	40	8	8	10	9	10	6	5
<u>Dry weight, mg.</u>								
Mean:	3.0	0.7	0.8	1.0	0.8	1.0	0.6	0.6
<u>Number of side roots</u>								
	20	4	8	15	3	5	0	5
	35	0	9	10	-	4	0	0
	30	2	4	0	6	5	0	0
	26	4	1	-	4	-	0	-
	33	8	0	0	1	4	1	0
	-	5	-	0	0	1	-	-
	26	0	0	3	1	11	0	0
	-	-	-	0	0	-	0	0
	31	8	0	-	5	-	0	0
	20	-	0	-	11	-	-	0
Mean:	28	4	3	4	4	5	0.1	0.6

¹None of the treatment means was statistically significant.

Table 22. Effect of phenylalanine, tyrosine, and arginine on roots grown in culture media containing 0.12 M NaCl. Experiment No. 36.

0.06 M + 0.00 NaCl		0.06 M + 0.12 M NaCl		0.06 M sucrose + 0.12 M NaCl + AA sucrose sucrose treatment.	
				M	
				10 ⁻⁶	10 ⁻⁶
				phenyl- alanine	tyro- sine
				10 ⁻⁶	argin- ine
<u>Increase in length, mm.</u>					
	130	66	184	200	144
	177	49	183	178	-
	224	102	134	254	158
	170	79	161	150	61
	454	135	220	111	176
	189	102	148	81	83
	320	58	172	83	172
	215	87	129	90	-
	-	91	109	-	-
	-	98	-	-	-
Mean:	235	87	160*	143*	132
± s	± 104	± 25	± 34	± 65	± 49
<u>Fresh weight, mg.</u>					
Mean:	39	11	27	19	17
<u>Dry weight, mg.</u>					
Mean:	3.0	0.8	1.6	1.4	1.2
<u>Number of side roots</u>					
	11	7	18	13	13
	23	4	17	17	-
	18	13	17	21	10
	19	8	18	12	4
	45	14	16	11	14
	27	12	16	4	9
	27	8	16	3	11
	25	12	13	5	-
	-	10	9	-	-
	-	9	-	-	-
Mean:	24	10	16	11	10

* Significant at 0.05 level with respect to the salt control (Dunnett's two-sided test).

Table 23. Effect of threonine, lysine, tryptophan, valine, cystine, and proline on roots grown in culture media containing 0.12 M NaCl. Experiment No. 37.

0.06 M sucrose + NaCl		0.06 M sucrose + NaCl		0.06 M sucrose + 0.12 M NaCl + AA treatment.				
+	+	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶	
0.00 NaCl	0.12 M NaCl	thre- onine	ly- sine	tryp- to- phan	val- ine	cys- tine	pro- line	
<u>Increase in length, mm.</u>								
412	14	103	79	64	64	55	41	
242	16	176	117	55	88	86	82	
265	14	18	28	37	69	99	117	
264	10	80	43	35	95	66	34	
257	90	162	37	35	148	69	54	
204	64	83	33	22	93	-	70	
458	35	189	55	51	39	219	53	
381	37	97	67	42	143	67	43	
168	85	44	104	51	31	48	18	
132	27	61	65	37	49	104	27	
Mean:	278	39	101*	63	43	82	90*	54
± s	± 107	± 30	± 57	± 30	± 12	± 40	± 60	± 30
<u>Fresh weight, mg.</u>								
Mean:	56.0	9	18	12	8	14	19	13
<u>Dry weight, mg.</u>								
Mean:	3.4	1.0	1.6	1.3	0.9	1.3	2.3	1.1
<u>Number of side roots</u>								
39	0	10	5	2	4	2	0	
31	0	16	10	5	8	6	4	
36	0	0	0	1	4	6	7	
35	0	4	1	0	8	4	0	
25	6	10	0	0	11	3	1	
27	4	5	0	0	8	-	4	
42	0	13	2	2	0	17	3	
35	0	8	3	1	10	4	3	
17	6	2	9	2	0	1	0	
17	0	3	5	2	3	5	0	
Mean:	30	2	7	4	2	6	5	2

* Significant at 0.05 level with respect to the salt control (Dunnett's two-sided test).

Table 20 shows the effects of valine, tyrosine, cystine, proline, glutamic acid, and arginine. Tyrosine and arginine stimulated root growth. Roots cultured in media containing either of these amino acids showed greater increase in length, fresh weight, and dry weight and formed more side branches than those cultured in media deprived of any amino acid. Statistical analysis indicated significant differences in the increase in length in both of these treatments as compared with the NaCl control. The effect of cystine, proline, valine and glutamic acid on root growth was not significant.

