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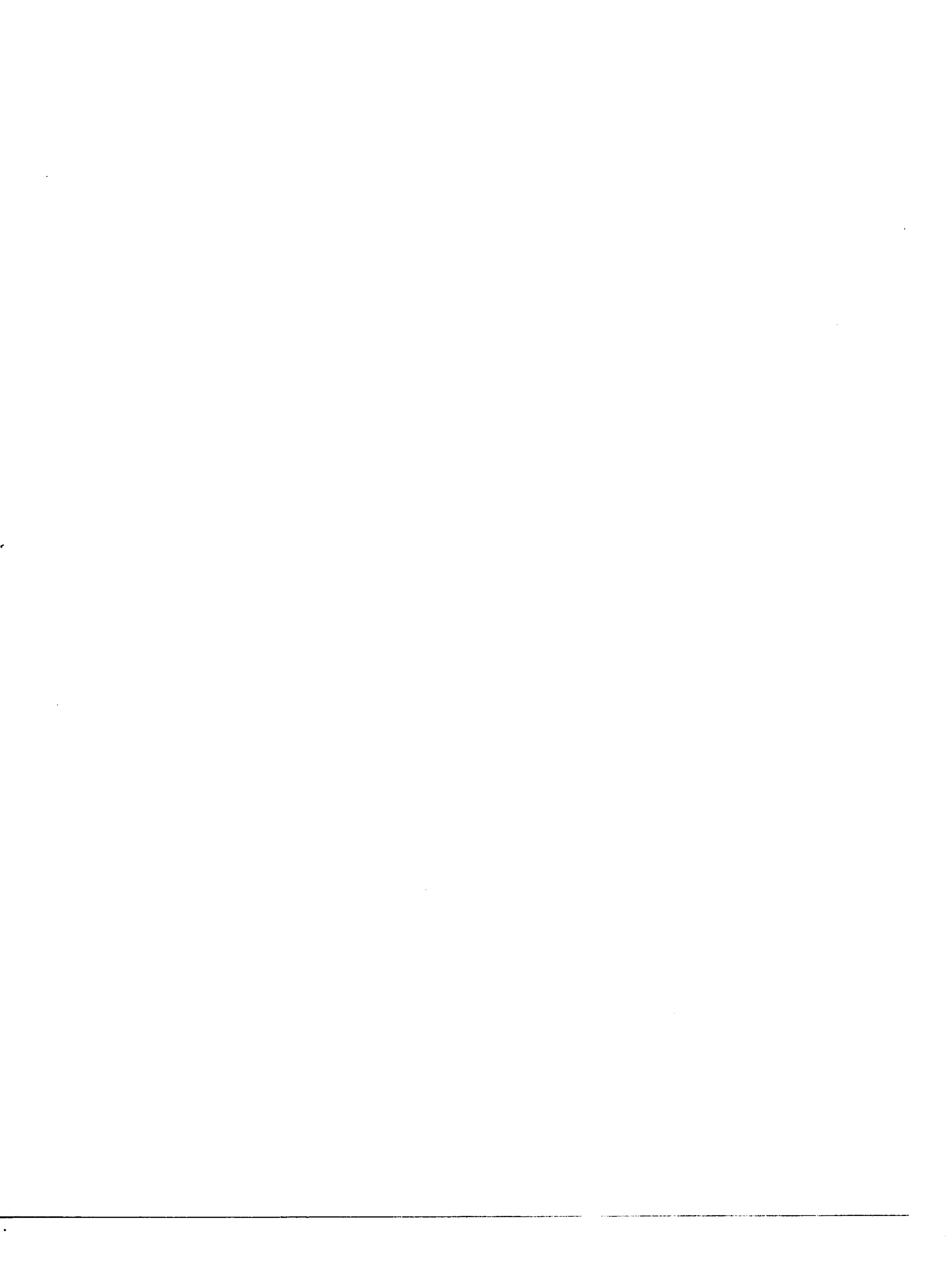

INTERACTIONS BETWEEN SALINE STRESS AND BENZYLADENINE ON CHILI PEPPERS (CAPSICUM ANNUUM L.)

by

Abreeza May Zegeer

A Thesis Submitted to the Faculty of the

DEPARTMENT OF PLANT SCIENCES

In Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE
WITH A MAJOR IN HORTICULTURE

In the Graduate College of

THE UNIVERSITY OF ARIZONA

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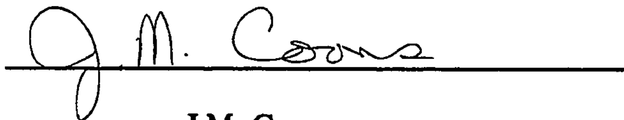

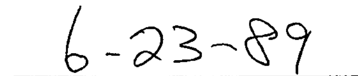

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ABSTRACT

Chili peppers (Capsicum annuum L.) were grown in salt stressed or nonstressed conditions in a greenhouse hydroponic system (0.00 to -1.25 MPa NaCl:$CaCl_2$, 3:1, w/w) and in the field (median range values of 4.6 vs 6.2 mmhos/cm). Salt inhibited growth of vegetative and reproductive tissues in chili peppers, resulting in fewer marketable fruit. Salt decreased leaf chlorophyll a, transpiration, and water potential, which corresponded to an increase in soluble solids.

When peppers were applied with microliter amounts of ^{14}C labelled benzyladenine (BA; 44,400 dpm l^{-1}), BA was translocated primarily acropetally from the site of application. Regardless of application site, translocated BA was ported primarily to expanding leaves, and BA was more readily absorbed by leaf as opposed to stem surfaces.

Exogenous application of BA (0, 50, 100 mg ul^{-1}) had no significant effects on tolerance of chili peppers to salt (-0.75 MPa NaCl:$CaCl_2$, 3:1, w/w) as measured by vegetative and reproductive weights, numbers of reproductive structures, transpiration and total chlorophyll.

INTRODUCTION

In semiarid regions worldwide such as Arizona, soil and water salinity cause serious problems in agricultural crops (Ehlig, Gardner and Clark, 1968). Salt stress, as with water stress, will reduce productivity and in extreme cases will cause death of the plant. To achieve yield increases in saline soils, plant responses to salt need to be modified.

Plant growth and development are governed by a plant's genes and a plant's environment and are regulated in part by plant growth substances. Plant growth can be defined as an irreversible increase in size and the term plant development refers to changes in the plant body that result from controlled processes of cell division, growth, and differentiation. Plant growth and development may be affected in less favorable environments such as saline waters and soils. Negative effects of environmental stress may be alleviated by the use of plant growth substances.

Plant response to salt stress induced on the root is expressed in a variety of ways. The primary effects of salt involve direct toxic effects and indirect effects. Such indirect effects include inhibition of growth, development, and metabolic disturbances, eg. reductions in photosynthesis, respiration, protein metabolism, and nucleic acid synthesis, as well as changes in specific enzyme activities (Levitt, 1980). Secondary effects of salt would include osmotic stress. When osmotic concentrations are high enough in the solution around the root, a

water stress develops, known as "physiological drought stress". Reduction in water uptake results in a reduction of nutrient uptake which is referred to as "deficiency stress".

Plants under stress respond to cytokinins differently depending on species and manner of application. Cytokinins exogenously applied are used widely to modify plant responses to plant stress. Significant changes in either production or transport or both of cytokinins can be triggered by stress and by changes in the plant development (Purohit, 1985).

In the following studies, objective one was to determine the effect of salts on chili pepper (Capsicum annuum) growth and development. Objective two was to determine if foliar applied cytokinin is incorporated into plant tissues, and if so, where does it move. Objective three was to determine if salt stressed chili peppers applied with exogenously applied cytokinin could overcome any growth related salt stresses.

LITERATURE REVIEW

In the following review, the effects of salinity, cytokinin, and the interaction of cytokinins with plant stress will be discussed. The scope of this review will deal primarily with plant stress (salt) responses, plant cytokinin responses, and cytokinin metabolism interactions with plant stress responses.

Salinity

Determining the nature of salt induced stress on any plant is difficult, because many plant stress response mechanisms are not clearly understood. Both primary and secondary salt stresses occur in plants; toxic responses are primary and osmotic and nutritional responses are secondary (Levitt, 1980). Toxic and osmotic stresses are hard to distinguish from one another because they often affect the plant simultaneously.

Injury due to toxic effects is specific to the type, ratio, and concentration of ions present in the root media. The toxicities of different salts is not constant for all plants under all conditions. Osmotic stress occurs, because salt added to water lowers its osmotic potential making it harder for the plant to absorb water. When a plant in low salt media is moved to a high salt media, osmotic dehydration may occur causing a decrease in plant volume, as well as osmotic and water potentials.

