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A Comparison of the Salt Hardiness of Barley, Petunia, and Tomato When Grown in Saline and Nonsaline Substrates

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A COMPARISON OF THE SALT HARDINESS OF BARLEY, PETUNIA, AND TOMATO WHEN GROWN IN SALINE AND NONSALINE SUBSTRATES

by

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James Louis Wright

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

INTRODUCTION

The excessive accumulation of salt in the root zone of plants is a problem which is widespread in area, increasing in magnitude, and limiting in its effect upon productivity. Accumulation of salt in the rhizosphere has an effect upon moisture availability for plant use, the nutritional balance of the essential elements, and causes a reduction in plant growth with toxicity often resulting. When plants grow on substrates high in salt, the salt content of the plant also increases. This uptake of salt often results in changes in the morphology of the plant. Accumulation of salt within the plant cells interferes with the protoplasmic activity.

Some plants survive or produce profitable yields under conditions that cause the death of other plants. The reason for their survival is the subject of much interest and research. It has been observed that this survival may be due to one or several characteristics of the plant. The uptake of salt increases the osmotic pressure within the plant and offsets the osmotic pressure of the salty substrate thus preventing physiological drought. Selective absorbtion of ions at the root surface prevents uptake of ions in excessive amounts. The plant may incorporate the absorbed salt into the nonprotoplasmic structures of the plant. Changes in morphology reduce the water requirement, or reduce the salt concentration within the cell through increased succulence, or the

salt is exuded through special anatomical structures. The plant has an inherent capacity of the protoplasm to resist the harmful effects of the accumulated ions. Salt tolerance, therefore, implies the ability of the plant to survive or produce by resisting the otherwise deleterious conditions.

Levitt (1956, p. 229) used the term "salt hardiness" but did not define it. However, in speaking of frost, heat, and drought hardiness, he used the term "hardiness" to denote the ability of the plant to survive an unfavorable internal environment. Along this line, protoplasmic salt hardiness could be defined as the ability of the protoplasm to resist the effects of salt accumulations within the cell. Salt hardening may be defined as an increase in salt hardiness under conditions of salt uptake. Salt tolerance does not specify the source of the resistance. Salt hardiness specifies that it is a protoplasmic resistance. Repp, McAllister, and Wiebe (1959) used protoplasmic salt resistance to mean nearly the same thing.

Salt tolerance ratings have been determined largely through field studies on artificially salinized plots. Such methods have the disadvantage of the time required and the facilities necessary. In some plants there is a relationship between the resistance of the protoplasm to excess salt accumulations within the cell and the salt tolerance of the plant. Two tests, one a plasmolytic test utilizing this resistance of the protoplasm and the other a staining technique using 2,3,5-triphenyl-2H-tetrazolium chloride (which shall be spoken of as TTC in this report), have been

developed to give salt tolerance ratings. However, to date it has not been determined if the protoplasm of a plant increases in resistance to salt as the salt accumulates within the cell when the plant is growing in high salt substrates. The methods have been used on only a limited number of plants.

The over-all purpose of this research was to make a comparison of the protoplasmic salt hardiness of tomato, barley, and petunia when grown in saline and nonsaline substrates and thus determine if salt hardening takes place. The research was divided into three areas of investigation: (1) comparison of the salt tolerance rating of the three plants as determined by plasmolysis studies and the TTC test with already established field tolerance ratings, (2) determination of the effect of the salt concentration of the root substrate on the protoplasmic salt resistance of the plant, and (3) examination of the effect of the salt concentration of the root substrate upon the validity of the salt tolerance rating of a plant as determined by the plasmolysis and TTC tests.

If plants do manifest salt hardening, the plasmolysis and TTC tests for rating salt tolerance must be carried out under specified conditions for valid results.

REVIEW OF LITERATURE

Salt Tolerance Ratings

The salt tolerance of a crop or plant as used by Hayward and Wadleigh (1949) and Hayward and Bernstein (1958) may be appraised according to three criteria: (1) the ability of the crop to survive on saline soils, (2) the yield of the crop on saline soils, and (3) the relative yield of the crop on a saline soil as compared with its yield on a nonsaline soil under similar growing conditions. Many of the first observations on salt tolerance were based on the criteria of the ability to survive; but this method has limited practical significance in irrigation agriculture. The third criteria was used by the Salinity Laboratory Staff (1954), pp 55-68) in investigating the salt tolerance of many species and varieties of crop plants. This criteria provides a better basis of comparison among diverse crops. In determining the salt tolerance rating, the salinity level at which a 50 percent decrease in yield was observed as compared to yields on nonsaline soil under comparable growing conditions was taken as the criteria. The level of salinity was expressed as the conductivity of the saturation extract at 25° C (EC_e).

Barley was given a relative rating of an EC_e of 16 mmhos/cm (millimhos per centimeter) by the Salinity Laboratory Staff (1954). This is the highest rating given to a field crop. Ayers, Brown

and Wadleigh (1952) found that barley was one of the most salt tolerant plants and produced grain satisfactorily at EC_e values of 12-14 mmhos/cm provided salinity during seedling development was in the range of 1-4 mmhos/cm. If the salinity level at germination was as high as 9 mmhos/cm, then salinity of 12-14 mmhos/cm in the later stages caused a serious decrease in yield of grain. Their work showed that vegetative growth and straw yields were much more sensitive to salinity than was grain production.

Ali Khan (1956) using artificially salinized solution cultures in the greenhouse found that barley survived a 9 atmosphere level of added salt. The barley did much better when the added salt was $CaCl_2$ than when it was NaCl. It yielded nearly as high when a 50:50 milliequivalent ration of NaCl and $CaCl_2$ was used to make up the 9 atmosphere level.

Tomato was given a rating of an EC_e of 10 mmhos/cm by the Salinity Laboratory Staff. This placed it at the top of the medium salt tolerant group. Hayward and Bernstein (1958) reported a considerable amount of work on the salt tolerance of tomatoes with the concensus that the vegetable is moderately tolerant. Hayward and Long (1943) working with tomatoes using solution-cultures found that the height of stems and fresh- and dry-weight of tomato vines were reduced as the concentration of the culture solution was increased. Hayward and Bernstein (1958) reported that Yankovitch determined that the lethal dose for tomatoes was 5.60 grams per killigram.

Petunia was not rated by the Salinity Laboratory.

Salinized Substrates for Salt Tolerance Studies

In studying the effect of salt upon plant growth, it is desirable to have a root substrate that can be controlled. One of the major problems with artificially salinized soil is that the distribution of the salt within the soil varies with the movements of the moisture. The salt may be accumulated on the surface of the soil by evaporation or moved to the bottom or out of the root zone by the downward movement of water.

Sachs in 1860 successfully grew plants to maturity using water as a substrate for the root growth with the added chemicals which plants were known to require at that time. Since the publication of Sachs' standard formula for growing plants in water-culture, many other formulas have been used with success by investigators in different countries. Hoagland proposed a formula in 1920. It has been widely used since that time (Hoagland and Arnon, 1950, p. 6-7). Broyer and Hoagland (1943) were some of the first to use solution-cultures in studying plant growth as affected by salt.

Ali Khan (1956) used Hoagland's No. 1 solution as the base nutrient solution for his studies on the growth and nutrition of plants as affected by different osmotic concentrations of $CaCl_2$ and NaCl. He used one solution as a control and added NaCl and $CaCl_2$ to the others in amounts sufficient to obtain the desired osmotic level in the nutrient solution. Seven-day-old barley seedlings were transferred to gallon-size pots which contained the nutrient solution. A one-week period was allowed for the

seedlings to get started in the nutrient solution after which time those which were to receive the added salts were given the initial increment of 1 atmosphere. Salt was added sufficient to raise the concentration by 1 atmosphere per day until the desired level was reached. The volume of the solution, its pH, and its concentration were periodically checked with the necessary adjustments being made.

Monk (1960) used a solution-culture method similar to that of Ali Kahn's with some exceptions. Seedlings were transferred to the solution pots at the time they were approximately three weeks of age. The base nutrient solution used was Hoagland's No. 2 at one-half strength. The pH of the solution was adjusted to 6.0 by using 0.1 N sulfuric acid. All cultures were aerated. The pH was adjusted every other day and iron chelate was added. The plants were allowed about three weeks to adjust to the new environment and then the salt treatments were begun. The time during which the salt treatments were brought to the total concentration was not reported.

Bernstein and Hayward (1958) in studying the osmotic pressure of the roots and leaves of several plants used sand cultures with graded additions of NaCl to the base nutrient solution.

Measuring Protoplasmic Salt Hardiness

Repp, McAllister, and Wiebe (1939) reported that the experimental and ecological investigations of plants of sea coasts, salt deserts, and salt stepees show that the most important physiological factor in salt tolerance is the salt resistance of the protoplasm.

It is well established that plants growing on substrates high in salt have a higher salt content than those growing under nonsaline conditions. Different species show differences in the relative amounts of different ions accumulated as shown by Collander (1941).

Plasmolysis Test

Work has recently been done on the resistance of protoplasm to salt as a test for salt tolerance. Correlation has been found to exist between the level of salt which the plant cell will tolerate and the over-all tolerance of the plant. The advantage of the physiology tests is that they require less time than the field experiments such as described by the U.S. Salinity Laboratory Staff (1954, p. 66).

Repp, McAllister, and Wiebe (1959) used a microscopic technique to detect the plasmolysis of cells by a slightly hypertonic glucose solution following an exposure to salt at various levels. They used freehand tangential sections of the epidermis and several subadjacent cell layers of the lower portions of the stem of plants growing in nonsaline substrate. The tissue sections were placed in the graded NaCl solutions for 24 hours and then transferred to slightly hypertonic sucrose for about 3 hours. The sections were then examined for plasmolysis under the microscope.

The appearance of normal plasmolysis was taken as an indication of life; whereas cells in which there were slightly visable changes in structure, mainly an increase in granularity, but in which the plasma membrane still possessed a smooth contour were considered slightly damaged. Cells which failed to plasmolyze were considered

as dead. The molal salt concentration in which 50 percent or more dead cells were observed was considered the critical concentration in relation to the salt resistance of the protoplasm.

It was pointed out that the condition of the vacuolar membrane must be investigated when studying salt resistance of the protoplasm. This is because the vacuolar membrane is more resistant to salt than the plasma membrane. In normal living cells, the relatively clear cytoplasm completely surrounds the shrunken vacuole. Death caused by salt gives the vacuole an appearance similar to that of a normally plazmolyzed cell except that the cytoplasm appears much more granular and is more or less concentrated in clumps bordering the vacuole. The vacuolar membrane may be entirely bare in places and as a result the cell may be mistaken for normal, living, plasmolyzed cell contents.

The results of the plasmolysis method agreed with field studies, and correlation was found to exist between the salt hardiness of the cells and the salt tolerance of the plants.

Monk (1960) used the technique as developed by Repp in studying the resistance of the protoplasm as related to salt tolerance of ornamentals and shrubs. He also used tissue from plants growing in nonsaline substrate for the test. Good correlation was found to exist between the plant species surviving in the artificially salanized field plots or the salinized nutrient solutions and the salt hardiness of the cell as determined by the plasmolysis test.

The plasmolysis test has the disadvantages of being tedious and adaptable to only plants or portions of plants with cells in

which plasmolysis can be detected.

TTC Test

Because of the disadvantages of the plasmolysis test, Monk (1960) developed a test for salt hardiness using the vital stain TTC (2,3,5-triphenyl-2H-tetrazolium chloride). Staining was used as a criteria of undamaged tissue rather than plasmolysis. In this method samples of the plant stem were split longitudinally and placed in a series of vials containing graded amounts of NaCl. Hoagland's solution at 0.1 strength was used as the base solution. The salt treatments covered the range from 0.10 molar NaCl to 1.25 molar NaCl in 0.25 molar steps.

The stem sections were allowed to remain in these solutions for 24 hours. After this period the salt solutions were decanted and the stem sections rinsed once with tap water. Then enough 0.05 percent TTC solution was added to each vial to cover the sections. They were left in these solutions for 24 hours. At the end of this period they were examined for red coloration of the tissue. If the sections appeared red or pink they were considered to be alive. The highest molar concentration in which they were considered as being alive was recorded.

Monk compared the results that he obtained from the TTC test with those which he obtained using the plasmolysis test and with the field or greenhouse results. He concluded that one method was as reliable as the other with the TTC technique being much easier to apply and less difficult to interpret. He also concluded that salt tolerance seemed to be manifest when either of the tests

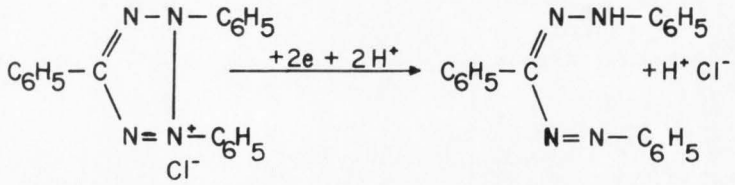
indicated viable tissue after being exposed to 0.75 molar or greater NaCl solutions.

TTC has been used by workers for several years as a vital stain. It was first prepared by Pechmann and Runge in Germany about 1894 (Smith, 1951). It is a white to pale yellow crystalline powder that darkens on exposure to light. It belongs to the family of tetrazoles.

Noller (1957, pp. 637-638) states that the reduction of tetrazolium salts to the formazan can be brought about readily by various reagents including biological systems. When tissues are treated with a tetrazolium salt, the colored insoluble formazan is deposited at the site where reduction is taking place.

Roberts (1950) surveyed tissues of vascular plants that would reduce TTC. He found that it differs from the majority of the redox indicators since, in the reduced state, it forms an insoluble formazan and the reaction is, therefore, irreversible. Small quantities give easily visible results and the reaction is very sensitive. He reported that it readily penetrates the majority of plant tissues. Once the formazan is formed it does not diffuse from the cell nor oxidize back to a colorless state.

Mattson, Jensen, and Dutcher (1947) conducted a preliminary survey of the use of TTC. The reaction is listed as follows. (See top of next page). They concluded that it was quite evident that enzyme systems are responsible when this reduction takes place in plant and animal materials, since tissues heated at 82° C or higher lose their ability to reduce the salt. They postulated that it is probable that the reduction is caused by dehydrogenase



2,3,5-Triphenyl-2H-tetrazolium chloride (colorless in solution)

Triphenylformazan (red in solution)

systems requiring coenzymes I or II. TTC has an apparent redox potential of about -0.08 volt. Thus it is possible for this compound to act as an electron acceptor from many pyridine nucleotide dehydrogenases. They stated that in all probability the reduction of these compounds by enzymes of living cells cannot be considered a general test for life. Nevertheless, the unusual properties of the compound suggest that it might be utilized in many types of biological research involving differences in tissue viability.