In experiment No. 35 (Table 21), alanine, serine, leucine, isoleucine, histidine, and dihydroxyphenylalanine (Dopa) were tested. The effects of these amino acids on root length were not significantly different from the salt control. Roots grown in media containing alanine, serine, leucine, or isoleucine showed greater fresh weight and dry weight than the NaCl control. However, root growth was inhibited by histidine and dihydroxyphenylalanine.

In experiment No. 36 (Table 22), phenylalanine, tyrosine, and arginine were applied again in media containing 0.12 M NaCl to confirm their stimulatory effects on the growth of roots. In this experiment, the roots again showed greater increase in length, fresh weight, and dry weight when treated with any one of these three amino

acids. Statistical analysis indicated that the difference in the increase in length between either phenylalanine or tyrosine treatments and the control was significant, and that the effect of arginine was not significant. The roots, however, formed more side branches in the phenylalanine treatment than in either the tyrosine or arginine treatments as compared with the control.

As shown in Table 23, threonine, lysine, tryptophan, valine, cystine, and proline were tested again in media containing 0.12 M NaCl. As it was pointed out earlier, lysine, valine, cystine, and proline showed some promise in stimulating root growth in saline media, especially when growth was expressed on fresh- and dry-weight basis. However, even the increase in length did not show any significant difference in experiments 32 and 34; accordingly, these amino acids were tested again. Threonine and tryptophan, which had previously shown significant effects, were included in this test. In this experiment, however, all the amino acid treatments, except that of tryptophan, showed greater increase in length, fresh weight, dry weight, and the roots formed more side roots than the control. Statistical analysis of these changes indicated that the difference in the increase in length between threonine, and cystine treatments and the control was significant; lysine, valine,

tryptophan, and proline treatments did not show any significant difference.

From the above results, it appears that certain amino acids from a number of available ones tested can counteract, by one way or another, the inhibitory effects of 0.12 M NaCl on the growth of tomato roots grown in vitro.

3. Using a mixture of amino acids

Results obtained from the preceding series of experiments suggested treatment of roots with a mixture of those amino acids which counteracted the inhibitory effects of 0.12 M NaCl on root growth.

In experiment No. 38 (Table 24), the six amino acids that showed significant effects were used in a mixture. Each was 10^{-6} M. The results indicated that the increase in length, fresh weight, dry weight, and number of side roots was greater in the amino acid medium than in the medium containing only 0.12 M NaCl, but statistical analysis indicated that the increase in root length was not significantly different.

In order to determine if the amino acid mixture could overcome growth inhibition by NaCl at other concentrations of NaCl, roots were cultured in media contained increasing amounts of NaCl with or without a mixture of eight amino acids (threonine, phenylalanine, tyrosine,

Table 24. Effect of an amino acid mixture (AA) on roots grown in culture media containing 0.12 M NaCl. The mixture contained threonine, phenylalanine, tyrosine, tryptophan, arginine-HCl, and cystine, each at 10^{-6} M. Experiment No. 38.

0.06 M sucrose - <th>0.06 M sucrose - <th>0.06 M sucrose -0.12 M NaCl- AA <th>0.06 M sucrose - <th>0.06 M sucrose - <th>0.06 M sucrose -0.12 M NaCl- AA </th></th></th></th></th>	0.06 M sucrose - <th>0.06 M sucrose -0.12 M NaCl- AA <th>0.06 M sucrose - <th>0.06 M sucrose - <th>0.06 M sucrose -0.12 M NaCl- AA </th></th></th></th>	0.06 M sucrose -0.12 M NaCl- AA <th>0.06 M sucrose - <th>0.06 M sucrose - <th>0.06 M sucrose -0.12 M NaCl- AA </th></th></th>	0.06 M sucrose - <th>0.06 M sucrose - <th>0.06 M sucrose -0.12 M NaCl- AA </th></th>	0.06 M sucrose - <th>0.06 M sucrose -0.12 M NaCl- AA </th>	0.06 M sucrose -0.12 M NaCl- AA
0.00 NaCl	0.12 M NaCl		0.00 NaCl	0.12 M NaCl	
<u>Increase in length, mm.</u>			<u>Number of side roots</u>		
265	53	138	36	3	12
180	78	147	26	4	13
322	45	76	41	2	8
99	81	191	17	2	19
237	144	128	40	13	14
344	107	142	45	7	13
154	72	68	16	5	5
-	19	122	-	1	10
196	117	118	26	8	9
191	61	190	27	5	15
295	77	44	52	8	3
224	81	26	37	7	1
360	22	67	60	1	4
117	-	40	28	-	1
202	73	27	31	4	0
323	-	27	41	-	0
174	52	28	25	3	0
267	16	34	43	1	1
182	99	-	34	4	-
202	-	32	20	-	0
Mean: 228	70	87 ¹	Mean:		
± s ± 75	± 35	± 57	34	5	7
<u>Fresh weight, mg.</u>					
Mean: 46	13	19			
<u>Dry weight, mg.</u>					
Mean: 2.7	1.0	1.5			

