THESIS

HYDROPONIC SCREENING OF STRAWBERRY FOR SALT TOLERANCE: CORRELATION WITH IN VITRO EVALUATIONS

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY FLOYD THOMAS WRIGHT ENTITLED "HYDROPONIC SCREENING OF STRAWBERRY FOR SALT TOLERANCE: CORRELATION WITH IN VITRO EVALUATIONS" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

Committee on Graduate Work

Advisor

Head of Department

ABSTRACT OF THESIS

HYDROPONIC SCREENING OF STRAWBERRY FOR SALT TOLERANCE: CORRELATION WITH IN VITRO EVALUATIONS

Strawberries (Fragaria x ananassa), as well as most agricultural crops, display sensitivity to increased salt concentrations in irrigation water and soil. Efforts to breed more tolerant crops have not been entirely successful in the past because many complicated factors are involved, but promising efforts continue.

'Fern' (F) and 'Douglas' (D) strawberries, two salt sensitive cultivars, were crossed with a salt tolerant beach strawberry selection, Fragaria chiloensis (C). Samples of the two crosses and cultivar parents were previously tested in 1989 and 1990 for salt tolerance in vitro as germinated seedlings. In 1991 and 1992, samples of the crosses and all parental types were hydroponically tested as mature plants for salt tolerance.

The in vitro and hydroponic findings were correlated. Significant salt tolerance was noted in the 'Fern' X F. chiloensis crosses both in vitro and in hydroponic testing, although the growth response was different between the two

methods. All genotypes tested in vitro grew better at the 0.2% salt concentrations when compared to control. At higher concentrations, there were varying degrees of growth reduction depending on the genotype.

Two types of hydroponic experiments were run. In type 1, salts were added in 0.3% increments over time in order to determine the highest total concentration each plant genotype could tolerate given time to adapt by metabolic alteration. In type 2, salt was added in one application at 0.2%, 0.5%, or 0.8% NaCl in order to determine tolerance to an osmotic shock.

Differences in relative growth were noted between the two hydroponic experiments. When lines were directly subjected to high salt, most plants were killed at the 0.5% level, and all plants were killed at the 0.8% NaCl level. When salt was gradually added, 1.3% was tolerated by the best adapted crosses.

Despite the differences in tolerance noted, the genotypes tested ranked similarly under both systems; F X C was superior to D X C, and both crosses were superior to the cultivars. Therefore, the hydroponic results validate the in vitro results.

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ACKNOWLEDGMENTS

I would like to express appreciation for the opportunity to acquire an education. Learning is a wonderful thing to apply oneself to, and it pays to seek until you find those things in life that truly light your fire--then go after them with all your heart.

Without knowledge, we see less; less good, and less evil. Education is, in a sense, a spoiling of innocence. With greater learning and increased knowledge, we increase our responsibility to the world, ourselves, our loved ones and those in our care. Once we acquire knowledge, we can never go back--our eyes are forever opened unless we choose to close them.

Education is something easily taken for granted in our country in this day and age because what we see and what we have is the accumulation of hundreds of years of work and thousands of years of the best and worst of mankind. Yet to envision the future as even a brighter place to live is what makes education a great privilege and opportunity.

When we think of the patience of so many people who have been close to us as we poured through the years of school, and the lives of those who have given their efforts to be

instruments of patient instruction, professors and mentors for example, how can we think of not returning the same to the next generation equally or greater?

Above all, I thank God for life, and for revealing through the eyes of science things about Himself concerning the unfathomable mysteries, complexities, and nature of His creation.

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CHAPTER 1

INTRODUCTION

Strawberries are a relatively minor crop considering total worldwide fruit production. This is partially due to the fact that strawberries are most commonly thought of as a delicacy, a dessert or gourmet crop rather than a staple. In 1989, global production was just under 2 million metric tons, which is only 2-3% of the global grape production of 60-80 million metric tons (Hancock et al., 1990). Strawberries are, however, the most widely distributed fruit in the world, even more so than grapes. They are found growing in every country from the tropics to the arctic (Galletta & Bringhurst, 1990).

The United States produces about one-quarter of the total world output. Approximately 80% of U.S. production occurs in California, an area of the country where saline waters and arid lands are widespread problems associated with crop production in saline soils. Developing plants with tolerance to salt may at some point become a common objective just as breeding for rust resistance in cereals is a prerequisite before other characteristics are considered (Greenway, 1973).

Most of today's strawberry cultivars are salt sensitive (Downton, 1984). Irrigation water in furrow systems may

commonly have sodium and chloride levels in excess of 100 ppm, with total dissolved salts near 1000 ppm (Nabors, 1985). In California, Brown and Voth (1956) found that 100 ppm NaCl consistently applied through irrigation water resulted in salt accumulation in the soil bed to the extent that yield decreased, though without visible plant injury.

Death occurs in many cultivated species, including strawberries, when subjected to a salt shock of approximately 2000 ppm (0.2% NaCl/l, or 34 meq.), and damage occurs when plants are grown continuously in water with much lower salt levels. Some wild ecotypes of F. chiloensis, however, may tolerate over 22,000 ppm (2.2% NaCl, or 380 meq. (Hancock and Bringhurst, 1979). This is equivalent to 67% seawater.

Salinity research has involved the use of various individual ions as well as combinations of ions. While plant response varies with the use of different ions, the differences are only slight. It would appear that results are sufficiently similar to say that total salinity is more important in reducing yield than differences attributed to specific ions. Growth depression is controlled largely by the total salt concentration, expressed in terms of osmotic pressure of the soil solution or the electrical conductivity of the saturated paste (Bernstein, 1980).

Most fruit crops are especially sensitive to chloride and sodium salts, and the relative amount of chloride or sodium can be as important as total salt levels (Bernstein, 1980).

Thus, NaCl is a reasonable representative salt to use in salinity research.

Most fruit crops are equally inhibited by effects of negative osmotic potential and the leaf injury caused by chloride and sodium accumulation (Bernstein, 1980). Strawberry growth, however, is apparently controlled more by the osmotic pressure of the saline solution than by specific ion toxicity since growth tends to be inhibited in the presence or absence of chloride leaf burn symptoms (Bernstein, 1980).

A substantial body of research on developing salt tolerant crops has been reported since the first paper on the subject was published in 1941 (Lyon). The promise of achieving the goal of increasing salt tolerance in crops appeared likely since F1 segregating populations between salt sensitive and tolerant tomato parents produced progeny with significant variability. Furthermore, the use of in vitro culture as a screening tool for NaCl tolerance has been widely used (Croughan et al., 1978; Dracup, 1991; Mathur et al., 1980; McHughen, 1987; Nieman and Shannon, 1976; Reddy and Vaidyanath, 1986; and Spiegel-Roy and Ben-Hayyim, 1985).

The value of hydroponic or nutrient culture has also been extolled as a screening method in which nutrition and salinity are easily controlled (Ells et al., 1991; Hall and Wilson, 1986; Jones, 1982; Nieman and Shannon, 1976; and Wilson, 1980).

The purpose of this study was to evaluate two strawberry crosses under hydroponic conditions and correlate the results with previous salt screenings obtained in vitro (Hughes et al., 1992; Volk et al., 1990).

CHAPTER II

LITERATURE REVIEW

Salt stress is one of the major problems facing agriculture around the world today. Over 10,000,000 hectares of land have been abandoned globally because of soil salinity according to the FAO (Anonymous, 1978 and 1980). Approximately 25% of the earth's arable land suffers from excess salinity (Nabors, 1985). This excess salinity, and subsequent loss in agricultural productivity may have led to the downfall of ancient civilizations such as Sumer in Mesopotamia (Downton, 1984).

In Colorado alone, about 35% of irrigated land is salt affected (U.S. Salinity Lab estimate). In the western U.S., salt is a major problem along the entire Colorado River drainage system. High levels of salt in the river come from natural sources such as Glenwood Hot Springs and shale deposits in western Colorado (Mancos shale), as well as from irrigation practices (Dale Tooker, Clifton, CO, water district, personal communication).

Water of the Western United States

The crux of the matter is that high quality crops depend on high quality water. Salts accumulate in waters because of

mineral weathering, water evaporation, soluble salts previously precipitated in soils, de-icer applied to roads, and human waste effluent (Donahue et al., 1983). About 50% of this problem is due to the practices of mankind and society, with the other half from natural sources.

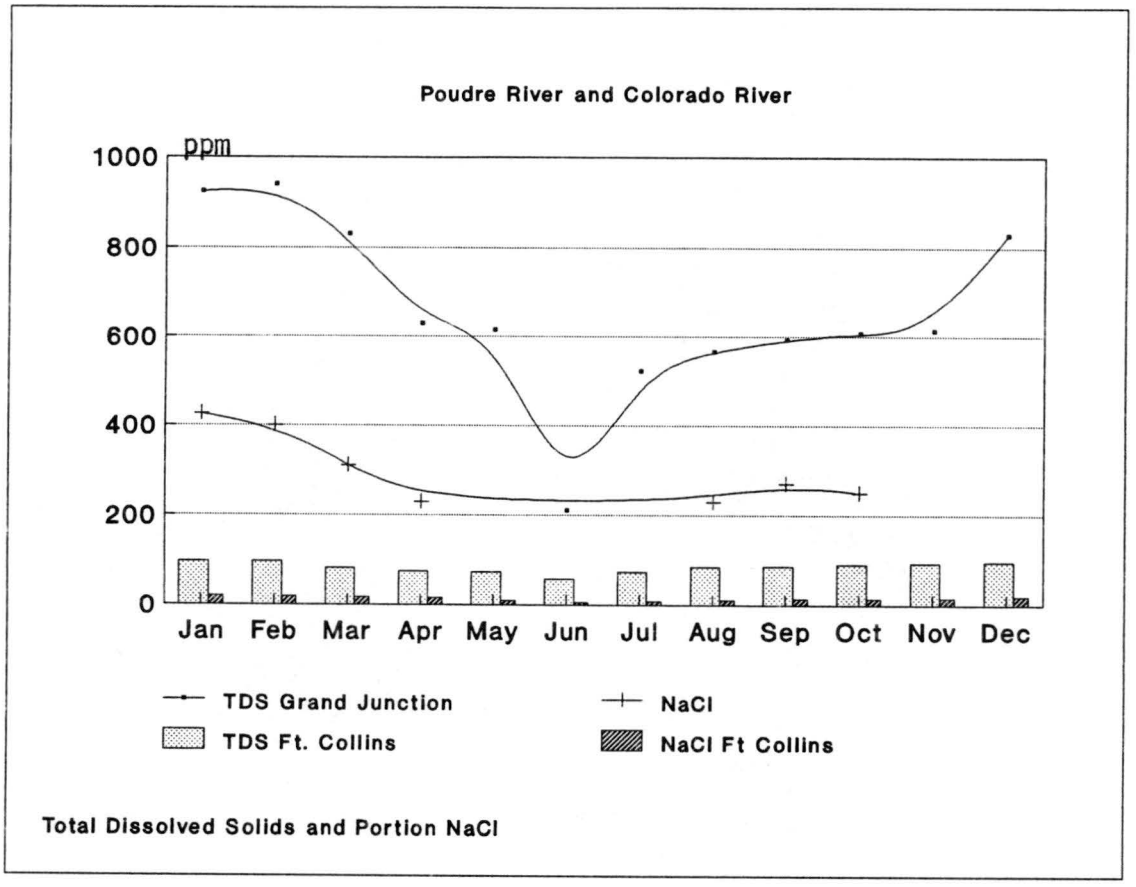
When snowmelt first leaves the headwaters of the Colorado River high in the Rocky Mountains, it has salinity levels of approximately 10 ppm NaCl and less and 70 ppm total dissolved salts (TDS). By the time it reaches the Gulf of California those totals have climbed to more that 100 ppm NaCl and 1000 ppm TDS (Nabors, 1985).