Waugh (1948) surveyed the reaction of TTC in stem tissues and concluded that the application will be of value in determining the viability of trees, shrubs, and cuttings.

Roberts (1950) used free hand sections of various tissues of the plant and applied TTC dropwise to the sections which had been placed previously on a microscope slide. A cover glass was added and the preparations allowed to stand for several minutes until reduction had occurred. The results showed that reduction had taken place in apical, lateral, and intercalary meristems. Lateral

meristems active in reducing tetrazolium were found in many vascular cambiums and, in some cases, in the cork cambium. In several plants certain zones in the cortex were found to be sites of reducing activity. Since these zones are physiologically separated from neighboring tissues by a greater reducing power, there is the appearance of a cortical meristem. When TTC was used, strong reduction sites were found in the nodes and meristems of leaves in monocots. Apart from this, Williams (1947) has reported a continued cell division in the cortex.

Roberts made the observation that staining must be completed and observed within 24 hours because reduction by microorganisms after this period of time may obscure the initial staining. Bacterial reduction can be readily identified since the tetrazolium solution itself is stained red. The sections stained by microbial activity are readily distinguishable from those stained by reactions within the tissue.

In a survey of the factors responsible for reduction of TTC in plant meristems, Roberts (1951) concluded that it was highly probable that the dehydrogenase enzyme systems are responsible for the oxidation of various substrates and the concomitant reduction of tetrazolium to formazan. He found the reducing agent to be heat liable but undamaged by freezing. It seems likely that a general redox potential level, maintained by the operation of several physiologically active systems, brings about the reduction of TTC.

There is, however, a diversity of opinion as to the enzymes or groups of enzymes responsible for the reduction of TTC. Mattson,

Jensen, and Dutcher (1947), Kun and Abood (1949), and Jensen, Sacks, and Baldauski (1951) have shown dehydrogenated DPN or TPN to be necessary for the reduction to take place. Brodie and Gots (1951) using highly purified lactic dehydrogenase, glycerol dehydrogenase, and phosphoglyceraldehyde dehydrogenase got reduction of DPN but not of TTC. Shelton and Schneider (1951) got no reduction of tetrazolium violet by lactic dehydrogenase or by alcohol dehydrogenase. It is possible that the use of impure enzymes in some investigations has caused the conflicting results.

Polarographic studies by Jambor (1954) have shown that, in media of pH lower than 6, the reduction of TTC yields chiefly a colorless product. In more alkaline solutions the reduction forms the red formazan. In strongly alkaline medium, TTC is reduced spontaneously without the action of light or any reducing agent.

Another important point to be considered is the influence of light on the enzymatic and nonenzymatic reduction of TTC. Jambor states that both types of reduction take place only in light; there is no reddening in darkness, except nonenzymatic reduction in strongly alkaline solution. These results clearly show that the conditions must be carefully controlled when TTC is used for quantitative experiments.

Mattson, Jensen, and Dutcher (1947) found that many other viable materials, in addition to seeds and yeast, reduce neutral solutions of tetrazolium salt: the fleshy parts of apples, oranges, and grapes; the fill area of mushrooms; carrot roots; white and sweet potatoes; young leaves; the stigmas and ovaries of certain

pollinated flowers, bull spermatozoa, and the blastoderm of hen's eggs. They stated that the use of TTC should have a distinct advantage over many indicators as a viability test, since it is one of the comparatively few organic compounds that is colored in the reduced state.

Brown (1954) studied the reduction of TTC in plant tissue as influenced by mineral nutrition. Corn, wheat, and cocklebur were grown in soils deficient in various minerals. When the plants reached early maturity, they were removed from the soil and immediately the cut ends or roots of the intact plants were placed in a brown bottle containing 0.5 percent TTC solution. The upward movement of the dye within the plant was observed by the presence of the red formazan. After the dye had reached the leaf tips, the stalks were cut open to determine to what extent and where TTC had been reduced. The reduction of TTC in corn and wheat plants could better be correlated with copper than with iron nutrition. In addition it was observed that the nodes of corn and barley stained whereas the internodes stained very little. Reduction of the compound seemed to be greatest in the meristematic regions. As corn and wheat advanced in maturity, the older nodes stained less.

Gall (1948) used tetrazolium salt to estimate the reducing activity of bean tissue cultured in the plant growth substance 2,4-dichlorphenoxyacetic acid. Pratt, Dufrenoy, and Pickering (1948) found it a valuable reagent in studies of cellular physiology. Gunz (1949) reported reduction of TTC by fresh brewers yeast; he also obtained reduction with a cell-free yeast extract, but heating

the yeast or the extract to 60° C inhibited the reaction.

Parker (1953), reporting on some applications and limitations of TTC, stated that a TTC solution with a pH of 7.8 to 8.4 gave the best results. Roberts (1951) reported the approximate optimum pH to be from 6.5 to 7.5 for tetrazolium reduction in normal tissues. Monk (1960) found that a 0.1 percent solution produced the best results for tissues from various woody species of plants, and that a 0.05 percent solution proved best for tissues from herbaceous plants.

Summary

In summary the review of literature indicates that barley, tomato, and petunia are all quite salt tolerant, as shown by field trials and greenhouse results. The plasmolysis and TTC tests have been used on petunia but not on tomato or barley.

In the plasmolysis and TTC tests that have been conducted, tissue samples from plants growing in nonsaline substrates were used. There is, therefore, no information on the salt hardening of plants growing in saline substrates.

A water-culture solution can be used to good advantage in studying the physiological effects of salt upon protoplasmic salt hardiness.

There are limitations to the use of the plasmolysis test since it depends upon the detection of plasmolysis of the cell contents. Salt injured cells can be mistaken for plasmolyzed cells, and the cells of some plants are too small for detection of plasmolysis.

The TTC test has advantages over the plasmolysis test since it is not so tedious and the results are less ambiguous. However, certain definite precautions must be maintained in the use of TTC for valid results.

EXPERIMENTAL PROCEDURE

Growth of Plants

The experiments reported herein were conducted between July 1, 1960, and May 1, 1961 in the greenhouse at Utah State University, Logan, Utah. It was equipped for solution-culture methods and provided a means of continuing the experiments during cold weather.

Five-gallon tanks were used for the culture-solution containers. Each tank was made from a five-gallon, tined honey can by laying the can on its side and cutting out the top side to form a lid. Holes were cut in the lid to accommodate the split corks which were used to hold the seedlings in place when they were transplanted to the tanks (figure 1). Two tubes, one-half inch in diameter and one inch in length, were soldered in holes which were cut in the handle end of the tank. The tubes were used for filling and draining the tanks. The tanks and lids were given two coats of black, water-tank paint to prevent tin toxicity and rust.

Several strands of cord were wrapped around each end of the tank to serve as a support for the lid which was placed on these strings and taped in place. Two gas diffusing stones were provided to each tank and attached to an air supply for aeration purposes. A section of five-eights inch rubber tubing two inches in length was attached to the inlet tube and a screw clamp was used to close

Figure 1. Five-gallon culture solution tank made from honey can showing the inlet and overflow tubes on the handle end, stone bubbler, lid with holes for split corks, and funnel bucket on stand attached to inlet tube for purposes of filling tank.

the tube. A funnel bucket supported on a stand (figure 1) was used to fill the tanks.

Barley, Hordeum vulgare L. Var. Bonneville, was selected as one of the crops because of its high salt tolerance and importance in agriculture. Tomato, Lycopersicon esculentum Mill. Var. V. F. Rutgers, was selected because of its medium-high salt tolerance and adaptability to greenhouse culture. Petunia, Petunia hybrida Vilm. Var. Mixed, was selected because it was used by Monk (1960) in salt hardiness studies and would thus serve as a check for this investigation.

The seeds were treated with Cerasan or Aresan and were germinated in flats containing a horticultural grade vermiculite. The flats were watered with tap water when needed. After the seedlings were one week old, they were watered twice a week with culture-solution. The seedlings were transferred to culture-solution tanks when the roots were of sufficient length to reach into the solution. Three tanks of tomato with 11 plants per tank, three tanks of petunia with 11 plants per tank, and three tanks of barley with 24 plants per tank were used.

Each of the tanks contained 16 liters of Hoagland's No. 2 solution, one-half strength (Hoagland and Arnon, 1950). Table 1 gives the recommended formula and the concentrations of the chemicals in the culture-solution. This was used as a guide in calculating the concentration of the stock solutions as they were used in this experiment (table 2).

The nutrient solution was prepared in a 55-gallon polyethylene barrel. At weekly intervals the old solution was drained from the

Table 1. Recommended formula for the preparation of a culture-solution showing the resultant concentration of the compounds in the root substrate[a]

Compound	Stock solution (gm/l)	Ml of stock solution per liter of culture-solution	Culture-solution (ppm)
KNO_3	101.10	6	606.6
$Ca(NO_3)_2 \cdot 4H_2O$	236.16	4	944.8
$MgSO_4 \cdot 7H_2O$	246.49	2	493.0
$NH_4H_2PO_4$	115.04	1	115.0
Chelate 330	55.00	0.1	5.5
Minor elements	----	1	---
H_3BO_3	2.86	-	2.9
$MnCl_2 \cdot 4H_2O$	1.81	-	1.8
$ZnSO_4$	0.22	-	0.22
$CuSO_4 \cdot 5H_2O$	0.08	-	0.08
$H_2MoO_4 \cdot H_2O$	0.02	-	0.02

[a]Hoagland's Solution No. 2 (Hoagland and Arnon, 1950, p. 31).

Table 2. Volume of stock solution used in making up one-half strength Hoagland's No. 2 culture-solution with the resultant concentration of the respective compounds in the culture-solution

Compound	Stock solution (gm/l)	Ml of stock solution per liter culture-solution	Culture-solution (ppm)
KNO_3	159.20	2	318.4
$Ca(NO_3)_2 \cdot 4H_2O$	474.20	1	474.2
$MgSO_4 \cdot 7H_2O$	246.50	1	246.5
$NH_4H_2PO_4$	57.50	1	57.5
Chelate 330	2.75	1	2.75
Minor elements	----	1	---
H_3BO_3	1.43	-	1.43
$MnCl_2 \cdot 4H_2O$	0.90	-	0.90
$ZnSO_4$	0.11	-	0.11
$CuSO_4 \cdot 5H_2O$	0.04	-	0.04
$H_2MoO_4 \cdot H_2O$	0.01	-	0.01

tanks and replaced with new solution. Twice weekly the solution was drained from each of the tanks into a graduated bucket. Tap water was added to bring the solution to 16 liters and the pH was adjusted to 6.0 using 1.0 N H_2SO_4 or 1.0 N NaOH. Only rarely was the pH below 6.0 before adjustment. At this time 20 milliliters of iron chelate solution (2,750 ppm chelate) was added to each solution.

The plants were given three weeks to adjust to the culture-solution and start growth before the salt treatments were begun. The salt was added to the new solution at the time it was drawn from the polyethylene barrel for the respective tanks.

Since it was desired to study the effect of saline substrates upon the salt hardiness of the protoplasm, a level of salinity was selected for each of the plants near the maximum under which they would still grow and produce. A 1:1 ration of NaCl and $CaCl_2$ on a me/l (milliequivalent per liter) basis was used to give the desired salinity levels, the combination being used to prevent as much as possible specific ion and toxic ion effects.

The maximum salinity level was calculated using USDA Handbook 60 (U.S. Salinity Laboratory Staff, 1954). Tomatoes are listed with a salt tolerance rating of $EC_e X10^3=10$. Reference to figure 6, page 15, of the handbook shows that a conductivity of 10 mmhos/cm is equivalent to an osmotic pressure of the saturation extract of 3.8 atmospheres. The handbook states (p. 17) that the osmotic pressure of the soil solution at the upper limit of the field-moisture range will be approximately double the value of the saturation extract. This gives an osmotic pressure of 7.2 atmospheres.

To be on the safe side, 6.0 atmospheres was chosen as the upper limit for the substrate treatment with tomatoes. The tables on osmotic pressures for various salts at given conductivities or concentrations were used to convert the osmotic pressure to me/l. Reference to figure 5 of the handbook shows that 6.0 atmospheres of NaCl gives a conductivity of 15 mmhos/cm and 6.0 atmospheres of $CaCl_2$ gives a conductivity of 20 mmhos/cm. For a conductivity of 15 mmhos/cm, figure 2 gives a concentration of 140 me NaCl/l and for a conductivity of 20 mmhos/cm, a concentration of 210 me $CaCl_2$/l. Using the lower value of 140 me/l and rounding up to 150 me/l gives a 1:1 ratio of 75 me/l of NaCl plus 75 me/l of $CaCl_2$. This is equivalent to 4.38 grams NaCl/l plus 5.51 grams $CaCl_2.2H_2O$/l. Using these values and calculating back to the osmotic pressure in the reverse order of above gives a value of 5.4 atmospheres. Using a base culture solution with an osmotic pressure of 0.5 atmospheres gives a total pressure of 5.9 atmospheres.

The same procedure was used to arrive at the maximum salt levels for barley and petunia. The salt tolerance rating of $EC_e X10^3$=16 was used for barley, giving a concentration of 100 me $CaCl_2$/l plus 100 me NaCl/l which is equivalent to an osmotic pressure of 8.0 atmospheres. The maximum salt level chosen for petunia was 90 me NaCl/l plus 90 me $CaCl_2$/l which is equivalent to an osmotic pressure of 6.4 atmospheres. The calculations of the maximum values for all three plants are tabulated and shown in table 3.

One tank of each of the plants served as a control. The salt level of one tank was increased gradually over a four-week period.

Table 3. Amount of salt used in formulation of maximum concentrations for substrate treatments[a]

Salt	Concentration (me/l)	Conductivity[a] (mmhos/cm)	Osmotic pressure[b] (atm)	Gm salt per liter	Gm salt per 16 liter
		Tomato			
NaCl	75	8.0	3.10	4.38	70.2
$CaCl_2 \cdot 2H_2O$	75	7.8	2.25	5.51	88.2
	150		5.35		
		Petunia			
NaCl	90	9.7	3.80	5.26	84.2
$CaCl_2 \cdot 2H_2O$	90	9.2	2.60	6.62	105.9
	180		6.40		
		Barley			
NaCl	100	10.1	4.10	5.84	93.5
$CaCl_2 \cdot 2H_2O$	100	10.1	3.90	7.35	117.7
	200		8.00		

[a] Based on figure 2, p. 10 USDA Handbook 60 (U.S. Salinity Laboratory Staff, 1954).

[b] Ibid., figure 5, p. 14.

