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**RESPONSE OF FOUR GREENHOUSE PEPPER
HYBRIDS TO NaCl SALINITY**

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RESPONSE OF FOUR GREENHOUSE PEPPER HYBRIDS TO NaCl SALINITY

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Abstract

The decline in availability of fresh water in the region of Ierapetra, Crete, Greece has resulted in the increased use of saline water for irrigation of greenhouse crops. The aim of this work is to study the responses of four greenhouse pepper hybrids, that are widely used in this region (*Capsicum annuum* L., Drago, Sammy, Kappy and Gracia), to NaCl salinity during in hydroponic culture (perlite) and culture in soil of young plants and during in vitro germination and seedling growth. Salinity treatments were imposed by adding NaCl either to the irrigation solution, or the substrate of the in vitro culture, at concentrations of 0, 12.5, 25, 50, 100, 150, 200 mM. High concentrations of NaCl affected the growth of the plants in hydroponic culture and in soil culture and the growth of seedlings in vitro in all four hybrids. Growth parameters such as height, number of leaves, fruits and flowers per plant and plant weight were significantly reduced in all hybrids. The percentage germination in the in vitro culture of the seeds was 100% for all hybrids on all NaCl concentrations.

The photosynthetic rate (Po), the stomatal conductance, the intercellular CO₂ concentration and the transpiration were significantly affected at high concentrations of NaCl in Drago and in Sammy. The Fv/Fm values of the leaves, which are related to the chlorophyll fluorescence, were significantly reduced at 100-200 mM NaCl, more in Kappy and Gracia and less in Drago and Kappy. The chlorophyll content in the leaves was negatively affected in all hybrids.

NaCl caused a decrease in the soluble proteins in all hybrids. The decrease was significant in Drago, Kappy and Gracia in the greenhouse experiment while in vitro the protein content was less affected by the increasing concentrations of NaCl.

Soil salinity positively affected the activity of GDH at 100 mM to 200 mM NaCl more in Kappy and Gracia and less in Sammy and Drago. In the vitro experiments there was a small increase in the higher concentrations of salt in all hybrids.

The presence of NaCl affected the antioxidant enzyme GPX in Sammy Kappy and Gracia, SOD in Drago and POD in Kappy while APX was not affected.

In general Sammy was the least affected by NaCl followed by Kappy , Drago, and Gracia.

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To my parents,
Efsthios and Niki
and to my young nephew,
Nikolas

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Notacion

%	per cent
°C	degrees Celsius
APX	Ascorbate Peroxidase
ANOVA	analysis of variance
B	Boron
BciP	Bromochloroindolyl Phosphate
BER	Blossom End Rot
BSA	Albumin Bovine Serum
C	Carbon
Ca ⁺²	Calcium
CaCl ₂	Calcium Chloride
Cl ⁻	Chlorium
CO ₂	Carbon Dioxide
Cu	Copper
d.f.	degrees of freedom
D.W.	dry weight
E.C.	Electrical Conductivity
<i>et al.</i>	and others
Fe	Iron
Fm	Maximum Fluorescence
Fo	Basal Fluorescence
Fv	Variable Fluorescence
F.W.	Fresh Weight
g.	grammar
GDH	Glutamate Dehydrogenase
GOGAT	Glutamate Synthase
GS	Glutamine Synthetase
GPX	Glutathione Peroxidase
Ci	Intercellular CO ₂ Concentration
K ⁺	Potassium

KCL	Potassium Chloride
KOH	Potassium Hydroxide
m ²	square meter
Mg ⁺²	Magnesium
mg/ l	milligram/liter
mg/ml	milligram/mole
MgCl ₂	Magnesium Chloride
min	minutes
mm	millimeters
mM	millimole
Mn	Manganese
Mo	Lead
Na ⁺	Sodium
NaCl	Sodium Chloride
NH ₄ Cl	Ammonium Chloride
NR	Nitrate Reductase
NRA	Nitrate Reductase
NiR	Nitrite Reductase
nm	nanometer
PSII	Photosynthetic System II
POD	peroxidase
pH	Power of Hydrogen (measure of the activity or basicity of a solution)
Po	Photosynthetic rate
ROS	Reactive Oxygen Species
S ⁻	Sulphur
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
sec	seconds
sig.	significance
gs	Stomatal Conductance
SOD	Superoxide Dismutase
TCA	Trichloroacetic acid
w/v	weight/volume
v/v	volume/volume

Chapter 1. Introduction

1.1. The Salinity Problem at the Region of Ierapetra

Ierapetra, a town of approximately 20,000 inhabitants, is located at the south east of Crete on the seaside of Libyan sea (Plate 1.1). The climatic conditions of the area favour the production of vegetables in greenhouses during winter. The temperature varies from 5 to 40 °C (mean 20 °C). Mean precipitation per year is 400 mm (240-670 mm). There are 150 to 400 hours of sunshine per month (mean 265 hours). The total area of greenhouses is slightly above 10,000,000 m² . A large part of them, 2,650,000 m² , is cultured with peppers, which is more than one third of the total area of pepper culture in greenhouses in Greece (6,392,000 m² –according to the archives of the Greek Ministry of Agriculture). Most of the greenhouses are high-roof, passively ventilated structures covered with polyethylene, which is replaced every 3 to 4 years.

The main problem of the agriculture in the area is the shortage of water. The last eight years were exceptionally dry. A big artificial lake, which gathers the run off water and is used for irrigation, does not suffice any more (Plate 1.2). In order to solve the problem the Water Management Committee of the area, decided to redirect the water of a saline spring into the lake. The resulting water is of varying quality. In summer and autumn the electric conductivity is rather high, and this is a problem for most vegetable crops. Pepper growers are complaining of reduced quality of the plants during that time and furthermore decrease of fruit quality.



Plate 1.1: The region of Ierapetra



Plate 1.2: The artificial lake

1. 2. *Capsicum annum* L. Culture at the Region of Ierapetra

Peppers (*Capsicum annum* L.) originate from central and South America where numerous species were used for many centuries. Although perennials, they grow as annuals in the greenhouses. They are sensitive to low temperatures and are relatively slow to establish.

At the region of Ierapetra the most common greenhouse pepper production season extends from mid- August or early September to May or late June depending on fruit prices and on the quantity and quality of the fruits harvested. High temperatures during July and August adversely affect production but are good for young plant growth. With some cultivars, percentages of unmarketable fruits increase during the late spring mainly due to a higher incidence of blossom-end rot and fruit cracking. Fruit set can also be low during the summer due to high rates of flower abortion under high temperatures. Cold weather during winter can also adversely affect the set of marketable fruits. In Ierapetra, optimum, or near optimum daytime temperatures required for pepper production can usually be achieved in winter while optimum night temperatures cannot, and therefore, heating during the night is necessary to increase fruit yield and improve fruit quality (according to experienced pepper growers of the region).

In the greenhouses of Ierapetra the most popular cultivars are producing either sweet bell fruits, which are sold green (mature but unripe stage of fruit development) or sweet long fruits-peppercorns, which are sold mainly ripe (yellowish or red depending on the cultivar). The production of the bell peppers is about 8 to 9 tons per 1000 m², while sweet peppercorns produce 10 to 15 tons per 1000 m², depending in both cases on the duration of the growing season.

Greenhouse production of peppers is based on indeterminate cultivars in which the plants continually develop and grow from new meristems that produce new stems, leaves, flowers and fruit. In comparison, field pepper cultivars are determinate; the plant grows to a certain size, produces fruit and stops growing and eventually dies. Indeterminate cultivars require constant pruning to manage their growth. In order to optimize yield, a balance between vegetative and generative growth must be established and maintained. At the beginning the plant has one stem. After a certain number of leaves has been formed, a node produces 2-3 stems and a flower which is usually

removed. After the secondary stems from two nodes, they produce two more stems and flowers at their axis, and this pattern goes on.

Water suitable for greenhouse crop production should not have electrical conductivity (E.C.) in excess of 1.0 mmhos/cm, but this is not always possible. During hot, dry weather, a mature pepper plant can use approximately 3.5 to 4.0 litres of water per day, while in cool weather 2 liters may be enough. This water always contains fertilizer.

The green bell peppers are sold in the Greek market and usually achieve higher prices than yellow long peppers. The yellow long peppers are usually exported while the red long peppers are sold mainly in the market of North Greece.

Most of the pests and diseases are chemically cured.

Since the ban of use of the Methyl Bromide soil treatment, most of the greenhouses are sun disinfected. The soil is thoroughly watered, covered completely with transparent plastic and then remains covered for at least two months (July and August). The results are good enough according to the growers.

Two of the most common physiological problems that reduce fruit quality are: Sunburns and Blossom End Rot (BER). The latter, according to the growers, increases with the use of saline water.

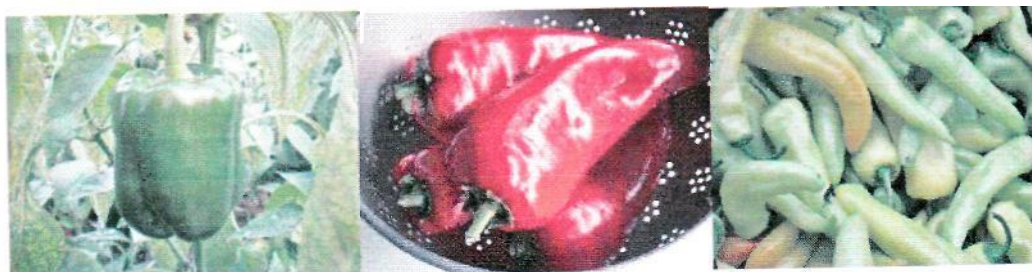


Plate 1.3: Pepper hybrids Drago, Kappy and Sammy

1.3. Description of the Experimental Work

In this work, growing different pepper hybrids on seven levels of NaCl salinity allowed a study of the effects of salinity on the certain hybrids. Two bell hybrids were used (Drago and Gracia) and two long hybrids (Sammy –yellow and Kappy -red), which have never been investigated before and which are very widely cultivated – specially Drago and Sammy are very popular among the growers of south Greece.

Three types of experiments were conducted:

a. **In the greenhouse in pots filled with perlite (hydroponics):** The young pepper plants were grown in pots filled with perlite and fed with macro and micro nutrients according to the literature (Navarro et al 2006, Martinez-Ballesta 2004), for two to three months or until the first fruit reached the marketable stage of development. The NaCl was added to the nutrient solution on a regular basis.

b. **In the greenhouse in pots filled with soil:** Young pepper plants were grown in pots filled with soil and fed with the standard fertilizers according to the usual practise. The NaCl was added to the soil on a regular basis.

c. **In vitro germination and in vitro growth of the seedlings.** The NaCl was added to the substrate only once, during its preparation. The seedlings were either germinated on NaCl substrates or transferred on NaCl substrates after the the first two leaves appeared.

In all experiments a concentration range of 0, 12.5, 25, 50, 100, 150, 200 mM NaCl was used.

In order to measure the effect of salinity on growth and development of the plants, plant height, number of leaves, flowers and fruits, fresh and dry weight of leaves, stems, flowers and fruits were all recorded. When feasible the fresh and dry weight of the roots was also measured.