¹The treatment mean was not statistically significant with respect to the NaCl control (two-sided "t" test).

tryptophan, arginine, lysine, cystine, and valine). Each amino acid was 10^{-7} M (Table 25). Statistical analysis indicated no significant difference in the increase in length between the amino acid-treated and the non-treated media when NaCl was not present. Again, when NaCl was in the concentration of 0.04 M, root growth was generally greater in the amino acid-treated media than in the non-treated media, particularly as far as the number of side roots is concerned, but statistical analysis of the increase in length between the two treatments was not significant. Further increase in NaCl concentration in the culture media to 0.08 M brought about less root growth than those containing either 0.04 M or no NaCl. Again, when the amino acid mixture was added to media containing 0.08 M NaCl, statistical analysis indicated no significant difference between the increase in root length in the amino acid-treated and the non-treated media. On the other hand, when NaCl was 0.12 M, the amino acid mixture restored a large percentage of root growth. The increase in length, fresh weight, and number of side roots was about three times greater in media treated with the amino acid mixture and the dry weight was about two and a half times greater. Statistical analysis for 0.12 M NaCl treatments indicated that the difference between the amino acid-treated and the non-treated media was highly

Table 25. Effect of an amino acid mixture (AA) on roots grown in culture media containing different concentrations of NaCl. The AA-mixture contained threonine, phenylalanine, tyrosine, tryptophan, arginine-HCl, cystine, valine, and lysine-HCl; each at 10^{-7} M. All cultures contained 0.06 M sucrose. Experiment No. 42.

0.00 NaCl		0.04 M NaCl		0.08 M NaCl		0.12 M NaCl		0.16 M NaCl		
-AA	+AA	-AA	+AA	-AA	+AA	-AA	+AA	-AA	+AA	
<u>Increase in length, mm.</u>										
420	373	251	483	455	335	42	240	52	22	
296	112	314	236	494	199	81	244	24	29	
302	194	173	161	234	204	49	177	17	17	
-	239	311	269	-	224	80	176	32	10	
298	293	175	757	373	313	68	83	15	19	
324	183	366	248	134	254	43	113	-	-	
293	311	479	328	91	494	22	207	11	53	
395	294	597	-	227	362	-	75	-	44	
247	312	316	507	-	337	79	167	34	26	
214	367	238	591	-	323	52	71	13	35	
Mean:	310	268	322	398	287	305	57	149**	25	28
± s	± 65	± 84	± 133	± 197	± 156	± 89	± 21	± 67	± 14	± 14
<u>Fresh weight, mg.</u>										
Mean:	41	47	54	56	34	47	9	23	5	5
<u>Dry weight, mg.</u>										
Mean:	2.4	3.0	3.1	3.4	2.0	3.0	0.7	1.7	0.4	0.4
<u>Number of side roots</u>										
48	62	18	32	35	25	3	23	4	0	
32	25	24	15	32	24	8	21	3	0	
45	32	17	13	14	11	2	6	2	1	
-	35	25	20	-	17	7	14	3	1	
27	36	16	82	30	22	7	8	1	3	
36	25	22	22	5	23	2	10	-	-	
55	44	47	33	6	30	1	19	3	5	
58	52	46	-	19	33	-	8	-	4	
26	30	35	50	-	20	7	12	3	1	
30	53	22	68	-	26	4	5	1	1	
Mean:	40	39	27	37	18	23	5	13	3	2

** Significant at 1% level (two-sided "t" test).

significant for the increase in root length. Further increase in the NaCl concentration in the culture media to 0.16 M brought about considerable inhibition and the application of amino acids ceased to show any anti-inhibition and no significant difference was indicated.

The effect of glycine on root growth in a medium containing 0.12 M NaCl was also studied (Table 26). Results obtained from this experiment indicated that glycine, one of the nutrients in every culture medium, should be added to the group of amino acids which counteracts the effects of NaCl on tomato roots.

4. Effect of an amino acid mixture on roots subcultured twice in media containing 0.12 M NaCl.

Tables 27 and 28 show the effect of amino acids in two concentrations (10^{-6} and 10^{-7} M) on the growth of tomato roots subcultured twice in media containing 0.12 M NaCl. Most of the roots were measured and then discarded at the end of the first subculture; a few roots were not measured and their tips were transferred to the second subculture (Table 28). In Table 27, which presents results from the first subculture, root growth was better in both amino acid mixtures as compared with the control. In the second subculture (Table 28) it is evident that the amino acids in these two concentrations still showed better

Table 26. Effect of glycine (4×10^{-4} M) on roots grown in two culture media, one without NaCl and the other with 0.12 M NaCl. Experiment No. 39.