Two weeks after the first increment of salt was added to this tank, the total amount was added all at one time to the third tank. The gradual versus the rapid addition was made to study the effect of each upon the salt hardiness of the plants.

The three substrate treatments given to each of the three types of plants used gave a total of nine treatments. The treatments were designated using T for tomato, P for petunia, and B for barley with the substrate treatments as a subscript. The nine treatments were:

Tomato:

T_1. Control--culture-solution only.

T_2. Gradual addition of salt--culture-solution plus 75 me NaCl/l plus 75 me $CaCl_2$/l added over a four-week period.

T_3. Rapid addition of salt--Same as T_2 except salt added all at one time.

Petunia:

P_1. Control--culture-solution only.

P_2. Gradual addition of salt--culture-solution plus 90 me NaCl/l plus 90 me $CaCl_2$/l added over a four-week period.

P_3. Rapid addition of salt--Same as P_2 except salt added all at one time.

Barley:

B_1. Control--culture-solution only.

B_2. Gradual addition of salt--culture solution plus 100 me NaCl/l plus 100 me $CaCl_2$/l added over a four-

week period.

B_3. Rapid addition of salt--Same as B_2 except salt added all at one time.

At the time the substrate solutions were changed each week; samples were taken of the old and of the new solutions from each of the tanks. The electrical conductivity of the samples was determined using a conductivity cell and resistance bridge. The conductivity values were used in estimating the osmotic pressure of the respective substrate solutions.

Two sets of plants were grown during the course of this investigation. The first plantings were made in July, 1960, and were discontinued in November. The second plantings were made in December and discontinued in April, 1961. The second set of petunias were transplanted into gallon jugs rather than the tanks. Three plants were placed in each jug and two jugs were used for each treatment giving a total of six jugs.

Salt Hardiness Studies

TTC Test

Solutions of NaCl from 0.0 to 1.25 molar in 0.05 molar steps, and from 1.30 to 2.00 molar in 0.10 molar steps, were prepared using 0.1 strength Hoagland's No. 2 solution as a base. Five milliliters of the desired solutions were placed in 10-ml vials at the time the test was made.

Three weeks after the root substrates in the tanks had been brought to the maximum salt level, stem-section samples were taken for the TTC test. One stem section was placed in each of the vials

and a cotton plug was inserted. The samples were left in the vials for varying amounts of time. In the method that was used during most of this investigation, the salt solution was then drawn off using a glass jet tube attached to an aspirator. The samples were then rinsed in the vials by adding tap water at room temperature. Following this, 5.0 ml of 0.05 percent TTC (2,3,5-triphenyl-2H-tetrazolium chloride), which had been made up using 0.1 strength Hoagland's No. 2 solution, was added to each vial. The vials were restoppered and placed in a dark place for 24 hours. The intensity of the red staining at the end of the 24-hour period was a measure of the reducing activity of the tissue. The intensity of staining was examined by pouring the solution and sample from the vials into a 10-inch porcelain extracting funnel. The filter paper in the funnel was sectioned so that a whole series of samples could be placed in the funnel at one time and the intensity of staining compared. Figure 2 shows the apparatus.

The intensity and characteristics of the staining were recorded. The point at which the greatest decrease in the intensity of staining occurred from one sample to the next was recorded. The point at which staining ceased was also recorded. For each of the various plants in question, several trial runs had to be made in establishing a range of salt concentrations that would bracket the staining of the tissue. After the range was established, several determinations were made and the results of one trial were photographed.

Time studies were made for each of the plants to determine the optimum amount of time to leave the stem sections in the salt solution before the addition of the TTC solution. Several types of stem section samples and various parts of the stem of each of the three plants in question were used in preliminary studies to determine which were satisfactory.

Towards the end of this investigation, a second method was tried. The samples were treated as before except that when the salt solution was drawn off, the samples were not rinsed and a 0.05 percent TTC solution containing the respective concentration of NaCl was added. Thus the samples were kept in a salt solution during the staining period of 24 hours in addition to the initial period of 24 hours. It was felt that this would eliminate osmotic shock which was a possibility of the first method where the samples were taken from the salt solution with a relatively high osmotic pressure to the TTC solution with a much lower osmotic pressure.

Plasmolysis Test

The plasmolysis test as developed by Repp, McAllister, and Wiebe (1959) and as used by Monk (1960) for determining salt hardiness of the protoplasm was used with some modifications in this investigation. Preliminary studies indicated that observation of the tissue after taking it from the sucrose solution did not give the full story on the plasmolysis of the cells. For this reason, the tissue was examined under the microscope before and after being transferred from the salt solution to the sucrose solution.

Three weeks after the root substrates in the solution-

culture tanks had been brought to the maximum salt level, the plasmolysis study was initiated. Free hand, tangential sections of the epidermis of the lower portion of the stem of tomato and petunia and of the sheath of barley were made using a sharp razor blade. These sections contained subadjacent chlorenchyma cells as well as the nonchlorophyll epidermal cells. The very thin tissue sections were transferred from the razor blade into a petri dish containing nutrient solution by dipping the blade into the solution. When an adequate number had been cut, approximately 10 sections each were transferred to a series of 10-ml vials containing 5-ml each of graded concentrations of NaCl solution. The vials were stoppered with cotton plugs and set aside for 24 hours.

As soon as this had been done, an investigation of the osmotic concentration of the tissue sections was begun. Sections of tissue were transferred from the petri dish to a series of microscope slides containing two drops of graded concentrations of NaCl and a series containing graded concentrations of sucrose. NaCl solutions from 0.15 molar to 1.0 molar in 0.05 molar steps and sucrose solutions from 0.25 molar to 1.0 molar in 0.05 molar steps were used. Ten slides were prepared at a time. As soon as the tenth was prepared, the first was examined for plasmolysis. The point at which incipient plasmolysis occurred was recorded.

At the end of the 24-hour period, two additional sets of vials corresponding to the ones containing the salt solutions were prepared. Tap water was added to the one set and 1.0 molar sucrose

was added to the other set. A few sections of tissue were transferred from each of the salt solution vials to the water vials and the sugar vials. The cells in the sugar solution were given one hour to plasmolyze before being examined.

The tissue sections in the salt solutions were examined under the light microscope at the end of the 24-hour period. The microscope slides were prepared by placing two drops of the respective salt solution on the slide and then placing two sections of the tissue on the slide. A cover glass was added, the slide identified, and examined under the microscope. A record was made of the condition of the cells. The type and degree of plasmolysis, if present, was observed and recorded. The point at which there was a marked difference in the appearence of the cells from one salt level to the next was recorded. Notations were made on the granularity of the cytoplasm, the smoothness of the ectoplast, the clumping of the chloroplasts, the presence or absence of cytoplasmic streaming, and the stranding of cytoplasm through the vacuole. The ability of the cells to deplasmolyze was examined by placing a few drops of water at the edge of the cover glass while the slide was under the microscope.

The tissue sections which had been transferred to the vials containing tap water were examined for evidence of deplasmolysis.

At the end of an hour, the tissue sections which had been transferred from the salt solutions to the vials containing the 1.0 molar sucrose were examined. Any increase in plasmolysis by the sucrose over that by the salt was noted and recorded. The ability of the cells to deplasmolyze was checked by the same

method as mentioned above.

Several trial runs were required to establish a range of salt concentrations that would bracket the range of plasmolysis. As soon as the range was established, the procedure was repeated several times to establish mean values and the results of one trial were photographed in color and in black and white using a photomicroscope.

RESULTS AND DISCUSSION

Growth of Plants

At the time the substrate treatments were begun, all plants of the same species were of the same size and making luxuriant growth. All control plants, substrate treatment No. 1, continued to make growth during the entire time of the experiment, and in all cases the growth of the control plants was much greater than that of the plants growing in the salt substrate.

Two of the petunia tanks which were used for the two salt substrate treatments developed leaks early in the experiment. In the repair of the leaks, toxic compounds were evidently introduced into the tanks which caused a reduction in the growth of the plants. For this reason the salt treatments were not initiated in the first set of petunia plants and the second set of petunias was transplanted into gallon-size jugs.

As a whole, the tanks were much more satisfactory to use and were more efficient than the jugs. It was relatively easy to construct mechanical support for the plants with the tanks.

Within a week after the initiation of the gradual addition of salt, substrate treatment No. 2, the difference in the rate of growth of these plants and those of the control was noticeable. At the end of the fourth week, after all four increments of salt had been added, the difference was quite pronounced but the plants

did survive and continued to make growth. All tomato and petunia plants receiving the gradual addition of salt survived; however, only 50 percent of the barley plants which received the treatment lived to maturity.

Substrate treatment No. 3, the rapid addition of salt was initiated two weeks following the initiation of treatment No. 2. For this reason the plants of treatment No. 3 were larger when they received the salt treatment than were the plants of treatment No. 2 when they received the first increment of salt. This difference in size was pronounced for nearly six weeks, but after that time the difference was not noticeable except in the case of barley. Approximately 70 percent of the barley plants receiving the rapid addition of salt lived to maturity; whereas, only 50 percent of those receiving the gradual addition of salt lived to maturity. This is in agreement with the findings of Ayers (1952) on the sensitivity of barley seedlings to salt.

Within five minutes of the time that the rapid addition of salt was initiated on tomatoes, the plants had lost turgor. The wilting reached a maximum after 30 minutes. However, at the end of 36 hours, all of the tomato plants had regained turgor though some of the very youngest leaves did not regain turgor and eventually died. The same thing was observed when the rapid treatment was initiated on petunia. In this case; however, all leaves did regain turgor after a 48-hour period. When the treatment was given to barley there was no evidence of wilting.

The petunia plants with salt in the substrate developed morphological changes very characteristic of plants growing

under saline conditions. The leaves had a thick waxy appearence and were dark blue-green in color. The internodes were much shorter than those of the control plants. This characteristic was not so noticeable in the case of the tomato and barley plants. However, the tomato plants receiving the salt treatment were much more brittle and not nearly so erect in growth habit as those of the control. Figures 3 through 8 show the plants prior to the initiation of the substrate treatments and two months later.

It was observed after cutting stem samples from tomato plants that the control plants would send out new growth; whereas, the plants growing in salt would not, even though several nodes may have been left. In these plants the existing leaves died and eventually the whole plant died. The same situation was also observed with barley. Petunia, however, would initiate regrowth in all treatments. In general, all of the plants receiving the salt substrate treatment seemed to lack the vigor of the control plants.

Table 4 gives the results of the conductivity determinations made on the new and old solutions each week. The conductivity is expressed in mmhos/cm ($EC_e X10^3$). Each value listed is the mean of all the values obtained for the respective solutions over the course of the experiment. The conductivity values were used to calculate the osmotic pressure (O.P.) of the solutions using figure 5, page 15, of USDA Handbook 60 (U.S. Salinity Laboratory Staff, 1954) which gives the relation between the conductivity and osmotic pressure of the soil solution. The results obtained for the plants of the two planting dates are listed separately.

Figure 3. Condition of tomato plants on January 10, 1961, prior to the initiation of substrate salt treatments.

Figure 4. Condition of tomato plants on March 1, 1961, showing effect of substrate treatments (NaCl plus $CaCl_2$) on growth. (1) Control; (2) treatment T_2, 150 me salt per liter; (3) treatment T_3, 150 me salt per liter.

Figure 5. Condition of petunia plants on January 10, 1961, two months after initiation of substrate treatments. (1) Control; (2) treatment P_2, 180 me salt per liter; (3) treatment P_3, 180 me salt per liter.

Figure 6. Condition of petunia plants on March 1, 1961, showing effect of substrate treatments (NaCl plus $CaCl_2$) on growth. (1) Control; (2) treatment P_2, 180 me salt per liter; (3) treatment P_3, 180 me salt per liter.

Figure 7. Condition of barley plants on January 10, 1961; two weeks prior to the initiation of substrate sale treatment.

Figure 8. Condition of barley plants on March 1, 1961, showing effect of substrate treatments (NaCl plus $CaCl_2$) on growth. (1) Control; (2) treatment B_2, 200 me salt per liter; (3) treatment B_3, 200 me salt per liter.

Table 4. Mean conductivities and calculated osmotic pressure values of the culture-solution for all substrate treatments for new solutions and for the same solutions at the end of one week (old)

	Substrate treatment					
	Control		Gradual salt		Rapid salt	
Planting	New	Old	New	Old	New	Old
			Tomato			
First						
$EC_e X10^3$	1.5	1.8	16.1	19.6	16.0	18.3
O. P.[a] (atm)	0.5	0.6	6.4	7.9	6.4	7.3
Second						
$EC_e X10^3$	1.5	4.3	15.4	17.9	15.4	16.9
O. P. (atm)	0.5	1.5	6.1	7.2	6.1	6.9
			Petunia[b]			
First						
$EC_e X10^3$	1.5	2.1	19.2	21.2	----	----
O. P. (atm)	0.5	0.7	7.7	8.6	----	----
Second						
$EC_e X10^3$	1.5	1.8	17.7	18.1	----	----
O. P. (atm)	0.5	0.6	7.0	7.2	----	----
			Barley			
First						
$EC_e X10^3$	1.4	1.5	20.6	20.4	19.9	20.9
O. P. (atm)	0.5	0.5	8.3	8.2	8.1	8.5
Second						
$EC_e X10^3$	1.7	2.6	19.8	20.7	18.7	19.4
O. P. (atm)	0.6	0.9	8.0	8.4	7.5	7.8

[a] Osmotic pressure calculated from $EC_e X10^3$ values using figure 6, p. 15 USDA Handbook 60 (U.S. Salinity Laboratory Staff, 1954)

[b] The conductivity samples for the rapid and gradual addition of salt to petunia substrates were combined in one.

The results show that the base culture-solution had a conductivity of approximately 1.5 mmhos/cm which is equivalent to an osmotic pressure of 1.5 atmospheres. In all cases, except treatment B_2, the conductivity of the solution at the end of the week was greater than the initial conductivity of the new solution. This was caused by the loss of water from the solution through transpiration and evaporation. However, observations indicated that loss of water by evaporation was not significant in comparison with the loss by transpiration. The calculations based on the conductivity and volume of the solution show that the total milliequivalents of salt per tank actually decreased during the week (table 5). However, the loss of water was greater than the uptake of salt and nutrients by the plant; therefore, the general trend was for the conductivity of the solution to increase slightly as the volume of the solution was diminished.

TTC Test

In general the time studies which were conducted for the three plants in question to determine the optimum length of time to leave the stem sections in the graded concentrations of NaCl before the addition of TTC, indicated that 24 hours was adequate. Time studies with petunia showed that a minimum of eight hours was required, but the results using 24 hours were equally as good. A minimum of 16 hours was required for tomatoes, and the results were nearly the same using 16-, 20-, or 24-hour periods. Barley time studies indicated that a 16-hour period was adequate but that the results were nearly the same when using a 24-hour period.