Colorado River salinity is a problem that first begins in Colorado near the city of Glenwood Springs on Colorado's western slope. Here is one of the world's largest natural mineral water hot springs which pours out of the ground and into the river. The problem is further aggravated by hundreds of miles of shale formations along the Colorado River and its tributaries which contribute still more salt.

By the time the Colorado River reaches Grand Junction, Colorado, the river's total salt load averages over 610 ppm. Total salts at Grand Junction (data from 1990) vary from a low of approximately 200 ppm in June when the river is at its peak flow and salts are the most diluted, to over 900 ppm in January (Figure 2.1), when the river's flow is low and salts are the most concentrated (Dale Tooker, personal communication). Since most of Colorado's fruit growing region

is on the western slope and is irrigated with Colorado River water, problems resulting from the use of saline waters appears likely.

Figure 2.1. A comparison of Colorado River water at Grand Junction, CO, where high salts occur naturally, and Poudre River water on the east slope, where salts are naturally low.



By the time the Colorado River reaches Mexico, it has concentrated salts from California's prime agricultural regions and large urban areas. As a result of this excess salt and associated problems, the U.S. and Mexico governments reached an agreement in 1973 regarding the salinity of water

reaching Mexico's border. This agreement stated that total salinity (TDS) was not to exceed 1500 ppm. After millions of dollars were spent correcting the problem, water was supplied to Mexico at 960 ppm in 1976 (Donahue et al., 1983).

As previously stated, approximately 50% of the Colorado River's salts come from human activities such as irrigation and municipal sewage. Today, California has regulations stating that municipal waste water discharge should not exceed 500 ppm. Some additional human-attributed sources of salts in water come from chlorine added to purify drinking water supplies, and sodium bicarbonate added to city water systems to decrease pipe corrosion (Dale Tooker, Clifton Water District, Colorado, personal correspondence).

Salinity and its Effects on Plants

Saline habitats are characterized by an excess of inorganic salts and occur mainly in the arid and semi-arid regions of the world. Salts usually occur in the upper layers of soil as the result of evapotranspiration of irrigation water or a result of rising ground waters high in salts. Saline soils are especially common in low lying areas and areas where ground water is near the surface (Mengel and Kirkby, 1987).

Salinity may increase in the soil after each cycle of irrigation. The water evaporates or is transpired and salts are left behind unless leached through the soil profile.

Salt stress slows plant growth and may lead to necrosis. Root growth is almost always less affected than shoot growth, so the root:shoot ratio increases as noted in tepary beans (Goertz and Coons, 1991). At low salinity, root growth may not decline at all while shoot growth declines. With time, transpiration brings large quantities of salt into the shoot which leads to necrosis (Munns and Termaat, 1986).

There are three primary effects of salt on plants. These are: 1) stresses from a negative osmotic potential which causes reduced water uptake, 2) specific ion toxicity, and 3) nutritional imbalances.

Osmotic Effects

Osmotic shock occurs to plants when the concentration of the soil solution is greater than the concentration of solute inside the plant roots. A concentration gradient in favor of the soil can form as the result of irrigation with saline water, or from the build up of mineral salts in the soil from fertilization and improper management practices. Osmotic effects can be very detrimental to the plant. These effects include plasmolysis of plant root cells (Cheeseman, 1988; Mengel and Kirkby, 1987), and decreasing water uptake and transpiration (Kannan and Ramani, 1988).

The degree to which a plant is affected by salts is determined by the total concentration of salts, length of exposure to salts, the plant species, and general health and

nutritional status of the plant. If a plant encounters a steep concentration gradient in a short time period, water uptake may be almost entirely inhibited and the plant can wilt and may die. If the external salt concentration increases gradually, as is often the case during the normal course of a growing season, some species of plants are able to overcome the gradient by metabolically altering their internal solute concentration.

There are two ways plants can alter their internal solutes to overcome increasingly negative osmotic potential--a process called osmoregulation. First, a plant can accumulate inorganic ions like Na^+, Cl^-, NO_3^-, and K^+ in the shoots (principally in the vacuoles), and the second is by synthesis of cytoplasmically compatible compounds (Flowers et al., 1977; Mengel and Kirkby, 1987). Some of these synthesized compounds include glycinebetaine, amino acids such as proline, (Hellebust, 1976), sugars, and organic acids (Hanson and Wyse, 1982; Gorham et al., 1985).

Although some plants are able to undergo a measure of adaptation without showing visible injury or signs of inhibition, any metabolic adjustment to osmotic gradient differences requires an energy expenditure on the part of the plant (Hellebust, 1976; Helal and Mengel, 1981). Wilting is the visible manifestation of a plant's inability to respond rapidly enough to maintain turgor when subject to sudden osmotic shock, while a yield or growth rate reduction may be

significant even when osmotic adjustment facilitates the continuous maintenance of turgor. O'Neill (1983) showed that strawberry leaves have the capacity for osmotic adjustment, but that it is an age-dependent factor related to individual leaves.

Specific Ion Toxicity

When there is specific ion toxicity, the plant takes up particular ions which have harmful effects on the normal functioning of the plant. Sodium and chloride are generally thought to be the two most toxic salt ions commonly found in soils and water supplies. Accumulation of either or both of these can cause severe leaf injury in the form of leaf burn and necrosis (Bernstein, 1980).

Adequate turgor pressure found in many plants growing on saline conditions implies that the detrimental effects of soluble salts on plant growth is a salt-induced physiological disorder above the effects of osmotic pressure alone. PEG (polyethylene glycol) is a compound of high osmoticum, yet not taken up by plants. In experiments comparing plants grown in PEG and NaCl of the same osmotic pressure, those in NaCl grew slower than those in PEG (Lagerwerff et al., 1961; Greenway and Munns, 1980).

Nutrient Imbalance

Salinity has an effect on many different metabolic processes including CO_2 assimilation, protein synthesis, respiration, phytohormone turnover, and uptake of many nutrient elements (Mengel and Kirkby, 1987). Any process where energy is required could be affected. Whether or not NaCl's effects on these processes are direct or not is unknown, but marked imbalances of ions in plant tissues occur.

Nutrient deficiencies occur even though the total salt concentration may not be toxic. This may be due to inadequate selectivity by carriers or dominance at entry sites (Yeo, 1983). Selectivity by membrane carriers may be a matter of preference or exclusion to other ions (Pitman, 1984).

Most harmful effects of salts are thought to be nonspecific, for example, darkening of leaves and an increase in leaf succulence. But some specific symptoms of plant damage may be recognized, such as leaf tipburn due to Na^+ or Cl^- toxicity.

Controversy surrounds whether or not, and how, calcium can be used to alleviate some of the effects of NaCl damage in plants since calcium deficiencies are noted when plants are subject to high salt concentrations. Bradfield and Gutteridge (1984) showed that tipburn of emerging strawberry leaves was caused by localized calcium deficiency, and that calcium foliar sprays alleviated leaf tipburn and increased berry firmness.

Calcium deficiency during salt stress has been noted by other authors studying a variety of plants. Awad and Nair (1989) noted that increases in Na^+ concentration within sporophores of Agaricus bisporus caused by high external concentrations of NaCl were accompanied by a large decrease in the concentration of calcium ions to a level of 0.02% dry weight, considerably lower than that normally required by a healthy high yielding crop (0.1%). Therefore, calcium deficiency may be an additional factor in decreasing growth and yield at high external salinities. These authors did note, from visual inspection, that the roots of plants in a high NaCl solution to which high calcium was added were whiter and more healthy looking.

Cramer and Spurr (1986) found that calcium concentrations in roots and shoots increased when additional calcium was added to the solution and that the absorption of Na decreased. However, "the addition of supplemental calcium did not affect salt tolerance" [in lettuce]. Cramer and Spurr also agreed that their work may or may not be applicable to other species, and that their work may contradict earlier held hypotheses.

The Genetics of Breeding for Salt Tolerance

Management practices can go a long way towards alleviating problems associated with saline soils. Some of these include 1) establishment of drain pipes, 2) leaching of salts (which is often impractical in the western U.S.), 3)

gypsum addition and leaching to get rid of excess sodium, 4) seed bed shape modification in furrow irrigation, and 5) drip and sprinkler irrigation practices (Donahue et al., 1983).

In addition to field practices that minimize the negative effects of salts, we can also develop crops more tolerant of adverse conditions. The research in this paper focuses on this approach--developing more tolerant plants.

Although a great deal is known about salt stress effects on plants and resulting physiological adaptations, overall, salt tolerance in crops is complex and still poorly understood.

Salt tolerance is not simply inherited as a one gene trait. Most likely, salt tolerance is the result of many multiple gene complexes working together. Although we don't understand the inheritance process of the many mechanisms involved, we can still make selections on an empirical basis. The efficiency of our breeding programs, however, should increase as our knowledge of the physiological, biochemical and genetic background increases (Tal, 1985).

Advances in releasing new salt tolerant crops are almost non-existent (Shannon, 1984; and Tal, 1985), although the high variability frequently observed in F1 populations indicates that success should be possible.

In crosses with coastal (tolerant) and inland (non-tolerant) ecotypes of Festuca aubra, Humphreys (1982) found that tolerance was dominant to non-tolerance at salt levels of

sea water strength. He concluded that salt-tolerance is heritable and dominant to non-tolerance.

While the promise of developing better crops is still appealing, research efforts are often clouded by problems from non-heritable influences such as plant microclimates, cultural practices, humidity, temperature, and other unknown factors.

In spite of these difficult influences, the first prerequisite of any breeding program must be adequate genetic diversity within crossable species, and the presence of salt tolerance traits (Tal 1985). Thus, strawberries are an excellent test subject because of their tremendous range of adaptability.

Most commercial varieties of strawberries are moderately to extremely salt sensitive. Yet within the species of one of its two original parents, Fragaria chiloensis, there are found ecotypes with very high salt tolerance. Some of these ecotypes grow along the Pacific ocean beaches and are watered with sea spray (Hancock and Bringhurst, 1979).

History

In 1941, Lyon wrote "it may be possible and desirable to select and breed plants for saline conditions" (Lyon). Lyon's paper was largely overlooked until 1961, when Epstein and Jefferies (1964) discussed genetic control over mineral ion transport and salt tolerant plants with special references to breeding economic plants more tolerant of salt than present

day crop species. This stimulated interest in further scientific studies in this area.

In 1962, Dewey reported investigations on salt tolerant cultivars of *Agropyron*. He was the first person to conduct a systematic study of a large number of strains in a given species, and then outline a breeding program for salt tolerance.

In 1964, Epstein and Jefferies recognized the importance of cultivar differences in salt tolerance. Furthermore, Wadleigh wrote in 1968 "as economic pressures on water supplies force agriculture to use more and more water of impaired quality with respect to salt loading, salt tolerant crops will increase in importance." In keeping with this idea, Mudie et al. (1972) examined the possibility of irrigating with highly saline water including seawater. H. Greenway (1973) expressed a profound sensitivity to the severity of salty soils in agriculture by stating that: "breeding of salt tolerant varieties should be a prerequisite for areas with saline waters, i.e., in the same way as cereals tolerant to rust has become a prerequisite before further selection of other characteristics."

By 1980, a survey conducted by Epstein et al. revealed that at least 61 researchers around the world were interested in the genetic aspects of salt resistance.