It is difficult to determine if reduced growth is caused by ion excess or water deficit in expanding tissues (Greenway and Munns, 1980). At decreased

water potentials (water stress) transpiration decreases, stomates close, and respiration increases (Greenway and Munns, 1980; Levitt, 1980; Morgan, 1984). Under water stress, corn plants almost immediately stop growth, which suggests growth is strongly affected by stress. However, if stress is continued, growth will resume after a lag period (Hsiao, 1973). The lag period increases and growth rate is decreased with the degree of stress. Growing zones were more sensitive to water stress and this was evident after 0.5 hours. Under nonstressed conditions, mature regions of corn plants respond mostly to transpiration, whereas, growing regions respond to both transpiration and growth (Westgate and Boyer, 1984). Xylem water potential gradient (transpiration stream) decreases through the day, while the water potential gradient between the transpiration stream and the growing tissues remains almost constant. Westgate and Boyer (1984) suggest that this response in growing tissues is due to decreases in water potential caused by assimilate accumulation (osmotic adjustment) in growing tissues required to maintain a good water potential gradient between the transpiration stream and the growing tissue.

Irrigation with salt waters may decrease the yields of vegetables. Papadopoulos (1986) found that irrigating with waters of increasingly higher sulfates also increased the soil salinity. The increased soil salinity significantly decreased yields in tomato and eggplant, but not in bell pepper. Yield decreases were due to a decrease in fruit size, not in the number of fruit. Peppers did not have reduced yields, but the fruit quality (marketability) deteriorated due to

misshapened fruit.

Peppers are moderatly salt sensitive (Ehlig et al., 1968; Fernandez, Caro, and Cerda, 1977; Levitt, 1980; Nieman, 1962), although the degree of salt sensitivity varies between cultivars. The above ground symptoms of salt injury on chili peppers are wilting, small leaves, and necrotic or burned portions on leaves. Below ground, the stem often dies or becomes girdled (Shannon and Cotter, 1985). This girdling happens more frequently in younger plants which are less tolerant than older plants to salt stress.

Khaddar et al. (1984) measured physiological responses of tomato plants to salt ($NaCl:CaCl_2$, 4:1 at 4, 8, 12 and 16 EC mho cm^{-1}) at various moisture regimes (20, 25, 30 and 35%, by weight of soil mass). Transpiration rate (g plant day^{-1} water) decreased with higher salinity and lower soil moisture. Transpiration was about 100 g water at zero salt and 30 g water at 16 EC; 120 g water at 35% soil moisture and 15 g water at 20% moisture. Ehlig et al. (1968) found in bell pepper that transpiration rate decreased in salt treated (50% NaCl, 38% $CaCl_2$ and 12% $MgCl_2$, w/w) soils and the decrease in transpiration was enhanced by reduced soil water potentials.

In bean (Phaseolus vulgaris) under external salt stress of about 125 mM (internal Cl^- concentration 250 mM) stomatal conductance (mol $cm^{-2}s^{-1}$) was less than 0.10 compared to no salt at 0.38 (Seeman and Critchley, 1985). This response indicates that increased salt stress reduces the conductance of water, or causes stomatal closure. Also decreases in photosynthesis correlated with external

and internal salt concentrations. Shoe and Gale (1983) found reduced photosynthates in the growing regions of lucerne (Medicago sativia L.) under sodium chloride stress. Shoe and Gale (1983) believe photosynthate is reduced, because of inhibition of photosynthesis due to stomatal closure, or the direct effect of salt on photosynthesis.

Nutritional deficiency stress is induced by salt ions competing with nutrient ions, and can be overcome by adding sufficient amounts of potassium to the root media (Ehret and Ho, 1986). A higher Na^+ concentration may decrease Ca^{+2} uptake. High sodium salts in the soil also will decrease Mg^{+2}, K^+, N and P in plants but will increase amounts of Na^+ in plants. These increases in Na^+ salts cause an increase in the water saturation deficit. Reduction of N, P or K^+ can affect synthesis of proteins, nucleotide pools and a wide variety of other compounds including growth regulators (Levitt, 1980; Turner, 1985).

Action of Cytokinins

Cytokinins are naturally occurring plant growth substances that promote cell division and differentiation in many plants. In the plant kingdom hundreds of natural active and inactive cytokinin-like substances have been isolated. Most of these are isopentyl adenine derivatives from the breakdown of tRNA (Lawrence, 1984). Cytokinins are produced in meristematic root tips and in some cases other meristematic areas, like the shoot apex (Chen et al, 1985). Endogenous cytokinins have been extracted from xylem exudate and differentiating meristematic cells.

In 1892 J.Weisner proposed the possibility of growth controlling chemical factors in plant cell division (Wilkins, 1984). Haberlandt (Wilkins, 1984) in the early 1900's noticed that applying wound rinses or crushed tissue from wound responsive plants to tissue that lacked the wound response would initiate cell division and callus healing. It was over 25 years later that Skoog and Tsui (1948) suggested the existence of a chemical inducing factor for cell division. In tobacco pith lacking vascular tissue, auxin promoted cell elongation but not cell division. However, when vascular tissue was in contact with the pith cell division and elongation occurred. Miller (1956) and Skoog and Miller (1957) first isolated kinetin from coconut milk, autoclaved (denatured) DNA, and extracts of malt, yeast and vascular tissue. They then provided evidence that growth and organ formation is regulated partially by a chemical growth regulator.

One of the most arduous jobs in plant physiology to understand in its entirety is the biosynthesis and metabolism of plant growth hormones. Cytokinin pathways of biosynthesis and metabolism are poorly understood. This difficulty stems in part from the lack of good analytical methods. Two theoretical pathways for biosynthesis are proposed; de novo synthesis from adenine monomers and via the hydrolysis or breakdown of polynucleotides (Maab and Klambt, 1980). Both pathways may be used in different plant species, or in different stages of development. These two pathways may be necessary because of the numerous plant responses induced by cytokinin and of the over 30 "free" and bound tRNA cytokinins.

Stomatal areas are affected by exogenous cytokinin in intact plant systems of Anthephora pubescens (Incoll and Whitelam, 1977) and Kalanchoe daigremontiana (Jewer and Incoll, 1981). Mitotic activity increased around stomates in hypocotyl embryos of Picea abies applied with a pulse treatment of cytokinin (Arnold and Gronroos, 1986).

Interrelationships between roots and cytokinin were studied using exogenous cytokinin applications and measuring endogenous levels of cytokinins. Apical or basal application of kinetin or benzyladenine (BA) reduced adventitious rooting in pea cuttings (Davis and Potter, 1981) and in Picea abies embryos (Arnold and Gronroos, 1986). Other evidence also suggests that inducement of root stresses, like water logging, osmotic stress or root decapitation (Ehret and Ho, 1986; Itai and Vaadia, 1971) reduces cytokinin content in the leaves and shoots. Cytokinin content, however is not reduced totally after root decapitation in Phaseolus vulgaris plants, suggesting another source of cytokinin (Carmi and Van Staden, 1983). Cytokinin content in the stem was higher than shoots or roots.

Nordstrom and Eliasson (1986) induced shoots of apple rootstock in vitro using benzylaminopurine (BAP) in culture media with different induction periods. They showed more shoots at the longer induction period (36 days BAP), while in the shorter in-duction period (6 days BAP + 30 days basal media) shoots were fewer in number but longer. In the shorter induction period shoots developed more fully than in the longer induction period which indicates growing shoots

produce their own cytokinin.

Chen et al. (1985) postulated that cytokinin is produced in cambial and meristematic areas of excised carrot roots and pea plants, respectively. Cytokinin-active ribonucleotides in root exudate of Phaseolus vulgaris L. (Palmer and Wong, 1985) were identified using high performance liquid chromatography and gas chromatography analysis. Endogenous levels of cytokinin in bean xylem exudates fell between 10-100 ng cytokinin ml^{-1}. Previous experiments from Palmer's lab have indicated that certain cytokinin derivatives, i.e., dihydrozeatin (DHZ) and glucoside (GDZ), are produced in shoot tissues or metabolized after reaching the shoot area because they are not found in root xylem exudate.