Table 5. Mean values of the quantity of salt and nutrients in milliequivalents total salt per tank for the new solution and for the same solution at the end of one week (old) for the second planting of plants

	Substrate treatment					
	Control		Gradual salt		Rapid salt	
	New	Old	New	Old	New	Old
			Tomato			
Me salt/tank[a]	241	142	3276	2930	3260	2844
			Petunia[b]			
Me salt/tank[c]	57	30	946	733	----	----
			Barley			
Me salt/tank	233	199	4298	4015	4192	4126

[a]Calculated by determining me salt per liter from conductivity values using figure 4, p. 13 USDA Handbook 60 (U.S. Salinity Laboratory Staff, 1954) and multiplying by the volume of solution in liters.

[b]The conductivity samples for treatments No. 2 and No. 3 were combined in one for petunia.

[c]Jugs with a volume of 3.8 liters were used instead of the 16-liter tanks for petunia. The given values are a mean value per jug.

Therefore a 24-hour period was used.

The studies that were conducted using various types of stem sections indicated that for barley, split node samples were the best; for tomato, radial sections of the nodes 1/8 inch thick were best; and for petunia, split stem sections 1/2 inch in length gave best results. It was found with tomatoes that the degree of staining of the nodes was higher than the internodes. The lower nodes of the barley colon gave better results than the upper nodes which contained more chlorophyll.

It was found that only one stem section should be placed in each vial. When two were placed in each vial, they often stuck together, thus preventing uniform absorption of salt or of TTC.

The decrease in staining of the stem sections with increased concentration of the tissue treatment is a gradual one. For this reason it is difficult to determine at which level of intensity the tissue should be judged inactive. Monk (1960) considered the tissue viable if the sections appeared red or pink, depending on the plant species and the amount of coloration found in comparison with the samples in the control solution. In this investigation, it was found that a break in the degree of staining occurred at lower levels than the actual cessation of staining. This point of marked decrease in staining was characterized by change from total staining to a staining of only parts of the sample. The staining between this point and the actual cessation of staining was limited mostly to the pith tissue and meristem. In some trials with tomatoes this pith tissue stained as high as 2.5 molar NaCl and there was no true cessation of staining.

As a whole the marked decrease in staining was more consistant from one trial to the next than was the cessation of staining. The position of cessation of staining from one trial to another varied as much as 0.25 molar NaCl, and the position of the marked change in intensity varied as much as 0.15 molar NaCl. This variation did not warrant the use of the 0.05 molar increments which were used. The genetic difference between plants may account for this variation. It may also be due to physiological differences between plants. The metabolic condition of the plant at the time of sampling may also be a factor.

In each series of samples analyzed, the intensity of staining was compared with the staining of the stem section of a control plant which had been subjected to zero NaCl in the tissue treatment. The intensity of staining of the other stem sections relative to this was indicated by a color value. The color values used and the corresponding abbreviations are listed below in order of decreasing intensity.

VVDR--Very, very, dark red

VDR--Very, dark red

DR--Dark red

R--Red

SR--Slightly red

VP--Very pink

P--Pink

SP--Slightly pink

VSP--Very, slightly pink

VLC--Very little color

G--Green, no staining

The results of approximately 20 trials on each of the nine treatments used in this experiment are summarized in table 6. The color values listed are the mean values of the several trials. The consistency of these mean values is not characteristic of a single trial. The point of marked decrease in staining and the point of cessation of staining are indicated in the table as horizontal lines between corresponding color values. The decrease in staining occurred between the color value Red and Slightly Red. This was the point at which staining became nonuniform. The cessation of staining occurred between the color values Very Slightly Pink and Very Little Color. The point of cessation of staining corresponded to the one used by Monk (1960). Figures 9 through 12 show in color the comparison of the staining of the tissue samples from control plants and plants receiving substrate treatments.

The salt concentration of the points of decreased staining and cessation of staining are listed separately for each of the substrate treatments in table 7. The range of the concentrations of the two points obtained in the several trials are so indicated. When the results of the three plants in question are compared it is observed that the point of marked reduction in staining does not parallel the cessation of staining.

In all cases the staining of stem sections from control plants was more intense than the staining of stem sections from plants growing in salt substrate. The intensity of staining of the control plants was a maximum at the lowest tissue treatment. However, in the case of the plants receiving one of the substrate

Table 6. Summary of results of salt hardiness as determined by staining with TTC using stem sections of tomato, petunia, and barley subjected to three substrate treatments

Tissue treatment (molar)	Substrate treatments								
	T_1	T_2	T_3	P_1	P_2	P_3	B_1	B_2	B_3
0.0	VVDR	P	SP	DR	SR	SR	VDR	DR	R
0.1	VVDR	SR	SR	DR	SR	R	VDR	DR	DR
0.2	VDR	R	R	R	R	R	VDR	DR	DR
0.3	DR	DR	R	R	R	DR	VDR	VDR	VDR
0.4	R[a]	DR	R	R	DR	R	VDR	VDR	VDR
0.5	SR	VDR	DR	SR	R	R	DR	VDR	VDR
0.6	P	DR	DR	P	R	R	R	DR	DR
0.7	P	DR	DR	SP	P	SR	R	DR	DR
0.8	SP	R	DR	VSP[b]	SP	SP	R	DR	DR
0.9	SP	R	R	VLC	VLC	VLC	SR	R	R
1.0	SP	SR	SR	G	G	G	SP	R	R
1.1	SP	VP	P	G	G	G	VSP	R	R
1.2	VSP[b]	SP	SP	G	G	G	VLC	SR	SR
1.3	G	G	G	G	G	G	G	VSP	SP
1.4	G	G	G	G	G	G	G	VLC	VLC
1.5	G	G	G	G	G	G	G	VLC	VLC
1.6	G	G	G	G	G	G	G	VLC	VLC
1.7	G	G	G	G	G	G	G	G	G

[a] Point of marked decrease in intensity of staining.

[b] Cessation of staining.

Table 7. A comparison of the points of marked decrease in staining and actual cessation of staining by TTC of stem sections of tomato, petunia, and barley

Treatment	Marked decrease in staining (molar NaCl)	Cessation of staining (molar NaCl)
Tomato		
T_1	0.45 ± 0.10	1.15 ± 0.25
T_2	0.95 ± 0.15	1.20 ± 0.20
T_3	0.95 ± 0.10	1.20 ± 0.20
Petunia		
P_1	0.45 ± 0.10	0.80 ± 0.10
P_2	0.60 ± 0.10	0.80 ± 0.10
P_3	0.60 ± 0.10	0.80 ± 0.10
Barley		
B_1	0.85 ± 0.10	1.10 ± 0.10
B_2	1.15 ± 0.10	1.35 ± 0.15
B_3	1.15 ± 0.10	1.30 ± 0.15

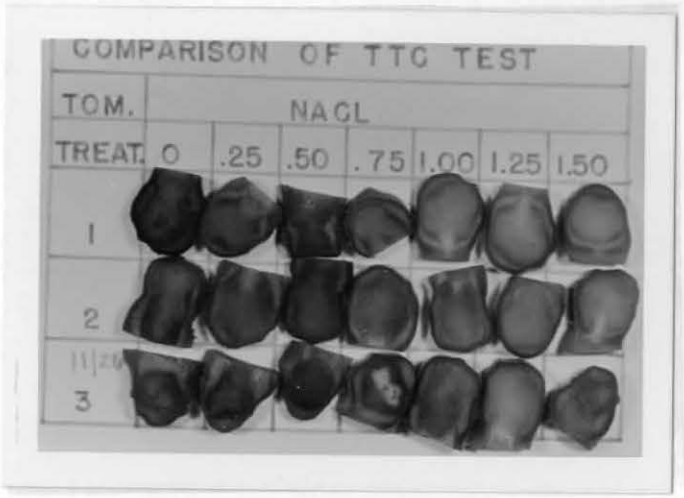


Figure 9. Comparison of TTC staining of tomato stem sections which had been subjected to graded concentrations of NaCl for 24 hours prior to staining. (1) Control, no added salt; (2) gradual addition of 90 me each of NaCl and $CaCl_2$ per liter of substrate; and (3) rapid addition of the same amount of salt to the substrate.

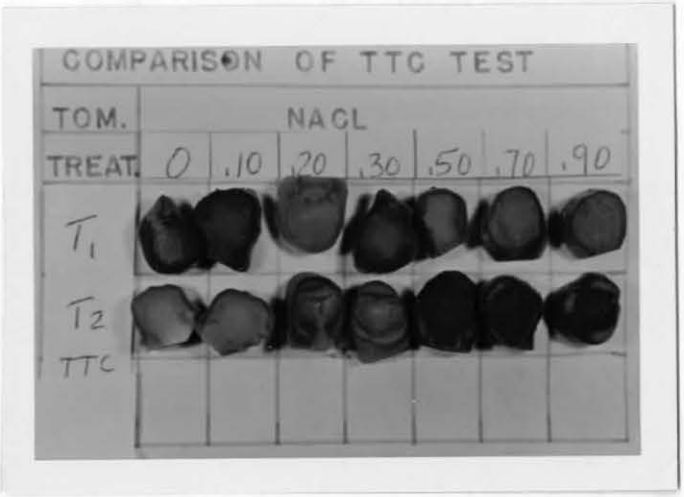


Figure 10. Comparison of TTC staining of tomato stem sections subjected to graded concentration of NaCl for 24 hours prior to staining. (1) Control, no added salt; and (2) gradual addition of 90 me each of NaCl and $CaCl_2$ per liter of substrate. Note is made of the lack of staining in the second row of tissue at the lower levels of NaCl in comparison with the staining of the control tissue.

Figure 11. Comparison of TTC staining of petunia stem sections subjected to graded concentrations of NaCl for 24 hours prior to staining. (1) Control, no added salt; and (2) gradual addition of 90 me each of NaCl and $CaCl_2$ per liter of substrate.

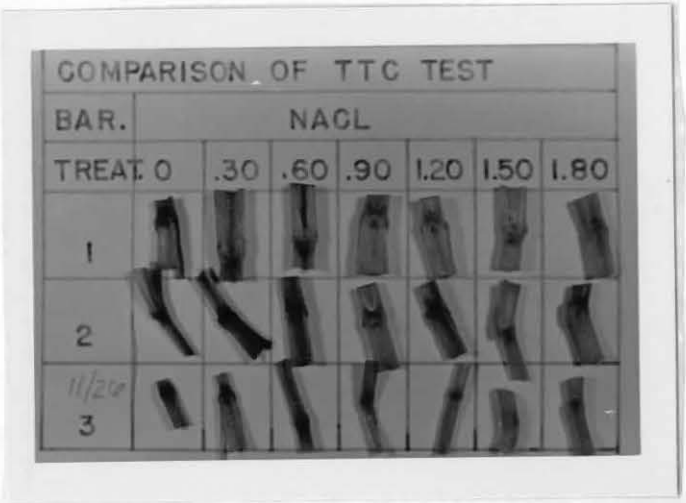


Figure 12. Comparison of TTC staining of split barley nodes subjected to graded concentrations of NaCl for 24 hours prior to staining. (1) Control, no added salt; (2) 100 me each of NaCl and $CaCl_2$ per liter of substrate, gradual addition; and (3) rapid addition of the same amount of salt to the substrate.

treatments, the intensity of staining increased from the lowest tissue treatment to a peak at a concentration higher than the lowest tissue treatment and then decreased as the concentration of the tissue treatment increased. The TTC test did not reveal any differences in salt hardiness between the gradual and rapid salt substrate treatments. There was, however, a great difference between the staining of control tissue and the salt treated plants.

A comparison of the results of the several substrate treatments seems to indicate that salt hardening did take place since the marked decrease in staining occurred at higher concentrations for the salt substrate treatments than for the control. Tomato showed the greatest difference of 0.50 molar NaCl between the point at which staining was markedly reduced with control tissue and the point at which the decrease occurred with stem sections from salt treated plants. The difference in the concentration at which cessation of staining occurred was not significant. Barley showed the next greatest increase with a difference of 0.30 molar NaCl between the point of reduced staining for control tissue and for tissue from plants in salt substrate. The difference in the point of cessation of staining was 0.25 molar NaCl for barley. Petunia showed the least increase with a difference of only 0.15 molar NaCl between the point of marked reduction of staining of stem sections of control and of salt treated plants. The difference in the point of cessation of staining was zero.

The occurrence of the point of cessation of staining for petunia control tissue at a concentration between 0.80 and 0.90 NaCl was just under the value of Monk (1960). He listed petunia

as staining pink at 1.00 molar salt.

The results of the TTC test with control plants are in line with salt tolerance ratings of the three plants in question as previously established. If the point of marked reduction in staining is used as the criteria, the relative ranking in increasing order of salt tolerance would be tomato, petunia, and barley. If the point of cessation of staining is used, the order would be petunia, tomato, and barley.

During the investigations with TTC, it was observed that the powder can deteriorate with time. The Merk Index states that the powder will turn from the normal bluish-white color to a yellow color upon exposure to light. The results of several trials of this investigation had to be discarded because the bottle of TTC which was being used did gradually deteriorate over a period of several months. Examination of the powder showed that it had turned yellow. So far as is known, this has not been previously reported in the literature.

In the second method of staining that was used, where the stem sections were placed in salt solutions containing TTC during the 24-hour staining period, some differences in the pattern of staining as compared to the first method were noted. When tomato stem sections from control plants were used, the staining at the lower concentrations of the tissue treatment were very similar to the results obtained by the first method. The point of marked decrease in staining corresponded to the position in the first method. Beyond this point the nature of the staining was much different. The pith did not stain beyond this point; however,

there was considerable staining of the meristematic tissue. The point of cessation of staining was not as evident as in the first method though there did seem to be a concentration beyond which the meristem did not stain (figure 13).

When tomato plants which had been subjected to salty substrate at the lower levels of tissue treatment were used, there was very little staining with either of the methods. However, in the first method the intensity of staining increased with increasing level of treatment until a peak was reached beyond which it decreased again. With the second method, there was never any staining of the pith, even in the position corresponding to the peak of the first method (figure 14).

A time study was conducted using this second method with tomato stem sections from control plants. It was found that the shorter the period of time during which the tissue remained in the salt solutions before the addition of the TTC solution, the less the staining of the pith tissue. When a six-hour period, or less, was used, the pith tissue did not stain in any of the tissue treatments but the meristematic tissue did stain. It seemed that the longer the tissue was subjected to salt before the addition of TTC plus NaCl, the more intense the staining of the pith at the lower levels of the tissue treatment. In the case of petunia, when the second method was used on tissue from both plants in saline and nonsaline substrate, the staining was more intense and occurred at higher tissue treatments than when the first method was used (figures 15 and 16).