In the greenhouse experiments, - as a measure of the effect of NaCl on photosynthesis- the chlorophyll content, the chlorophyll fluorescence, photosynthesis rate, stomatal conductance, transpiration and CO₂ mesophyll concentration were all measured.

Tissue analysis was also conducted to determine the activities of the antioxidant enzyme system in relation to NaCl in the greenhouse experiments, and to measure the total leaf proteins, and also the activity of GDH. Although there are not clear biochemical indicators of salt tolerance or sensitivity, the latter are considered as quite reliable for possible salt tolerance indication (Ashraf and Harris 2004).

1.4. Thesis Structure

This thesis is arranged in six chapters

- Chapter 1 covers the background, the salinity problem at the region of Ierapetra, and the pepper culture there.
- Chapter 2 is a literature review on the problem of salinity and its effects on plants with respect to growth, development, photosynthesis, antioxidative enzyme systems etc. There is also a review on the effect of salinity on pepper plants. The aim and the objectives of this work and the structure of the thesis are also covered in this chapter.
- Chapter 3 contains the description of the methods and the materials used, the descriptions of the experiments, the culture methods, the experimental design, the measurements and the analytical methods for the evaluation of the physiological and biochemical parameters, and the methods of statistical analysis of the data.
- Chapter 4 contains the results of all the experiments. It is divided into three units, one for each type of experiments conducted: A. The results of the greenhouse experiment of culture in hydroponics, B. The results of the greenhouse experiment of culture in soil and C. The results of the in vitro culture experiments.
- Chapter 5 contains a critical discussion of presented results. It is divided in three units following the presentation of the results. At the end of the chapter there is a general discussion and comparison –of all the experiments
- Chapter 6 contains the conclusions drawn from the intergrated results of this work. In this chapter are recommendations for future work of research and the possible practical value of this results for pepper growers.

Chapter 2. Literature Review

2.1. Salinity

On Earth, oceans are the main source of salt as their water contains Na^+ and Cl^- in concentrations of 460 mM and 540 mM respectively. Salinity affects 19.5% of the irrigated and 2.1 % of the not irrigated agricultural land of the globe (FAO, 2000; Yamaguchi and Blumwald, 2005).

The reasons for salinity in soils are mostly natural and are due to either the erosion of the rocks which contain Na^+ , Ca^{+2} , Mg^{+2} but also S^- , C , and Cl^- , or the transfer of salts mainly NaCl from the oceans to the land by wind or rain. Other secondary reasons of salinity are due to the use of brackish water for irrigation and the over fertilization of cultured lands (Munns, 2002).

In the future the salinity problem is expected to worsen (Wang et al., 2003) In the Mediterranean area in particular the lack of water is expected to be one of the most important factors affecting agricultural development. It is estimated that in the next decades the demand for good quality water for irrigation and municipal and industrial purposes will be increased. Therefore in some areas the use of marginal quality waters will be a way to overcome the problem of water shortage. However the use of such water for irrigation will bring certain problems in agriculture which will not be managed unless there is a better understanding of the effect of salts on plant physiology (Hamdi et al., 1995; Correia, 1999).

Good quality water is considered to be water with an electric conductivity (EC) of less than 2 dS.m^{-1} , while sea water has an EC 44-55 dS.m^{-1} . In nature sodium chloride salt is the most common factor causing salt stress in plants (Veneti, 2005).

2.2. Salinity Effects on Plants

Salt stress affects plants in three ways:

- reduces water potential
- causes ion imbalance or disturbances in ion homeostasis
- causes toxicity.

As a result each of the above, but also the combinations of them, many adverse effects are observed on the growth, development, productivity, (physiological), but also biochemical and molecular parameters of the plants (Asraf and Harris, 2004). Suppression of growth occurs in all plants, including halophytes, (naturally salt tolerant plants which are able to complete their life cycle on a NaCl rich substrate (Schimper, 1891), but the lethal concentrations of salt and the rate of growth reduction differs greatly among species (Flowers et al., 1977; Greenway and Munns, 1980; Parida and Dash, 2005). Parida and Dash (2005), in a review article, list many effects of salinity on plants besides growth including; water relations, leaf anatomy, photosynthetic pigments and proteins, lipid metabolism, ion levels in the plant parts, antioxidative enzymes and antioxidants, nitrogen metabolism, malate metabolism, chloroplast ultrastructure, gas exchange and photosynthesis itself.

Salt tolerance is the ability of plants to complete their life cycle on a substrate that contains high concentrations of soluble salt (Flowers et al., 1977). On an agricultural point of view, plant salt tolerance or resistance is accessed by the inherent ability of the plant to withstand the effects of high salt concentration in the soil without significant adverse effect (Shannon and Grieve, 1999). Research conducted in recent years has shown that most tolerant and not so tolerant plant species tolerate salinity by similar strategies and analogous processes. But the inherent tolerance mechanisms show changes in time as the plant organs change in form and function and depend on the genes that are expressed at certain developmental stage of that plant or plant organ. So, the whole plants' ability to withstand or to react to salinity stress, changes in time. Besides salt, environmental conditions affect the degree of tolerance (Hasegawa et al., 2000b; Asraf and Harris, 2004; Flowers, 2004). Plant response to salinity consists of numerous processes that must function simultaneously and in coordination in order to alleviate effects of the increase of the osmolarity in the cells and the results of the cellular ion imbalance (Yokoi et al., 2002) Salt tolerance is considered to be a complex characteristic which is controlled by many genes (Shannon, 1996; Flowers, 2004).

Plants develop many mechanisms (biochemical and molecular) to tolerate salt stress, which may act additively and synergistically (Flowers, 2004). Parida and Das (2005) categorize the biochemical ways used by the plants to achieve salt tolerance in

the following: (i) selective accumulation or exclusion of ions, (ii) control of ion uptake by roots and transport into leaves, (iii) compartmentalization of ions at the cellular and whole-plant levels, (iv) synthesis of compatible solutes, (v) change in photosynthetic pathway, (vi) alteration in membrane structure and permeability, (vii) induction of antioxidative enzymes, and (viii) induction of plant hormones.

2.2.1. Effects of Salinity on Growth

At the initial stages of salinity stress the effects on growth are actually caused by the water stress which is due to the osmotic effect of the salt in the soil. The reduced water absorption results in less water tension in the cells and consequently in smaller cells and a generally smaller plant (Greenway and Munns, 1980; Muns, 2002). The reduced amount of water entering the plant because of the low osmotic potential of the soil, leads to stomata closure in order to minimize plant transpiration. Stomatal conductance and the consequent restriction of the available CO₂ for carboxylation lead to the reduction of the photosynthetic CO₂ assimilation, and the production of enough assimilates for growth (Brugnoli and Lauteri, 1991; Geissler et al., 2009). Later, as salt ions enter the plant and built up, toxic effects will appear in the leaves which will become yellowish and senesced. Photosynthesis will be reduced, less assimilates will be produced and subsequently, less assimilates will be available for the growing points of the plant resulting in limited growth.

Many experiments have been conducted in different plant species to discriminate the salt-induced reductions in net photosynthetic rates due to stomatal/mesophyll CO₂ limitations compared to biochemical limitations. It was found that in salt-treated olive, photosynthetic processes is drastically reduced by stomatal and mesophyll limitation to CO₂ diffusion in the leaf (Cimatoa et al., 2010 and references therein). In *Plantago coronopus* (L.) the salt load in the leaves did not cause irreversible damage to the photosynthetic apparatus, but only changes in stomatal conductance (Koyro, 2006). In rice reduced photosynthetic rate is due to stomatal closure and decrease in intracellular CO₂ concentration though the magnitude of the phenomenon is species dependent (Nakamura et al., 2002). Reduced photosynthetic rate due to stomatal closure and decrease in intracellular CO₂ concentration is also reported for fourteen barley genotypes (Jiang et al. 2006), *Phaseolus vulgaris* and *Gossypium hirsutum* (Brugnoli and Lauteri, 1991). In some *Sorghum bicolor* varieties, photosynthesis was affected mainly through stomatal closure, and only to a smaller extent through interference with

the photosynthetic apparatus which happens later in salinity stress (Netondo et al., 2004). Photosynthesis also decreased because of the reduced leaf area expansion and hence reduction of light interception, which is caused early at the beginning of the salt stress in many species like Pea (Hernandez et al., 1995), tomato (Mohamad et al., 1998), *Raphanus sativus* (Marcelis and VanHooijdonk, 1999) *Amaranthus tricolor* (Wang and Nil, 2000), sorghum (Netondo et al., 2004). Growth is decreased quicker and for longer time than photosynthesis in tomato (Cuartero, 1999)

Another reason for the decrease in photosynthetic rates could be the leaf chlorosis, which is because of reduction in chlorophyll content which is reported in many species as well as changes in the chlorophyll a/b ratio (Singh and Dubey, 1995; Delfine et al., 1999; Shabala et al., 2005).

In some cases of halophytes, it has been reported that salinity, at low concentrations of NaCl, causes an initial increase in fresh and dry weight of roots and shoots, but further increase in salinity restricts growth and eventually leads to death (Flowers et al., 1977; Kurban et al., 1999; Wilson et al., 2000). Often salt stress also results in a decrease in the fresh and dry weights of leaves, stems, and roots in many plant species. Some examples are pea (Hernandez et al., 1995); *Raphanus sativus* (Marcelis and VanHooijdonk, 1999); cotton (Meloni et al., 2001); maize seedlings (Hua et al., 2007); *Aster tripolium* (Geissler et al., 2009) pepper (Chartzoulakis and Klapaki, 2000; Navarro et al., 2003 and Lycoskoufis et al., 2005) although in certain tomato genotypes the dry weight of the aerial and ground plant parts do not differ following salinity tolerance response - in the tomatoes (Dasgan et al. 2002)

Reductions in plant height, number of leaves per plant, leaf area, root length, and root surface area also have been reported in some plants like pepper (Chartzoulakis and Klapaki, 2000), tomato (Mohammad et al., 1998), *Phaseolus vulgaris* (Wignarajah, 1990), *Cucumis sativus* (Duan et al., 2008), *Solanum melongena* (Savvas and Lenz, 2000; Unlukara et al., 2008) and other vegetables (Shannon and Grieve, 1999), olive cultivars (Chartzoulakis et al., 2002), and citrus cultivars (Storey and Walker, 1999).