Cytokinin stimulates chlorophyll synthesis, promotes chlorophyll differentation and prevents leaf senescence (Nowak, Mlodzianowski, and Szweykowska, 1986; Purohit, 1985; Zhang et al.1987). Cytokinin induced chlorophyll synthesis and cell differentation are researched widely regarding senescence and chlorophyll retardation. Nowak et al.(1986) used chlorophyll free carnation callus to test induced chlorophyll synthesis and differentiation by the cytokinins, BA and kinetin. BA application to chlorophyll free callus resulted in higher chlorophyll per dry weight content than kinetin application, but the reverse was true in terms of fresh weight measurements. Microscopic analysis shows that callus cultured 1.5 years on BA developed normal chloroplasts. When callus was cultured on cytokinin free medium or with kinetin added, the chloroplast thylakoids developed poorly and the starch grains were larger and more abundant

than in the BA culture. This observatiion implies that cytokinins differ in their activities.

Potassium can interact with cytokinins to affect chlorophyll. Potassium ions (K^+) activate many enzymes that are essential for photosynthesis and respiration, and also activate enzymes needed to form starch and protein. K^+ abundancy in the plant makes it a major contributor of osmotic potential in cells. Arnold and Fletcher (1986) observed chlorophyll synthesis in excised and intact cucumber cotyledons with potassium (40 mM) and BA (1 mg l^{-1}, 4.4 uM). In excised cotyledons, BA stimulated chlorophyll synthesis after 4 and 8 hours of illumination, but not 24 hours. K^+ continued to stimulate chlorophyll synthesis after 24 hours and to reduce the lag phase of chlorophyll synthesis. When both BA and K^+ were applied, chlorophyll production and cotyledon expansion were maximum. In intact cotyledons, K^+ was not limiting because foliar and soil treatments did not increase greening and BA stimulated chlorophyll synthesis when foliarly applied but inhibited it when applied to the soil. BA (30 mg l^{-1}) application to intact bean plants increased chlorophyll content for 2 weeks after initiation and chloroplast DNA was increased over controls (Kinoshita and Tsuji, 1984).

In studies where light is a factor chlorophyll content decreased when cytokinins were used in the dark (Weidhase et al.,1987). Weidhase and colleagues looked at barley leaf segment response to BA and a BA inhibitor, jasmonic acid methylester (JA-Me). They found that chlorophyll and protein

content were reduced in the dark with BA and in control leaf segments. Chlorophyll and protein content of leaf segments were not affected in the dark JA-Me treatment. When BA and JA-Me were combined, chlorophyll was decreased in the light, but began to recover after 3 days. In the dark, BA/JA-Me reacted the same as JA-Me alone. In contrast, Lehmann and Parthier (1985) found in detached pumpkin cotyledons that chlorophyll increased with increased concentrations of BA to 10 mg BA l^{-1}, but chlorophyll was not affected by light or dark regimes. The authors believe that cytokinin might stimulate plastid development in the dark and at low light intensities by means of a phytochrome gene mediated response in which ribulose-1,5,-bisphosphate (carboxylase) protein is synthesized. Ribulose-1,5,-bisphosphate is a normal substrate to which carbon dioxide is added to form phosphoglyceric acid in the dark reaction of photosynthesis.

Exogenous cytokinin attracts assimilates into leaf tissues, and may have an influence over fruit development as far as partitioning is concerned. The ability of cytokinins to attract assimilates and to induce mitosis has an important role in plant flowering and senescence. Bernier et al.(1977) measured the mitotic index in intact Sinapis alba plants (long day-LD) and found that applications of BA (1, 5, and 10 ug l^{-1}) and zeatin (1, 10, and 20 ug l^{-1}) directly to apical buds induced mitosis at nearly the same rate as plants induced to flower by a 20 hour photoperiod, but did not induce flowering. Havenlange, Bodson, and Bernier (1986) used intact Sinapis plants grown at two floral induction periods (short day

induction, SDI and long day induction, LDI) with BA (43 uM) to test BA's ability to induce the photoperiodism response in Sinapis. They found that SDI plants were induced by cytokinin to produce some of the responses found in LDI plants ,e.g., increased starch content, increased number and decreased size of vacuoles, rise in mitotic activity, and synchronization of meristematic cell division in G1 phase of mitosis. Increases of soluble carbohydrates and acid invertase that occur in LDI plants did not occur in treatments of SDI plus BA.

Cytokinins may be involved in regulation of proton extrusion resulting in cell wall loosening. Kappler and Kristen (1986) applied cytokinins (kinetin, zeatin, and 6-benzylaminopurine) each at 0.46 or 4.65 uM to the root tip cortex of corn, and induced separation of cortical cells or the expansion and isolation of cortical cells. They believe that this expansion and isolation in corn root cells may result from localized cell wall loosening in the growing root. The authors agree that cytokinin action may be involved in regulation of proton extrusion, as far as cell expansion growth, wall extensibility, wall acidification, and H+ generation and extrusion.

As stated at the begining, cytokinin can induce a variety of physiological and biochemical responses in plants. No hard evidence indicates exactly where cytokinins are actively produced or what their effect is on the overall status of the developing plant.

Cytokinin Interactions with Plant Stresses

Plant stresses most commonly are measured in terms of the changes in

water status, stomatal aperture, and metabolites of the plant (Greenway and Munns, 1980; Levitt, 1980). Stomata will open when the turgor pressure in guard cells increases. This increase in turgor is an osmotic effect due to the accumulation of inorganic and organic ions in the vacuoles of the guard cells. Livine and Vaadia (1965) proposed that stomatal opening is conditioned by normal leaf metabolism, the growth hormones (kinetin and gibberellic acid), and whatever osmotic mechanism responsible for stomatal movements are closely linked to and/or controlled by leaf metabolism. They also proposed that under root water stress stomatal closure reduces translocation of naturally occurring root factors (hormones) to the shoot.

Waterlogged tomato plants have reduced stem growth, chlorosis of lower leaves, epinastic curvature of leaves, production of adventitious roots, wilting, and thickening of the stem (Railton and Reid, 1973). Many of the above characteristics of waterlogging are typical of toxic levels of auxin. BA (50 mg l^{-1}) applied prior to waterlogging partially alleviated reduction in stem growth and stem thickening.

Root growth restriction (root trimming) of bean (Phaseolus vulgaris) plants caused dwarfing (e.g., smaller leaves and shorter internodes). Foliar applied BA did not affect trifoliolate leaves or internode length, but did increase the growth of primary leaves (Carmi and Heuer, 1981). When a combination of GA_3 and BA were used, internode and primary leaf growth was improved. However this combination of hormones did not restore totally normal growth.

Xylem fiber formation was decreased in sunflower plants by lowering transpiration rate (by increasing the humidity) or by root removal (Saks, Feigenbaum and Aloni, 1984). In the presence of kinetin (0.5 ug ml^{-1}) the fiber formation of the above treatments was increased considerably. Higher concentrations of kinetin were recorded to cause inhibitory effects.

Endogenous cytokinins from xylem sap were increased in tomato and tobacco when plants were disbudded to alter their sink-source relationship (Colbert and Beever,1981). Chlorophyll content and chloroplast integrity were maintained in disbudded plants, whereas the opposite was found in plants that were not disbudded. The increase in cytokinins in the xylem sap may be a wounding response to disbudding. Disbudded plants (plants kept in the vegetative stage) have the ability to prevent leaf senescence because other sinks (buds) have been removed.

Cytokinins play a role in regulating plant response to root water stress (Itai and Vaadia, 1971). They suggest that an inactivation of cytokinin is in process during stress, either due to inhibition in accumulation of cytokinin during stress or transformation of the cytokinin molecule.

Benzioni, Mizrahi and Richmond (1974) showed that kinetin applied to roots of salt stressed (NaCl) tobacco caused accumulation of NaCl in the shoots, but also prevented leaf necrosis. Kinetin also dwarfed plants and reduced plant dry weight significantly. They suggested that kinetin reduces cell breakdown (cellular senescence) rather than preventing salt accumulation as a means of

reducing salt induced leaf necrosis.

The cytokinin, kinetin, reduced effects of salt stress on plants when foliarly applied. Salinity reduces growth and essential oil formation in spearmint and marjoram (El-Keltawi and Croteau, 1987). Foliar applied cytokinins, diphenylurea (10 ppm) and kinetin (4 ppm), partially alleviated growth and oil reductions incurred by salt, but without salt, kinetin impaired growth. Naqvi, Ansari and Khanzada (1982) used wheat seedlings grown in the dark with salt stress (100 and 170 mM NaCl) and kinetin (1 and 5 uM) foliar applications, and found kinetin without salt and salt without kinetin treatments reduced shoot and root growth. When kinetin and salt were added simultaneously the decreases in growth were reversed partially.

Katz, Dehan and Itai (1978) used tobacco leaf discs floating on 0.1 % NaCl (w/v) solution to test the response of kinetin and ABA on salt induced stress. Kinetin alleviated the stomatal stress effects by 10 percent, but was still only approximately 60 percent of the control. Carbon dioxide fixation was increased to the level of the control discs and chlorophyll content was 90 percent of the control in the salt plus kinetin treatment.