The second method was not used with barley because it was

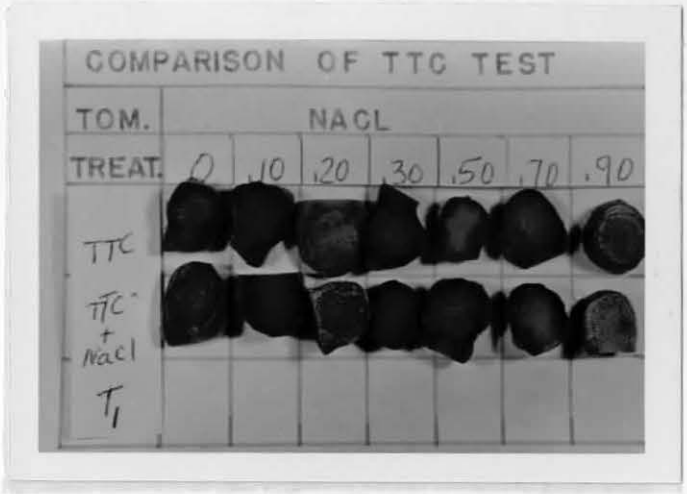


Figure 13. Comparison of TTC staining of tomato stem sections, from control plants, subjected to graded concentrations of NaCl for 24 hours prior to staining. Two separate staining methods are shown. The first row of tissue samples were placed in a TTC solution during the 24-hour staining period. The second row of samples were placed in TTC solution containing the respective concentration of NaCl. Note differences in the staining of the pith tissue at the higher levels of NaCl tissue treatment.

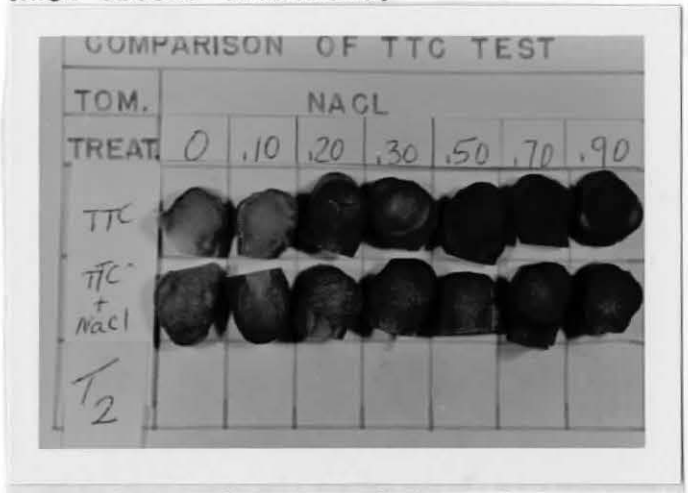


Figure 14. Comparison of TTC staining of tomato stem sections, from plants grown in salty substrate, subjected to graded concentrations of NaCl for 24 hours prior to staining. The results are of two separate staining methods. The top row of tissue samples were placed in a TTC solution during the 24-hour staining period. The second row of samples were placed in TTC solution containing the respective concentration of NaCl. Note differences in the staining of the pith tissue at the higher levels of NaCl tissue treatment. Also note failure of pith tissue to stain at any level in the second row of samples

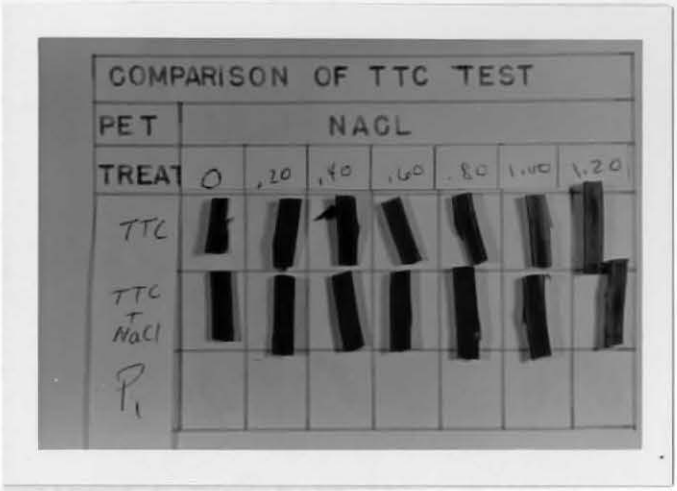


Figure 15. Comparison of TTC staining of petunia stem sections, from control plants, subjected to graded concentrations of NaCl for 24 hours prior to staining. The top row of tissues was stained with a TTC solution, the second row with a TTC plus the respective concentration of NaCl solution. Note the greater intensity of staining with the second method.

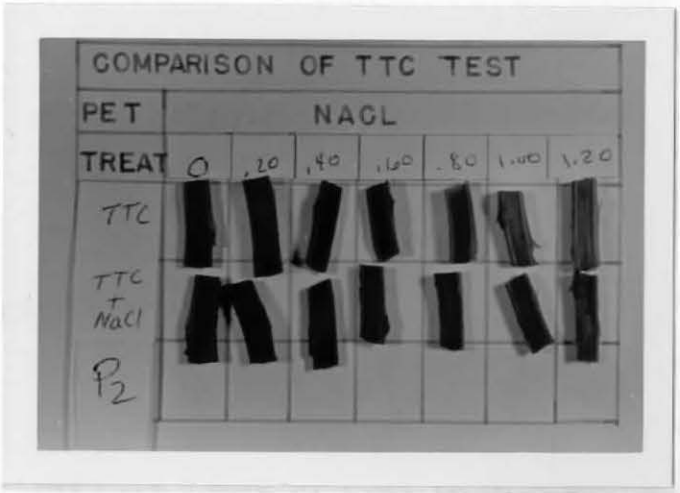


Figure 16. Comparison of TTC staining of petunia stem sections, from salt substrate plants, subjected to graded concentrations of NaCl for 24 hours prior to staining. The top row of tissues was stained with a TTC solution, the second row with a TTC plus the respective concentration of NaCl solution. Note the greater intensity of staining with the second method.

mature at the time the test was conducted.

There was not sufficient time to explore the possibilities of this second method further, but it appears that with some refinements it may have some advantages over the first method.

Plasmolysis Test

Several months of examination of tissue samples under the microscope were required before the characteristics of salt injury and plasmolysis could be fully recognized. It was soon found that above certain concentrations of NaCl, the tissues were already plasmolyzed before being placed in the sucrose solution. This is as expected since above the osmotic concentration plasmolysis occurs. The criteria of the plasmolysis test as developed by Repp, McAllister, and Wiebe (1959) and as used by Monk (1960) does not mention this initial condition of the tissue samples following the salt treatment and prior to being placed in the sugar solution.

The osmotic concentrations of the tissues as determined using both NaCl and sucrose plasmolyzing solutions are summarized in table 8. The values listed represent the highest concentration in which no plasmolysis occurred. The osmotic concentrations of all three plants in question receiving all three substrate treatments were within the range of 0.25 to 0.35 molar NaCl and 0.45 to 0.50 molar sucrose. In most cases the osmotic concentration of the tissue from plants receiving substrate treatments No. 2 and No. 3 were 0.05 molar higher than the control as determined by the plasmolysis method. This also is as would be expected since plants

Table 8. The osmotic concentration of epidermal tissue sections of tomato, petunia, and barley as measured by NaCl and sucrose plasmolyzing solutions

Substrate treatment	Osmotic concentration[a]	
	NaCl (molar ± 0.05)	Sucrose (molar ± 0.05)
Tomato		
T_1	0.25	0.45
T_2	0.30	0.50
T_3	0.30	0.50
Petunia		
P_1	0.25	0.45
P_2	0.30	0.50
P_3	0.30	0.50
Barley		
B_1	0.30	0.45
B_2	0.35	0.45
B_3	0.35	0.50

[a]The highest concentration of plasmolyzing solution in which cells remained turgid.

growing in salty substrate accumulate more salt.

The results of the plasmolysis study of epidermal tissues from control plants after 24 hours in salt solutions followed by one hour in sucrose solutions are presented in tables 9 through 11. Photomicrographs of one trial corresponding to the results presented in table 9 are shown in figures 17 through 26. The results obtained with the tissue from plants receiving the substrate treatments are not presented in table form since the pattern is similar to that of the control plants even though the critical concentrations are different. The results of all treatments are summarized in table 12. The osmotic concentration and the concentration beyond which less than 50 percent of the cells would not deplasmolyze are listed. As can be seen by comparing tables 8 and 12, the initial osmotic concentrations as determined with NaCl are the same as the osmotic concentrations after 24 hours in the tissue treatment.

Use of the results of the plasmolysis test with control tissue in predicting salt tolerance, would put tomato highest with petunia and barley equal and somewhat lower. When the criteria of Monk is used, all three plants would be considered to have some salt tolerance since the critical concentration exceeds 0.75 molar NaCl. However, the high rating of tomato above barley does not agree with field results. The resistance to salt of the protoplasm of plants receiving salt substrate treatments is less than that of the control plants. The decrease in the critical concentration is 0.50, 0.30, and 0.15 molar NaCl for tomato, barley, and petunia respectively. This decrease is parallel and equal to the

Table 9. Condition of epidermal sections of barley sheath tissue from control plants (B_1) after 24 hours in graded concentrations of NaCl and also after being transferred to 1.0 molar sucrose for one hour. Figures 17 through 26 correspond to these data.

NaCl (molar)	Barley tissue treatment		
	Salt (24 hours)	Sucrose (1 hour)	Water
0.2	Turgid (figure 17)	Plasmolysis (figure 18)	Deplasmolysis (figure 19)
0.3	Turgid	Concave Plasmolysis	Deplasmolysis
0.4	Plasmolyzed-clumped[a] (figure 20)	Increase in plasmolysis (figure 21)	Deplasmolysis (figure 22)
0.5	Plasmolyzed-clumped	Increase in plasmolysis	Deplasmolysis
0.6	Plasmolyzed-clumped	Small increase in plasmolysis (figure 23)	Deplasmolysis (figure 24)
0.7	Plasmolyzed-clumped	No change	Deplasmolysis
0.8	Disorganized[b] (figure 25)	No change, no plasmolysis (figure 26)	No deplasmolysis

[a]Plasmolyzed-clumped: ectoplast visible with chloroplasts concentrated in a ball within the cytoplasm.

[b]Disorganized: no membranes visible within cell contents. Cell may appear plasmolyzed but is dead.

Table 10. Condition of epidermal sections of tomato tissue from control plants (T_1) after 24 hours in graded concentrations of NaCl and also after being transferred to 1.0 molar sucrose for one hour

NaCl (molar)	Tomato tissue treatment		
	Salt (24 hours)	Sucrose (1 hour)	Water
0.2	Turgid	Concave	Deplasmolysis
0.4	Plasmolyzed-clumped[a]	Increased plasmolysis	Deplasmolysis
0.6	Plasmolyzed-clumped	Increased plasmolysis	Deplasmolysis
0.8	Plasmolyzed-clumped	Increased plasmolysis	Deplasmolysis
1.0	Plasmolysis	Some increase in plasmolysis	Deplasmolysis
1.2	Irregular plasmolysis[b]	Little increase in plasmolysis	Complete deplasmolysis
1.4	Ringing[c]	Less than 50 percent plasmolysis	Incomplete deplasmolysis

[a]Plasmolyzed-clumped: ectoplast visible with chloroplasts concentrated in a ball within the cytoplasm.

[b]Irregular plasmolysis: contour of ectoplast not smooth.

[c]Ringing: cell contents concentrated in center of cell but no ectoplast visible.

Table 11. Condition of epidermal sections of petunia tissues from control plants (P_1) after 24 hours in graded concentrations of NaCl and also after being transferred to 1.0 molar sucrose for one hour

NaCl (molar)	Petunia tissue treatment		
	Salt (24 hours)	Sucrose (1 hour)	Water
0.2	Turgid	Concave plasmolysis	Deplasmolysis
0.3	Plasmolyzed-clumped[a]	Increased plasmolysis	Deplasmolysis
0.4	Plasmolyzed-clumped	Plasmolysis more evident	Deplasmolysis
0.5	Plasmolyzed-clumped	Plasmolysis more evident	Deplasmolysis
0.6	Plasmolyzed-clumped	Plasmolysis more evident	Deplasmolysis
0.7	Plasmolyzed-clumped	Plasmolysis less evident	Deplasmolysis
0.8	Mostly plasmolyzed	Over 50 percent plasmolysis	Incomplete deplasmolysis
0.9	Ringing[b]	No change	No change

[a]Plasmolyzed-clumped: ectoplast visible with chloroplasts concentrated in a ball within the cytoplasm.

[b]Ringing: cell contents concentrated in center of cell but ectoplast not visible.

Table 12. Summary of results using plasmolysis test to determine salt hardiness of tissues of barley, petunia, and tomato showing the concentrations of NaCl causing plasmolysis and the concentration causing death of the cells after 24 hours

Substrate treatment	Osmotic concentration[a] (molar NaCl ± 0.05)	Critical concentration[b] (molar NaCl ± 0.05)
Tomato		
T_1	0.25	1.30
T_2	0.30	0.80
T_3	0.30	0.80
Petunia		
P_1	0.25	0.80
P_2	0.30	0.65
P_3	0.30	0.65
Barley		
B_1	0.30	0.80
B_2	0.35	0.50
B_3	0.35	0.50

[a] Osmotic concentration: the highest concentration of plasmolyzing solution in which cells remained turgid.

[b] Critical concentration: the highest concentration of plasmolyzing solution in which 50 percent of the cells were still plasmolyzed.