2.2.2. Effects of Salinity on Photosynthetic Pigments

Under prolonged salt stress the leaves develop chlorosis - a change in the leaf colour due to reduction or loss of chlorophyll, and membrane deterioration- which start from the older leaves and move up. The leaves often develop premature senescence and fall off. The phenomenon of chlorosis in species or even cultivar dependent and is often less or slower developed in salt tolerant species (Lutts et al. 1999) For example sodium chloride stress reduced leaf chlorophyll content in red raspberry at concentrations of 10 mM NaCl and stomatal conductance at 15 mM (Neocleous 2007), while in the halophytic *Paspalum*, chlorophyll content decreased significantly only at electrical conductivity of water (EC) 50 – almost seawater- (Lee et al. 2004) Further, in the leaves of the halophytic *Plandago coronopus* the carotene content increased, but the chlorophyll content decreased following increase in soil salinity (Koyro 2006). The phenomenon, apart from the severity, depends on the duration of the stress as well. Delfine et al., (1999) reported that there was no significant difference in the chlorophyll content of spinach leaves after a 20 days salt stress, but found that the chlorophyll content was highly reduced after 40 or 50 days of stress. They assumed that the reduction of the absorbed light by the stressed leaves was due to the reduction of the chlorophyll content. Reduction of the chlorophyll content has been reported in tomato (Khavarinejad and Mostofi, 1998; Debuba et al., 2006) and *Triticum aestivum* leaves (Wang et al., 2007). However, Wang and Nil (2000) have reported that chlorophyll content increases under salinity stress in *Amaranthus*.

2.2.3. Effects of Salinity on Soluble Proteins

In the leaves of many plants, soluble protein contents have been reported to decrease in response to salinity, as in *Cicer arietinum* (Soussi et al., 1998), *Vicia faba* (Gadallah, 1999), *Amaranthus* (Wang and Nil, 2000) in *Raphanus* leaves (Muthukumarasamy et al., 2000), tomato (Debuba et al., 2006), and *Triticum aestivum* leaves (Wang et al., 2007). In leaves of potato plants soluble protein content decreased by 10.4% in response to salt stress while in roots and tubers it increased by 50,4% compared to the control (Texeira and Pereira, 2007). Agastian et al. (2000) have reported soluble protein increases at low salinity and decreases at high salinity in mulberry.

Differences in soluble proteins between aerial and ground parts of the plants are considered to be signs of differential salt responses of leaves and roots (Texeira and Pereira, 2007).

2.2.4. Effects of Salinity on Photosynthesis

(a) Chlorophyll Fluorescence

The light energy which is absorbed by the chlorophyll molecules in a leaf will either:

- be used for photosynthesis
- be dissipated as heat to the environment
- or be re-emitted as light –chlorophyll fluorescence

The above described processes are competing with each other. Any increase in the efficiency of one will cause a decrease in the others. (Maxwell and Johnson, 2000). It is considered that the fluorescence signal originates primarily from PSII. Therefore if the function of PSII is affected, chlorophyll fluorescence will also be affected. The measurement of chlorophyll fluorescence is considered to be a sensitive method of assessing the efficiency of PSII and the changes in photosynthesis caused by environmental effects (Lichtenthaler, 1988;). Fluorescence induced in dark adapted leaves by rapid exposure to actinic light can be measured in the field. The ratio of the variable to the maximum components of fluorescence (F_v/F_m) is correlated with the quantum yield of photosynthetic O_2 evolution or CO_2 assimilation under light-limiting conditions (Krause and Weis, 1991; Mulkey and Pearcy, 1992; Schreiber et al., 1995; Krause and Winter, 1996). This ratio is a measure of the capacity of photosynthetic system II (PSII), which is sensitive to a variety of environmental factors inducing different kinds of stress. The method can show signs of degradation of the PSII before other symptoms are visible and therefore can trace changes in chlorophyll function which take place before changes in chlorophyll content. (Krause and Weis, 1991; Figueroa et al., 1997; Flexas and Medrano, 2002).

The effect of salinity stress on the maximum quantum yield of PSII (F_v/F_m) depends on the salt tolerance among the species or even among the genotypes. Some researchers report that in the early stages of the salinity stress there are no major changes in the maximum quantum yield because salinity initially decreases stomatal conductance, which reduces photosynthesis, leaving PSII unaffected (Baker and Rosenqvist, 2004; Morales et al., 2005). Other studies have shown little to no effect of

salinity on Fv/Fm at low to mid-salinity levels (Morant-Manceau et al., 2004 in *Triticale*, Netondo et al., 2004 in *Sorghum*, Nawmann et al., 2007 in halophytes *Myrica cerifera* and *Phragmites australis*) even when leaf growth and gas exchange were reduced. Other researchers (Belkhodja et al., 1994 and Jiang et al., 2006 in barley, Lee et al., 2004 in *Paspalum*, report adverse effects on Fv/Fm at high levels of salinity reaching very low values for example only 49.8% of control in certain barley genotypes (Jiang et al., 2006). The way salinity affects Fv/Fm may depend on the genotype (Lee et al., 2004; Jiang et al., 2006).

In short; in order to measure chlorophyll fluorescence, pulsed light is used to excite chlorophyll. If the intensity of this pulse is low so that electron transport and CO₂ fixation do not occur, the basal fluorescence yield (F_o) is measured. Then the leaf is exposed for about 0.8sec to saturating actinic light (typically 5,000-10,000 10,000 μmol photons m⁻²s⁻¹) and the maximum fluorescence yield (F_m) is measured. (F_m-F_o) = F_v is called variable fluorescence. (F_m-F_o)/F_m which is also referred as F_v/F_m represents the efficiency of energy capture (photon yield) by PSII. In non-stressed leaves the value of F_v/F_m ranges in 0.75-0.85. If lower values are measured then it can be assumed that PSII has been reversibly or irreversibly damaged due to the imposed stress (Calatayud and Barreno 2004).

b. Photosynthetic Rate

The effects of salinity stress on photosynthetic rate and the photosynthetic apparatus has been researched and there is a general opinion that salinity and drought may have similar effects on photosynthesis (Flexas and Medrano, 2002; Flexas et al., 2004; Parida and Dash, 2005). But there are conflicting reports suggesting that these stresses limit photosynthesis through either diffusive resistance (i.e., after stomatal closure) or metabolic impairment (Flexas et al., 2004). It seems that at the beginning of stress photosynthesis is affected mainly by stomatal closure (Brugnoli and Lauteri, 1991; Nakamura et al., 2002; Jiang et al., 2006; Koyro, 2006). As stress progresses, other factors become more important in limiting photosynthesis. For example mesophyll resistance (Renou et al., 1990), ribulose phosphate regeneration (Escalona et al., 1999), rubisco (Carboxylase of Diphosphoribulose) activity (Antolin and Sanchez-Diaz, 1993), and photochemical activity (Souza et al., 2004) which are referred as non-stomatal factors. In grapevines (Medrano et al. 2002), and other species (Delfin et al 1999 in spinach, Neocleous and Vasilakis 2006 in red mulberry), it has been reported

that stomatal conductance values correlate with several photosynthetic and steady-state chlorophyll fluorescence parameters.

2.2.5. Effects of Salinity on Antioxidative Enzymes and Antioxidants

Many environmental stresses, specially salt and draught stress, result in oxidative stress because of the formation of reactive oxygen species (ROS) such as superoxides and hydroxy and peroxy radicals which cause membrane dysfunction and cell death (Bohnert and Jensen, 1996, Wang et al. 2009). This happen because stresses deregulate the electron transport in chloroplasts and mitochondria and activate the plasma membrane-bound NADPH oxidases, the cellwall-bound NAD(P)H oxidase-peroxidase, and possibly the amine oxidases (Papadakis and Roubelakis-Angelakis, 2005; Paschalidis and Roubelakis-Angelakis, 2005b). The plants in order to detoxify these ROS increase the activities of certain antioxidative enzymes such as catalase, peroxidase, glutathione reductase, and superoxide dismutase, which scavenge reactive oxygen species (Sairam et al 2002). There is evidence that efficient scavenging of ROS is important in the osmotic stress tolerance of plants caused either by draught or by salt (Hasegawa et al., 2000b).

There are several reports shown that salt tolerant cultivars generally have enhanced antioxidant enzyme activities under salt stress when compared to sensitive cultivars. This was observed in wheat (Meneguzzo *et al.*, 1998), tomato (Gueta-Dahan *et al.*, 1998; Rodriguez *et al.*, 1999), citrus (Gueta-Dahan *et al.*, 1997), rice (Dionisio-Sese and Tobita, 1998), fox-tail millet (Sreenivasulu *et al.*, 1999) Mulberry (Sudhakar *et al.*, 2001), weat (Sairam et al., 2002), lentil (Isin- Nur, 2004) mustard (Khan et al., 2009) etc.

2.2.6. Effects of Salinity on Nitrogen Metabolism

Nitrate reductase (NR) catalyses the reduction of nitrate to nitrite, which is further reduced to ammonia by nitrite reductase (NiR). Nitrate reductase activity (NRA) of leaves decreases in many plants under salt stress *Zea mays* (AbdElBaki et al., 2000); tomato (Flores et al., 2000 ; Deboubaa et al., 2006), Vitis (Loulakakis et al., 1996). The cause of reduction of NRA in the leaves associated with the presence of Cl^- salts in the external medium, is due to a reduction in NO_3^- uptake and consequently a lower

NO_3^- concentration in the leaves. There is also the suggestion that Cl^- , has a direct effect of on the activity of the enzyme (Flores et al., 2000).

Assimilation of ammonia into amino acids takes place with the help of enzymes, glutamine synthetase (GS) and glutamate synthase (GOGAT) (Deboubaa et al, 2006). Alternatively, NADH-dependent glutamate dehydrogenase (NADH-GDH) can also catalyze the reductive amination of 2-oxoglutarate (2-OG) and the oxidative deamination of Glutamate (Kumar et al., 2000; Skopelitis et al., 2006). The function of the alternative GDH pathway is operative not only under conditions of ammonia limitations, but also under a variety of stresses (Loulakakis et al, 1996) Under stress conditions, including salinity, there is an increase in proteolytic activity. This condition leads to increased intracellular hyperammonia which must be removed in order to avoid toxicity (Lutts et al., 1999). Intracellular hyperammonia increases aminating GDH activity and can be caused by exogenous ammonium (Loulakakis and Roubelakis-Angelakis, 1992), or senescence which leads to proteolytic activities (Loulakakis et al., 2002) or abiotic stress (Lutts et al., 1999). In the senescing leaves, there is a decrease in GS and GOGAT activity, GDH protein accumulation, and increased aminating activity (Loulakakis et al., 2002).

According to Loulakakis and Roubelakis-Angelakis, 1991, 1992 the GDH holoenzyme has a hexameric structure consisting of two subunit polypeptides, a and b, with small but distinct differences in their molecular mass and charge. The two subunits are associated in an ordered ratio so that isoenzymes 1 and 7 are homohexamers of the b- and a-subunits, respectively (Loulakakis and Roubelakis-Angelakis, 1990a, 1990b Figure 2.1).

Skopelitis et al 2006 tested the hypothesis that GDH is a stress-responsive protein that may reflect an additional/alternative route to the GS/GOGAT pathway for ammonia assimilation under intracellular hyperammonia conditions, and they suggest that their results support the antistress physiological role and the aminating activity of the anionic isoenzymes of GDH, since they found out that gene expression, protein accumulation, and in vivo aminating activity were induced by salinity in a dose-response and time response manner.

There are some reports where aminating GDH activity increases with increasing salt stress in the salt-tolerant, and decreases in the salt-sensitive cultivars in rice, (Kumar et al., 2000), in wheat (Wang et al. 2007) and potato (Teixeira and Pereira 2007).