	0.06 M sucrose + 0.00 NaCl+ glycine	0.06 M sucrose + 0.00 NaCl- glycine	0.06 M sucrose + 0.12 M NaCl+ glycine	0.06 M sucrose + 0.12 M NaCl- glycine
	<u>Increase in length, mm.</u>			
	330	301	105	100
	222	337	66	-
	275	337	146	32
	265	301	116	-
	344	466	178	13
	279	315	186	55
	-	271	57	83
	-	354	202	38
	-	-	113	88
	-	-	-	76
Mean:	286	335	130	61
\pm s	\pm 45	\pm 59	\pm 52	\pm 31
	<u>Fresh weight, mg.</u>			
Mean:	52	72	20	10
	<u>Dry weight, mg.</u>			
Mean:	4.0	5.0	1.4	0.9
	<u>Number of side roots</u>			
	34	30	9	10
	21	60	7	-
	40	54	13	1
	27	58	9	-
	46	55	19	1
	48	48	20	7
	-	51	5	10
	-	43	20	2
	-	-	11	7
	-	-	-	5
Mean:	36	50	13	5

Table 27. Effect of an amino acid mixture (AA) on roots grown in culture media containing 0.12 M NaCl. The amino acid mixtures contained threonine, phenylalanine, tyrosine, tryptophan, arginine-HCl, cystine, valine, and lysine-HCl; each at the concentration indicated. Experiment No. 41 (First subculture).

	0.00 NaCl	0.02 M NaCl	<u>0.12 M NaCl + AA</u>	
			10 ⁻⁷ M AA	10 ⁻⁶ M AA
	<u>Increase in length, mm.</u>			
	261	86	101	125
	243	41	59	80
	127	106	125	113
	-	76	69	-
	368	50	68	93
	262	45	59	92
	290	-	104	85
	249	-	57	66
Mean:	257	67	80	93
± s	± 71	± 26	± 26	± 20
	<u>Fresh weight, mg.</u>			
Mean:	34	10	12	14
	<u>Dry weight, mg.</u>			
Mean:	2.7	0.9	1.2	1.5
	<u>Number of side roots</u>			
	31	4	8	9
	28	1	4	5
	10	8	8	9
	-	6	6	-
	32	3	4	8
	30	3	3	6
	29	-	8	7
	26	-	3	6
Mean:	27	4	6	7.0

Table 28. Effect of an amino acid mixture (AA) on roots grown in culture media containing 0.12 M NaCl. The amino acid mixtures contained threonine, phenylalanine, tyrosine, tryptophan, arginine-HCl, cystine, valine, and lysine-HCl; each at the concentration indicated. Experiment No. 43 (Second subculture).

	0.00 NaCl	0.12 M NaCl	0.12 M NaCl + AA	
			10 ⁻⁷ M AA	10 ⁻⁶ M AA
			<u>Increase in length, mm.</u>	
	182	4	11	61
	321	5	10	22
	257	7	16	53
	-	-	14	55
	125	10	23	108
	-	-	-	-
	-	12	15	72
Mean:	221	8	15	62
± s	± 86	± 3	± 5	± 28
			<u>Fresh weight, mg.</u>	
Mean:	22	2	3	11
			<u>Dry weight, mg.</u>	
Mean:	1.6	0.2	0.3	0.8
			<u>Number of side roots</u>	
	22	0	0	6
	41	0	0	0
	28	0	0	3
	-	-	0	4
	12	0	0	10
	-	-	-	-
	-	0	0	9
Mean:	26	0	0	5

growth than the control, except no side roots were formed in either the control or in media containing 10^{-7} M amino acids. However, when amino acids were at a concentration of 10^{-6} M, roots showed an eight-fold increase in length, five-fold increase in fresh weight, and four-fold increase in dry weight, as compared with the control (Fig. 1).