Moshe Tal (1985) proposed four prerequisites for improving salt tolerance for a specific crop. These were:

1) Identification of plants that display vigorous growth on saline substrates.
2) The genus must have species with suitable genetic variability in cultivars or wild relatives to conduct a breeding program.
3) Suitable methods for screening large numbers of plants for salt tolerance.
4) Establishment of diagnostic criteria to easily identify segregating populations

Since strawberries are geographically widely distributed, the genus contains many unique ecotypes. The native range of strawberries are from high mountain slopes to coastal regions and from pole to pole. In the genus Fragaria, there are at least 16 species scattered in various regions of the world. There are diploids, tetraploids, hexaploids, and octaploids. However, plants with ploidy levels of 9x, 10x, and even 16x are also known. The most common species represented in world commerce today is Fragaria x ananassa, a recognized and stable cross originating about 250 years ago by a chance cross of F. chiloensis and F. virginiana (Scott and Lawrence, 1975; Sjulin and Dale, 1987). Fragaria x ananassa is highly crossable with either of the two original parents, thus, wild germplasm as a source of traits is extremely valuable. Recent plant

collection expeditions to South America have brought back hundreds of accessions of F. chiloensis. These were collected from sea level to high mountain meadows and from the equatorial regions to near the continent's southern tip (Sjulin, 1992; personal communication). Many of these accessions are currently being evaluated at the National Clonal Germplasm Repository in Corvallis, Oregon.

The natural range of F. chiloensis is along the Pacific coast from Alaska to California and along the beaches of Chile. They also range inland to the Andes Mountains and are found in Hawaii as well.

Certain genotypes of strawberries, like most plants, have some adaptive mechanisms for coping with salt stress. There are large differences in these abilities within the genus which results in substantially greater growth of some types under salt stress than others.

Testing and Evaluation of Plants for Salt Tolerance

Salt tolerance may be defined generally as sustained growth of plants in an environment of NaCl or any combination of mixed salts. Levitt (1972) described salt tolerance as an absence of negative effects on growth in plants that accumulate salt in their tissues. He distinguishes that from plants with salt avoidance mechanisms.

Plant tolerance to salinity may be evaluated in several ways: 1) absolute plant growth or yield, 2) the ability of a

plant to survive on saline soils, and 3) relative growth on saline soils compared to non-saline soils (Maas, 1986).

In practice, salt tolerance and resistance are used interchangeably to define cytoplasmic resistance to salinity in terms of avoidance by compartmentation. Cytoplasmic resistance is the ability of a plant to maintain a low salt concentration in the cytoplasm by sequestration within cellular compartments such as the vacuole (Kramer, 1984).

Tolerance may also be defined as the difference in plant productivity between stress and non-stress environments. Slow growing wild tomatoes, for example, are defined as tolerant because they exhibit only slight inhibition under saline conditions. Selection for tolerance may only be worthwhile when an increase in yield in stress environments is the objective. Otherwise, such selection will decrease both productivity and yield in non-stress environments (Rosielle and Hamblin, 1981).

Relative salt tolerance is the percentage of biomass obtained under saline conditions compared with that under non-saline conditions. However, there are problems with this definition. A slow growing plant may do well in that salinity doesn't reduce its growth much. A very vigorous plant may be highly affected by salinity, but still out yield a slow growing plant little affected by salinity. So absolute growth may be more important to a grower, and relative yield may be more important to the breeder and geneticist. Relative

plasticity, the ability to grow well on a range of saline and non-saline environments, is the desired characteristic, but may be more difficult to achieve. To complicate matters more, it has been noted that selecting for high stress tolerance may be incompatible with high yield under non-stress conditions (Shannon, 1984).

In developing a more salt tolerant cultivar, an evaluation of salt tolerance may be accomplished in vitro, in hydroponics, or under field conditions using different soils, and using different evaluation criteria. Testing can and should also be done during the different stages of plant growth, for example, at germination, the seedling stage, and as mature plants.

In research on wheat and barley at ICARDA, it has been demonstrated that reaction to salt stress varies with the stage of plant development, and that a given cultivar may be tolerant at one stage and sensitive at another. Tolerance at seed germination may differ from the adult plant in many species, but in barley, salt tolerance during germination is known to reflect the tolerance of mature plants (Srivastava and Jana, 1984).

Some lines tested at ICARDA exhibited salt tolerance both in field screening tests as well as in germination tests indicating apparent association between field response and seedling survival. However, only some cultivars appeared tolerant at all stages. Some lines looked promising on the

basis of a field test, but were only moderately so in the germination tests. The reverse was also true: lines selected at germination failed field tests. These results confirm the importance of utilizing both seedling and mature plant screening. One can breed for resistance at one or the other stage, depending on the perceived need and breeding objective. It is recognized that different genetic systems appear to control tolerance at different stages of plant growth (Srivastava and Jana, 1984; Shannon, 1984).

Salinity affects plants at all stages of development, but sensitivity sometimes varies from one growth stage to another. A comparison of salt tolerance during germination and emergence with later growth stages is difficult because different criteria must be used to evaluate plant responses. Tolerance at emergence is based on survival, whereas tolerance after emergence is based on decreases of growth or yield. Most crops are as tolerant, if not more so, at the germination stage as at later stages. There are exceptions, however. For example, wheat, barley, and sugarbeet are more salt sensitive at the seedling stage (Maas, 1986). Grain sorghum is significantly more tolerant at the germination stage than later stages of growth (Francois et al., 1989).

The Future of Salt Tolerance Studies

The study of salinity effects on plants is a complex undertaking. Many factors influence the final ability of a

plant to yield well in the field under saline conditions. These include the species under consideration, total concentration of salts, age and general health of plants, and environmental conditions such as duration of stress, temperature levels, humidity and light intensity. The contributions of these factors are poorly understood and needs further study.

"Other essential requirements [to developing better crops] that have not generally been emphasized enough are greater use of the physiological knowledge and breeding experience acquired for stresses other than salinity, and greater appreciation for the ecological strategies of stress tolerant species" (Tal 1985). Drought stress is an example of a stress which is similar to salt stress in that plants respond similarly to increasingly negative osmotic potentials. Also needed is a better understanding of metabolic energy costs of tolerance mechanisms (Shannon, 1984).

CHAPTER III

MATERIALS AND METHODS

The plant materials used in the hydroponic research consisted of a beach strawberry clone, Fragaria chiloensis, collected in 1988 near Orick, California (Volk et al., 1990), and Fragaria x ananassa cultivars Fern and Douglas. Crosses were made between the individual cultivars and the beach strawberry and F1 populations were established. The parental clones were maintained in a greenhouse and propagated by runners.

The same plant genotypes were used in the in vitro work as in the hydroponic work except that the F. chiloensis clone was not tested in vitro.

Two different hydroponic experiments were conducted. In experiment 1, salts were added in 0.3% increments over time in order to determine the highest total concentration each plant genotype could tolerate given the time to adapt by metabolic alteration.

Experiment 2 was repeated in the 3rd experiment. In these experiments, various salt levels were added at one time in order to determine survival at high osmotic shock.

The strawberry cultivars Fern and Douglas were each used as female parents in crosses to the beach strawberry, F. chiloensis. Reciprocal crosses using the beach strawberry as female were unsuccessful in that seed failed to set when either self-pollinated or when pollen was applied from a cultivar even though both are octoploids (2n=8x=56).

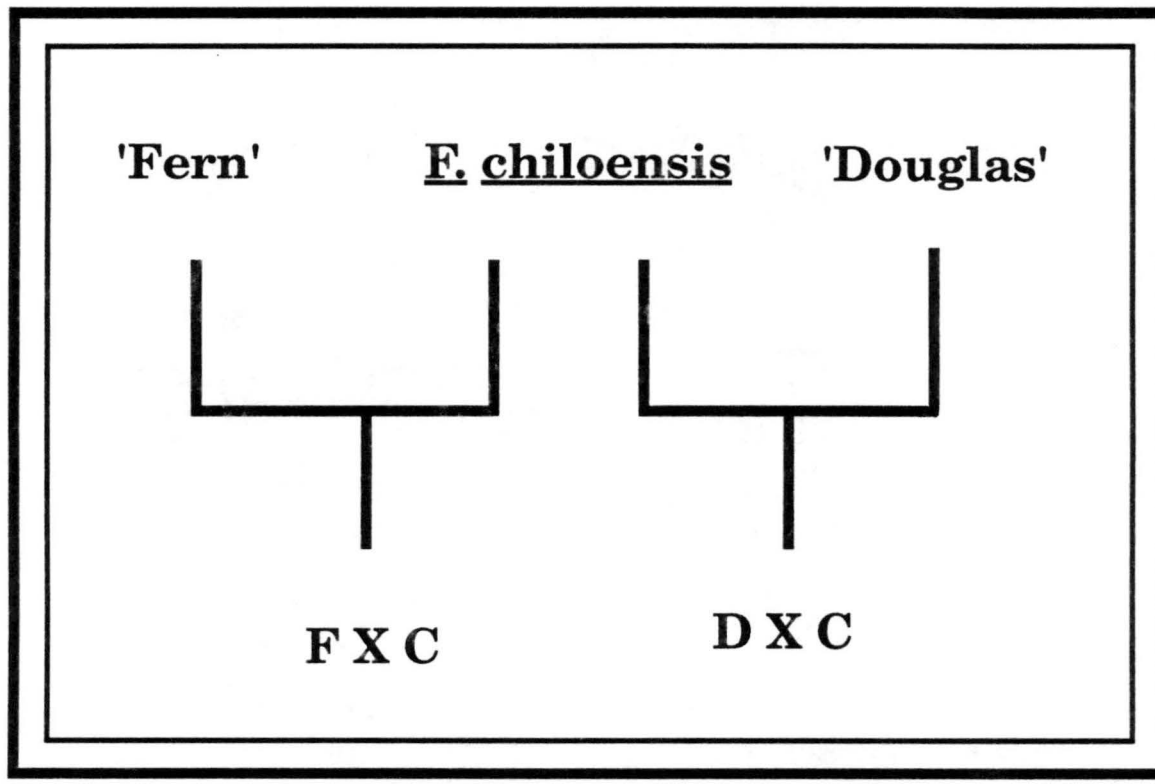


Figure 3.1. Pedigree of plant materials used.

F. chiloensis flowers in the greenhouse on a seasonal basis from December through early spring although it rarely sets fruit in this area (one fruit was noted on a clone within in a two year period).

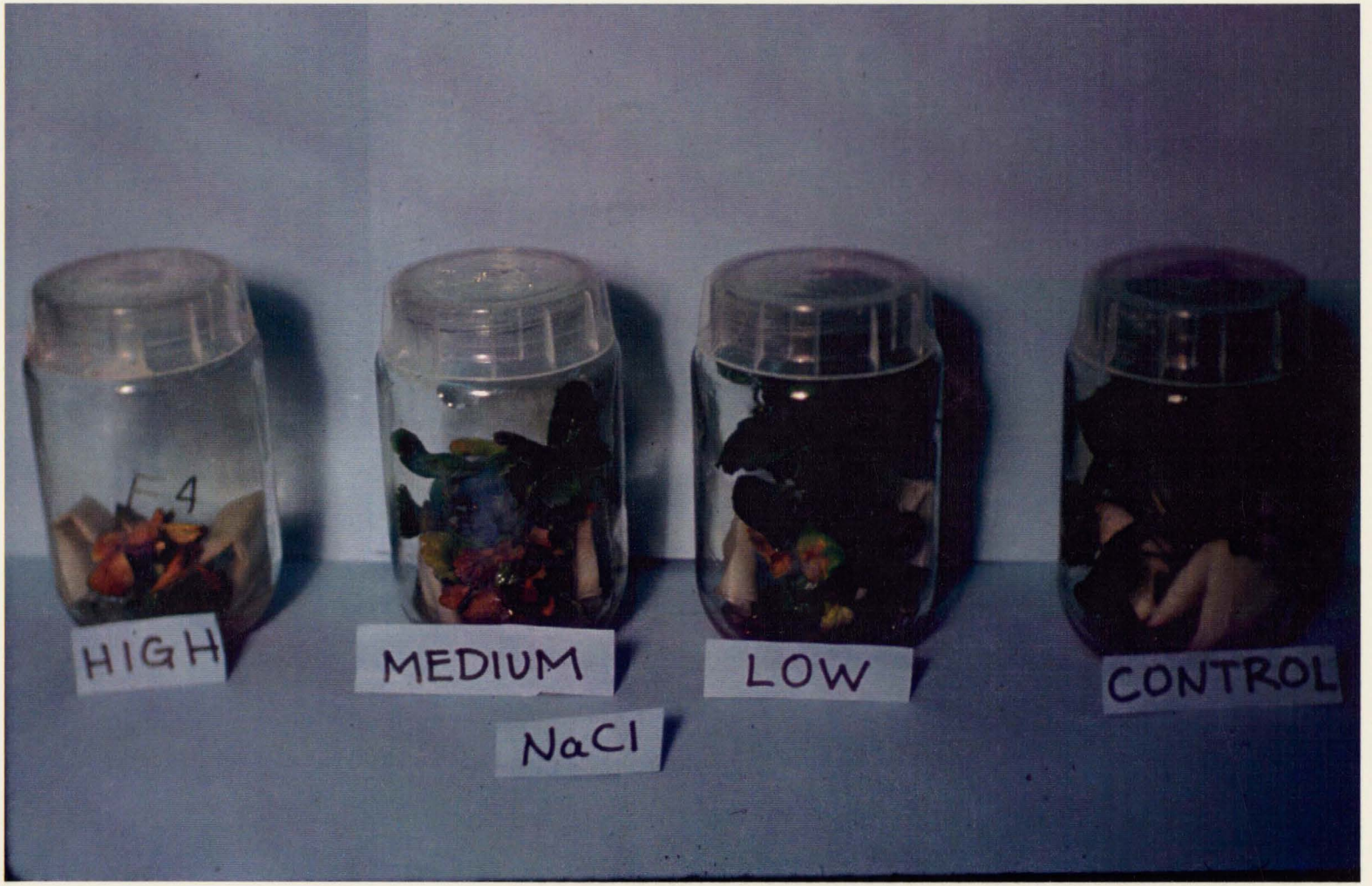
After pollination and seed set, mature fruit were collected from 'Fern' and 'Douglas'. Fruits were homogenized using a Waring blender and seed separated on a fine mesh screen by washing with water. The seeds were then placed in cold storage for several months. Seeds from crosses were used in the in vitro work and also to establish an F1 population in the greenhouse.