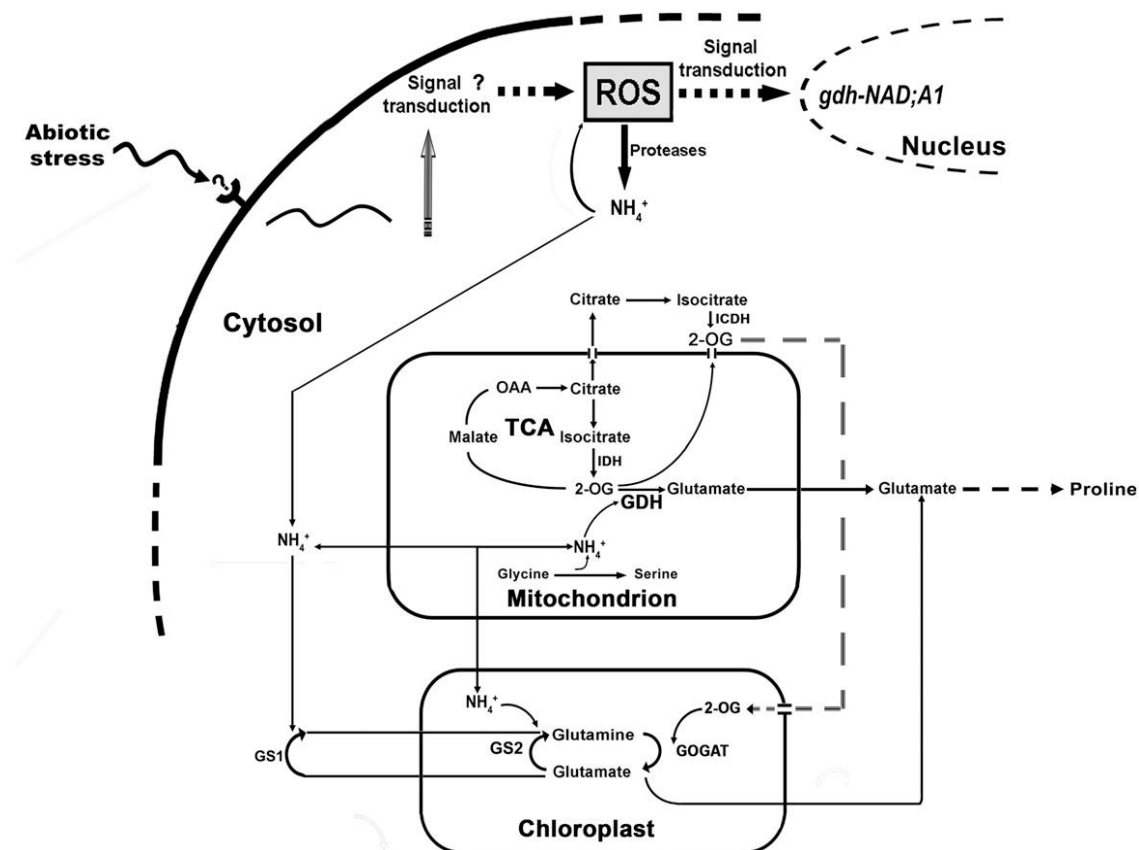


Figure 2.1: Potential Role of GDH under Stress Conditions.

Abiotic stresses result in ROS generation, inducing protease activity, increases in intracellular ammonium ions, and expression of *gdh-NAD;A1*, which participates, along with the GS/GOGAT pathway, in NH_4^+ assimilation. The supply of 2OG for Glu synthesis is provided by isocitrate deamination catalyzed by I(C)DHs. Glu synthesis under stress conditions by GDH is shifted to Pro synthesis. (Skopelitis et al., 2006).

2.2.7. Effects of Salinity on Ion Levels

Higher NaCl uptake competes with the uptake of other nutrient ions, especially K^+ , leading to K^+ deficiency. Increased NaCl salinity induces increases in Na^+ and Cl^- and decreases in Ca^{2+} , K^+ , and Mg^{2+} levels in a number of plants like *Halopyrum mocroronatum* Khan et al., 1999 and *Haloxylon recurvum* Khan et al., 2000a; *Suaeda fruticosa* Khan, 2000b, and *Capsicum* (Chartzoulakis and Klapaki, 2000; Lycoskoufis et al., 2005; Actas et al., 2006). The magnitude of the accumulation and the plant part where accumulation occurs (e.g. leaves, stems fruits, roots) depends on

the plant species or variety, and is connected to salt tolerance or susceptibility or the certain genotype – excluders of Na^+ and Cl^- have been considered more tolerant (Chartzoulakis and Klapak, 2000; Aktas et al., 2006)

2.2.8. Effects of Salinity on Water Relations

Water potential and osmotic potential of plants become more negative with an increase in salinity, whereas turgor pressure increases with increasing salinity, as has been found in *Argyranthemum* (Morales et al., 1998); pea plants, (Hernandez et al., 1999) cotton (Meloni et al., 2001) tomato plants (Romeroaranda et al., 2001), pepper plants (Navarro et al., 2003).

2.2.9. Effects of Salinity on Leaf Anatomy

Salinity causes increases in epidermal thickness, mesophyll thickness, palisade cell length, palisade diameter, and spongy cell diameter in leaves of bean, cotton, and Atriplex (Longstreth and Nobel, 1979). In leaves of potato, salt stress reduces chloroplast number and results in smaller intercellular spaces (Bruns and Hecht-Buchholz, 1990). In tomato leaves salinity causes reduction of leaf area and stomatal density (Romeroaranda et al., 2001).

2.3. The Effect of Salinity on Pepper Plants

The effect of saline irrigation on pepper plants has been little investigated. Some articles have referred to the results of saline water on fruit yield and quality of the fruits. In some cases the incidence of blossom-end rot (BER) was investigated in association with fruit quality and quantity (Rubio et al., 2009; Aktas et al. 2005) and the role of Ca^{2+} concentration of the fruit (Rubio et al., 2010) on the severity of the phenomenon was discussed. Other parameters improving blossom-end rot appearance have been investigated such as potassium and calcium fertilization (Rubio et al., 2009) or manganese, zinc and calcium fertilization (Aktas et al., 2005) or calcium and high root zone temperature (Cabañero et al., 2004). It seems that the effects of salinity on the marketable yield and fruit quality are more pronounced towards the end the harvest season, something the growers of Ierapetra have noticed, probably because of the combination of stresses (high temperature and dry conditions) which may act synergistically with high salinity (Rubio et al., 2010). The marketable fruit yield reduction by salinity is mainly due to the increase in the BER incidence. K^+ although

reduced the percentage of BER did not improve the yield quantity (Rubio et al., 2009). It is suggested (Actas et al., 2005) that generation and scavenging of oxygen free radicals in the apoplast may contribute to the appearance of BER symptoms in pepper fruits under saline conditions. Manganese may serve as an antioxidant in pepper fruit and may alleviate BER symptoms (Actas et al., 2005) while supplementary KH_2PO_4 can partly mitigate the adverse effects of high salinity on fruit yield and also whole plant biomass (Kaya et al., 2003). Calcium supply will alleviate the negative effect of salinity, with respect to tissue calcium concentration and water relations, on a whole plant level (Cabañero et al., 2004). Fruit number per plant and fruit weight were both reduced by the salinity (Chartzoulakis and Klapaki, 2000) in two greenhouse pepper hybrids as total fruit yield was reduced at salinities higher than 10 mM NaCl, the reduction being 95% at 150 mM NaCl.

Few reports are dealing with the effect of salinity on fruit quality and dietary value. Walker et al., (1980) found that salinity reduced both fruit Ca^{2+} and ascorbic acid concentrations in a correlated manner. Navarro et al., 2006, found that the use of a moderately-saline water was beneficial if the peppers were harvested mature, by increasing ascorbic acid and lycopene in fruits, with no significant effects on other parameters.

Few reports have dealt with the comparison of different genotypes in their response to salinity. Van der Bee and Ltifi, 1991 compared 9 tunisian varieties of peppers in terms of yield, mortality and root weight and found significant differences among them. Chartzoulakis and Klapaki (2000) compared two greenhouse pepper hybrids with respect to salinity at different growth stages and they found the hybrid 'Lamuyo' more sensitive to salinity than the hybrid 'Sonar'. Actas et al. (2006) compared 120 pepper genotypes for their tolerance to NaCl, based on the severity of leaf symptoms only. They chose six tolerant and six sensitive varieties, which in further experiments, showed small or higher dry matter reduction respectively under saline conditions, while shoot Na concentration was at a higher level in the sensitive compared to the tolerant genotypes. The authors believe that it is likely that exclusion of Na from roots into growth medium plays a critical role in expression of high Na tolerance in pepper, and similar opinion is expressed by Azuma et al., (2010).

There are also few papers where authors attempt to evaluate the effect of salinity on pepper plants and the response of pepper plants at a physiological and biochemical level. Martinez-Ballesta (1986) in order to assess the ability of pepper plants to adjust

to the osmotic imbalance caused by NaCl and KCL salinity, measured leaf water relations, gas exchange, osmotic adjustment of the plants, root hydraulic conductance, ion analysis and organic solute analysis of the xylem sap. They observed reductions in root hydraulic conductance, stomatal conductance and net assimilation of CO₂. Cl⁻ and NO₃³⁻ and sucrose accumulation were used by the plants to achieve the osmotic adjustment and regulation. The authors suggest that the increase in sucrose content and the decreases in leaf water potential and stomatal conductance under saline conditions show that, although plants may have been adjusted osmotically, water uptake could not balance water loss and so turgor decreased. Navarro et al (2003), in a similar experiment found that turgor of NaCl-treated plants was maintained at low EC (3 and 4 dS m⁻¹) probably due to the maintenance of water transport into the plant (decrease of stomatal conductance).

Lycoskoufis et al., (2005) and Azuma et al., (2010) found that both the stem and the root dry weights were markedly restricted by salinity. Net photosynthesis leaf chlorophyll content, stomatal conductance and transpiration rate were reduced. The leaf Na and Cl concentrations were raised. Salinity reduced the leaf K, Ca, and Mg uptake but not to levels that could cause nutrient deficiencies. The authors agree with Navarro (2003) and Martinez –Balestra (1986) that pepper is susceptible to high salinity, because of reduced stomatal conductance, but they also suggest that growth suppression after longer exposure to salinity is also because of inhibition of photosynthesis at chloroplast level. Salinity is reported (Azuma et al., 2010) to reduce the photosynthetic rate (Po) and related parameters such as, stomatal conductance (gs), and intercellular CO₂ concentration (Ci) in pepper plants.

Azuma et al (2010) also reported changes in lipid peroxidation as a result of salinity stress, assessed by the degree of accumulation of MDA which significantly increased in both leaves and fruits, due to toxic reactive oxygen species. Moreover salinity stress caused a decrease in Superoxide dismutase (SOD) activity an important substance in the cellular defence system against oxidative stress (Azuma et al 2010). It has also been reported that membrane permeability in leaf cells (as assessed by electrolyte leakage from leaves) was impaired by NaCl application (Kaya, et al, 2003). Chartzoulakis and Klapaki (2000) tested the salt tolerance of two bell-pepper hybrids in terms of seed germination rate. They found out that salinities up to 50 mM delayed germination but not the final germination percentage, while at 100 and 150 mM NaCl the reduction was significant in both hybrids.