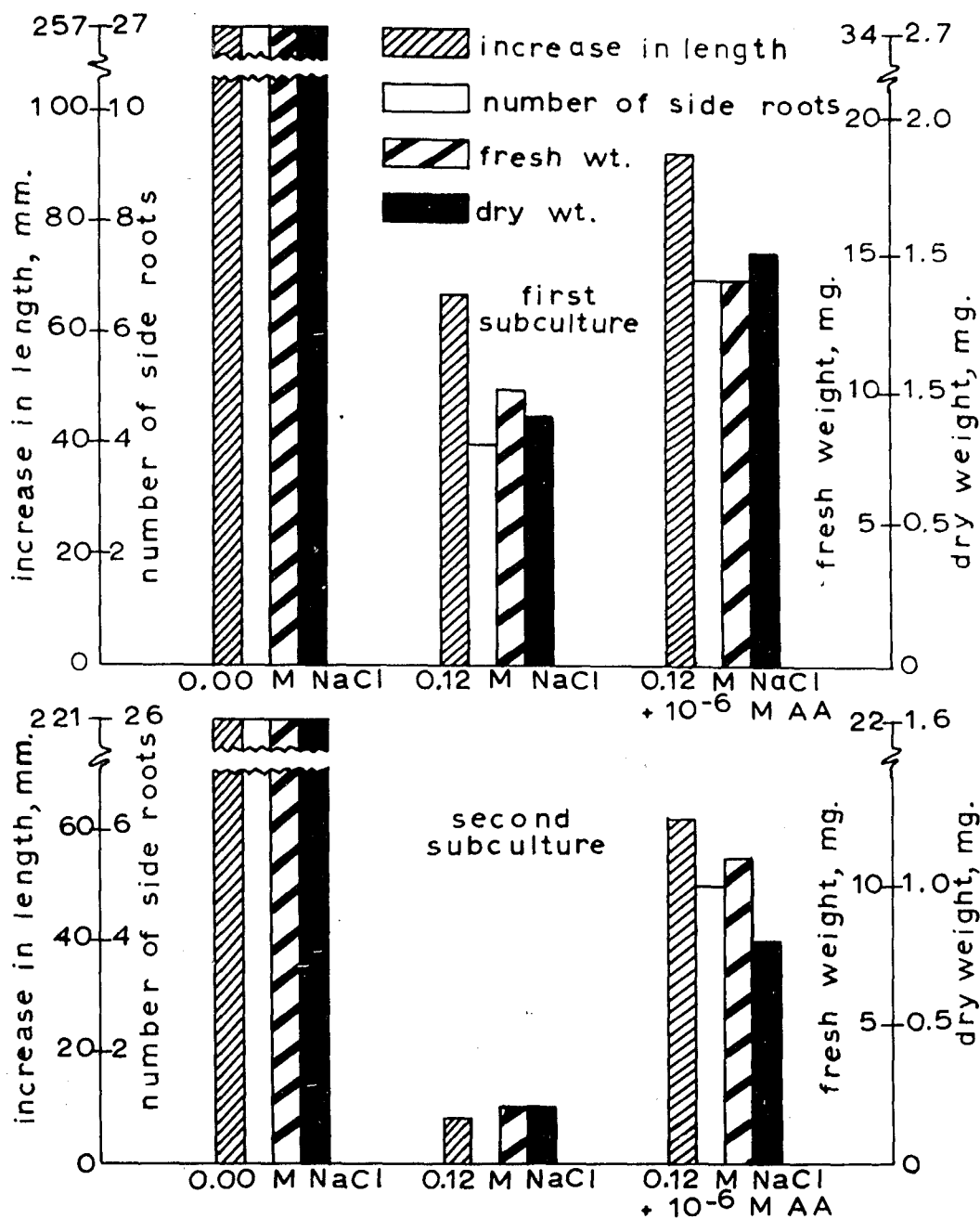


Fig. 1. Effect of an amino acid mixture (AA) on roots subcultured twice in media containing 0.12 M NaCl. The amino acid mixture contained threonine, phenylalanine, tyrosine, tryptophan, arginine, cystine, valine, and lysine, each at 10^{-6} M. Experiment No. 41 (first subculture) and No. 43 (second subculture).

DISCUSSION

Several workers have studied the relationship between drought and plant metabolism. Ter-Karapetyan (1957) noticed that lysine, threonine and glycine were missing in the 80% alcohol extract of leaves from Goebelia alopecuroides grown under saline conditions, and Prusakova (1960) found more tryptophan, alanine, and tyrosine and less proline in the leaves of wheat grown under sufficient water supply than if the water supply were insufficient.

In our work with tomato roots, it is evident that a number of amino acids are required for these roots to show better growth in media containing inhibitory concentrations of NaCl. It is interesting to note that the amino acids found most effective, threonine, phenylalanine, tyrosine, tryptophan, arginine-HCl, and cystine are in part those found by Ter-Karapetyan (1957) and Prusakova (1960) to be depleted in plants growing under saline and drought conditions.

In higher plants there is considerable information indicating effects of the ionic environment on the activity of both particulate and soluble enzyme preparations. Massey (1953) reported that chloride, bromide,

thiocyanate, and iodide, in increasing order of effectiveness, inhibit the activity of fumarase either in the presence or absence of activating anions and that the extent of inhibition is markedly dependent on the pH. Hiatt and Evans (1960) studied the influence of a variety of salts on the activity of malic dehydrogenase from spinach leaves. The effect of chloride salt of sodium on the enzyme activity is different from that of other anion salts of sodium. In their work, as the NaCl concentration was increased the enzyme activity increased until a maximum was reached at a concentration of 0.04 M. Above this concentration enzyme activity decreased with increasing increments of NaCl until a concentration near 0.05 M was reached. Further increases in NaCl concentration produced another increase in enzyme activity with a second peak in the curve near 0.07 M NaCl. At concentrations greater than this the effect of salt was to inhibit enzyme activity.