Osmotically stressed plants have reduced water uptake and cytokinin can alter this stress response in plants. In a study using excised germinated cotyledons of sponge gourd (Luffa cylindrica), BA (25 uM) application increased the fresh weight, soluble solids, and amino acids, while decreasing the osmolality in stressed (-0.5 MPa PEG-6000) and nonstressed cotyledons (Virk et al 1985).

The authors attribute this response to an increase in the capacity for water uptake by modifying the cell wall characteristics.

Endogenous levels of cytokinins, other hormones and compounds change during salt stress. Tobacco plants were evaluated for their abscisic acid (ABA) and cytokinin contents under high (100%) and low (less than 10%) humidities with and without salt (Mizrahi et al.,1971). Their findings differ from Itai and Vaadia (1971), in that Mizrahi and coworkers found no detectable decrease in leaf cytokinins in the high humidity or salt stressed treatments, whereas Itai and Vaadia found a decrease in cytokinin in the stem exudate and leaf tissue of salt stressed plants. On salt stressed barley, BA inhibited wilt induced proline accumulation, whether it was applied pre- or post-salt stress (Stewart, Voetberg and Rayapati, 1986). However, ABA accumulation and leaf wilting were not prevented. Walker and Dumbroff (1980) found reductions in endogenous cytokinins, trans-zeatin and cis-zeatin after 1 day of salt stress (-0.6 MPa), whereas the ribosides of trans-zeatin and cis-zeatin increased. After 4 days of stress, levels of endogenous cytokinins returned to normal.

Plant response grown under hydroponic conditions vary greatly when drought or heat shocked. In aeroponically grown sunflower, endogenous levels of cytokinin and other hormones were measured (Caers et al., 1985). Plants were drought stressed to -1.23 MPa by reducing water sprays to the roots. Plants showed a minimal change in GA_3 and ethylene; ABA 32 times higher; and cytokinin half the level of non-stressed (-0.37 MPa) plants.

The level of specific hormones may not be important as the ratio between the hormones (Hubick, Taylor and Reid, 1986). Caers et al.(1985) dipped the roots of light and dark grown corn plants in 47.5°C water for 2 minutes. The heat treated excised leaf segments had reduced shoot and root growth, photosynthetic activity, and chlorophyll accumulation. Etiolation of plants also showed inhibition of chloroplast development. BA (1uM) applications could reverse all the above parameters except for growth. Endogenous cytokinins were reduced in green heat stressed plant segments, but cytokinins were not detectable in any of the etiolated plant segments. This finding was probably due to low levels and methods for extracting cytokinins.

MATERIALS AND METHODS

Four different experiments were conducted to study the effects of salt and the cytokinin, labelled benzyladenine (BA) on chili pepper (Capsicum annuum L.) cv.'New Mexico 6-4' plants (courtesy of New Mexico State University). The first was a study of the incorporation and movement of labelled BA in the plant. The second was a study to find the appropriate salt concentration to cause significant reduction in growth for chili peppers hydroponically grown at one of the University of Arizona campus greenhouses.The third was exogenously applied benzylaminopurine (BAP) to salt stressed and nonstressed chili pepper plants grown hydroponically. The fourth was a field trial at the University of Arizona experimental farm in Safford, Arizona. Each experiment was a completely randomized design.

Labelled Cytokinin Studies

Plants were grown in 8 inch long plastic rooting tubes in a vermiculite:peat:perlite (1:2:1 v/v/v) mixture at one of the University of Arizona campus greenhouses. Three seeds were planted per tube, watered every 2 days, and fertilized every week with Peters 20-20-20 (N-P-K), using the manufacturer's recommended rate for vegetables. Plants were grown to the second leaf stage (cotyledons fully expanded but first leaves not) or to the third leaf stage (cotyledons senescing, first leaves fully expanded, and second leaves present).

Labelled benzyladenine (benzyl 8-14 adenine, ^{14}C-BA) was acquired from Amersham Corporation (Amersham,UK). The ^{14}C-BA had a molecular weight of

227 grams at a specific activity of 57 mCi $mmol^{-1}$ (2.11 GBg $mmol^{-1}$). The amount applied was determined by reviewing Clark and Hackett (1979), who used a 10 ul drop of ^{14}C, BA at 65,000 dpm (1 mg BA^{-1}). In our case we increased it to 88,880 dpm for ease of preparation. Our vial of ^{14}C-BA contained 10 uCi in a powder form which was dissolved in 0.5 ml of 85% ethanol (v/v). This labelled solution is equivalent to 44,440 dpm ul^{-1} (calculations and equivalencies are given in Appendix A).

To avoid non-specific adsorption, all glass material coming in contact with ^{14}C-BA was silylated before use by the following procedure. Silylation breaks the -OH bond by removing the hydrogen and replacing it with $Si(CH_3)_3$. The silylating solution consisted of Sigmacote (Sigma, St.Louis, MO) stock SL-2, diluted 10% into toluene. The silane and toluene are highly volatile and toxic, and all silylation is performed under a laboratory hood. Treated surfaces of glassware was placed in contact with the silylating solution for five minutes. The glassware was then rinsed with methanol and washed with soap and water.

Two plants were used for each treatment of 8 and 24 hours. Using a 1 ul Hamilton syringe, 2 ul application of ^{14}C-BA was made at midday in a room with the lights off (to prevent breakdown of BA) at either the cotyledon, stem, or first true leaf. The ^{14}C-BA was kept in place using lanolin dams (Fisher Scientific Co.,Fair Lawn, NJ). Plants were kept in rooting tubes and left in the dark for 20 minutes. Plants then were rinsed with 5-6 ml of deionized water and washes were pooled in scintillation vials for analysis. Plants were then illuminated under fluorescent lights at room temperature for either 8 or 24 hours, after which plants

were excised and separated into different parts using a disposable scalpel.

Plant samples were placed in plastic scintillation vials with 1 ml toluene tissue solubilizer (TS-1, Appendix A, Research Products International, Mount Prospect, IL) for 24 hours. Bleaching solution (Appendix A) about 5 ml was then added and samples were left under fluorescent lights for 24 hours. Fifteen ml of liquid scintillation counting solution (Handiflour, Scintillar, Div. of Malinkrodt Inc., St.Louis, MO, Appendix A) was added to plant samples and washes. All samples were counted in a Beckman LS 8100 liquid scintillation counter. Five blanks containing solubilizer, bleaching solution, and scintillation solution were placed ahead of the samples within the counter. The blanks insured that the plant samples had time to defluoresce prior to machine reading of the samples (approx. time 50 minutes).

Calculations from counts per minute (cpm) to disintegrations per minute (dpm) are given in Appendix A using a quench correlation curve for ^{14}C toluene. The percent of ^{14}C per plant part was calculated on the bases of the total ^{14}C recovered.

Salt Hydroponic Studies

Prior to transfer to a hydroponic system, cultural growing conditions for plants in this study were the same as in the experiment using labelled BA. Twenty-four plants at the third or fourth leaf stage were transferred to 12 tubs (2 per tub) in a hydroponic system. The hydroponic system consisted of 12 plastic 25 x 33 x 15 cm tubs containing 8 liters of water each with Hoagland's standard nutrient solution (Appendix B). Aquarium air pumps, Secondnature Challenger

II, attached with tygon tubing (1/4 inches outside diameter) and glass air stones were used for aeration of water. One air pump was used to aerate four tubs. A 0.75 inch thick piece of styrofoam, floating on the nutrient solution in each tub, served to support the plants. Foam squares (3.8 x 3.8 cm) slit diagonally half way to fit into holes (2.54 x 2.54 cm) cut into the styrofoam. Plants were placed in the foam slit and put into the styrofoam holes. A homemade trellis suspended from the ceiling of the greenhouse was used to support the plants as they matured.

At the time of transfer to the hydroponic system, the temperature was measured with a mercury thermometer. The air temperature was 15°C and the hydroponic solution temperature was 19oC. During the seedling's first 2 weeks in hydroponic's the air temperature fluctuated from 14°C (night) to 24°C (day) and water temperature between 18°C (night) to 20°C (day). Daylength during the course of the experiment ranged from 9 to 11 hours.

Beginning ten days after transferring the seedling to hydroponics, the salt concentration of the hydroponic solution of selected tubs was gradually increased. Concentrations of NaCl: $CaCl_2$ (3:1, w/w) at 3 g l^{-1} (-0.25 MPa equivalent) were added every 3 to 7 days. Twenty-five days after the start of the salt additions, the hydroponic solution of two tubs had the following salt concentrations: 0, 3, 6, 9, 12, and 15 g l^{-1} (-0.00, -0.25, -0.50, -0.75, -1.00 and -1.25 MPa, respectively). The pH remained at acceptable levels between 6.5 and 7.3 during the course of the experiment.