2.4. The Use of in Vitro Culture for Screening of the Pepper Hybrids for Salt Tolerance or Sensitivity

Tissue culture is an easy and cheap tool for developing stress-tolerant plants. This technique can spare time and space, and allows a better control of the environment and the imposed stress (Jain, 2001; Lutts et al. 2004). It also allows a better understanding of the effects of salt at a cellular level and allows the ability to assess salt tolerance and survival by looking at parameter other than whole plant growth which is not always reliable (Gulati et al., 1992; Lutts et al., 2004).

Two types of selection methods has been suggested: (a) long-term treatment, in which cultures are exposed to low stress level for a long time with gradual increase and (b) shock treatment, in which cultures are directly subjected to a 'shock' of high concentration .(Rai et al., 2010).

Identification, isolation, and regeneration of salt tolerant genotypes from cell cultures have been proposed as an efficient method of breeding for salt tolerance in many plants like tobacco (Binzel et al., 1985), rice (Smith et al., 1981), alfalfa (*Medicago sativa*) (Shah et al., 1993) *Vigna radiata* (Gulati et al., 1993) and tagetes (Mohamed et al., 2000) although the researchers stress the need of further in vivo investigation. Cell culture techniques have been used for many plants but not in all cases were positive correlations for callus (or other tissue) salt tolerance and whole plant salt tolerance found (Starvarek and Rains, 1984).

Many authors have evaluated salinity stress effects on plant (not callus) growth in vitro. Work has been carried out on wheat (Maddock et al., 1983), potato (Morpurgo, 1991; Naik and Widholm, 1993) , citrus rootstock *Poncirus tritoliata* (Beloualy and Bouharmont, 1993), prunus (Rajashekar et al., 1995), tagetes (Mohamed et al., 2000), and jojoba (Russos et al., 2007)

The results are not clear because although in most cases tolerance in vitro followed tolerance in vivo, sometimes in vitro tolerant lines were not as tolerant in the field (Zhang, 1998).

2.5. Aim and Objectives of the Present work

AIM

The effect of salinity on the pepper production of Ierapetra is a major problem for the growers of Ierapetra. The aim of this PhD project is to examine the effect of NaCl in the irrigation water (or the substrate in cultures in vitro) on four greenhouse pepper hybrids, by studying the effects of salinity on different physiological and biochemical parameters of their growth and development. Two bell hybrids (Drago and Gracia) and two long hybrids (Sammy –yellow and Kapy -red) were used, which have never been investigated before.

OBJECTIVES

- To study the effect of NaCl on the growth and development of four of the most popular pepper hybrids in order to understand the problem.
- To assess the impact of salinity on major physiological and biochemical parameters of the growth and development of each hybrid.
- To compare the level of salinity on the severity of the symptoms and the effect on the measured parameters.
- To collect evidence on the tolerance or sensitivity of the four pepper hybrids by evaluating the symptoms and comparing the results of salinity on the measured parameters.
- To gather more information on the mechanisms involved in the plant tolerance to NaCl.
- To consider the possibility of using any - physiological or biochemical parameters as an indicator of salinity tolerance in pepper.

In figure 2.2 is summarized the general plan of this work

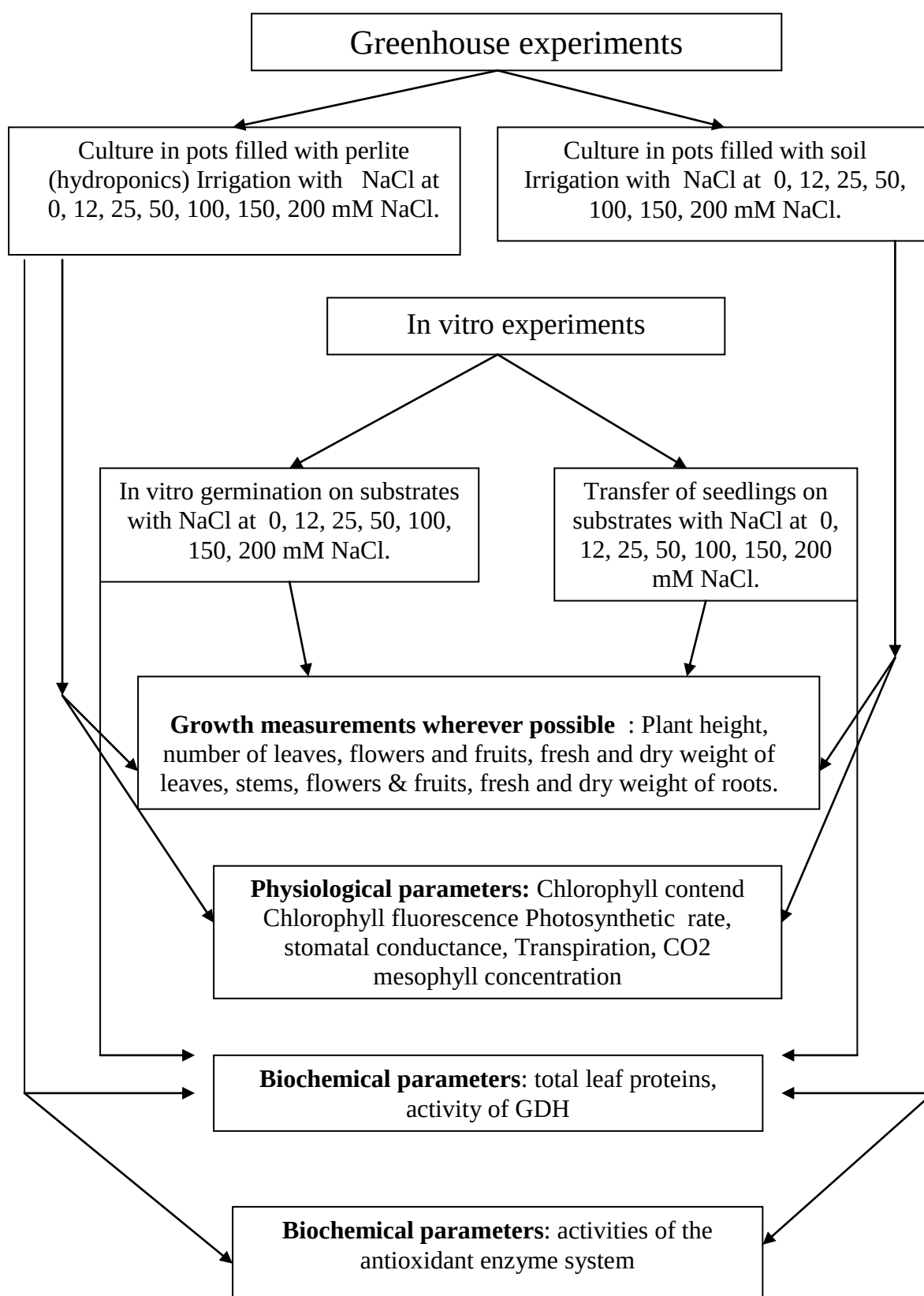


Figure 2.2: Diagram of the experimental work carried out for this thesis

Chapter 3. Materials and Methods

This study is concerned with the growth of pepper (*Capsicum annum*) in vivo and in vitro with respect to salt tolerance. It was decided to use and compare the most popular sweet bell pepper hybrids: Drago (HELAMCO) and Gracia (AGROSYSTEM) and the most popular long sweet peppers Sammy (AGROSYSTEM) and Kappy (AGROSYSTEM).

3.1. Greenhouse experiments

The in vivo experiments were conducted in a heated greenhouse at the farm of the Technological Educational Institute of Crete- in Heraklion – Greece. The minimum night temperature during all experiments was 12 °C.

The experiments in perlite (hydroponic) were conducted in autumn – winter 2008 to. In October 2009 started the experiments of pepper plants cultured in soil and finished in January 2010. The duration of the experiments was approximately 3 months. The plants used were 5 weeks old and of about 10cm height. They were obtained from a commercial nursery at the region of Ierapetra and were transplanted in pots. During the experiments the plants were treated with the pesticides and fungicides usually used by the local growers whenever necessary.

3.1.1. Hydroponic culture

The young plants transplanted in pots with perlite after their roots were washed in tap water so that most of the soil was removed. The pots were placed in plastic plates 3 cm in depth and twice as wide as the bottom of the pots. The plants were watered with a dilution of nutrient solution every day, or other day (depending on the amount of the run off water in the plate). Every pot received a quantity of 150 ml. The nutrient solution used for irrigation had the following macronutrient composition (Navarro et al 2006, Martinez-Ballesta 2004), (mM): NO_3^- , 14; H_2PO_4^- H_2PO_4^- , 1.5; SO_4^{2-} , 1; Ca^{2+} , 4; K^+ , 7.5; Mg^{2+} 1. Micronutrient concentrations were (mg/ l): Fe, 1.0; Mn, 0.5; B, 0.25; Cu, 0.02; Mo, 0.01. The pH was adjusted to 5.6 by the use of HCl.

Salinity treatments consisted of seven NaCl levels (0 mM, 12,5 mM, 25 mM, 50 mM, 100 mM, 150 mM and 200 mM) and started two weeks after the transplanting. Salt was added gradually on eleven consecutive days to the base nutrient solution to

avoid osmotic shock. The first day all plants, with the exception of those watered with 0 mM, received 12.5 mM NaCl. The third day all plants were treated with 25 mM NaCl with the exception of those treated with 0 mM or 12.5 mM NaCl. The fifth day, the appropriate plants were watered with 0 mM, 12.5 mM or 25 mM NaCl, while the rest were watered with 50 mM NaCl. On the seventh day the 100 mM treatment was introduced, on the ninth day the 150 mM NaCl treatment and on the eleventh the 200 mM NaCl was introduced. During the experiment the amount of the solution was increased by 50 ml every three weeks to follow the increase of the size of the plants.

In the experiment 16 plants were used per treatment in the cases of Drago, Gracia and Kappy and 12 plants per treatment for Sammy distributed in 4 blocks with 4 replications respectively in a completely randomized block design.

3.1.2. Culture in soil

The young plants transplanted in pots with commercial compost (Terahum). The plants were watered every other day with the nutrient solution. Every pot received a quantity of 150 ml. The nutrient solution used for irrigation was the same as the one used for the hydroponic culture.

Salinity treatments consisted of seven NaCl levels (0 mM, 12.5 mM, 25 mM, 50 mM, 100 mM, 150 mM and 200 mM) and started two weeks after the transplanting. Salt was added gradually to the nutrient solution to avoid osmotic shock in the same manner as it was done for the hydroponic experiment. During the experiment the amount of the solution was increased by 50 ml every three weeks to follow the increase of the size of the plants.