If we compare this information with results we obtained on tomato roots grown in vitro, it seems evident that when NaCl was in concentrations ranging from 0.0 to 0.08 M NaCl the increase in root length did not show any significant difference between those cultured in media containing amino acids and those cultured without amino acids. On the other hand, when the NaCl concentration was

increased to 0.12 M, certain amino acids were required in the medium in order to restore an appreciable amount of growth. As the concentration of NaCl was increased to 0.16 M, the addition of amino acids did not restore any significant amount of growth.

In elucidating the mechanism of salt inhibition of enzyme activity, Hiatt and Evans suggested that the action of NaCl is upon some point other than the active site of the enzyme. However, Walaas and Walaas (1956) studied the effect of anions on the activity of D-amino acid oxidase and concluded that negatively charged anions are bound to positively charged groups in the apoenzyme. They found almost complete inhibition by chloride ion at pH 9.2 and suggested it is competitive with flavine-adenine-dinucleotide.

It seems reasonable to suggest that either the enzymes required for the synthesis of the effective amino acids in the cells of tomato roots were inactivated under saline conditions, or it might be possible that another enzyme system in the cell was inactivated, or both may have occurred at the same time. Since certain amino acids restored only part of the normal root growth, it may be that another substance or substances became necessary, and these requirements became progressively more numerous when the NaCl concentration was increased. These

suggestions are in agreement with the proposed concept outlined by Kurtz (1960) who suggested that the ability of a plant to adjust or adapt to an environment depends upon the organism not having a deficiency of some essential enzyme or metabolite. These suggestions may be compared also with proposed mechanisms of temperature effects reported by Koffler (1957) and Koffler et al. (1957), Kurtz (1958) and Langridge (1963). It is evident from this information that the ability of the organism to grow at high temperatures depends to a large extent on the formation of enzymes with increased thermal stability. Langridge and Griffing (1959) detected high temperature lesions in Arabidopsis thaliana by growing 43 races at temperatures of 25, 30, 31.5°C. Five of these races were depressed in growth and morphologically abnormal when cultured at 31.5°C. They found that in two races the temperature lesion was completely prevented if biotin was supplied in the medium, while in a third race the temperature lesion was alleviated in the presence of cytidine; the other two races did not respond to supplements.

These results may be compared with the depression of the growth of tomato roots under saline conditions. When these roots were supplied with certain amino acids, a considerable amount of growth was restored. It may be

assumed that plants growing in saline habitats (halophytes), or those growing under water stress (xerophytes), may possess proteins that are more adapted to the environment than are comparable proteins from mesophytes.

Another possible interpretation of our data is based on an idea postulated by West (1962) and Kessler and Frank-Tishel (1962). West found a significantly higher ratio of guanosine monophosphate and uridine monophosphate to cytosine monophosphate and adenosine monophosphate, as well as a quantitative reduction of proteins and nucleotides in corn during germination under water stress. Kessler and Frank-Tishel found not only an accumulation of ribonucleic acid but also an increase in the guanine and cytosine/ adenine and uracil ratio in the leaves of drought resistant olive as compared with leaves from the non-drought resistant ligustrum when both were subjected to dehydration.

It seems likely that since the determination of sequence, cross-linking, and folding of peptide chains in proteins must involve ribonucleic acid, any alteration in the composition of ribonucleic acid will alter the structure of the enzyme protein. The base ratios in ribonucleic acid so far reported indicate the same kind of complementarity as in deoxyribonucleic acid. If the sequence of the code in the protein molecule is

determined by the sequence of purines and pyrimidines of the deoxyribonucleic and ribonucleic acids, any shift in the ratio of these bases will change the sequence of amino acids in the enzyme protein. Accumulation of ribonucleic acid molecules with higher ratios of guanine and cytosine/ adenine and uracil, as found by the above authors in the dehydrated leaves of the drought resistant olive, could be an adaptation of drought resistant plants to such conditions.

From the above discussion it can be suggested that whether certain sites of the enzyme proteins involved in the synthesis of certain amino acids were inactivated by salt or whether the salt ions affect enzymes indirectly during their synthesis, the application of specific amino acids was required to counteract partly the inhibitory effect of NaCl on growth. Because root growth was only partly restored by certain amino acids, there must be other sites of salt inhibition. Based upon these results, it might be possible to counteract further the inhibition of salt by supplying other metabolites to roots already supplied with the amino acids.

CONCLUSION

A number of experiments were made to provide some information about the mechanism of the inhibition of root growth under conditions of physiological drought. The methods involved in vitro culture of excised tomato roots obtained originally from a single root tip.