Both prior to salt additions and 31 days from placing the seedlings in hydroponics, transpiration and stomatal conductance of the youngest fully expanded leaf were measured at midday with a Li-Cor steady state porometer (LI-1600, Li-Cor, Inc., Lincoln, NE). At the later time of measurement one fully expanded leaf per plant was also taken for estimation chlorophyll content. Leaf fresh weight (g) and area (cm^2) were taken prior to extraction of chlorophyll. Chlorophyll was extracted and measured using the technique of Knudson, Tibbitts, and Edwards (1977), except 95% ethanol was used instead of 100% ethanol to extract the chlorophyll. Plants were harvested 7 weeks and 5 days after transferring plants to hydroponic culture, when shoots and roots were separated. Fresh and dry weights were recorded, with plant material dried in 60°C oven.

Cytokinin Salt Studies

Pepper seeds were planted in soil in an evaporative-cooled greenhouse on February 1, 1987. At 31 days from sowing (third to fifth leaf stage) seedlings were transferred to a hydroponic system as described above. Growing conditions were the same as the previous experiment except that the mean temperatures (air and hydroponic solution) were about 7°C warmer.

All of the twelve tubs contained standard Hoagland's nutrient solution. Six of these contained, in addition, 9 g l^{-1} (-0.75 MPa) salt (same ratio and type as previously mentioned). Which was gradually brought to this concentration as described previously. This salt concentration was selected on the basis of plant response in the salt hydroponic studies.

Benzyladenine (225.2 grams m.w., lot 15F-0739 from Sigma Corporation, St.Louis, MO) was dissolved in 10 ml 1 N KOH and warmed in an oven at 50°C for 20 minutes. A solution of 1% (v/v) Triton X-100 in distilled water was used as a surfactant with and without BA. Solutions were applied with plastic spray bottle at three different concentrations of 0, 50, and 100 mg l^{-1} on March 6, 1987 at 3:30 pm. The entire above portion of the plants were applied foliarly with BA until run-off occured. Brown paper bags were cut to confine foliar sprays to target areas and to prevent runoff into root systems. Experimental design was a 2 by 3 factorial replicated twice with two levels of salt (0 and 9 g l^{-1}) in the hydroponic solution and three levels of BA (0, 50, and 100 BA mg l^{-1}) applied foliarly to the plants.

Parameters measured were the same as in the previous experiment, ie., transpiration, stomatal conductance, and chlorophyll. Additional measurements taken included stem length, axil number, flower bud number, and flower number while in the hydroponic culture. Plants were harvested 83 days from the application of BA and 116 days after seed sowing. At harvest, fresh weights, fruit number, and fruit weight were recorded. Dry weights were recorded after air drying for 8 days followed by oven drying for 20 minutes at 60°C.

A electrical shortage in the greenhouse just prior to harvest made it necessary to move all plants to another greenhouse at the University of Arizona, Campus Agricultural Center. Plants were returned to the original greenhouse one day later, but some were severely injured due to the rough handling and the lack of aeration to the roots. The lack of air caused full or partial defoliation to

plants in four of the hydroponic tubs. Hence, data on shoot weight (fresh and dry) is actually stem weight, because prior to harvest measurements, leaves were removed from all plants in order for treatments to be equivalent.

Salt Field Studies

Peppers were direct seeded on April 24, 1986, at the University Arizona, Safford experimental farm. The bed spacing was 40 inches (1.02 m) with 120 feet (36.6 m) rows and a planting rate at 4 pounds per acre (4.48 kg ha^{-1}). Preplant irrigation and subsequent irrigation as needed for optimal crop estabishment were used. Fertilizer, herbicide, etc. were applied as commercially practiced for chili peppers. Plants were watered with high saline water in the salty plot and low saline water in the nonsalty plot. Soil was a sandy loam type and soil samples were taken at approximately 6 inches (0.15 m) soil depth. The salinity of the high salt plot was 5.00 to 7.50 mmhos cm^{-1} (high to very high salinity) and the low salt plot was 2.50 to 6.75 mmhos cm^{-1} (medium to very high salinity). Stand establishment was good in some areas and poor in others. Thus, thinning and transplanting from areas of heavy to light stand were performed to establish uniform stands on June 12, 1987. This attempt was effective, although plant stands remained uneven and irregular in the plots. Due to the poor and uneven stands, applications of BA were not performed. However, data was gathered to compare the differences between pepper response on the high and low salt plots.

Transpiration and stomatal resistance were measured as previously mentioned on a fully expanded leaf on all plants about midday. A fully expanded leaf was used to measure water potential with a preasure bomb (PMS-600,

Corvalis, OR) on July 24, 1986. At this time, the average mature leaf weights were determined by removing 5 mature leaves from 2 plants in both the high and low salt plots.

Two plants, about 2 feet (0.61 m) tall from each of the high and low salt plots, were dug at midday and brought back to campus on August 28, 1986. Stem diameter above the first node, leaf weight, and fruit number were measured. Fruit size and weight were based on standard classification (Appendix C).

Peppers were harvested from 2 plants in both the high and low salt plots on the following dates: September 3, September 10, and October 1, 1986 (different plants were used for each harvest date). On September 10 at midday, soluble solid analysis was performed by crushing fully expanded leaves with pliers and letting plant exudate drip on the refractometer (Bausch and Lomb range 0 to 60 %). This was done in ten different areas throughout both the high and low salt plots. The harvested fruit were critiqued for marketability on the latter three harvest dates (see Appendix C for marketability standards).

RESULTS

Labelled Cytokinin Studies

Figure 1 shows uptake of ^{14}C-BA in the plant at three different application sites and two stages of growth. At the first true leaf stage ^{14}C-BA was applied on the stem or on the cotyledons, and at the second true leaf stage ^{14}C-BA was applied to the first true leaf. When ^{14}C-BA was applied to the stem, ^{14}C-BA largely remained at the point of application (76.15%), with percentages of recovered isotope found in the first true leaves of 11.23, and in the cotyledons of 5.18. The dispersion of ^{14}C was greater in plants where the label was applied to the cotyledons. The first true leaf had 35.37% of the isotope and the apical tip had 3.45%. This percentage is more than a three-fold increase over that of the stem site application at the same stage of growth. When ^{14}C-BA was applied to the first true leaf (plant at second true leaf), the label dispersed very little. Overall, the ^{14}C was translocated least from application sites on the stem and petiole tissue, and most from application sites on the leaf. In general, translocation of ^{14}C-BA was acropetal to the point of application.

Salt Hydroponic Studies

Results of the salt experiment are shown in Table 1, where the parameters measured are growth and transpiration. A significant decrease in dry weight was seen first between 0.00 and -0.25 MPa. A further decrease in dry weight and the first significant decrease in fresh weight and transpiration were observed between -0.50 and -0.75 MPa. At this level of salt stress, reductions in root dry