In the experiment 12 plants were used per treatment of each hybrid Drago, Sammy and Kappy and were distributed in 4 blocks with 3 replications respectively in a completely randomized block design. Gracia was not used as was not available seed or seedlings.

3.1.3. Measured Parameters

a. Morphological Parameters

At the beginning of the salt treatments the following parameters were measured: plant height, number of leaves, and number of fruits. These were measured once per week for three months. At the end of this period all the plants were sampled by

separating the roots, the stems, leaves and the fruits. The fresh and dry weight of each part, and the length of the roots were measured in all cases. Immediately after the harvest, 1,5g of leaves of each plant was separated and without any other preparation were kept at -80°C in a ThermoForma – 86 ULT freezer to use for tissue analysis.

b. Physiological Parameters

The first youngest fully shaped leaf from the top of the plant was used to measure the chlorophyll content the chlorophyll fluorescence, the photosynthesis and stomatal conductance every two to three weeks, depending on the weather conditions.

1.Chlorophyll Content

For the chlorophyll content, a SPAD-502 portable counter (Minolta) (Plate 2.1) was used, which performs quick measurements of the chlorophyll content of leaves without damaging the leaf. (www.konicaminolta.com)

The SPAD-502meter utilizes two light-emitting diodes, 650 and 940 nm, and a photodiode detector to sequentially measuring transmission through leaves of red and infrared light. The light source, which is 2 LEDs, one for the red and one for the infrared light, is in the head of the instrument and transmits the light when it is closed. The measuring area where the light is transmitted is only 2×3 mm, allowing measurements of even small leaves. In all measurements the chlorophyll content was recorded by measuring the first fully expanded leaf from the top of the plant.



Plate 3.1: SPAD-502 chlorophyll counter

2. Chlorophyll Fluorescence

Chlorophyll fluorescence was measured by an OS-30p Chlorophyll Fluorometer (OPTI-SCIENCES) (Plate 2.2) which is optimized for rapid field measurements. Two top, fully developed leaves of each plant were used. Measures were made at 660 nm after 10 min dark acclimation.



Plate 3.2: OS-30p chlorophyll fluorescence meter

3. Photosynthesis, Conductivity, Intracellular CO₂ concentration (C_i) and Transpiration

For the photosynthesis, conductivity, transpiration and intracellular CO₂ concentration (C_i) measurements, a LI-6400 (LI-COR) (plate 2.3) portable counter was utilized which performs quick measurements of the chlorophyll content of leaves without damaging the leaf. The LI-6400 is an open system, which means that measurements of photosynthesis and transpiration are based on the differences in CO₂ and H₂O in an air stream that is flowing through the leaf cuvette. The LI-6400 compared to traditional open systems has the gas analyzers in the sensor head. This eliminates plumbing-related time delays, and allows tight control for responding to leaf changes.



Plate 3.3: LI-6400

All measurements for an experiment were conducted on the first fully expanded leaf on all plants in the same day in the order the plants were placed in the blocks. The measuring apparatus allowed for independent control of CO₂ concentration (using an integrated CO₂ mixer), relative humidity, and flow rate at levels of 370 $\mu\text{mol mol}^{-1}$, 15%, and 400 $\mu\text{mol s}^{-1}$, respectively. Light intensity was set to 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Ambient temperature varied between 23 and 30°C during all measurements. Leaf temperature was at 25°C by the heating/cooling system of the measuring apparatus

c. Biochemical Parameters

1. Protein Extraction and Protein Determination by using the Modified Lowry Method.

The frozen tissue samples were pulverized to a powder in liquid nitrogen with a mortar and pestle. The powder was mixed with the extraction buffer which contained: 100 mM Tris-HCl pH 8, 2 mM EDTA, 3 mM MgCl₂, 0,2% Glycerol, 0,2% Triton X 100 and 5 mM DTT. After homogenization in an Ultra-Turrax (three times, 30 sec each) and incubation on ice for 15 min, debris was removed by centrifugation using a Sigma laboratory 4K15 centrifuge at 24000 rpm for 20 min and the supernatants were used for protein determination and enzyme assay. All steps were carried out at 4°C.

The protein content of the extracts was determined following trichloroacetic acid (TCA) precipitation by the method of Lowry et al. (1951) with bovine serum albumin as the protein standard.

2. Determination of Glutamate Dehydrogenase (GDH) activity

Aminating GDH activity was determined following the NADH-dependent absorption change at 340 nm (Loulakakis and Roubelakis - Angeleakis 1996). The standard

amination reaction mixture contained 100 mM Tris-HCl, pH 8.0, 20 mM α -ketoglutarate, 200 mM NH_4Cl , 1.0 mM CaCl_2 , 0.2 mM NADH, enzyme solution and deionized water to a final volume of 1.0 ml. All assays were performed at 30°C. The absorption change at 340 nm was measured using a UV/VIS spectrophotometer. One unit of GDH activity was defined as the amount needed to oxidize 1.0 μmole per min at 30°C.

3. Assessment of Glutamate Dehydrogenase (GDH) isoenzymic profile by Polyacrylamide gel electrophoresis and immunoblot analysis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the Bio-Rad mini Protean II Dual Slab Cells gel apparatus as previously described (Loulakakis et al. 1994). The analysis of GDH was performed on 10% acrilamide gels. For Western blot analyses the electrophoretically separated proteins were transferred onto nitrocellulose filters (0.2 μm pore size; Schleicher and Schuell, Keene, NH., USA) and immunodetected as described by Loulakakis and Roubelakis-Angelakis (1990b). After blocking the sites with 2% Albumin Bovine Serum (BSA) in PBS the filters were incubated for 2 h at room temperature with the GDH (Loulakakis and Roubelakis-Angelakis 1990b) antibody and the alkaline phosphatase-conjugated second antibody, both diluted 1:2,000 in PBS containing 1% w/v BSA and 0.5% w/v Tween-20. GDH antibody complexes were visualized by the bromochloroindolyl phosphate (BciP; 0.05 mg/ml) colour development reaction in 100mM anolamine (pH 9.6) and 0.1 mg/ml nitroblue tetrazolium.



Plate 3. 4: Electrophoresis apparatus



Plate 3.5: Apparatus for Western Blot method

4. Antioxidant Enzymes Extraction and Assay

The activity of ascorbate peroxidase (APX), glutathione peroxidase (GPX), peroxidase (POD) and superoxide dismutase (SOD) was determined in fresh leaf (0.2 g) which was homogenized with a mortar and pestle in 1.6 ml of ice-cold 50 mM NaH_2PO_4 extraction buffer pH=6.5, containing 1% (w/v) Polyvinylpolypyrrolidone PVPP and 1M NaCl. The homogenate was centrifuged at 20000 rpm for 20 min and the supernatant was used for the enzyme assays. All steps were carried out at 0-4°C.

The reaction mixture for each enzyme was: a) for APX 50µl of the enzyme extract in 0.5 mM ascorbate and 0.1 mM H_2O_2 in 50 mM phosphate buffer (KH_2PO_4) (pH 7). The activity was determined by following the rate of the decrease of absorbance at 290 nm using UV spectrophotometer every 20 sec from 0 to 280 (Pinhero, G.R., Rao, M.V., Paliyath, G., Murr, D.P. and Fletcher, R.A, 1997). b) for GPX, 100µl of the enzyme extract in 0.024 mM guaiacol and 327 mM H_2O_2 in 50 mM phosphate buffer (KH_2PO_4) (pH 6). The activity was determined by following the rate of the increase of absorbance at 470 nm. Every 20 sec from 0 to 140 . (Pinhero, G.R., Rao, M.V., Paliyath, G., Murr, D.P. and Fletcher, R.A, 1997) c) for POD, 10µl of the enzyme extract in 0.5% (w/v) pyrogallol and 0.027% (w/w) H_2O_2 in 14 mM phosphate buffer (KH_2PO_4) (pH 6). The activity was determined by following the increase of absorbance at 420 nm every 20 sec from 0 to 180 (Chance B, Maehly AC 1995). d) for SOD, while the determination was done by using the xanthine oxidase-cytochrome c method²⁶ at pH 10. We used 10µl of the enzyme extract and the activity was determined by following the increase of absorbance at 550 nm every 20 sec from 0 to 280 (Mylona, P.V., Polidoros, A.N. and Scandalios, J.G. 1998). All measures in the UV

spectrophotometer (Hitachi U-2000) were done every 20 seconds until the end of the reaction which was the end of the change in absorbance

3.2. In vitro Experiments

The in vitro experiments were conducted during 2007 and 2008 in the lab of Tissue culture of the Technological Educational Institute of Crete. Four hybrids were used: Grazia and Drago, Kappy and Sammy. There were two types of experiments:

- The seeds were placed for germination and seedling growth onto a substrate containing NaCl at the following concentrations: 0 mM, 12.5 mM, 25 mM, 50 mM, 100 mM 150 mM and 200 mM. The experiment was repeated after one year. It was used the same lot of seeds – the remaining from the previous experiments. The seeds were cultured in vitro on a substrate containing NaCl at the following concentrations: 0 mM, 12.5 mM, 25 mM, 50 mM, 100 mM, 150 mM and 200 mM.

- The seeds were first germinated on a NaCl – free medium and 2 weeks and afterwards they were aseptically transplanted into a NaCl – containing substrate.

The substrates, with or without salt, contained half strength (2,15g/l) Murashige and Skoog salt mixture (SIGMA) with macro and micro nutrients as described by Murashige and Skoog (1962) and agar at 7 g/l. pH was adjusted to 6,5 by the use of 1N KOH. The medium was distributed into culture tubes (aprox. 15ml per tube), and they were autoclaved at 121⁰C, for 20min.

The seeds were obtained from the companies that trade them. Before their use the seeds were washed with tap water to remove the fungicide that covers them, and then they were surface sterilized for 15 sec in aqueous alcohol (80% v/v), then for 10min in 10% commercial bleach. The commercial bleach contains 5% NaOCl. Then they were washed 3 times for 5, 10 and 15min with deionized and sterilized water. The seeds were aseptically placed on the substrate, each seed in one tube. 16 seeds were prepared at each concentration. The tubes were placed in a growth room with standard temperature of 23 ⁰C and 16 hour light of 2800 lux. in four blocks, each treatment in four replications.

3.2.1. Measured Parameters

a. Morphological Parameters

After 10 weeks culture on the saline medium all the plants were separated into roots, stems and leaves. The weight of each part was measured on an analytical scale (Gibertini E42S), and 1,5g of leaf tissue was stored at -80 °C freezer (ThermoForma – 86C ULT) to be used for the tissue analysis. Germination was also recorded.

b. Biochemical parameters

Protein extraction and protein determination by using the modified Lowry method, determination of Glutamate Dehydrogenase (GDH) activity and assessment of Glutamate Dehydrogenase (GDH) isoenzymic profile by Polyacrylamide gel electrophoresis and immunoblot analysis were also carried out following the procedures described in 3.1.3.c

Chapter 4. Results

4.1 Hydroponic Culture in Perlite

The greenhouse experiments lasted approximately three months. Below, the parameters that were measured during the experiments are presented. The height of plants, the number of leaves, and fruits were measured weekly. Chlorophyll content, chlorophyll fluorescence, photosynthesis, conductivity, transpiration and intercellular CO₂ concentration (Ci) were measured every 3 weeks if the environmental conditions were favorable. At the end of the experiments, the fresh and dry weight of the roots, the fresh and dry weight of the above ground part of the plants and then separately the fresh and dry weight of leaves and fruits were all measured. The results are presented below.