In Vitro Evaluations

Seeds taken from fruit of F X C and D X C crosses were surface sterilized in 15% bleach and Tween 80 for ten minutes followed by three rinses in sterile deionized water. The seeds were then placed into sterile petri dishes, 20 per dish, dampened with blotter paper and sealed with parafilm. Incubation was at 26° +/- 2°C with an 18/6 (light/dark) photoperiod until germination to ensure that only viable seeds were used (the determination of seed viability was essential--non-viable seed would skew the results by appearing to be seed killed as the result of high salt). Seedlings were transferred to growth medium after emergence.

Plantlets were grown in jars containing 50 ml of Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) with 6.5 g/L agar, and a pH of 5.7 without vitamins or hormones. Salt was added to the basal medium at the onset of the experiment in concentrations of 0, 0.2%, 0.5%, and 0.8% NaCl (Figure 3.2).

Figure 3.2. In vitro tissue culture of plants grown in control, 0.2%, 0.5%, and 0.8% NaCl for 30 days.


HIGH
MEDIUM
LOW
CONTROL
NaCl

Four seedlings were placed in each magenta cube with 8 to 32 replications per salt and cross or self combination. The difference in replication numbers were due to differing germination rates. Plants were grown for 67 days and evaluated for total fresh weight, shoot and root fresh weight, dry weights, and leaf area.

Hydroponic Evaluations

Once plants were established in the greenhouse, runners were allowed to grow from each plant type. When sufficient runners were available, they were rooted in potting soil for one week, cut from the mother plants, washed free of soil, and placed in hydroponic solution. Plants were inserted into 30 X 35cm styrofoam boards so that the crown was just above the solution level (Figure 3.3).

Since some plants appeared wilted for a few days after transplanting due to a partial loss of root mass in handling, plants were given one week to acclimate in hydroponic culture before salt was added.

While the F1 populations of F X C and D X C consisted of 30 potted plants each, only about 15 of each formed runners at any one time. Therefore, the experiments were carried out with a sample of three rooted runners from each of 15 F1 plants.

Plants were distributed within each tub in five rows (one for each genotype) with three plants of each genotype per row, for a total of 15 plants per tub.

Figure 3.3. Hydroponic culture of strawberries to determine salt tolerance.

A sample population's total growth was determined by taking the average initial weight of plants and the average final weight and determining the average percentage of growth increase. Percentage of growth increase was compared between genotypes instead of actual total plant weight since average initial weights of sample populations varied.

Research was conducted in a fiberglass greenhouse on the Colorado State University campus with north south oriented benches. Automatic temperature controls maintained the greenhouse at 16°C at night and 27°C (± 4°C) during the day. The experiments were conducted in the summer and temperature control was maintained with a fan and evaporative cooling system. No CO_2 was injected into the greenhouse. The Hoagland fertilizer solution strength was in the range of 600-800 ppm (Hoagland and Arnon, 1952). This was the rate of application used throughout the entire greenhouse range.

Experimental Design

Fifteen nine-liter plastic tubs (30 X 35 cm) were arranged in three rows of five. Styrofoam floatboards were cut to fit tightly into each tub level with the top. Eight liters of Hoagland's solution were used per container and was sufficient to touch the bottom of the floatboard.

Each styrofoam board had five rows of three holes. Each hole was approximately one square centimeter. Plants were inserted into the holes root first with the plant's crown

fitting tightly into the hole so that only the roots were in solution.

Three large aquarium air pumps were used, each attached to a five-way gang-valve. Tubing ran from the gang-valves to each tub and supplied oxygen to the water via a fine pore air-stone.

A split-split plot design was used. Each column of three tubs contained one of each treatment (Figure 3.4). The three treatments evaluated (experiments 2 and 3) were: 1) control (0 NaCl added), 2) 0.2% (2 g/L, 16 g total) NaCl, and 3) 0.5% (5 g/L, 40 g total) NaCl.

Conductivity was measured with a Corning hand-held conductivity meter to ensure that the desired osmotic potentials were reached and maintained.

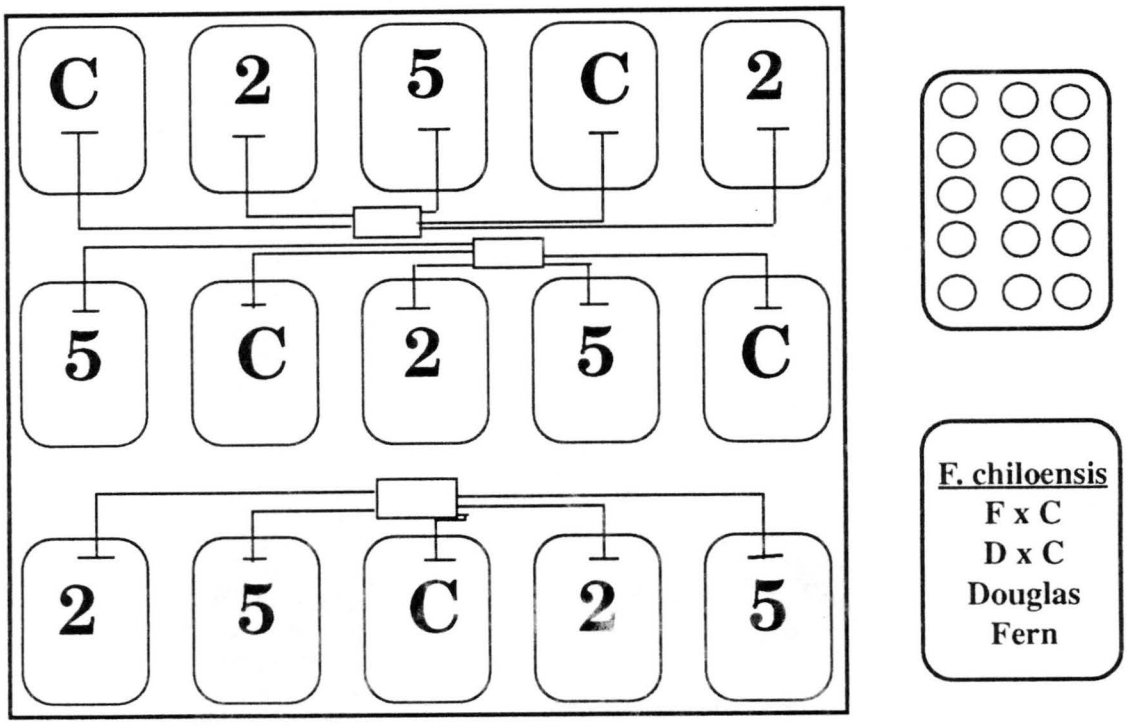


Figure 3.4. Split-split plot design of hydroponic cultures. C = Control (0% NaCl), 2 = 2 g/L (0.2%) NaCl, 5 = 5 g/L (0.5%) NaCl. Fifteen plants per tub, 3 plants per row per genotype.

Experiment 1

In the first experiment, salt levels were increased in increments of 0.3% gradually in order to determine the highest concentration plants could survive.

Salt was initially added to ten of the fifteen tubs at the 0.2% concentration. Ten days later, the solution was changed and salt was added to five tubs at 0.2%, and five tubs were increased to 0.5% NaCl. Every ten days thereafter, the water was changed and the salt level was increased by 0.3% in all treatment tubs until all plants died. The two groups of treatment tubs were thus maintained at a 0.3% NaCl difference

so that when the final treatment killed the last plants, the lower treatment tubs held leaf tissue which could be analyzed.

Experiments 2 and 3

Runners of F. chiloensis, F X C, D X C, 'Douglas', and 'Fern' were rooted in soil. These were subsequently cut from the mother plant, washed, weighed and placed in styrofoam boards. They were grown in nutrient solution for one week to acclimate before salt was added. Flowers and excess runners were pinched from the plants, but were allowed to grow back once the test began.

After one week, salt was added at the rate of 0.2% and 0.5% NaCl to appropriate tubs. Salt levels were measured weekly and tubs were drained and washed every other week and new salt and fertilizer solution added.

Hoagland's solution in control treatment tubs was rapidly lost due to transpiration of the actively growing plants and required filling twice weekly. Water in the 0.2% tubs declined slightly and needed weekly adjustment to maintain 0.2% NaCl concentration. The water level in the 0.5% tubs did not change appreciably, indicating slow growth, low water uptake and transpiration. Salt concentrations were maintained at their desired level using conductivity meter measurements.

One plant at random from each genotype and each tub was harvested at two week intervals from the time salt was initially added. Measurements were taken for fresh weight,

shoot, root, leaf weight and leaf area. Dry weights were also determined. Total leaf area was measured and the percentage of live to dead leaves was determined. This experiment was repeated. The second time was from 7/20/92 through 9/9/92. All plant types except D X C were similar in both experiments. D X C was not similar because it was vulnerable to root rot which was not adequately controlled in experiment number 2.

Root rot was controlled by Ridimil fungicide at the rate of 6 ppm (0.2 ml per 8 liter tub). Even with this protection, 'Douglas' adjusted poorly to hydroponic culture. Some crosses with Douglas (D X C) did not adapt, while others readily adapted.

Insects, primarily two-spotted spider mites, thrips, and aphids were sprayed with mild insecticides such as Safer's soap and nicotine sulfate because of regular handling of plants. The stock populations of potted plants were dipped in horticultural oil which provided excellent long term insect control.

CHAPTER IV

RESULTS

In Vitro Results

During the in vitro evaluations, conducted by a former student, plants grown in NaCl and KCl were compared for growth differences (Volk et al., 1990). The test showed that differences between high and low levels of salt are greater than differences between different types of salts. Since wilting occurs when plants are subjected to salt shock, this may indicate that growth is reduced as a result of osmotic potential.

All seedling genotypes in vitro showed increased growth at 0.2% NaCl level over the control. Growth decreased at the 0.5% NaCl concentration treatment.