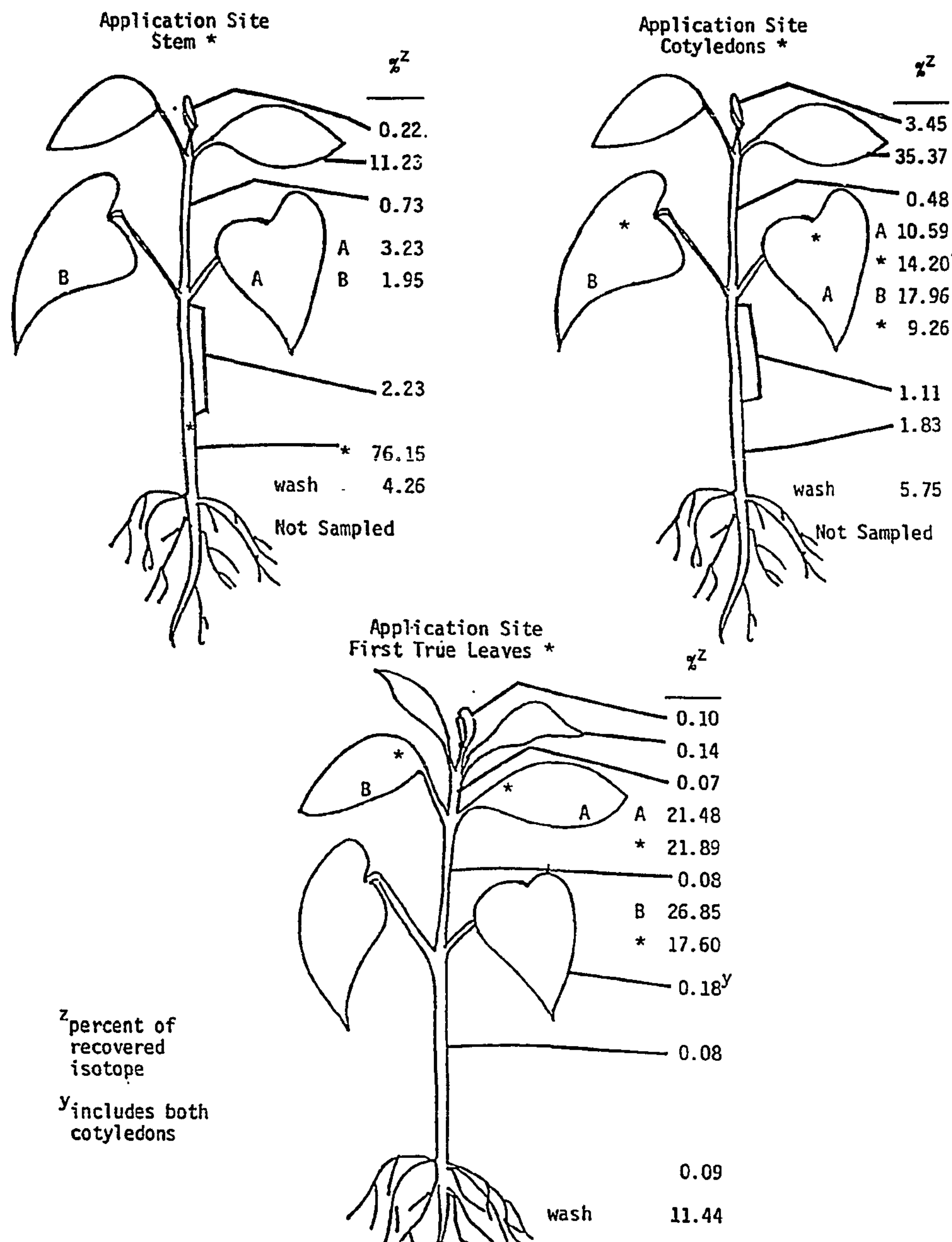


Figure 1. Application points and uptake of labelled benzyladenine applied to three different sites and two stages of growth of greenhouse grown chili pepper plants.

Table 1. Effects of various concentrations of NaCl and $CaCl_2$ (3:1, w/w) on whole plant weights and transpiration of hydroponically grown chili pepper plants. The measurements were taken six days after salt concentrations were added to hydroponic solution. The salt mixture was gradually added over a 2-3 week period.

Salt Conc. (MPa)	Fresh Wt	Dry Wt	Transpiration ($ug\ H_2O\ cm^{-2}s^{-1}$)
	----------grams----------		
0.00	70.71 a[z]	8.70 a	10.43 a
-0.25	60.59 a	6.70 b	10.89 a
-0.50	54.89 a	5.95 b	10.35 a
-0.75	24.53 b	2.85 c	7.10 b
-1.00	9.79 b	1.65 c	5.95 b
-1.25	8.80 b	1.40 c	4.85 b

[z]Mean separation within columns by LSD at the 5% level.

weight, shoot dry weight, and chlorophyll content were also significant (Table 2).

The greatest reductions in biomass (Tables 1 and 2) were found in the plant dry weights rather than the fresh weights at all levels of salt stress. A significant difference is seen first at -0.25 MPa (Table 2) in the dry weight of shoots. Dry weight loss is due mostly to shoot dry weight loss. Chlorophyll reductions also can be observed at -0.25 MPa. Since significant reductions were found in all parameters measured at the -0.75 MPa salt, this level of salt was chosen for the cytokinin studies.

Cytokinin Salt Studies

Transpiration, stomatal resistance and chlorophyll concentration were measured 24 hours after BA application (Table 3). Table 4 shows the harvested biomass at the end of the experiment. For rates twenty-four hours after application, BA had no significant effect on transpiration, stomatal conductance, and chlorophyll content (Table 3). No significant interactions were present between the factors of salt and BA. There was, however, a significant effect of salt on transpiration and stomatal conductance 24 hours after the application of the BA treatments (Table 3). Transpiration decreased and stomatal resistance increased as a result of salt stress, but salt had no significant effect on chlorophll concentration.

Harvest data indicate significant reductions in fruit weight and plant dry weight due to salt stress plants (Table 4). However, fresh weights were not different between stressed and nonstressed plants, probably due to the removal of all leaves prior to weighing.

Table 2. Effects of various concentrations of NaCl and $CaCl_2$ (3:1, w/w) on dry weights of shoots and roots, and chlorophyll a content at harvest of hydroponically grown chili pepper plants. The salt mixture was gradually added over a 2-3 week period.

Salt Conc.	Shoot DW	Root DW	Chlorophyll a[z]
(MPa)	--------grams-------		(ug/mg DW)
0.00	6.45 a[y]	2.30 a	2.03 a
-0.25	4.90 b	1.85 a	1.55 b
-0.50	4.20 b	1.80 a	1.42 b
-0.75	2.00 c	0.85 b	0.96 c
-1.00	1.10 c	0.53 b	0.59 c
-1.25	0.88 c	0.50 b	0.67 c

[z]Total chlorophyll showed a significant difference which was due to chlorophyll a since chlorophyll b was not significantly different among treatments.

[y]Mean separation within columns by LSD at the 5% level.

Table 3. Effects of a mixture of NaCl and $CaCl_2$ salts (3:1, w/w) on the transpiration, stomatal resistance, and chlorophyll content of hydroponically grown chili pepper plants. Measurements were taken 32 days after transfer of 12-day-old seedlings to hydroponic culture. The salt mixture was gradually added over a 2-3 week period.

Salt Conc. (MPa)	Transpiration ($ugH_2O\ cm^{-2}\ s^{-1}$)	Stomatal Resistance ($s\ cm^{-2}$)	Chlorophyll (ugChl/mg DW)
0.00	74.15 a[z]	0.07 a	8.51 a
-0.75	59.94 b	0.19 b	8.66 a

[z]Mean separation within columns by LSD at the 5% level.

LSD's$_{0.05}$ given in Appendix D.

Table 4. Effects of a mixture of NaCl and $CaCl_2$ salts (3:1, w/w) on whole plant harvested biomass (stem, roots, or fruit fresh weight) of hydroponically grown chili pepper plants. Measurements were taken 102 days after transfer to hydroponic culture. The salt mixture was gradually added over a 2-3 week period.

Salt Conc. (MPa)	Fresh Wt.	Dry Wt.	Fresh Fruit Wt.
	----------grams----------		
0.00	205.00 a[z]	31.73 a	201.84 a
-0.75	174.48 a	21.41 b	112.34 b

[z]Mean separation within columns by LSD at the 5% level. LSD's$_{0.05}$ given in Appendix D.

No significant effect of BA applications on alleviating dry weight biomass reductions induced by salt were measured (Table 5). A trend appeared for plant root and shoot dry weights in the -0.75 MPa : 100 mg l^{-1} BA to show some relief to the salt stress, but these trends were not significant. A slight inhibitory effect to the whole plant dry weight was noted when BA was applied to a nonstressed plant. Also biomass in the salt stressed plants applied with the higher concentration of BA appeared to recover somewhat, but not at the lower concentration of BA.

Buds were counted at 3 days, fruit set at 35 days, and fresh fruit weight at 67 days after BA foliar sprays (Table 6). The BA sprays showed no statistical differences on the number of bud or the number of fruit set on plants subjected to salt stress. Non-stressed plants treated with 100 mg l^{-1} BA had twice the number of set fruit as plants not receiving the BA treatment. Total fruit weight was increased by BA at the highest concentration in both stressed and nonstressed plants.

Effects of salt on number of flower buds, flowers, and fruit set, as well as fruit size are shown in Table 7. A significant difference was found between the 0.00 and -0.75 MPa salt treatments in all parameters measured. These findings show an 18% decrease in fruit number, 44% decrease in total fruit weight and a 39% decrease in average weight per fruit at 67 days.

In Table 8, significant differences were found in chlorophyll concentration and leaf density (fresh and dry) between the salt stressed and nonstressed plants. The application of BA did not affect any of the above mentioned parameters

Table 5. Effects of a mixture of NaCl and $CaCl_2$ salts (3:1, w/w) and benzyladenine on dry weight biomass yields (g) of hydroponically grown chili pepper plants. Measurements were taken 102 days after transfer of 12-day-old seedings to hydroponic culture. The salt mixture was gradually added over a 2-3 week period.