4.1.1 Morphological parameters

The height and the morphology of leaves were not effected from salt in the four first weeks. Till then the plants at all salt concentrations were in the same height and leaves were in a very good condition, without any signs of wilt or toxicity. Afterwards it was noticed that in the higher concentrations the height of the plants stopped to increase and toxicity symptoms started to appear on the leaves. The lower leaves became yellowish and there were marginal necroses. At the end of the experiment the lower leaves fell off earlier than the lower leaves of the control. Later the toxicity symptoms appeared on the upper leaves, the blossoms and fruits (Plates 4.1 and 4.2 Appendix A).

a.Height of plants

In Figures 4.1, 4.2, 4.3 and 4.4 the height of plants as affected by the salt treatments is presented. In all cases the plants did not grow very much, regardless of salt concentration. In Drago (Fig.4.1) there was a slow increase of the height until the ninth week, after that it remained stable. In the cases of Sammy, Kappy and Gracia the height stopped increasing at the fourth to fifth week, in all NaCl treatments (Figures 4.2, 4.3 and 4.4 respectively).

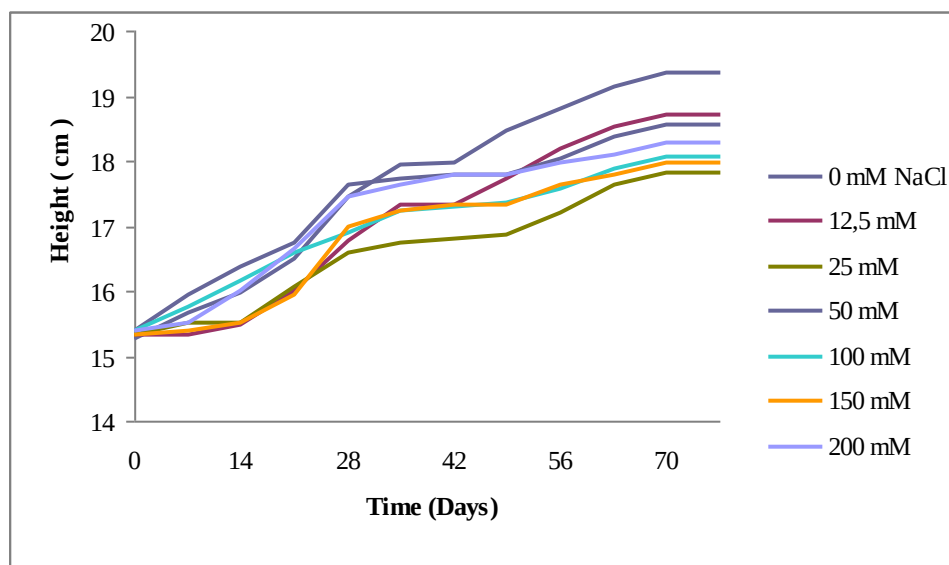


Figure 4.1: Effect of NaCl on the height of Drago pepper plants cultured in perlite.

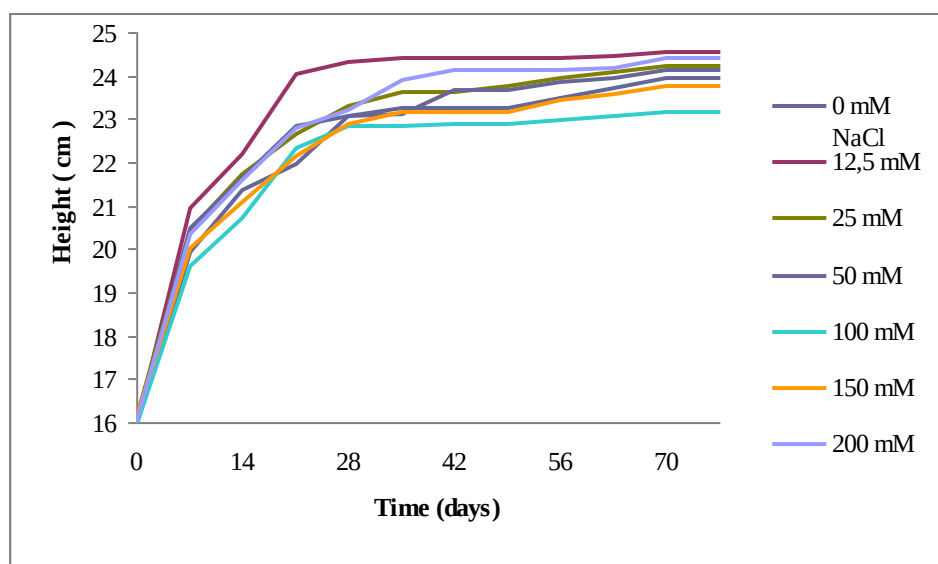


Figure 4.2: Effect of NaCl on the height of Sammy pepper plants cultured in perlite.

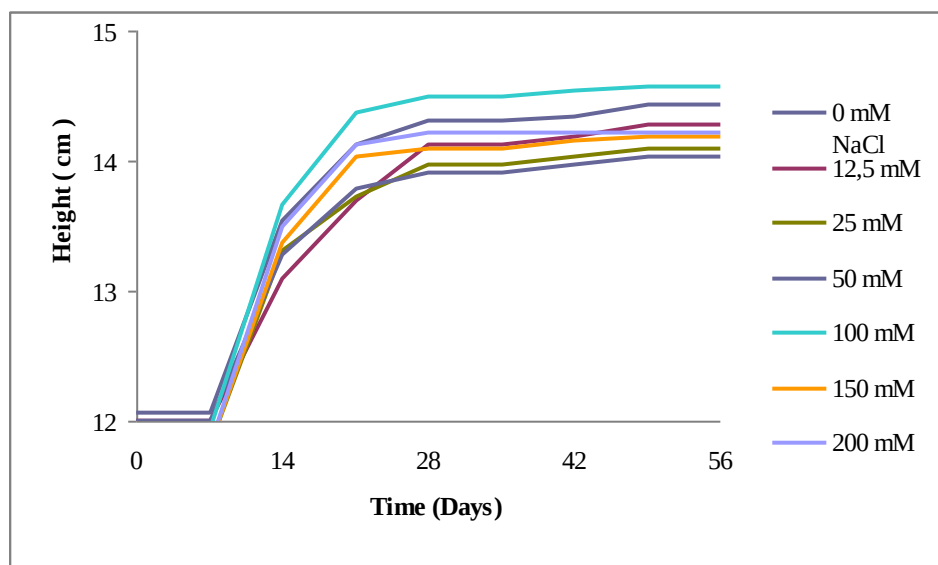


Figure 4.3: Effect of NaCl on the height of Kappy pepper plants cultured in perlite.

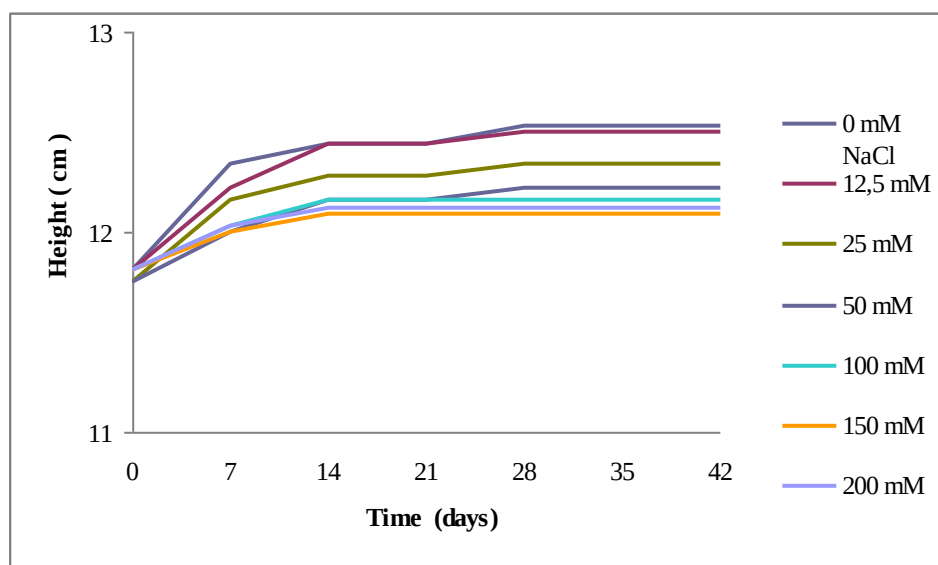


Figure 4.4: Effect of NaCl on the height of Gracia pepper plants cultured in perlite.

At the end of the experiment, 12 weeks after the start of the NaCl treatments, the height of the plants of each treatment and each hybrid was measured. The mean height is presented in Figure 4.5. In all hybrids the height of the plants was not affected by the salt, with the exception of Drago where the plants cultured without NaCl were

significantly higher than those cultured with the highest concentrations, specially 150 and 200 mM. Sammy plants are the highest, followed by Drago, Kappy and Gracia.

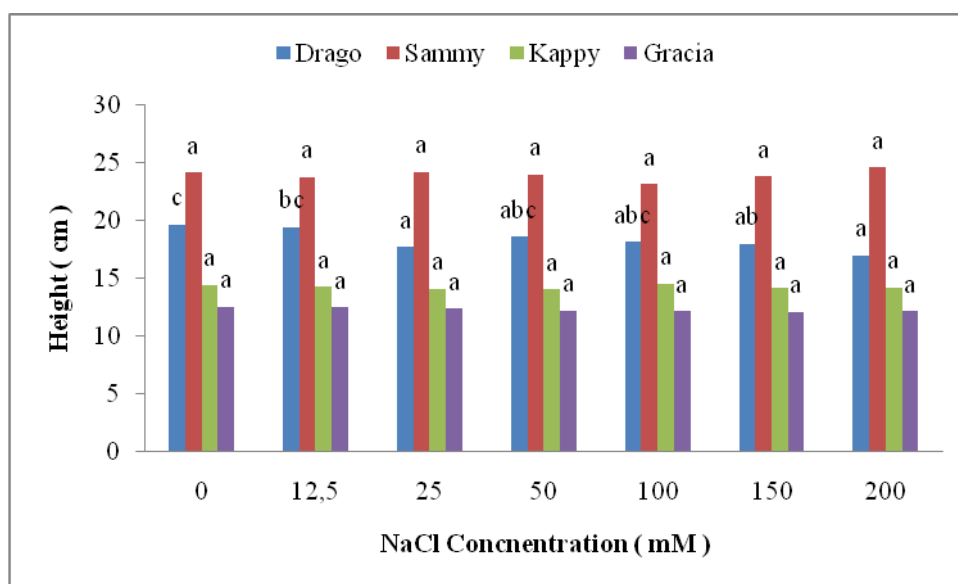


Figure 4.5: Effect of NaCl on the height of pepper plants after culture in perlite for 12 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

b. Number of leaves per plant

The number of leaves per plant of Drago and Sammy increased continuously during the experiment, in all salt treatments (Figures 4.6 and 4.7). In the cases of Kappy and Gracia the number of leaves stopped to increase at the fifth and third week respectively in all salt treatments (Figures 4.8 and 4.9) Later some leaves abscised at the 200 mM NaCl for Kappy and at the 100, 150 and 200 mM NaCl for Gracia.