Leaf area also decreased, although the crosses were again superior to cultivars (Figure 4.1). Statistical differences were noted at the 0.2% NaCl concentration for dry, fresh, stem and root weights (Table 4.1).

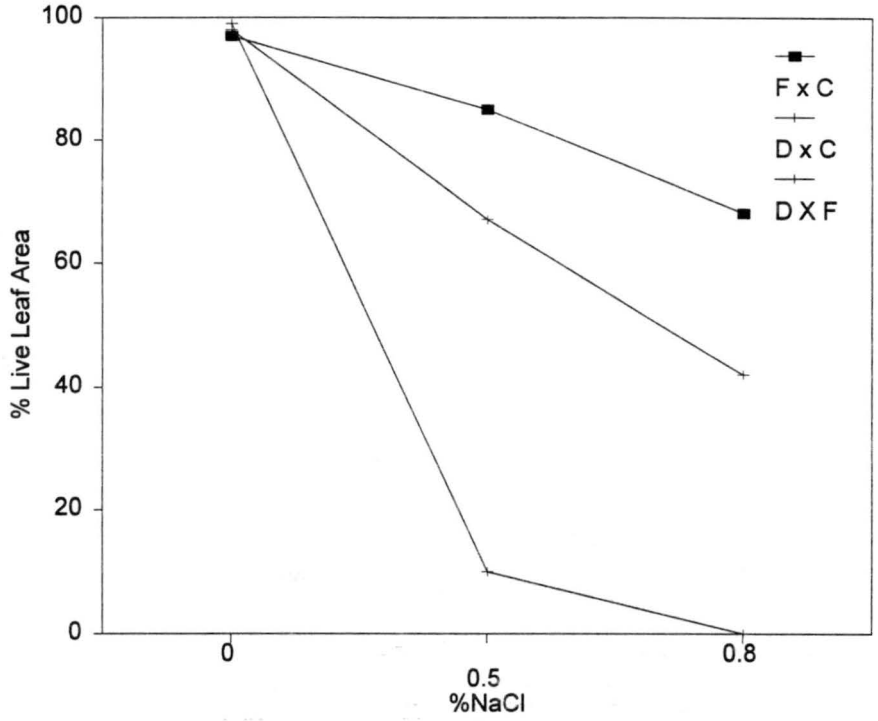


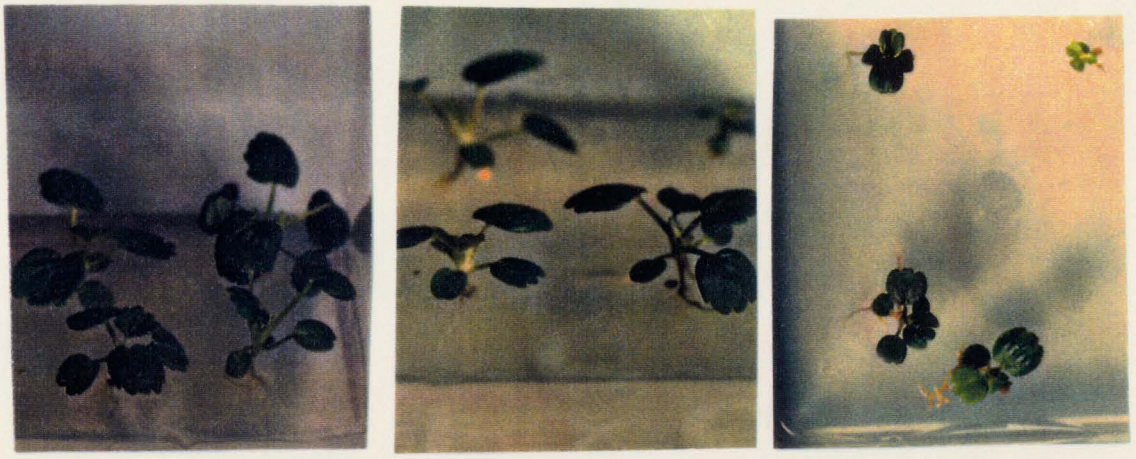
Fig. 4.1. Percentage of live leaf area to total leaf area of plants grown in vitro for 30 days at various salt concentrations.

Table 4.1. Dry, total fresh, fresh stem, and fresh root weights (gms) of seedlings of four strawberry crosses grown in vitro with 0.2% NaCl for 67 days.

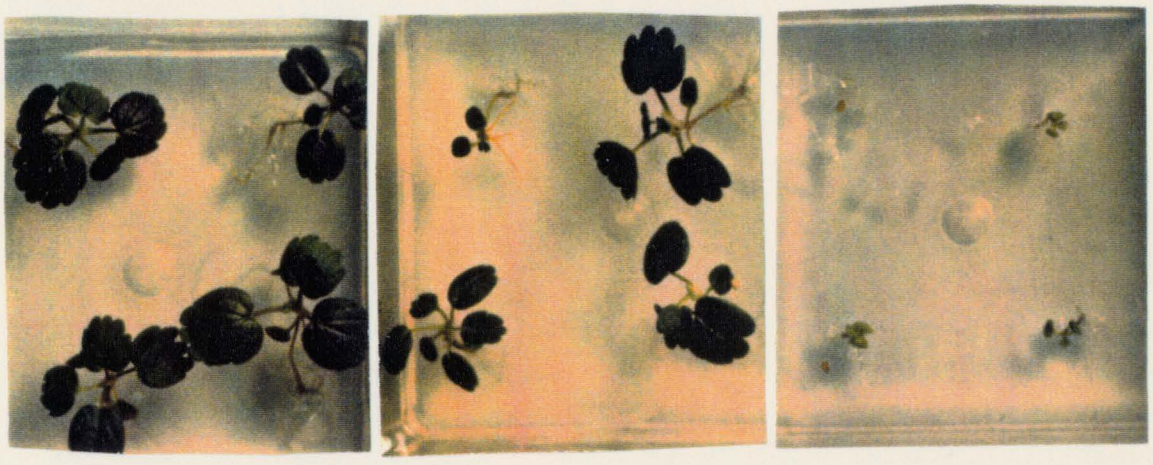
Genotype	Dry Wt.	Fresh Weight		
		Total Wt.	Stem Wt.	Root Wt.
F X C	0.171 a	0.231 a	0.192 a	0.039 a
D X C	0.013 b	0.152 b	0.134 a	0.018 b
F X D	0.007 bc	0.066 c	0.061 b	0.006 b
D X F	0.003 c	0.024 c	0.023 b	0.001 b

Mean separation within columns by Duncan's multiple range test, P=0.05.

A B C



F X C in vitro. Left to right, Control, 0.2%, and 0.5% NaCl



D E F

D X C in vitro. Left to right. Control, 0.2%, and 0.5% NaCl.

Figure 4.2. Relative growth of seedlings in vitro with 0, 0.2%, and 0.5% NaCl.

Hydroponic Results

Experiment 1. Gradual salt exposure.

The F. chiloensis clone was evaluated in experiment 1 and found to be tolerant to approximately 295 meq. of NaCl (17 g/L) when allowed to acclimate to salt stress gradually, and slightly less than 140 meq. NaCl (8 g/L) when subject to direct shock (experiment 2). Hancock and Bringhurst (1979) tested many F. chiloensis ecotypes and found tolerance ranged from 195 to 380 meq. NaCl.

Fig. 4.3. Relative injury of F. chiloensis, F X C, and D x C, (no injury, left to right) and 'Douglas' and 'Fern' (dead) when subjected to direct 0.2% NaCl shock.

Soon after adding the initial 0.2% NaCl to treatment tubs, 'Fern' and 'Douglas' cultivars displayed severe leaf

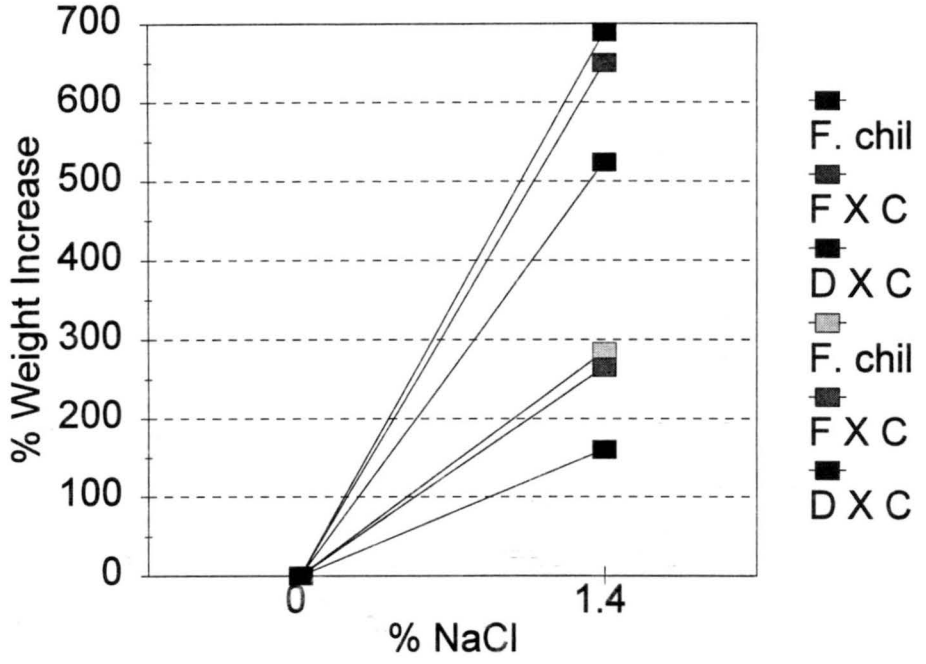


Fig. 4.4. Total fresh weight growth increase with gradual sequential increase in salt concentration. Experiment was harvested after 60 days. The top three lines represent average growth increase of control plants, and the lower three lines are the average growth increase of plant samples harvested from 1.4% NaCl treatment.

burn symptoms and wilting. Within 10 days, most of these two cultivars had wilted and died, Fig. 4.3.

When plants were subjected to gradual salt exposure, adequate turgor was maintained. This would seem to indicate that the detrimental effects of soluble salts on plant growth observed in these treatments may result from a salt induced disorder rather than osmotic effects per se (Mengel and Kirkby, 1987).

F. chiloensis, F X C and D X C displayed no visible symptoms of salt injury at 0.2% and continued growth. Since

no destructive harvests were made during this experiment, only visual observations were made concerning growth and salt stress factors. Obvious visible growth differences between control and treatment tubs of crosses and F. chiloensis did not occur until the 0.8% and 1.1% NaCl levels were reached. At this point, differences were obvious in terms of the size of the plants, although few signs of leaf burn and necrosis were apparent. As NaCl levels increased beyond 1.1%, leaves and plants begin dying. The D X C plants were the first to show evidence of necrosis and dying, followed by F X C. All crosses were dead at 1.7% NaCl. F. chiloensis was harvested at 1.7% NaCl. Although these were still alive, their growth was severely stunted and it appeared that the next higher application of salt (2.0%) might have resulted in death of the clone.

Since the crosses obviously have traits of salt tolerance, it appears that the F. chiloensis parent used in this experiment transmits the traits of salt tolerance to its progeny. Backcross breeding efforts to enhance both salt tolerance and fruit qualities would provide some interesting experiments for future research.

Another interesting addition to this experiment would be to analyze the leaves and roots of cultivars and crosses for NaCl content. This should aid in a better understanding of how salt uptake mechanisms are inherited, and what role NaCl storage in roots may have on root growth.

Advancement of the best F X C F1 plants into another stage of a breeding program would be useful. A selection of potential value that would combine both tolerance to high salts and high quality as well as quantity of fruit would seem possible. In addition to backcrossing, the recurrent selection breeding method commonly employed in strawberry breeding could be used.

Leaves of F. chiloensis were analyzed at the CSU soil testing lab for nutrient content at 0% NaCl added (control), and 1.7% NaCl. Large differences in many nutrients between the two samples were observed (Table 4.2). These differences indicate that plant disfunction may be related to nutrient imbalances and/or nutrient deficiencies. The F. chiloensis used in this experiment appears to be capable of taking up large quantities of salt and safely sequestering it while maintaining reasonable nutrition.