BA	Whole Plant		Shoot		Root	
	Salt[z]		Salt		Salt	
(mg l^{-})	-	+	-	+	-	+
0	34.58[y]	20.61	22.83	12.97	11.75	7.61
50	31.90	16.03	20.82	9.28	11.09	6.74
100	28.70	27.85	18.02	16.05	10.68	11.80

[z]Concentrations of salt mixture in hydroponic solution as follows: -, no salt; +, 9.0 gm l^{-} NaCl:$CaCl_2$ (3:1, w/w).

[y]No significant interactions Between salt and benzyladenine treatments. LSD's$_{0.05}$ given in Appendix D.

Table 6. Effects of a mixture of NaCl and $CaCl_2$ salts (3:1, w/w) and benzyladenine on budding, fruit set, and fresh fruit weight of hydroponically grown chili pepper plants. The salt mixture was gradually added over a 2-3 week period.

	Number of Buds (3 days)		Number of Fruit Set (35 days)		Fresh Weight of Fruit (g) (67 days)	
BA	Salt[z]		Salt		Salt	
(mg l⁻)	-	+	-	+	-	+
0	15.0[y]	14.5	2.5	1.5	175.2	83.0
50	17.3	10.5	4.8	1.3	179.8	64.1
100	15.8	12.55	5.0	1.3	232.5	189.3

[z]Concentrations of salt mixture in hydroponic solution as follows: -, no added salt; +, 9.0 gm l⁻ $NaCl:CaCl_2$ (3:0, w\w).

[y]No significant interactions between salt and benzlyadenine treatments. LSD's$_{0.05}$ given in Appendix D.

Table 7. Effects of various concentrations of a mixture of NaCl and $CaCl_2$ salts (3:1, w/w) on the number of flower buds, flowers, fruit set and fruit harvest of hydroponically grown chili pepper plants. Measurements were taken 102 days after transfer of 12-day-old seedlings to a hydroponic culture. The salt mixtures were gradually added over 2-3 week period.

Salt	Number of Buds		Number of Flowers		Number of Fruits		Total Fresh Fruit Wt.	Average Fruit Wt.
	---per Plant---						---grams---	
	DAYS[z]							
(MPa)	3	17	23	35	35	67	67	67
0.00	7.5a[y]	16.0a	2.8a	7.5a	4.1a	46.3a	201.9a	14.7a
-0.75	6.3b	12.5b	1.58b	4.1b	1.5b	38.0b	112.3b	9.0b

[z]Time in days after benzyladenine application.

[y]Mean separation within each column by LSD at the 5% level. LSD's$_{0.05}$ given in Appendix D.

Table 8. Effects of a mixture of NaCl and $CaCl_2$ salts (3:1, w/w) on leaf characteristics after benzyladenine application of hydroponically grown chili pepper plants. Measurements were taken 35 days after benzyladenine application. The salt mixture was added gradually for 2-3 weeks after transfer of plants to the hydroponic culture.

Salt Conc. (MPa)	Chlorophylls (ug Chl mg^{-1} DW)		Fresh	Dry
	Chla	Chlb	-----Density---- (mg tissue cm^{-2})	
0.00	1.85 a[zy]	2.57 a	25.23 a	4.18 a
-0.75	2.89 b	3.87 b	25.07 a	3.01 b

[z]Means are averaged across benzyladenine treatments.

[y]Mean separation within columns by LSD at the 5% level.

within the 5% LSD level.

Salt Field Studies

Fruit harvest (Table 9) showed significant differences in individual fruit weight, marketability and non-marketability between the low and high salt plots. The largest percent difference was found in the non-marketable fruit weights in low and high salt plots. Also this characteristic had the highest variability in the actual numbers recorded (Appendix C).

Table 10 shows plant status in the field when chili peppers were grown under low and high salt levels. In the low salt plot, transpiration rates were higher with lower stomatal resistances, but differences were not significant. The high salt plot had significantly lower water potential and higher soluble solids.

Table 9. Effects of salt on fruit yield and marketability 166 days after planting on chili pepper grown under field conditions.

Salt[z] Level	Average Wt. per Fruit (g)	% Marketable (g)		% Non-Marketable (g)	
		By Wt.	By No.	By Wt.	By No.
Low	29.5 a[y]	70.8 a	71.6 a	29.2 a	28.4 a
High	36.6 b	58.1 b	55.8 b	41.9 a	44.2 b

[z]Salt levels for the low salt area, 2.50-6.75 mmhos cm^{-1} and high salt area, 5.00-7.50 mmhos cm^{-1} were acquired at a depth of 15 cm from 4 sample areas throughout both field plots at the time of planting.

[y]Mean separation within columns by LSD at the 5% level.

Table 10. Effects of salt on transpiration, stomatal resistance, water potential, and soluble solids 140 days after planting chili pepper under field conditions.

Salt[z] Level	Transpiration ($ug\ H_2O\ cm^{-2}\ s^{-1}$)	Stomatal Resistance ($s\ cm^{-1}$)	Water Potential (MPa)	Soluble Solids (%)
Low	32.39 a[y]	0.67 a	-1.26 a	14.6 a
High	26.77 a	0.88 a	-1.66 b	18.0 b

[z]Salt levels are the same as in Table 9.

[y]Mean separation within columns by LSD at the 5% level.

DISCUSSION

Differences were first seen at lower salt concentrations for dry weight than fresh weight. Initially, when a plant is salt stressed, osmotic potential is reduced making it harder for the plant to absorb water and nutrients. The reduction in water will lower plant volume, and reduction of nutrients (e.g., N, P, K^+) can effect the synthesis of proteins and other compounds (Levitt, 1980; Turner, 1985). Salt stress can reduce photosynthates by direct salt effects or by reducing photosynthesis through stomatal closure (Shoe and Gale, 1983). Plants under moderate salt stress will adjust osmotically by changing (lowering) their internal water potentials. Considering the above statements, salt stressed plants may adjust osmotically better but have reduced biosynthetic metabolism. This reduction would explain the lower dry weight. Also, since shoot dry weight was less than root dry weight at a lower salt level, this would imply that shoot metabolism is more sensitive.

Leaf chlorophyll was reduced significantly at the same salt level as dry weight. Reduction in photosynthesis and stomatal closure will reduce chlorophyll synthesis and development. Reduction of potassium uptake in the plant due to salt may cause reduction in chlorophyll synthesis (Arnold and Fletcher, 1986). Application of BA had no significant effect on alleviating chlorophyll decreases caused by salt. This observation is contrary to the findings of others. Nowak et al. (1986) using chlorophyll free carnation callus, found that kinetin and BA could increase chlorophyll content in their treatments. Katz et al. (1978) also found

that exogenously applied cytokinins increase chlorophyll content in NaCl stressed tobacco leaf discs.

The salt field studies had significant differences in harvested fruit weight and fruit number. Papadopoulos (1986) found opposite results in bell pepper fruit grown under high sulfates. This may be due to the difference between the pepper varieties or that soil sulfates affect pepper fruit development different than the salts in the soils in this study. The increase in misshapened chili pepper fruit (unmarketable) in the high salt plot agrees with Papadopoulos (1986) findings for bell pepper.

In salt stressed plants osmotic adjustment occurs in the xylem water transpiration stream and between the transpiration stream and growing tissues (Westgate and Boyer, 1984). This adjustment in growing tissues is due to assimilate accumulation. Soluble solids, which are mostly sugars, are the major assimilates in osmotic adjustment in stressed plants. In the high salt plot soluble solids are significantly higher indicating osmotic adjustment, which is supported by the lower water potential of plants grown in the high salt plot.

With applications of ^{14}C-BA, ^{14}C moves within the plant, and movement depends on tissue of application, age of tissue and application site relative to the position on the plant. Stem application of BA had the least movement through the plant, possibly due to lack of sufficient infiltration. The lack of stomates on the stem might inhibit BA movement into the stem. The greatest percentage of recovered label that moved from the point of application ocurred when BA was applied to cotyledons and moved into the first true leaves. Whether the ^{14}C was

still in BA or metabolized to another compound is not known.

Application of foliar BA to the whole plant had no significant effect on salt stressed or nonstressed chili pepper plants. The following reasons may explain this. The first reason may be that whole plant applications of BA cause multi-sink areas. In addition to assimilates being attracted to more desirable developing areas (e.g., new flowers or fruits), there would be assimilate movement into developing leaves, as my study has shown. It is feasible that whole plant application may cause assimilate movement into matured or senescening areas also. Application of BA to select plant organs may have an effect that whole plant application does not. Another reason may be due to the breakdown in the hydroponic system previously mentioned which inhibited growth in all treatments causing reductions in harvest data. The third is that BA does not significantly enhance biomass production in either stressed or nonstressed chili pepper plants. The final reason may be the experimental design, since only two replications with two plants were utilized. A larger sample number may have showed treatment differences between individual plant responses.