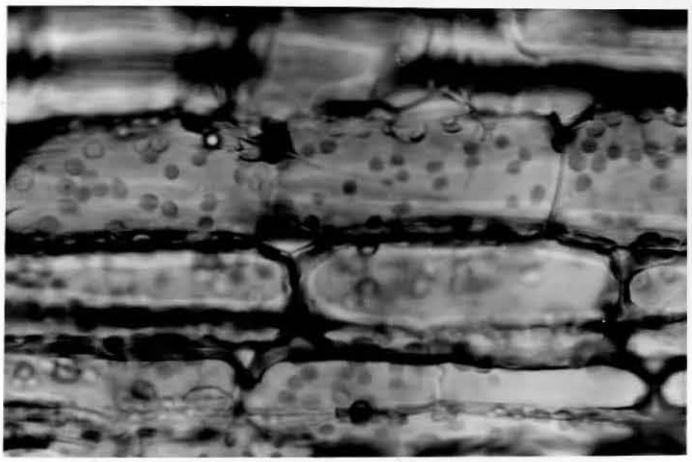


Figure 17. Turgid cells of barley sheath tissue subjected to 0.20 molar NaCl for 24 hours. Note the uniform distribution of the chloroplasts and their separate identity. (385 X)

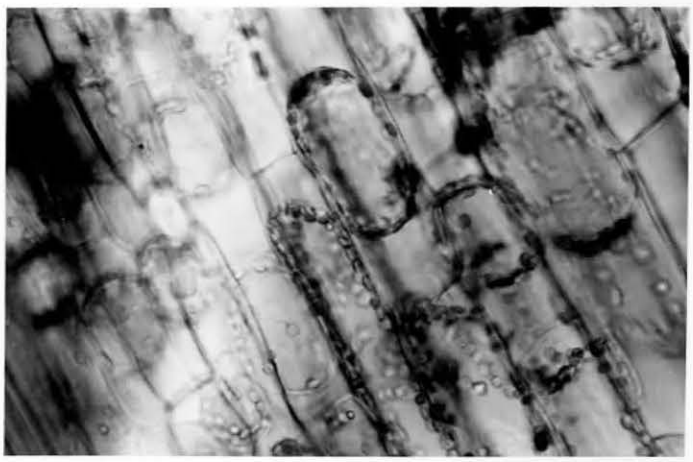


Figure 18. Plasmolyzed cells of barley sheath tissue subjected to 0.20 molar NaCl for 24 hours followed by one hour of 1.0 molar sucrose. Note the elliptical nature of the plasmolysis. (385 X)

Figure 19. Deplasmolyzed cells of barley sheath tissue subjected to 0.20 molar NaCl for 24 hours, 1.0 molar sucrose for 1 hour, and tap water for several minutes. Note the deplasmolyzed condition of the cell. (385 X)

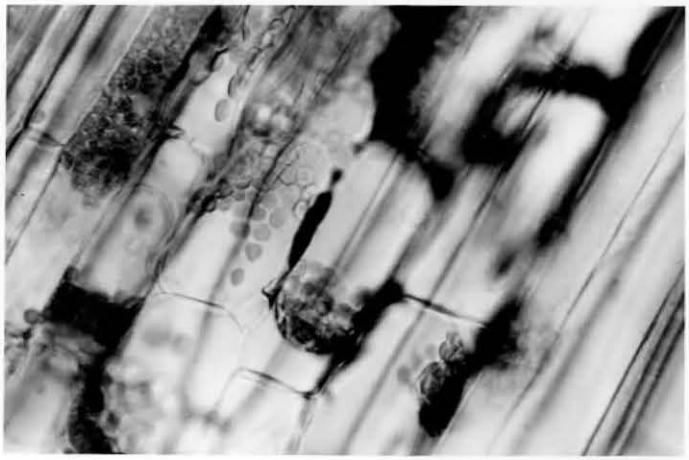


Figure 20. Plasmolyzed-clumped cells of barley sheath tissue subjected to 0.40 molar NaCl for 24 hours. Note the visible ectoplast and the clumped (concentrated) condition of the chloroplasts near the end of the plasmolyzed cytoplasm. (385 X)

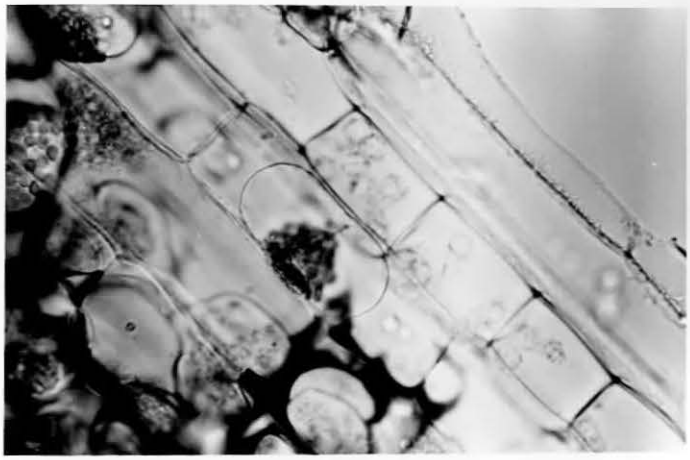


Figure 21. Plasmolyzed-clumped cells of barley sheath tissue subjected to 0.40 molar NaCl for 24 hours followed by 1.0 molar sucrose for one hour. Note the distinct outline of the ectoplast with the chloroplasts concentrated within. (385 X)

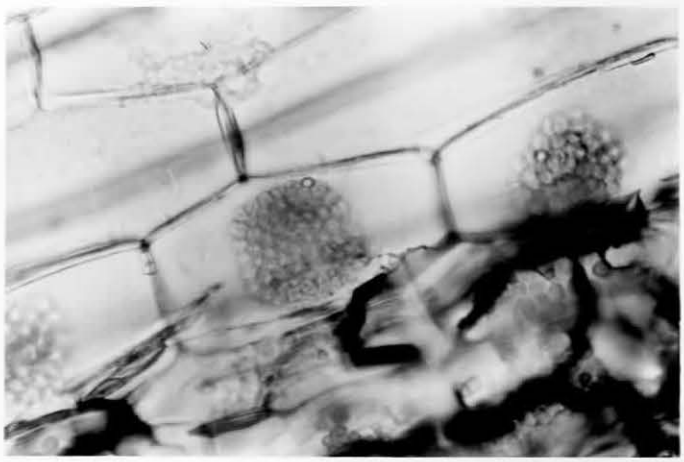


Figure 22. Deplasmolyzed plasmolyzed-clumped cells of barley sheath tissue subjected to 0.40 molar NaCl for 24 hours, 1.0 molar sucrose for one hour, and tap water for a few minutes. Note the deplasmolyzed condition of the cell and the chloroplasts concentrated near the center of the cell. (385 X)

Figure 23. Severe plasmolyzed-clumping of cells of barley sheath tissue subjected to 0.60 molar NaCl for 24 hours followed by 1.0 molar sucrose for one hour. Note the very visible ectoplast and the extreme clumping of the chloroplasts near the end of the plasmolyzed cytoplasm. (385 X)

Figure 24. Deplasmolysis of severely plasmolyzed-clumped cells of barley sheath tissue subjected to 0.60 molar NaCl for 24 hours, 1.0 molar sucrose for one hour, and tap water for a few minutes. Note the deplasmolyzed condition of the cell and the disorganized condition of the chloroplasts. (385 X)

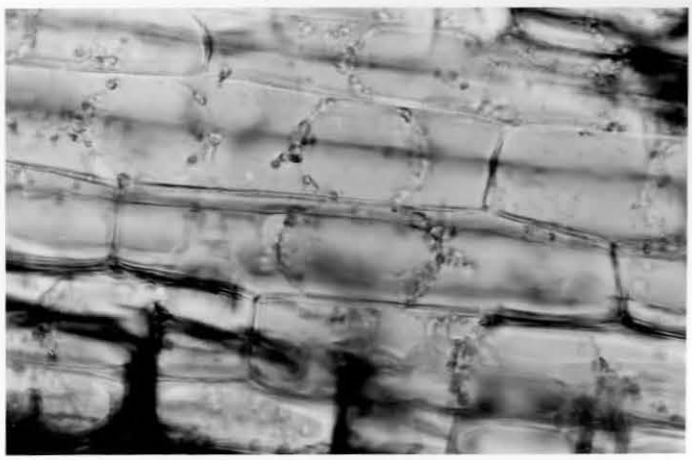


Figure 25. Inactive cells of barley sheath tissue subjected to 1.00 NaCl for 24 hours. Note the apparent plasmolyzed condition of the cell and the irregular contour of the outer boundary of the cell contents. These cells would not deplasmolyze when placed in water. (385 X)

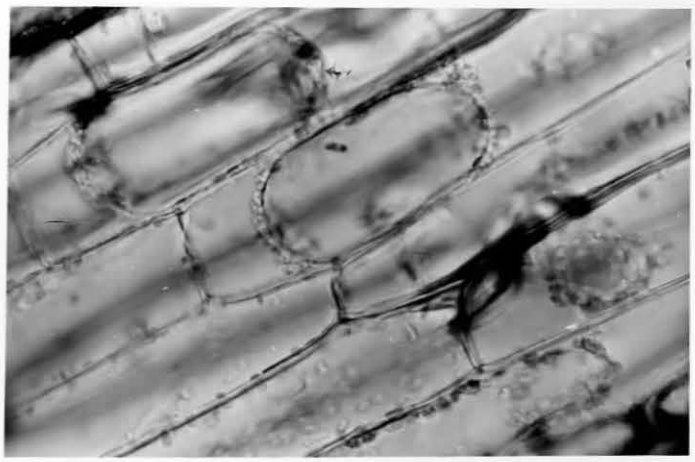


Figure 26. Inactive cells of barley sheath tissue subjected to 1.00 NaCl for 24 hours followed by 1.0 molar sucrose for one hour. Note that these cells have not changed in appearence over those above (figure 25). These cells would not deplasmolyze when placed in water. (385 X)

increase obtained with the TTC test. It is possible that the effects are additive and that the cells having already accumulated salt are less resistant to further exposure to salt.

The determination of the osmotic concentration was not difficult. However, with increasing concentration beyond this point the degree of plasmolysis increased gradually and there was not a definite point beyond which all plasmolysis ceased. Some cells remained active in tissue that contained a high proportion of inactive cells. For each of the three plants in question, the characteristics of plasmolysis were different.

Repp, McAllister, and Wiebe (1959) reported a difference between a salt injured cell and a sugar plasmolyzed cell. In this investigation it was possible to duplicate each type of plasmolysis obtained with salt concentrations (above the osmotic concentration but below the critical concentration) by using sugar. The length of time the tissue was allowed to remain in the plasmolyzing solution had a large influence upon the appearence of the plasmolysis. The plasmolysis of tissues subjected to either slightly hypertonic sugar or salt solutions for 24 hours appeared very similar. Highly hypertonic solutions of salt caused death of the cells as evidence by failure to deplasmolyze; whereas, cells subjected to highly hypertonic sugar solutions for an equal length of time deplasmolyzed when placed in water. For this reason deplasmolysis seemed to be a better criteria of cell activity than the plasmolysis itself.

By way of information, the types of plasmolysis and the steps involved in identification of the critical level are presented in this section. The method of obtaining the results is of equal

importance with the results.

Photomicrographs of turgid cells of barley and petunia are shown in figures 17 and 27. The chloroplasts in turgid cells were uniformily distributed and had separate identity. Subjection of such cells to hypertonic solutions caused a shrinking of the protoplasm. As it was drawn away from the cell wall, the ectoplast became evident. The membrane itself was too thin to be visualized under the light microscope but its properties caused a refraction of the light passing through it so that its presence was indicated by a thin dark line.

The initial plasmolysis was concave or elliptical depending upon the shape of the cell and the plant tissue being observed. With sugar as the plasmolyzing solution, the initial plasmolysis was often concave though concave plasmolysis was also observed with salt (figures 28 and 29). Elliptical plasmolysis was observed with both salt and sugar solutions (figures 18 and 30). No concave plasmolysis was observed in tissues that had been subjected to slightly hypertonic solutions for 24 hours (figure 31).

As the ectoplast was drawn in, the chloroplasts were pulled along with it and tended to concentrate along the membrane. An equilibrium point was reached beyond which there was no further shrinking of the cell contents. However, it was observed that the chloroplasts continued to concentrate until they were clumped in a small area (figure 23). This condition was spoken of as plasmolyzed-clumped in this investigation. The clump of chloroplasts sometimes formed near the end of the plasmolyzed cell contents and at other times seemed to form a bud on the contour of the cytoplasm

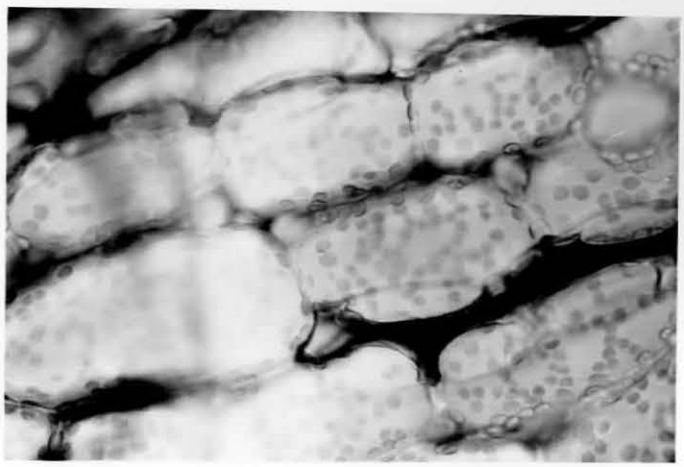


Figure 27. Turgid cells of petunia epidermal tissue from plants grown in salty substrate. (385 X)

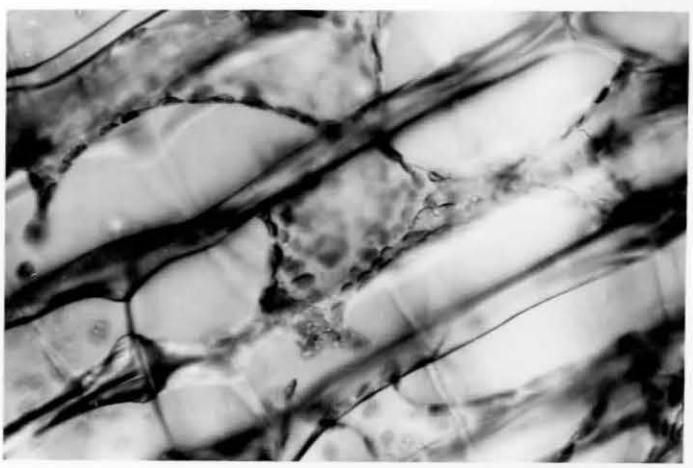


Figure 28. Concave plasmolysis of cells of petunia epidermal tissue subjected to 0.00 molar NaCl for 24 hours followed by 1.0 molar sucrose for 15 minutes. Note the concave nature of the plasmolysis and the concentrated nature of the chloroplasts along the edge of the ectoplast. (385 X)

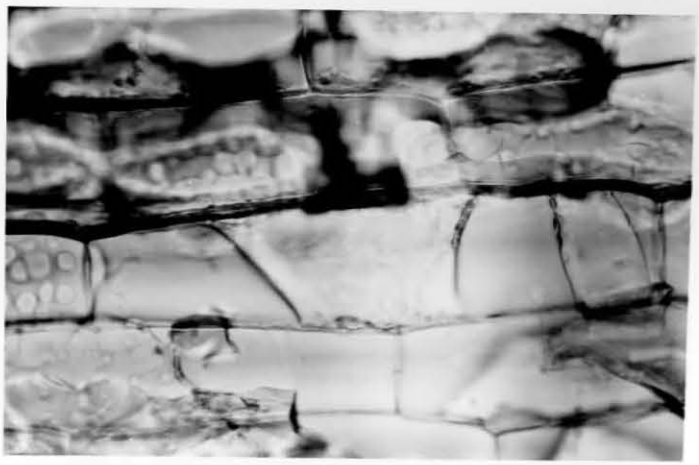


Figure 29. Concave plasmolysis of cells of barley sheath tissue subjected to 0.30 molar NaCl for 24 hours followed by 1.0 molar sucrose for 15 minutes.