Further experimentation would be useful to test cultivars at salt levels lower than 0.2% in order to evaluate mechanisms of salt tolerance and/or uptake in them.

Experiment 2. Salt shock tests.

Just as in experiment 1, within a few days after directly applying 0.2% NaCl, cultivars Fern and Douglas wilted and died. In the 0.5% NaCl treatment, significant salt stress symptoms also were apparent in the other plants. F. chiloensis plants showed little visible injury even at 0.5%,

Table 4.2. Leaf tissue analysis of F. chiloensis harvested after six weeks growth in control and in hydroponic cultures which were sequentially increased from 0% to 1.7% NaCl.

Nutrient	**Control**	**1.7% NaCl**
Ca	.852 %	.661 %
Mg	.19	.19
Na	.135	2.136
K	3.92	2.70
P	.435	0.548
Al	18.1	13.1
Fe	47.8	48.5
Mn	17.2	20.4
N	2.8	2.7
Mo	1.38 mg/kg	1.38 mg/kg
Cd	.52	.40
Cr	2.07	1.06
Sr	32.5	23.0
B	94.8	75.0
Ba	8.7	8.98
Pb	<2.5	<2.5
Si	23.1	19.9
Ti	2.75	2.03
Cu	13.5	9.76
Zn	60.5	40.0
Ni	2.10	1.65
V	2.37	2.00
Cl	4185.00	38896.0

while the progeny, F X C and D X C, showed substantial variability. This variability in tolerance to salt stress was greatest in the crosses since they were an F1 segregating population from heterozygous parents. They represented the range of tolerances among the parents.

'Fern', in the 0.5% NaCl treatment, wilted and died within a few days. In the 0.2% treatment, 'Fern' showed severe leaf burn symptoms and moderate wilting. Within two weeks, most 'Fern' plants were dead in both treatments, and by four weeks, all 'Fern' plants treated were dead. 'Douglas' responded similarly to 'Fern'.

At 0.2% NaCl few crosses showed leaf burn symptoms and most did not show any signs of leaf necrosis. At the 0.5% level many crosses had visible symptoms and several died.

None of the F. chiloensis died at 0.2% and less than 10% died at a level of 0.5% NaCl. In a separate test (data not shown), all plant types died when exposed to 0.8% NaCl with direct application, including F. chiloensis.

While ion toxicity plays an important role in salt stress, it appears that the effects of a sudden osmotic shock are more important since this kind of stress kills plants at a much lower level than with a gradual increase in salt level. It is therefore important to avoid severe stress caused by sudden large decreases in water potential of the nutrient solution or irrigation water.

Variability within plant genotypes was so great that error bars have been graphically displayed on separate growth curves. This variability may in part be due to the initial differences in daughter plant weights. Figures 4.5 through 4.8 illustrate the variability of individual genotypes at each salt concentration. Five plants were harvested at each date and each concentration. The average also does not fall in the middle of the error bars because some plants were disproportionately larger than others of their type.

Relative growth as expressed as a percentage of the growth that occurred in the control treatments at the 0.2% NaCl treatments ranged from 90% in the F. chiloensis to 52% in F X C, 46% in D X C, and -7% in 'Fern'. The negative number in 'Fern' was due to the leaves which dried up before growth occurred, thus the weight of the harvested plant was less than that of initial plants.

The relative growth of plants in treatment at 0.5% NaCl as compared to the control ranged from 67% in F. chiloensis to 22% in F X C and D X C, and -21% in 'Fern'.

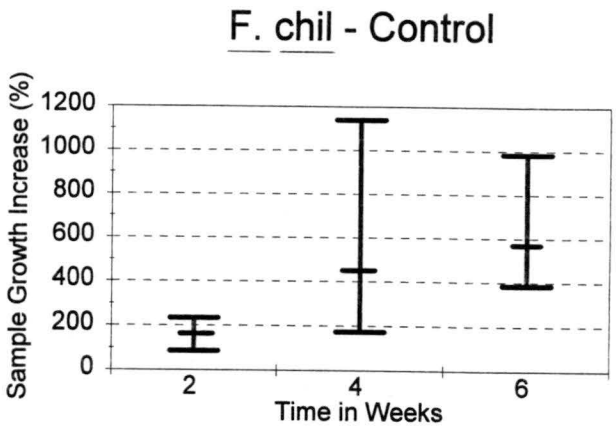
Although growth on the average was reduced in all treatments, many F X C plants were vigorous, and growth of specific plants exceeded even the growth of cultivars under control conditions (except that they produced less fruit and more runners).

Data for 'Douglas' is missing in most cases due to its failure to adapt to hydroponic culture. Stock plants were

runnering during the first two experiments but failed to survive in hydroponic culture. For the third experiment, the 'Douglas' stock plants were not runnering so it was not used in that experiment. With the exception of D X C, results in both experiments were similar. The results of D X C varied because of the susceptibility of this genotype to root rot.

In no case did a treatment average show greater growth than that of control, unlike the in vitro work where 0.2% NaCl resulted in the best growth.

It is interesting to note that the average growth increase (expressed as a percentage) of the F X C and D X C sample population falls near, although not on, the midpoint of the two parents (Figure 4.10).



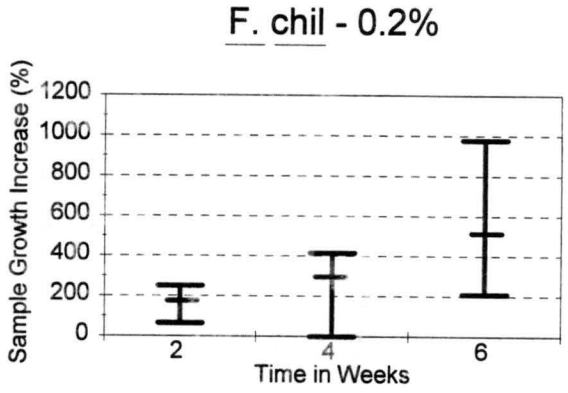
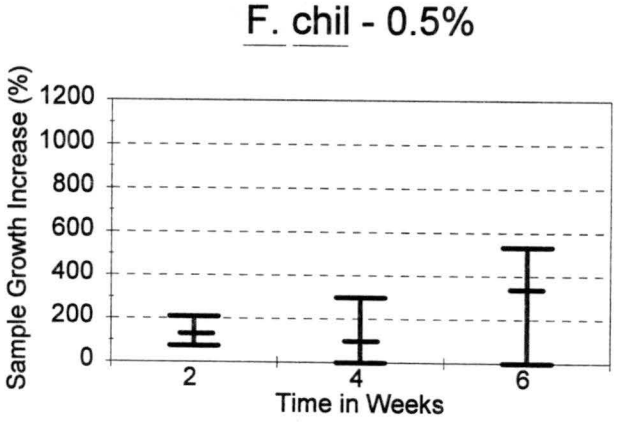
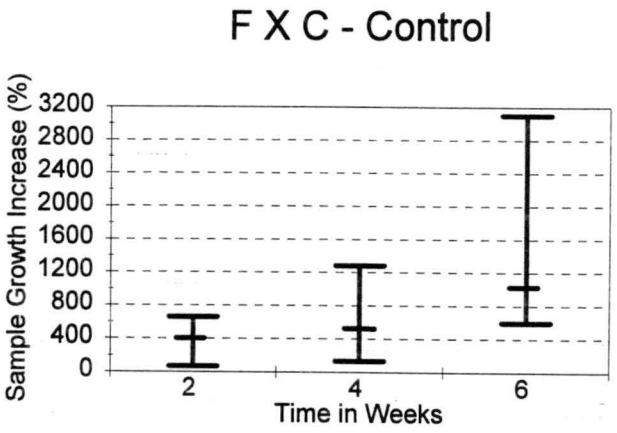
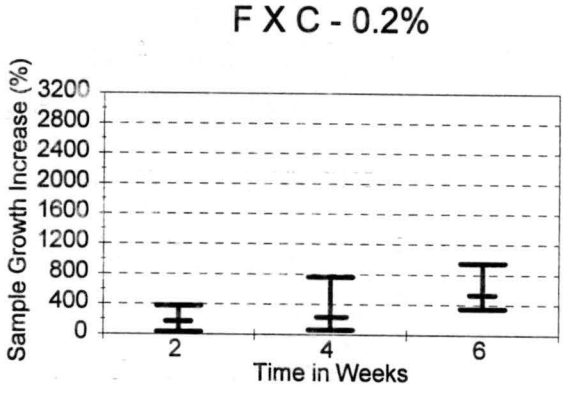


Fig. 4.5. Relative fresh weight increase of F. chiloensis subjected to direct application of 0%, 0.2%, and 0.5% NaCl in hydroponic solution.







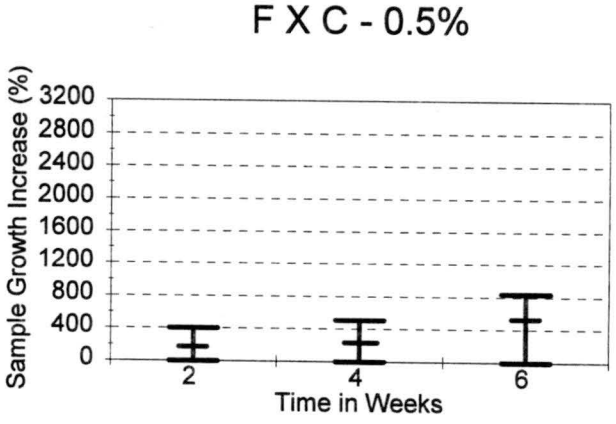
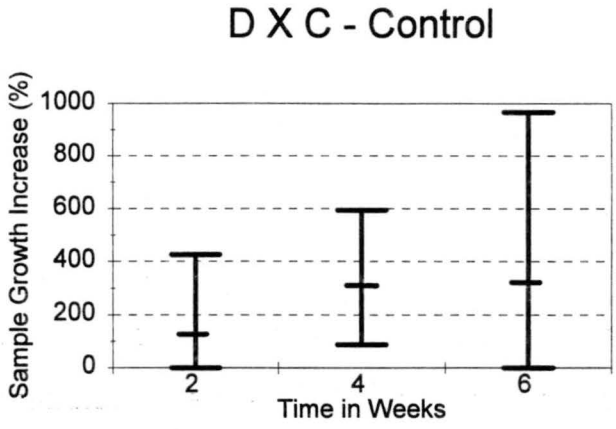


Fig. 4.6. Relative fresh weight increase of F X C subjected to direct application of 0%, 0.2%, and 0.5% NaCl in hydroponic solution.