Root growth was inhibited by the addition of increased amounts of either sugar (sucrose) or salt (NaCl) to the culture media. The degree of inhibition was increased as the amount of either sucrose or NaCl was increased.

A number of compounds was added to the media in which roots were inhibited either by elevated concentration of sucrose (0.20 M) or NaCl (0.12 M), in chemically similar groups, one at a time. These groups included amino acids, vitamins, purines, pyrimidines, organic acids, and plant alkaloids. It was shown that there was some stimulation with amino acids when roots were inhibited by either sucrose or NaCl, and some stimulation with pyrimidines and plant alkaloids when roots were inhibited by sucrose. Vitamins, purines, and organic acids did not lessen inhibitory effects; on the contrary, they increased the extent of the inhibition. Because of the

variability of the results obtained with amino acids, a detailed study was centered on those roots inhibited by only NaCl. Certain amino acids supplied individually to roots (threonine, phenylalanine, tyrosine, tryptophan, arginine, and cystine) restored a significant proportion of root growth inhibited by 0.12 M NaCl. When the inhibited roots were treated with a mixture of these amino acids at different NaCl concentrations, statistical analysis indicated that the effect of this mixture on the increase in root length was highly significant only at 0.12 M NaCl concentration. Finally, the effect of this mixture on roots was most pronounced when they were subcultured twice in media containing 0.12 M NaCl. In the second subculture, when amino acids were at a concentration of 10^{-6} M, roots showed an eight-fold increase in length and fresh weight and a five-fold increase in dry weight as compared with the salt control (Figure 1).

From these results it was suggested that either the enzyme systems involved in the synthesis of these amino acids, or other enzymes containing these amino acids, or both, were inhibited under saline conditions. The mechanism of this inhibition might involve some physical change in the enzyme protein or a competitive inhibition. Amino acids were required to counteract this change and consequently to restore partly normal root growth under saline conditions.

SUMMARY

1. By using in vitro culture methods with tomato roots, a salt-intolerant plant, evidence has been obtained that growth was impaired by higher concentrations of either sugar (sucrose) or salt (NaCl).

2. When a number of metabolites at various concentrations was added either in groups, or individually to salt-inhibited roots, growth was either inhibited further or stimulated.

3. Amino acids were the most promising group of substances to restore root growth under high concentrations of either sucrose or NaCl.

4. The study was centered on roots inhibited by salt (NaCl), and it was possible to identify a number of amino acids that restored growth of roots inhibited by salt. These amino acids were threonine, phenylalanine, tyrosine, tryptophan, arginine, and cystine.

5. Various mechanisms of salt inhibition were discussed, these mechanisms were biochemical rather than the more classical physical theories of salt inhibition.

6. Because it was possible to induce partial salt tolerance of roots from the salt-intolerant tomato

plant by supplying them with certain amino acids, the mechanism of salt tolerance or intolerance seemed to be metabolic.

7. Since the growth of the inhibited roots was restored partly by amino acids and was significant only for certain NaCl concentrations, it may be that another substance or substances became necessary and these requirements became progressively more numerous when the NaCl concentration was increased to higher levels.

8. Further work is needed to test the effects of a large number of metabolites at different concentrations on roots inhibited by different NaCl concentrations, and supplied with amino acids.

9. Since the mechanism of adaptation of roots to saline conditions is determined by the extent of enzyme action and function, studies on the effects of temperature, pH, and other factors of the root environment are needed.

10. Despite the fact that in vitro root culture has several advantages over other methods, especially for investigations of this kind, the excised root is expected to differ in its physiology from the intact seedling root. Therefore, application of the metabolites should also be applied to the intact plants.

LITERATURE CITED

- Bernstein, L. and Pearson, G. A. 1954, The Influence of Integrated Moisture Stress Achieved by Varying the Osmotic Pressure of Culture Solutions on Growth of Tomato and Pepper Plants. Soil Sci., 77: 355-368.
- Boll, W. G., 1954. Inhibition of Growth of Excised Tomato Roots by Desoxypyridoxin and its Reversal by Pyridoxin. Science, 120: 991-993.
- Boszormenyi, Z. and Cseh, E., 1962. The Effect of L-amino Acids on the Uptake of Glycine-C¹⁴, and Methionine-S³⁵ by Excised Wheat Roots. Acta Botan. Acad. Sci. Hung., 8: 39-49.
- Hiatt, A. J. and Evans, H. J., 1960. Influence of Salts on Activity of Malic Dehydrogenase from Spinach Leaves. Plant Physiol. 35: 662-672.
- Kessler, B. and Frank-Tishel, J., 1962. Dehydration-Induced Synthesis of Nucleic Acids and Changing of Composition of Ribonucleic Acids, a Possible Protective Reaction in Drought Resistant Plant. Nature, 196: 542-543.
- Koffler, H., 1957, Protoplasmic Differences between Mesophiles and Thermophiles. Bact. Revs. 21: 227-240.
- Koffler, H., Mallett, G. E. and Adye, J., 1957. Molecular Basis of Biological Stability to High Temperatures. Proc. Natl. Acad. Sci., 43: 464-477.
- Kurtz, E. B., 1958. Chemical Basis for Adaptation in Plants. Science, 128: 1115-1117.
- _____, 1960. Biochemistry of adaptation in Plants to Environment. Amer. Naturalist. 94: 237-243.
- Langridge, J., 1958. An Osmotic Mutant of Arabidopsis thaliana, Australian J. Biol. Sci. 11: 456-470.