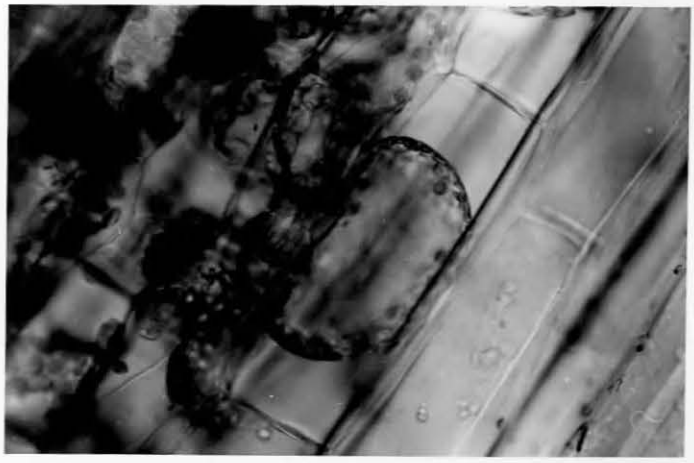


Figure 30. Elliptical plasmolysis of cells of barley sheath tissue subjected to 0.60 molar NaCl for 15 minutes. Note the concentrated nature of the chloroplasts along the edge of the ectoplast.

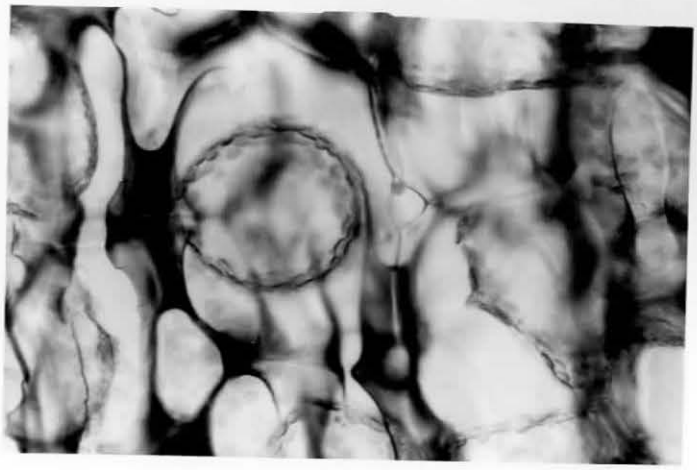


Figure 31. Elliptical plasmolysis of cells of petunia epidermal tissue (from plants grown in salt substrate) subjected to 0.20 molar NaCl for 24 hours followed by 1.0 molar sucrose for one hour. (385 X)

(figures 32 and 33). In cells which contained many chloroplasts, the concentration appeared to be less severe and the outer boundary of the chloroplasts more nearly conformed to the contour of the ectoplast (figure 34). Exposure of such cells to 1.0 molar sucrose usually resulted in a slight increase in the shrinking of the ectoplast.

The ectoplast of the clumped cell was clearly evident as can be seen by referring to the above figures. It often had a halo appearance as is shown in figure 35. Upon gradual deplasmolysis this membrane returned completely to the wall of the cell but the chloroplasts remained somewhat concentrated (figure 22). At highly hypertonic concentrations where the identification of plasmolysis became difficult, the use of the halo appearance of the ectoplast was very helpful. The plasmolyzed contents of an active cell also had a glossy appearance. When focused up and down with the fine adjustment of the microscope, the active cells could be distinguished from the inactive by the presence of the halo and the dark appearance of the plasmolyzed contents. This light effect was no doubt caused by differences in refraction and transmittence of light through the plasmolyzed cell contents which would have different properties than the cell contents outside of the ectoplast (figure 36). Such cells deplasmolyzed when placed in water. The structure and organization of the cells with inactive cytoplasm (figure 37) appeared similar to that of the active cells, but there was no differentiation in the transmittence of light, and the structure did not change when the cells were placed in water.

Figure 32. Plasmolyzed-clumping of cells of barley sheath tissue subjected to 0.50 molar NaCl for 24 hours followed by 1.0 molar sucrose for one hour. Note the bud appearence of the concentration of chloroplasts on the contour of the ectoplast. (385 X)

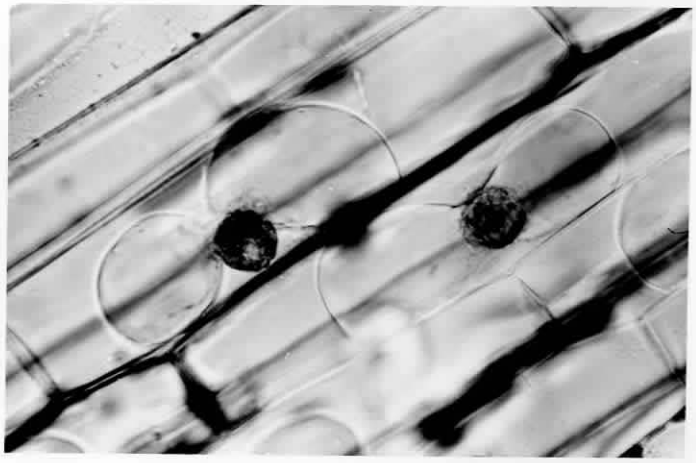


Figure 33. Plasmolyzed-clumped cells of barley sheath tissue subjected to 0.50 molar NaCl for 24 hours followed by 1.0 molar sucrose for one hour. Note the bud formation near the center of the ectoplast. (385 X)

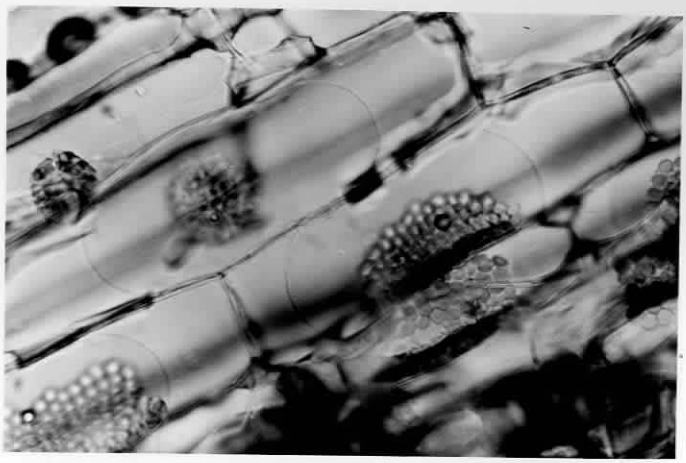


Figure 34. Plasmolyzed-clumped cells of barley sheath tissue subjected to 0.50 molar NaCl for 24 hours followed by 1.0 molar sucrose for one hour. These cells contained more chloroplasts than the cells in figures 32 and 33. Therefore the clumping was not severe though it did occur. (385 X)

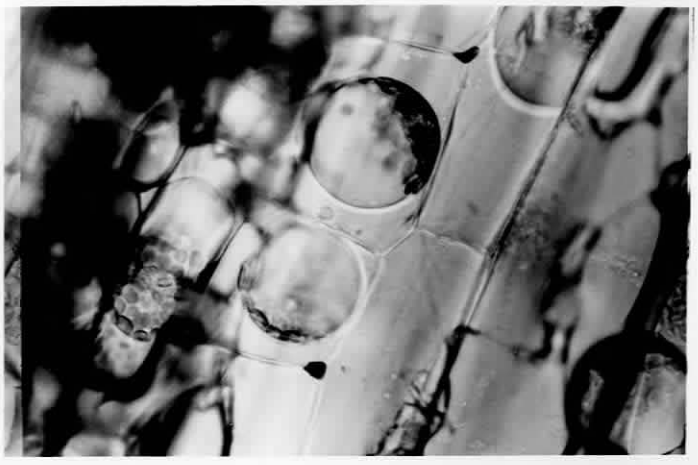


Figure 35. Plasmolyzed-clumped cells of barley sheath tissue subjected to 0.40 molar NaCl for 24 hours followed by 1.0 molar sucrose for one hour. Note the halo appearance around the ectoplast caused by the refraction of light by the membrane. This is an indication of active cytoplasm. These cells deplasmolyzed when placed in water. (385 X)

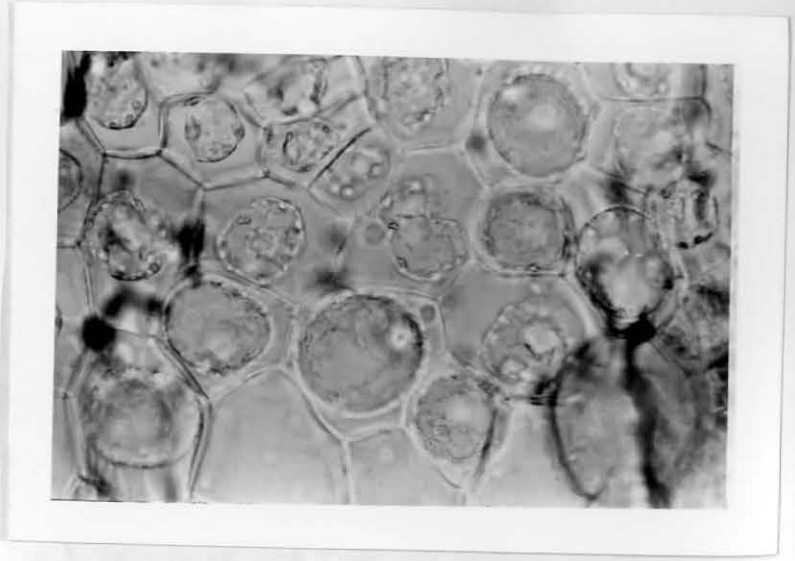


Figure 36. Plasmolyzed cells of tomato epidermal tissue subjected to 1.0 molar NaCl for 24 hours followed by 1.0 molar sucrose for one hour. This level of NaCl treatment was higher than the critical concentration (less than 50 percent active cells). The active cells have a halo around the ectoplast and the plasmolyzed cell contents appears darker than the surrounding medium. The microscope was placed slightly out of focus to give this effect. (385 X)

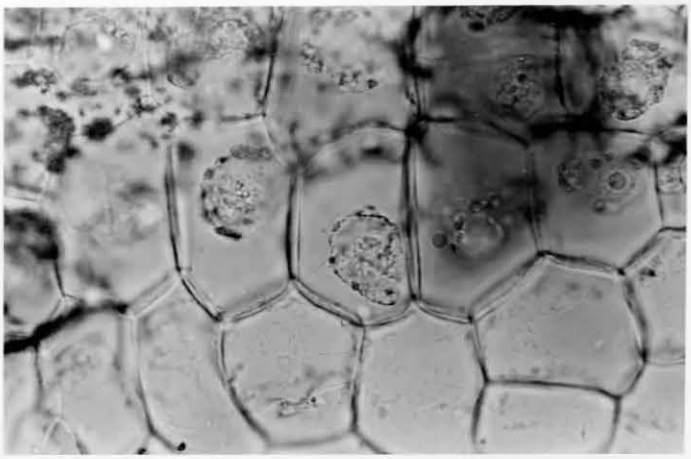


Figure 37. Dead cells of tomato epidermal tissue subjected to 1.50 molar NaCl for 24 hours followed by 1.50 molar sucrose for one hour. Note the concentration of the cell contents within the cell but the apparent absence of the ectoplast. These cells will not deplasmolyze when placed in water. (385 X)

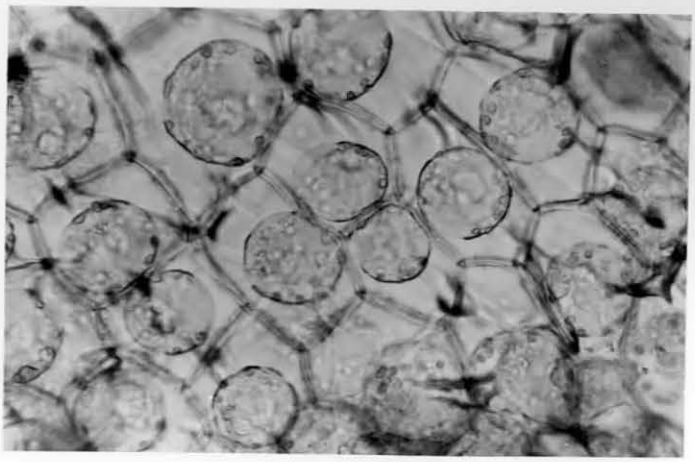


Figure 38. Active, plasmolyzed cells of tomato epidermal tissue subjected to 1.00 molar NaCl for 24 hours. (385 X)

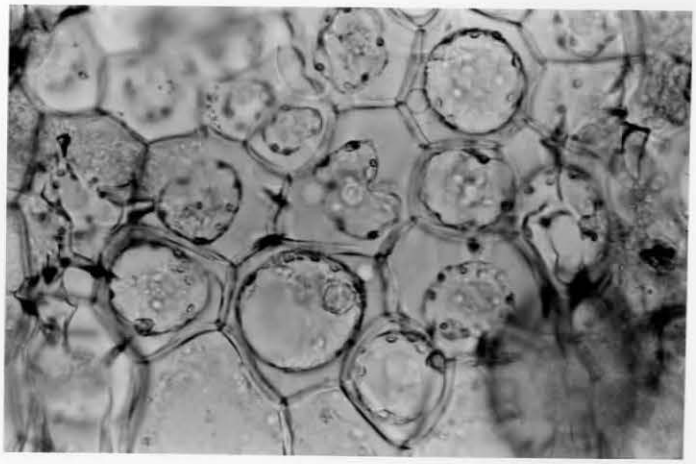


Figure 39. Active, plasmolyzed cells of tomato epidermal tissue subjected to 1.00 molar NaCl for 24 hours followed by 1.0 molar sucrose for one hour. Note the intensification of the halo and dark area of these cells in comparison with those above (figure 38) which were not placed in sucrose. (385 X)

The subjection of plasmolyzed cells to sugar sometimes intensified the halo and the dark area (figures 38 and 39) while it did not change the appearance of inactive cells. Figure 36 shows tissue containing active and inactive cells.