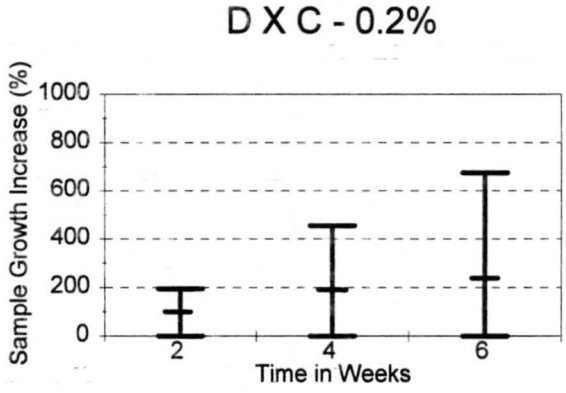
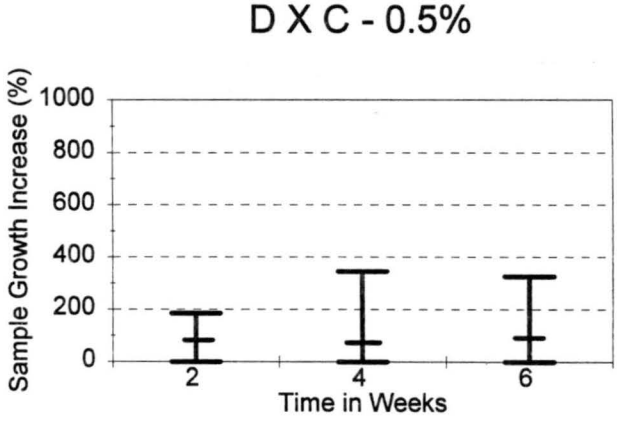
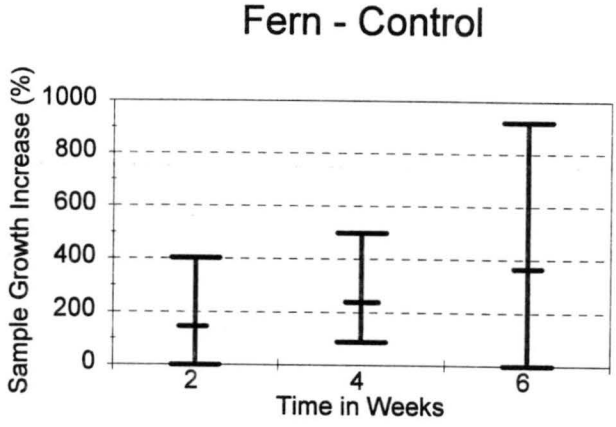


Fig. 4.7. Relative fresh weight increase of D X C subjected to direct application of 0%, 0.2% and 0.5% NaCl in hydroponic solution.





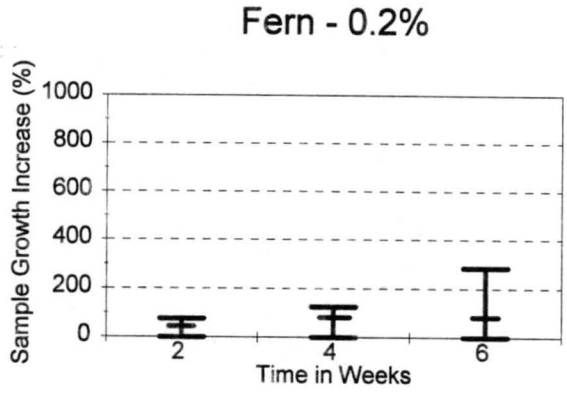


Fig. 4.8. Relative fresh weight increase of 'Fern' subjected to direct application of 0%, 0.2%, and 0.5% NaCl in hydroponic solution.

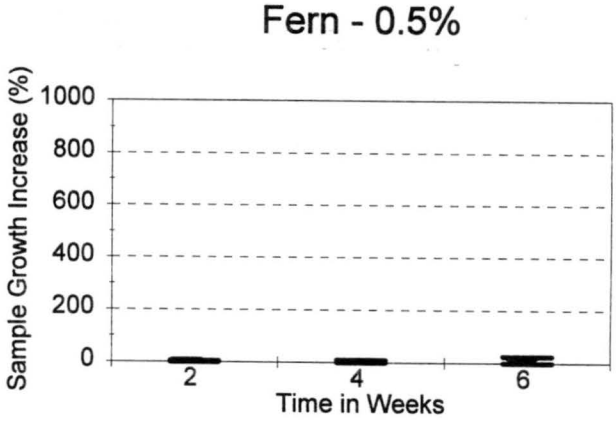


Table 4.3. Average dry weight, total fresh weight, stem and root weights (gms), and root:shoot ratio. Experiment 3.

Treatment/ Genotype	Dry wt.	Fresh Weights			R:S
		Total wt.	Stem wt.	Root wt.	
F. chiloensis					
Control	0.77	4.40	3.20	1.20	.38
0.2% NaCl	0.84	4.42	3.10	1.30	.42
0.5% NaCl	0.73	3.53	2.23	1.30	.58
F X C					
Control	1.32	7.78	5.30	2.46	.46
0.2% NaCl	1.10	5.40	3.40	2.00	.59
0.5% NaCl	1.04	5.54	2.80	2.56	.91
D X C					
Control	1.60	10.27	6.88	3.40	.45
0.2% NaCl	1.35	8.10	5.25	2.80	.53
0.5% NaCl	0.78	4.37	2.30	2.00	.87
'Fern'					
Control	1.60	8.60	5.20	3.40	.65
0.2% NaCl	1.08	5.10	2.70	2.40	.89
0.5% NaCl	0.85	3.30	1.93	1.38	.72

Note increasing root:shoot ratio with increasing salt concentrations.

Coefficient of Variability Analysis

Uniformity of plants introduced into hydroponic culture was difficult to control. For example, runners from the F X C plants were larger and more robust than either parent. Although plants were selected before they were placed into hydroponics for maximum uniformity, variability nonetheless was high. When large differences exist within the initial sample population weights of the genotypes tested, one way to critically look at these differences is through coefficient of variability (CV). CV is the standard deviation (s) expressed as a percentage of the sample mean (y). It is a relative measure of variation expressed by CV = 100s/y.

In general, when the CV was determined from the data of experiments 2 and 3 (Tables 4.4, 4.5), it was higher in the final weights of the crosses (F X C and D X C) than in the two parents. This is expected of an F1 population.

In most, but not all cases, variability tended to increase from initial to final weights and from control to the highest NaCl concentrations. In the case of the crosses, this is because F1 populations naturally have greater variability and parents tended to be more uniform and consistent.

Table 4.4. Coefficient of variability among initial and final plant weights of plants subjected to 0%, 0.2%, and 0.5% NaCl in experiment 2 (final harvest data only).

	Control		0.2% NaCl		0.5% NaCl	
	Initial	Final	Initial	Final	Initial	Final
F. chiloensis						
X	4.51	27.30	5.75	31.57	4.14	18.70
S	1.35	9.16	2.13	9.28	2.11	7.40
CV	30%	34%	37%	39%	51%	40%
F X C						
X	4.86	63.40	6.89	41.67	5.16	29.80
S	2.55	40.00	2.74	5.45	1.65	17.20
CV	52%	62%	40%	13%	30%	58%
D X C						
X	8.88	17.30	8.10	8.73	5.86	7.28
S	2.98	19.20	2.79	7.29	2.43	4.58
CV	34%	111%	34%	82%	42%	63%
Fern						
X	9.40	24.70	8.40	8.86	8.96	7.30
S	1.30	12.00	3.00	2.70	3.15	3.50
CV	14%	49%	36%	30%	35%	48%

X = Average weight in grams from a sample of five plants, S = standard deviation, and CV is coefficient of variability.

Table 4.5. Coefficient of variability among initial and final plant weights of plants subjected to 0%, 0.2%, and 0.5% NaCl for experiment 3.

	Control		0.2% NaCl		0.5%NaCl	
	Initial	Final	Initial	Final	Initial	Final
F. chiloensis						
X	1.77	11.45	1.81	10.79	1.95	8.68
S	0.50	2.90	0.51	2.86	0.45	3.80
CV	28%	25%	27%	23%	23%	44%
F X C						
X	2.75	24.57	3.48	19.99	4.35	5.64
S	0.78	11.10	1.61	8.47	0.15	3.66
CV	28%	45%	46%	42%	3%	65%
D X C						
X	3.87	22.67	4.22	21.16	3.897	7.32
S	0.66	12.25	0.92	13.10	0.82	5.10
CV	17%	54%	22%	62%	21%	70%
Fern						
X	6.16	15.48	3.02	2.27	3.31	1.8
S	3.31	7.65	0.58	1.43	1.18	0.53
CV	54%	50%	19%	62%	36%	30%

X = Average weight in grams from a sample of five plants, S = standard deviation, and CV is coefficient of variability.

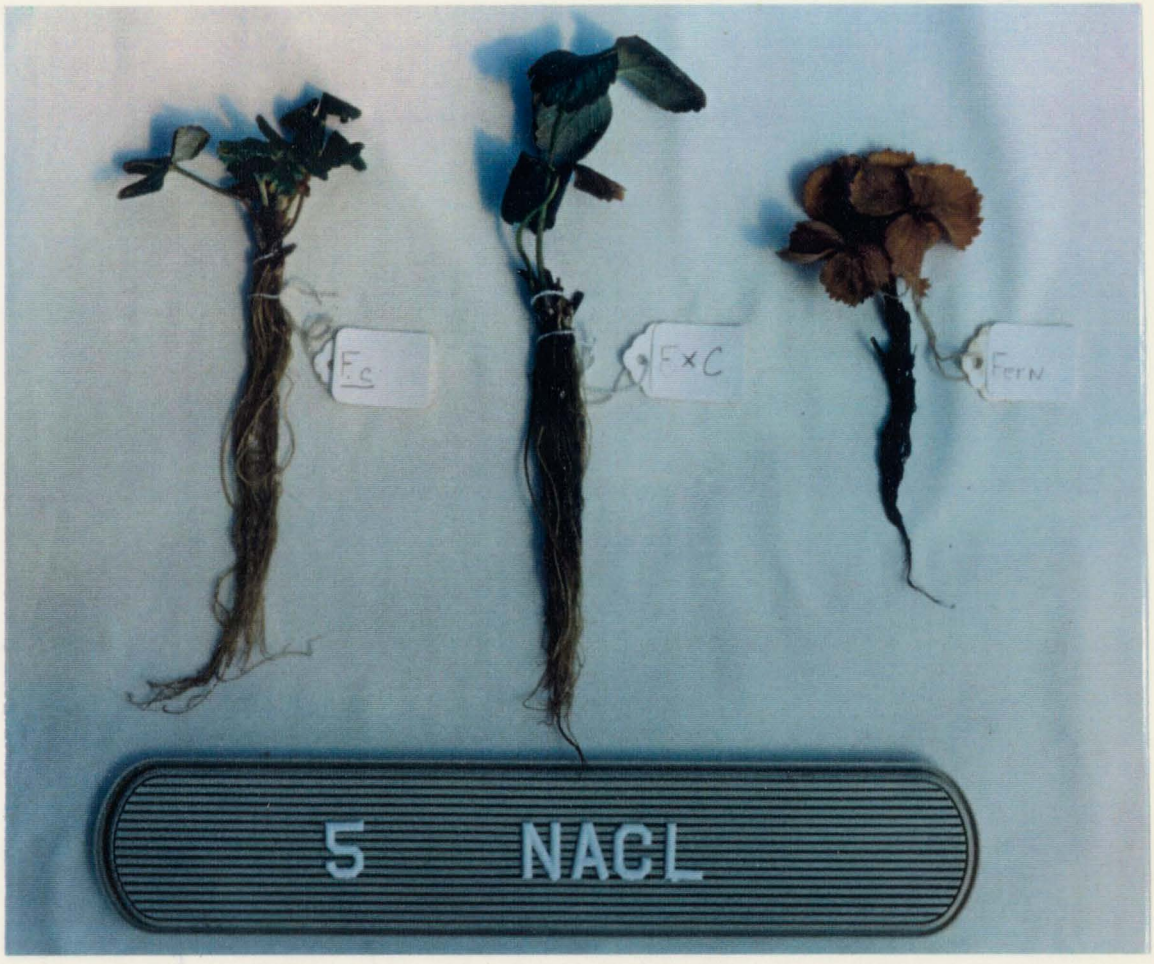


Figure 4.9. A comparison of plant growth of F. chiloensis, F X C and 'Fern' at 0.5% NaCl. The hybrid F X C is more vigorous than F. chiloensis at high salt levels, though it was reduced in growth to a greater extent. All 'Fern' died within two weeks.

Comparison of In Vitro and Hydroponic Results

The results of this study indicate that strawberry plants may be screened for salt tolerance both in vitro and in hydroponics. This was evident in that hydroponic evaluations were similar to in vitro observations in that the genotypes which showed greater growth at the higher salt levels in vitro showed similar growth in hydroponic culture. In both cases, the trend was that F X C and D X C crosses responded better than the cultivars. Therefore, one may use the in vitro system for rapid screening of crosses exhibiting salt tolerance.