- Langridge, J., 1963. Biochemical Aspects of Temperature Response, Ann. Rev. Plant Physiol. 14: 441-462.
- _____, and Griffing, B., 1959. A Study of High Temperature Lesions in Arabidopsis thaliana. Australian J. Biol. Sci., 12: 117-135.
- Laties, G. G., 1954. The Osmotic Inactivation in situ of Plant Mitochondrial Enzymes. J. Exp. Bot., 5: 49-70.
- Massey, V., 1953. Studies on Fumarase. II. The Effects of Inorganic Anions on Fumarase Activity. Biochem. J. 53: 67-71.
- Nickell, L. G., and Burkholder, P. R., 1950. Atypical Growth of Plants. II. Growth in vitro of Various Tumors of Rumex in Relation to Temperature, pH, and Various Sources of Nitrogen, Carbon, and Sulfur. Amer. J. Bot. 37: 538-547.
- Nieman, R. H., 1962. Some Effects of NaCl on Growth, Photosynthesis, and Respiration of Twelve Crop Plants. Botan. Gaz., 123: 279-285.
- Paris, D. and Duhamet, L., 1953. Action d'un Melange d'acides Amines et de Vitamines sur la Proliferation des Cultures de Tissus de Crown-Gall de Scorsonere: Comparaison Avec l'action du Lait de Coco. C. R. Ac. Sc. 236: 1690-1692.
- Pettersson, M. L. R., and Gray, G. E. J., 1958. Further Inherent Characters of Plant Species of Wetter and Drier Habitats. Nature. 182: 450-451.
- Prusakova, L. D., 1960. Influence of Water Relations on Tryptophan Synthesis and Leaf Growth of Wheat. Fiziol. Rast. 7: 170-180.
- Riker, A. J. and Gutsche, A. E., 1948. The Growth of Sunflower Tissue in vitro on Synthetic Media with Various Organic and Inorganic Sources of Nitrogen. Amer. J. Bot. 35: 227-238.
- Sandstedt, R. M., and Skoog, F., 1960. Effect of Amino Acid Components of Yeast Extract on the Growth of Tobacco Tissue in vitro. Physiol. Plant. 13: 250-256.

- Steel, R. G. D., and Torrie, J. H., Principles and Procedures of Statistics. McGraw-Hill Book Company, 1960. 1960. pp. 73-113.
- Smirnov, A. M., Stogonov, B. P., and Shevyakova, N. I., 1960. Study of the Salt Tolerance of Plants by a Method Involving Planting Isolated Roots. Fiziol. Ustoichivosti Rast. Sb. 1960: 767-770.
- Sutton, D., Scott, E. G., and Street, H. E., 1961. Studies of the Growth in Culture of Excised Wheat Roots. II. The Growth-Promoting Activity of Amino Acids. Physiol. Plant. 14: 712-724.
- Ter-Karapetyan, M. S., 1957. Variation in Amino Acid Content of the Leaves of Goebelia Alopecuroides under Conditions of Saline Soils. Akad. Nauk. Armianskoi SSR Dok. 25, No. 3:117-120.
- Walaas, E., and Walaas, O., 1956. Kinetics and Equilibria in Flavoprotein System. V. The Effect of pH, Anions and Partial Structural Analogues of the Co-enzyme on the Activity of D-amino Acid Oxidase. Acta Chemica Scandinavica 10: 122-133.
- Wadleigh, C. H., and Ayers, A. D., 1945. Growth and Biochemical Composition of Bean Plants as Conditioned by Soil Moisture Tension and Salt Concentration. Plant Physiol. 20: 106-132.
- West, S. H., 1962. Protein, Nucleotides, and Ribonucleic Acid Metabolism in Corn during Germination under Water Stress. Plant Physiol. 37: 565-571.
- White, P. R., 1943. Nutrient Deficiency Studies and an Improved Inorganic Nutrient for Cultivation of Excised Tomato Roots. Growth, 7: 53-65.
- _____, 1954. The Cultivation of Animal and Plant Cells, New York: The Ronald Press Co.
- _____, 1963. The Cultivation of Animal and Plant Cells, New York: The Ronald Press Co., pages: 23-141.