BA sprayed on the entire plant may cause sinks to develop throughout the plant, thus reducing the movement of assimilates to priority areas (e.g., developing plant organs). Reports show that mitotic activity increases (Arnold and Fletcher, 1986; Bernier, et al.,1977) and both chlorophyll synthesis and differentiation is enhanced (Arnold and Fletcher, 1986; Nowak, et al., 1986). Fresh weight, soluble solids, and amino acids increase (Virk et al., 1985) when endogenous cytokinins are applied to plants of Picea abies and

Sinapis alba or callus of carnation and sponge gourd. The increases in plant activity due to cytokinin mentioned above are a result of application to specific plant organs or tissues.

The conclusions that can be drawn by these studies are limited because the effect of cytokinins on plants is not clear. However, it would be safe to deduce that cytokinin use in the private sector is not recommended at this time. There are no conclusive reports that this author has reviewed or that this research has found that would suggest the use of cytokinin as a way of modifing plants response to stress. More research into the mode of action, biosynthesis and incorporation of cytokinin in the plant is needed before this can be considered for economic use in the alleviation of plant stress.

APPENDIX A

EQUIVALENCIES AND CALCULATIONS FOR ISOTOPE STUDIES

A vial of Benzyl(^{14}C)adenine contains 10 uCi (370 KBaq) at a specific activity of 57 mCi $mmol^{-1}$.

10 uCi = 22.2 * 10^6 dpm.

^{14}C-BA was dissolved in 500 ul 85% ethanol.

Application rate calculation:

22.2 * 10^6 dpm / 500 ul = 0.0444 * 10^6 dpm ul^{-1}

= 44,400 dpm ul^{-1}

2 ul per plant = 88,800 dpm application per plant.

Toluene Tissue Solubilizer (TS-1)

TS-1 (Research Products International, Mount Prospect, IL>) solution made to 0.33 N with Toluene.

Bleaching Solution

70 mg benzoyl peroxide in 0.5 ml toluene.

5 ml added to each vial for tissue bleaching.

Liquid Scintillation Counting Solution

Handifluor (PPO, Scintillar Division of Malinkroot, Inc. St. Louis, MO.), a toluene-based scintillation solution with concentration of approximately 6 g l^{-1}. 15 ml of counting solution was added to each vial.

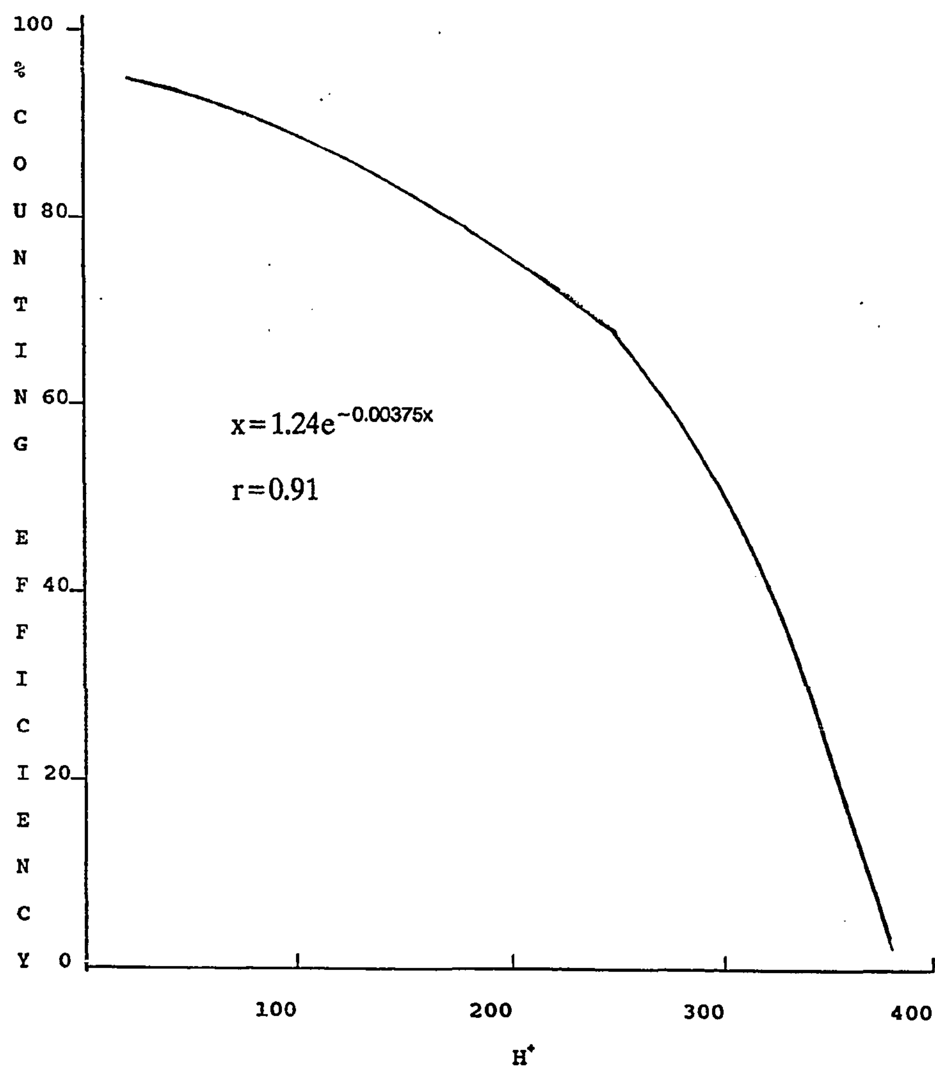
APPENDIX A (cont.)

Quench Correlation Curve for ^{14}C Toluene

Quench curve for ^{14}C

Based on Beckman LS

"H" number



APPENDIX B

STANDARD HOAGLAND'S NUTRIENT SOLUTION

Macro Nutrients	Grams/Liter
$Ca(NO_3)_2 \cdot 4\ H_2O$ 2	68.8
Fe 330	11.3

Add distilled water to 1 liter volume.

20 ml to each hydroponic tub on the first day, then 10 ml per week thereafter.

Micro Nutrients	Grams/Liter
KNO_3	90.1
$MgSO_4$	112.4
KH_2PO_4	60.8
H_3BO_4	0.64
$MnCl_2 \cdot 4\ H_2O$	0.50
$CuCl_2 \cdot 2\ H_2O$	0.056
MoO_3	0.01
$ZnSO_4 \cdot 7\ H_2O$	0.27

Add distilled water to 1 liter volume.

add 50 ml to each hydroponic tub on first day only.

APPENDIX C

MARKETABILITY STANDARDS

Marketability Standards to Evaluate Chili Peppers Grown in High and Low Salt Field Studies.

Green, Red, or Deformed*	Fruit Size (cm)
Large	≥ 16
Medium-Large	$< 16 \geq 13$
Medium	$< 13 \geq 10$
Small	< 10

* Extremely large or small chili peppers are undesirable for fresh or processed markets.
* Red chili peppers are also non-marketable, unless used for dried products.
* Deformed chili peppers are not desirable due to difficulties in processing, i.e., skin peeling is not uniform due to the curvature(s) of deformed chilis.
* All fruit red or deformed are classified nonmarketable.
* Fruit too large or too small, but not red or deformed are classified as marketable.

APPENDIX D

MEAN SEPARATIONS, LSD AT THE 0.05 LEVEL

Table #	Table Headings	Salt	BA
3	Chlorophyll	0.335	0.410
	Stomatal Resis.	0.078	0.096
	Transpiration	7.880	9.651
4	Fruit Fresh Wt.	82.756	101.355
	Plant Dry Wt.	8.498	10.408
	Plant Fresh Wt.	67.267	82.385
5	Root Dry Wt.	3.502	4.289
	Shoot Dry Wt.	5.635	6.901
	Plant Dry Wt.	8.498	10.408
6	Buds Number(3d)	2.215	2.713
	Fruit Set	2.539	3.109
	Fresh Fruit Wt.	82.756	101.355
7	Weight per Fruit	3.693	4.523
	Fruit Number(35d)	2.539	3.109
	Fruit Number(67d)	11.946	14.630
	Flower Number(23d)	1.019	1.249
	Flower Number(35d)	4.083	3.957
	Bud Number(3d)	2.215	2.713
	Bud Number(17d)	3.038	3.721

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