In vitro plants produced the most vigorous growth at 0.2% NaCl. In hydroponics, no treatment responded with growth greater than that of the control (Figures 4.10 and 4.11). Under both situations, however, a salt level of 0.5% NaCl reduced growth. Also, some seedlings survived 0.8% NaCl in direct salt shock in vitro, whereas none survived in hydroponics. This indicates that strawberry plants may be screened for salt tolerance at a level of 0.5% NaCl since growth decreased in both screening systems.

Shannon (1984) and others have completed studies that indicated that no relationship exists between salt tolerance at the germination stage and later growth stages. If selection is determined at the seedling stage only, valuable germplasm may be eliminated from a breeding program and the results of plants selected could be disappointing at the mature stage. Likewise, if selection is made at an adult

stage, material may not be selected which is tolerant during germination and early seedling growth. The objectives of a breeding program thus must be weighed as to which stage tolerance is most important. Since strawberries are propagated and planted as mature plants into the field rather than as seedlings, it would seem that the better procedure is to test strawberries as mature plants. Mature plants can also be tested in vitro, although it is further removed from real life field conditions by factors such as light, temperature, and humidity, all of which play an important role in determining plant tolerance.

One weakness of this study is that the plants of crosses (f X C and D X C) used in hydroponic culture were not the exact plants taken from the in vitro work, although they were from the same set of seeds taken from fruit of controlled crosses. We don't know whether the same individual plant is tolerant at both stages of growth. We only know that independently, selection for tolerance can be made, and that similar results are indicated.

A more valuable approach would be to identify those plants tolerant in vitro, label them, and test them as mature plants in order to determine if genotypes of the crosses which are tolerant in vitro show similar tolerance in hydroponics. If this is true, then a rapid screening for salt tolerance could be accomplished at the seedling stage.

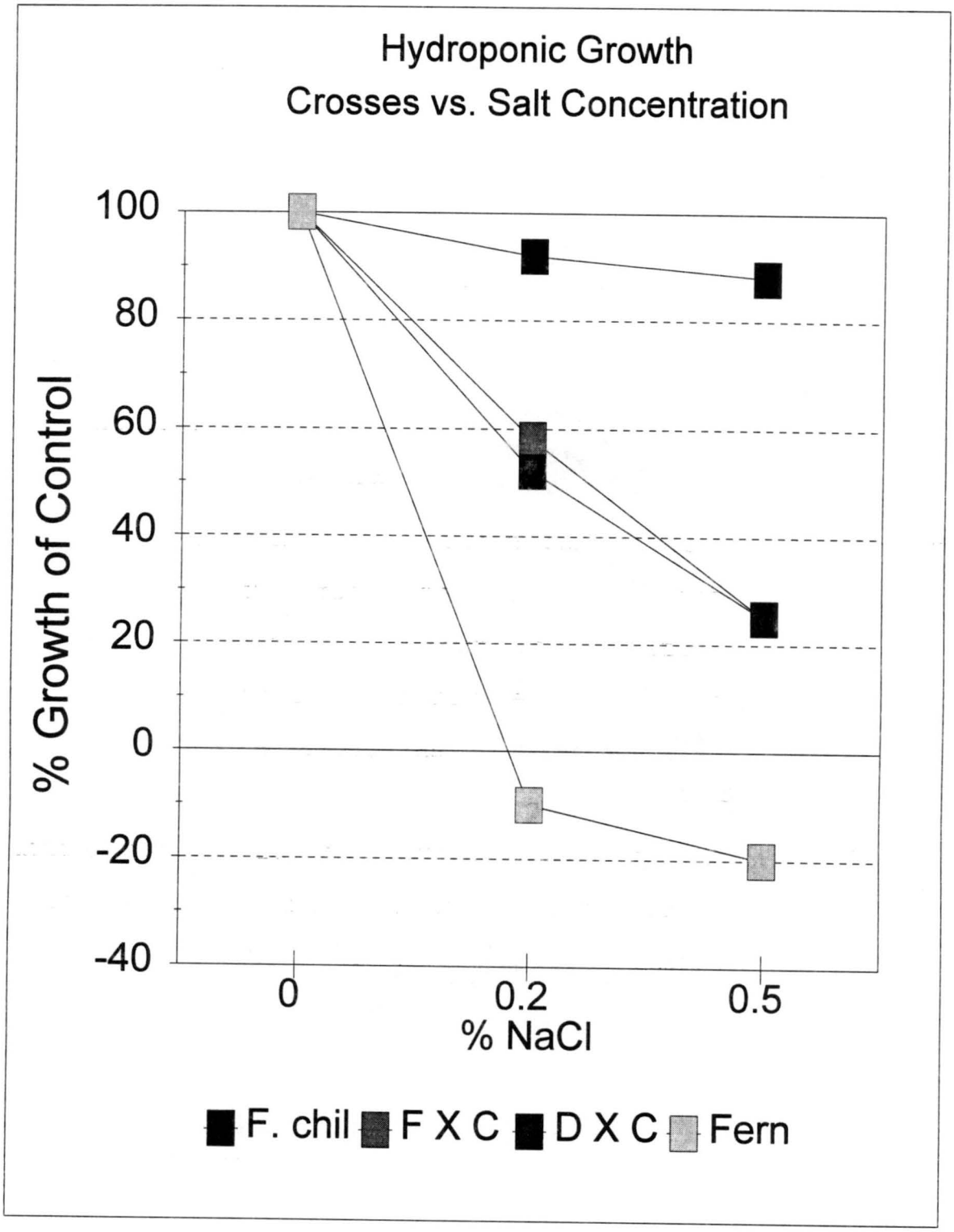


Figure 4.10. Relative growth of strawberry plants in hydroponic culture at various salt levels.

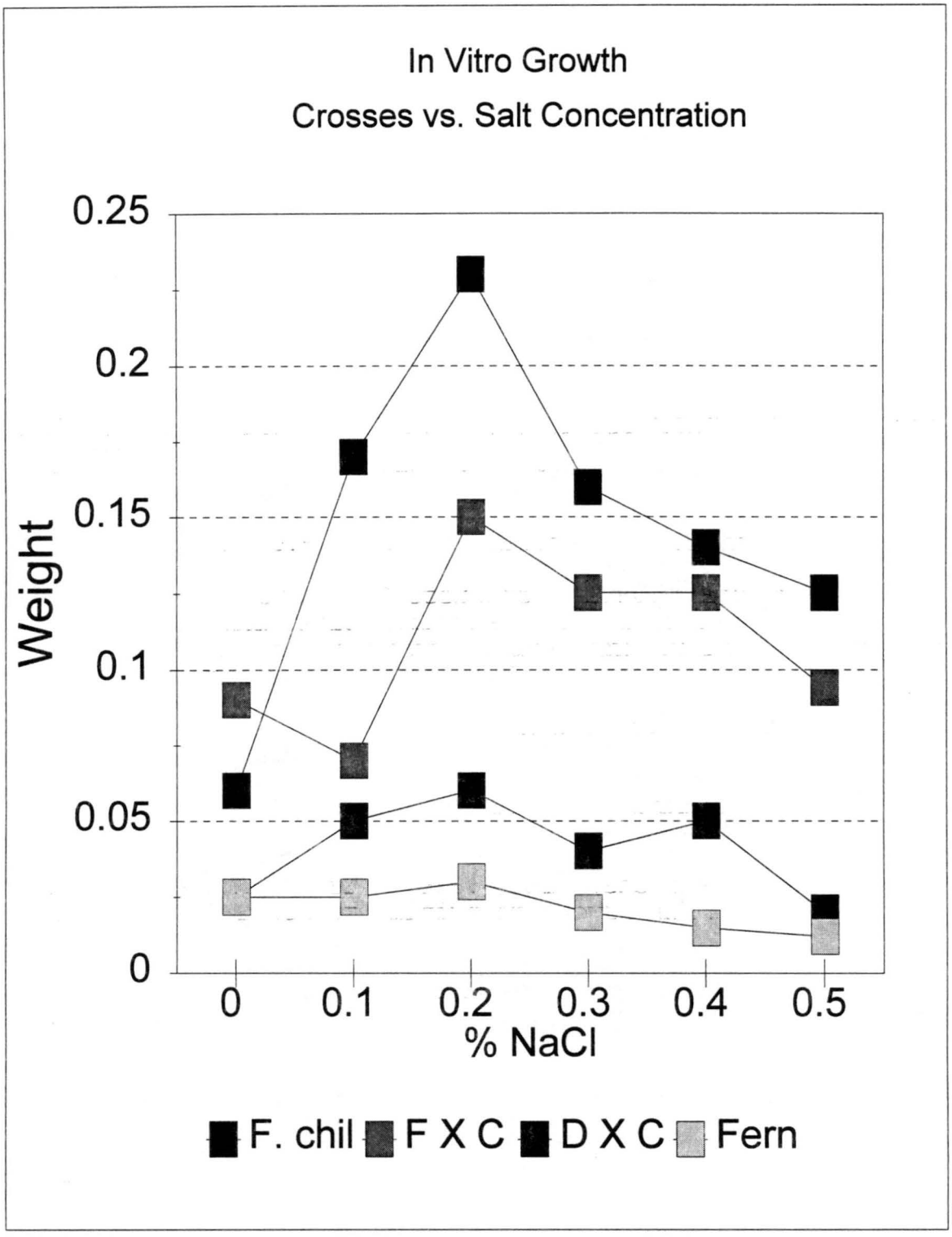


Figure 4.11. Relative growth of strawberry plants in vitro at various salt levels (F X D = 'Fern' X 'Douglas', D X F = 'Douglas' X 'Fern').

CHAPTER V

CONCLUSIONS

The problems with salinity in agriculture are as old as agriculture itself. Most of the major crops of the world are sensitive to salts at some stage of growth and are exposed to salts in many growing regions of the world. Strawberries, as well as most fruits crops, are especially sensitive to increased salt concentrations in irrigation water and soil. Efforts to breed more tolerant crops have not been entirely successful in the past because many complicated factors are involved, but promising efforts continue.

Crosses made between salt sensitive cultivars Fern and Douglas and a salt tolerant beach strawberry selection, Fragaria chiloensis, also indicate that the development of more tolerant strawberries is promising. Various screening methods bear witness to the fact that increased tolerance is noted in F1 generations. These F1's can then be entered into further breeding cycles to select for both increased fruit qualities and salt tolerance.

When the in vitro and hydroponic findings were correlated in this study, significant salt tolerance was noted in the 'Fern' X F. chiloensis crosses both in vitro and in

hydroponic testing. The growth response, however, was different between the two methods. It appears that seedlings of strawberries are more tolerant to salts than mature plants, which may or may not be the result of inherent differences between the in vitro and hydroponic systems. Further experiments could clarify these differences.

All genotypes tested in vitro grew better at the 0.2% concentrations when compared to the control. At higher concentrations, there were varying degrees of growth reduction depending on the genotype.

All genotypes tested in hydroponic culture grew best in the control treatment without NaCl added, and showed decreasing growth with increasing salts, although the reduction in growth was highly genotype dependent.

When salt exposure was compared in hydroponic culture between gradual salt build up and sudden salt exposure, differences in relative growth were noted between the two experiments. Sudden salt exposure resulted in the death of plants at a much lower level of salts than when a gradual increase was used. Crosses and F. chiloensis were able to tolerate more than twice as much salt when given time to acclimate.

Despite the differences in tolerance noted, genotypes tested ranked similarly under both systems. Therefore, the hydroponic results validate the in vitro results. In vitro selection thus may be useful for rapid and efficient screening

of strawberries for salt tolerance, assuming evaluations of seedling salt tolerance is similar to adult plants. This remains to be studied.

Figure 5.1.

Fruit size comparison.

Fern (left), F X C (right).

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