The contents of cells which were dead had a ringed appearance caused by the initial shrinking of the cytoplasm concentrating the cell contents in the center of the cell. The cytoplasm, however, became inactive after prolonged subjection to the high salt solution and the membrane disappeared. The chloroplasts in such cells had a diffuse appearance. They had to a large extent lost their separate identity, and the whole mass had an amorphous appearance. This condition is shown in figure 40 and 41.

The nature of the plasmolysis of tomato cells was much different than that of petunia or barley cells. The contour of the ectoplast was not smooth, even at salt concentrations just above the osmotic concentration as is shown in figure 42.

It was found in this investigation that the same results were obtained after subjecting the tissue sections to the graded salt solutions for only eight hours as when a 24-hour period was used. To check this out cells were subjected to salt concentrations just above the critical level and observed under the microscope. Plasmolysis was almost instantaneous, the cell contents being quickly drawn into the center of the cell (figure 43). The contour of the cytoplasm was very irregular. After an hour the cells were placed in water and very few would deplasmolyze. In contrast cells were subjected to salt concentrations just under the critical level and deplasmolysis would take place as long as 36 hours later.

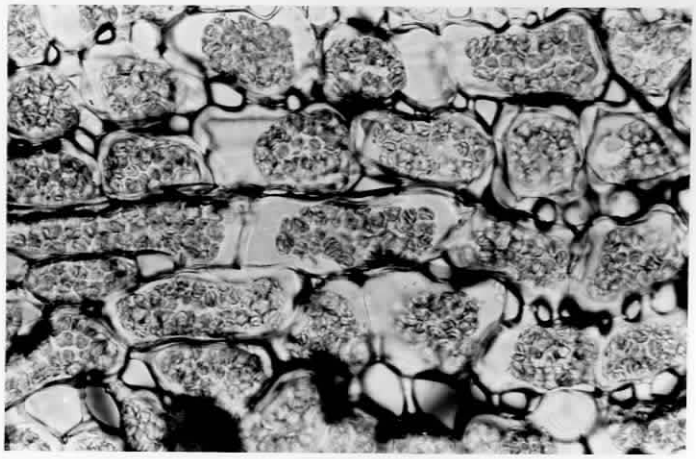


Figure 40. Ringing of cells of tomato epidermal tissue subjected to 1.50 molar NaCl for 24 hours followed by 1.50 sucrose for one hour. Note the similarity in appearance to a plasmolyzed cell. The chloroplasts are concentrated but the ectoplast is not present. These cells will not deplasmolyze in water. (385 X)

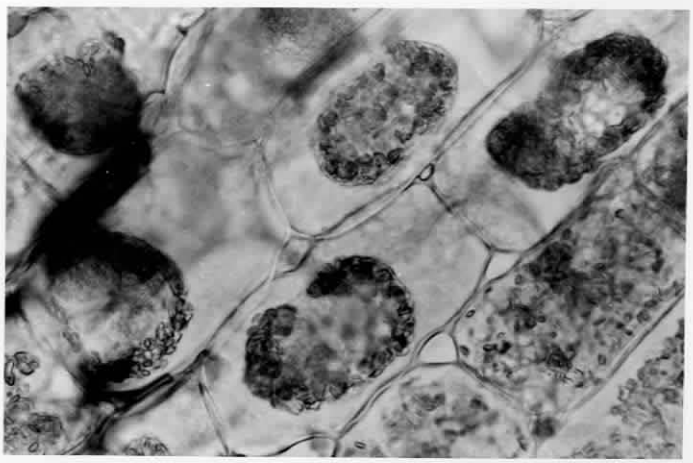


Figure 41. Ringing of cells of petunia epidermal tissue subjected to 1.50 molar NaCl for 24 hours. Note the granular appearance of the cell, the concentration of the chloroplasts, and the absence of an active ectoplast. These cells will not deplasmolyze in water. (385 X)

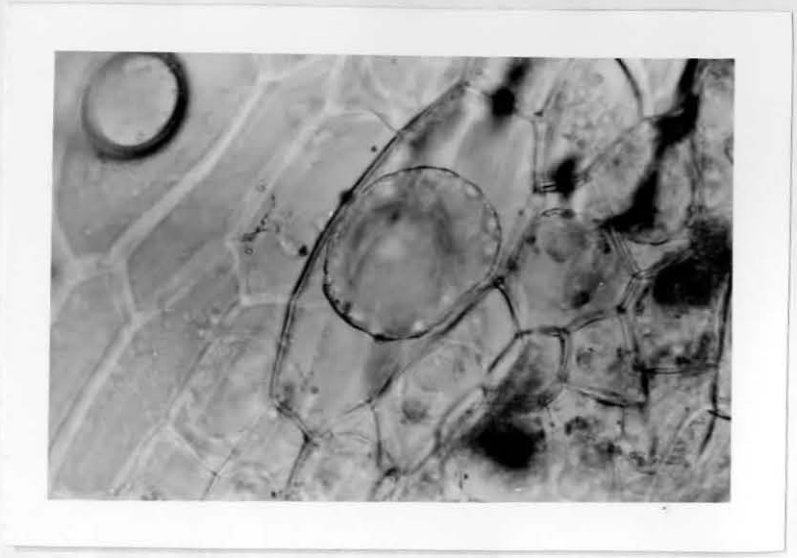


Figure 42. Irregular plasmolysis of cells of tomato epidermal tissue subjected to 0.75 molar NaCl for 24 hours. Note the unsmooth contour of the ectoplast. These cells did deplasmolyze when placed in water. (385 X)

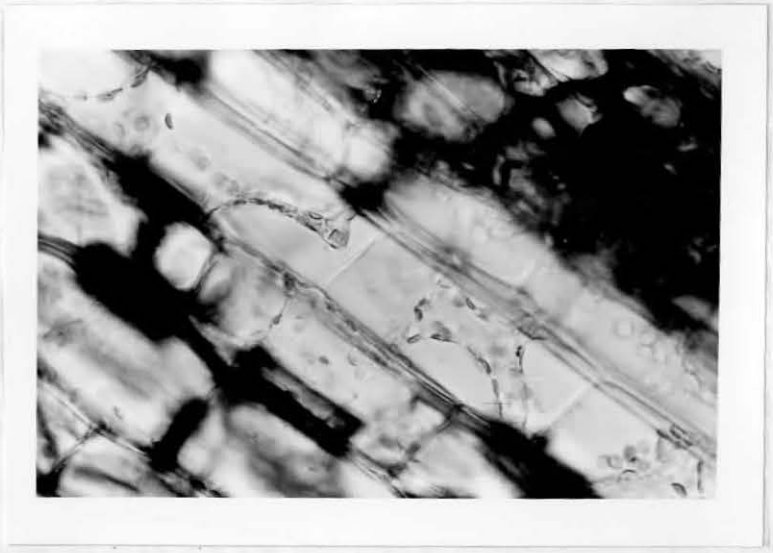


Figure 43. Severe plasmolysis of cells of barley sheath tissue subjected to 1.50 molar NaCl for 15 minutes. Note the extreme shrinking of the cytoplasm and the formation of peaks in the contour of the ectoplast. These cells would not deplasmolyze when placed in water an hour after initiation of treatment. (385 X)

It was further found that examination of the condition of the cells after being subjected to the salt prior to subjection to sucrose was sufficient to determine the critical concentration. As was previously mentioned, the active cells were sometimes more apparent in a sucrose solution; but, as a whole the effect of the sucrose on the cells could be predicted from their previous condition. Cells that did not already have a plasmolyzed appearance would not plasmolyze in sugar (except those treated with salt solutions below the osmotic concentration). Deplasmolysis in water served as the best check on the activity of the cells.

Results of the investigation also showed that an hour was sufficient time to leave the tissue sections in the sucrose solution. Observation of cells under the microscope after being placed in sucrose revealed that there was little initial plasmolysis of turgid cells or increased plasmolysis of already plasmolyzed cells after a 15-minute period.

Longevity of viability did not seem to be a problem. Tissue samples allowed to remain in concentrations of salt below the critical level for 36 hours would still deplasmolyze rapidly. Tissue subjected to salt treatment for 24 hours followed by subjection to 1.0 molar sucrose for 36 hours would still deplasmolyze. Tissue samples kept in tap water for periods of 48 hours after being sliced from the stem section would plasmolyze within 15 minutes when placed in either hypertonic salt or sucrose solutions and would deplasmolyze when placed back in water. In most cases, osmotic shock was observed when the tissue sections were placed back in water. Deplasmolysis proceeded so fast that the ectoplast

ruptured and the cytoplasm spewed into the cell cavity. Within two seconds after the rupture, the ectoplast had disappeared. For normal deplasmolysis to take place, the concentration of the plasmolyzing solution had to be diluted gradually.

Thin sections of epidermis from mature tissue of tomato and petunia gave the best results because the cells were larger. With barley, best results were obtained by using thin sections of the sheath tissue of the first or second leaf from an area below the ligule. The largest cells were found to be located in tissue that was light green in color. However, it is difficult to observe plasmolysis in white tissue since there are few chloroplasts.

Repp, McAllister, and Wiebe (1959) reported that in dead or nearly dead cells the vacuole appears normally plasmolyzed but the cytoplasm appears much more granular and is more or less concentrated in clumps bordering the vacuole. At places the vacuolar membrane is entirely bare. They further reported that cells which possessed normal plasmolysis were considered alive; whereas cells in which there were slight visible changes in structure, mainly an increase in granularity, but in which the plasma membrane still possessed a smooth contour were considered slightly damaged.

In this investigation it was difficult to distinguish the tonoplast from the ectoplast in a plasmolyzed cell. In cells plasmolyzed by salt concentrations just under the critical level, streaming of cytoplasm was observed in the cytoplasmic strands through the vacuole. This indicates that the ectoplast and tonoplast were still intact and separate. After the observations which were made during this investigation, it is felt that the cells

which they referred to as being slightly damaged are those cells that were already plasmolyzed before being placed into the sugar solution. The cells below the osmotic concentration would only have been plasmolyzed for four hours prior to examination in comparison to those above that level which would have been plasmolyzed for 28 hours before examination.

As a whole, the criteria of plasmolysis is a very difficult one since there are so many factors that can influence it.

Comparison of Results

The plasmolysis and the TTC tests were employed in this investigation to measure protoplasmic salt hardiness of tissues from plants grown in saline and in nonsaline substrates. The object was to determine if the protoplasm increased in salt hardiness when the plant was subjected to salt substrate. Such an increase would be an indication of salt hardening.

A comparison of the results of the two tests indicate that they do not measure the same property of protoplasmic resistance. With the TTC test, salt hardening seemed to take place since tissue from plants in salt substrate stained at higher NaCl levels than the tissue of control plants. Results of the plasmolysis test indicated negative salt hardening since the critical concentration of tissue from plants in saline substrate was lower than that of plants in nonsaline substrate. In the case of the TTC test, tomato showed the greatest increase (0.50 molar NaCl), followed by barley (0.30 molar NaCl), which was followed by petunia (0.15 molar NaCl). This is just opposite to the results of the

plasmolysis test where tomato showed the greatest decrease (0.50 molar NaCl), followed by barley (0.30 molar NaCl), which was followed by petunia (0.15 molar NaCl).

SUMMARY AND CONCLUSIONS

In order to compare the protoplasmic salt hardiness of plants grown in saline versus nonsaline substrates, barley, petunia, and tomato were grown in solution-culture in the greenhouse. Each of the three plants in question received one of three substrate treatments: (1) no addition of salt (control), (2) a gradual addition of salt, or (3) a rapid addition of salt.

Live tissue samples were subjected to graded concentrations of NaCl and the salt hardiness was measured by the vital stain 2,3,5-triphenyl-2H-tetrazolium chloride (the TTC test) and by plasmolysis studies.

The research was divided into three areas of investigation: (1) comparison of the salt tolerance rating of the three plants as determined by each of the salt hardiness tests with the salt tolerance rating as determined by field studies, (2) determination of the effect of saline substrates upon the protoplasmic salt resistance of the plant, and (3) examination of the effect of saline substrates upon the validity of the salt tolerance rating of a plant as determined by the plasmolysis and TTC test.

The following observations and conclusions are noted:

1. In all cases the growth of the control plants was much greater than that of the plants receiving the salt substrate treatment. Growth of plants receiving rapid addition of salt was

equal to that of those receiving a gradual addition with the exception of barley in which case plants receiving the rapid addition did better than those receiving gradual addition.

2. Results of the TTC test using control tissue showed all three plants to be salt tolerant with the order of increasing salt hardiness to be: petunia and tomato equal, with barley somewhat higher, which is in general agreement with the order of ranking as determined by field trials.

3. The TTC test showed no difference in the salt hardiness of the plants receiving the rapid versus the gradual addition of salt. However, the test indicated an increase in salt hardiness of these plants over the control plants. The order of decreasing salt hardening was tomato, barley, and petunia.

4. Observations during the plasmolysis test revealed that examination of the tissue samples following the NaCl tissue treatment prior to the sucrose treatment was adequate to determine the critical salt concentration. It was further found that tissue sections subjected to salt concentrations above the osmotic concentration were already plasmolyzed before being subjected to the sucrose solution. It was also found that in some cases the sugar treatment served to make identification of plasmolysis easier but as a whole the results of this treatment could be predicted from the first observations. Subjecting the tissue sections to the NaCl solutions for an eight-hour period gave the same results as for a 24-hour period. Deplasmolysis with water served as the best check on the activity of the plasmolyzed cell contents.

5. The plasmolysis test showed all three plants to be salt

tolerant. There was no difference in the salt hardiness of the tissue sections from plants receiving the rapid salt or the gradual salt treatment. There was a decrease in the critical concentration for tissues from plants receiving the salt substrate treatment in comparison with the control. The decrease shown by tomato was highest followed by barley and petunia.

6. The results of the TTC test on control plants are more in agreement with salt tolerance ratings previously determined by field studies than are the results of the plasmolysis test. The TTC test is much easier to use and interpret.

7. There is not conclusive evidence that salt hardening did take place. The enzyme system which reduces TTC seemed to show salt hardening but the over-all activity of the cell showed a reverse of salt hardening.

8. The salt content of the root substrate has a definite effect upon the validity of the salt tolerance rating of a plant as predicted from either the plasmolysis or the TTC test results. A salty substrate gives a lower rating when determined by the plasmolysis test and a higher rating when determined by the TTC test. For example, the level of salt hardiness of tomato plants in saline substrates as determined by the TTC test is higher than that of barley in nonsaline substrates. Therefore, for valid results when using these tests in predicting salt tolerance, plants must be grown under nonsaline conditions.

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