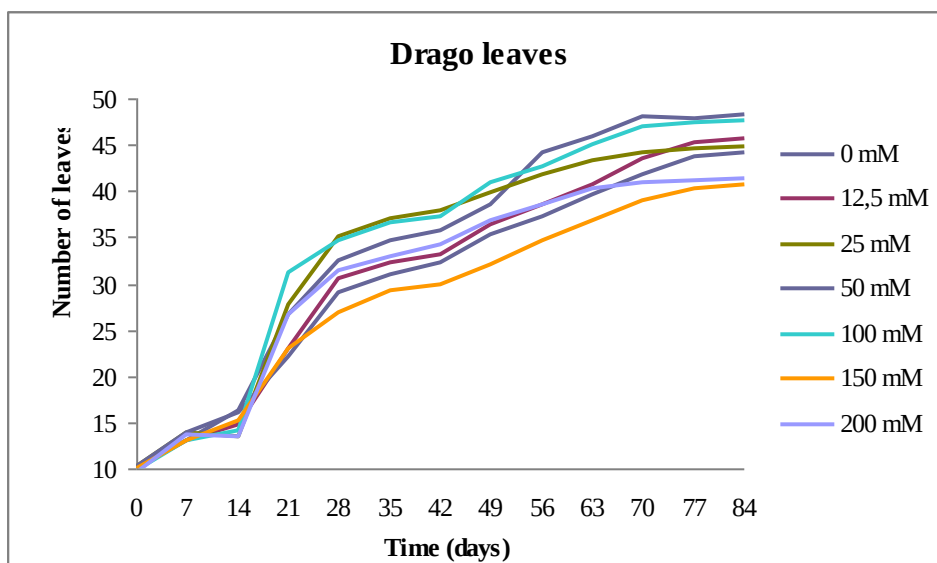


Figure 4.6: Effect of NaCl on the number of leaves per plant of Drago during culture in perlite for 12 weeks.

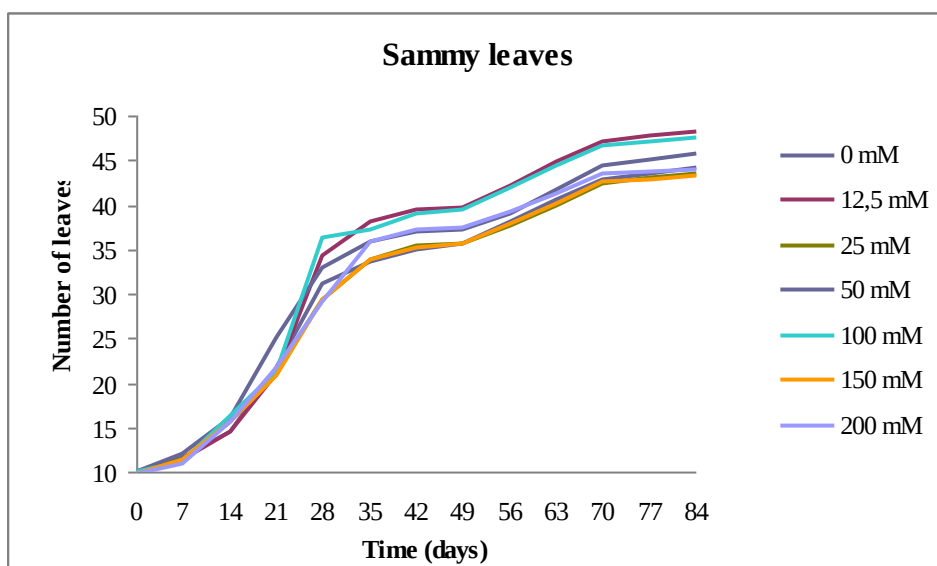


Figure 4.7: Effect of NaCl on the number of leaves per plant of Sammy during culture in perlite for 12 weeks.

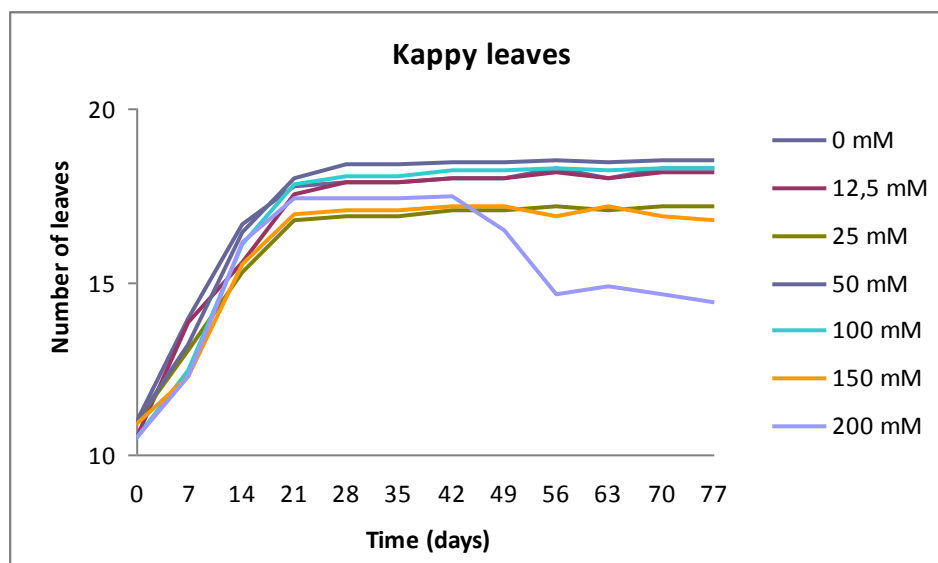


Figure 4.8: Effect of NaCl on the number of leaves per plant of Kappy during culture in perlite for 12 weeks.

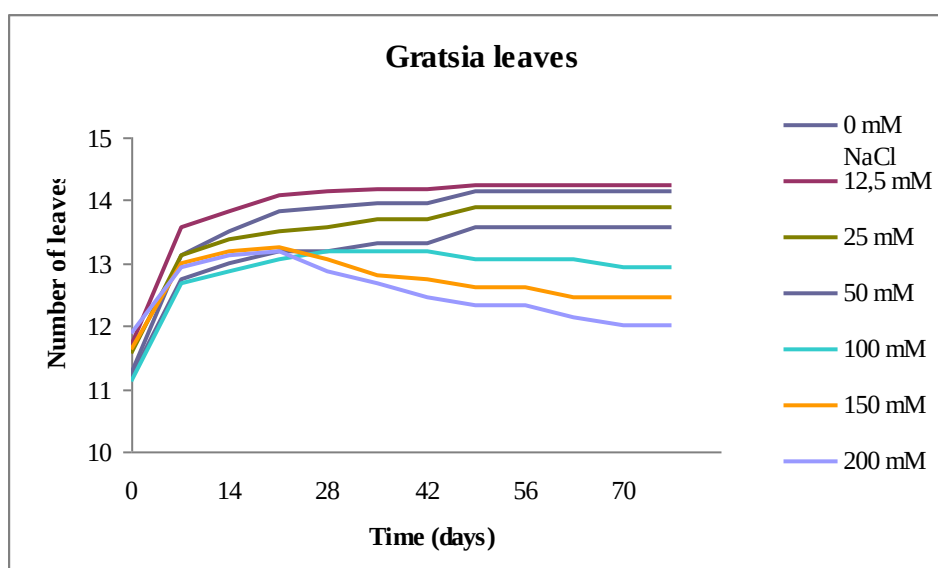


Figure 4.9: Effect of NaCl on the number of leaves per plant of Gracia during culture in perlite for 12 weeks.

The mean number of leaves per plant of all hybrids, in all NaCl concentrations, at the end of the experiment is presented in Figure 4.10. In Sammy the number of leaves per plant was not significantly affected by the NaCl stress. In Drago, NaCl salinity negatively affected the number of leaves per plant only at the concentrations of 150 and 200 mM NaCl. The decrease was small but statistically significant. Similarly in Kappy and Gracia statistically significant though small decreases were observed in the highest

concentrations of NaCl . In general Drago and Sammy plants were much more leafy than Kappy and Gracia plants.

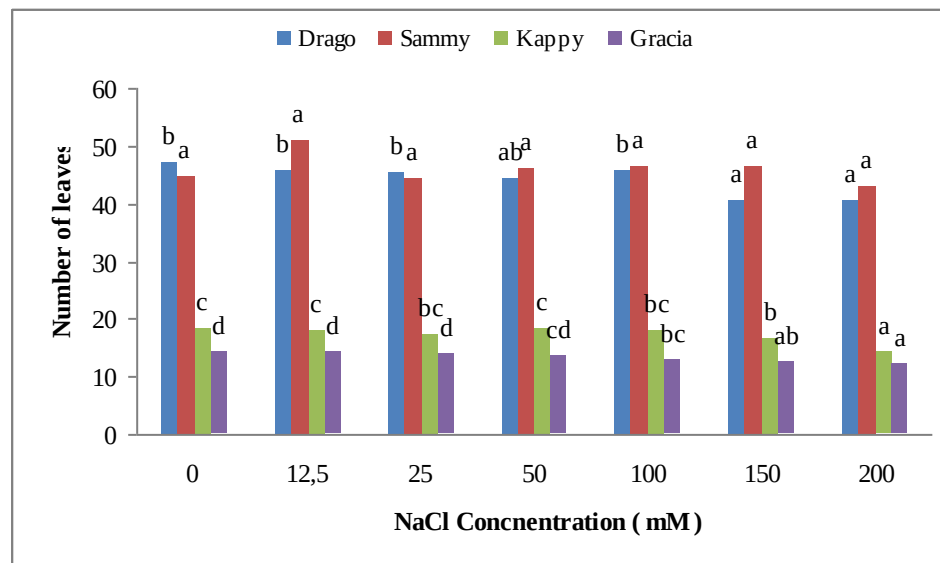


Figure 4.10: Effect of NaCl on the number of leaves per plant of all pepper hybrids cultured in perlite, for 12 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

c. Number of fruits per plant

The mean number of fruits per plant in Kappy (was 1.5) was not significantly affected from the salt stress (Fig. 4.11). In Drago and Gracia , there was a gradual decrease in the number of fruits per plant following the increase in NaCl salinity. In Drago from 4.5 fruits per plant in 0 and 12.5 mM, to approximately 3.5 fruits in the concentrations of 25 and 50 mM NaCl, and 3 fruits in 100, 150 and 200 mM NaCl was observed. In Gracia from 1.5 fruits per plant in 0 NaCl to aprox 0,5 fruits per plant in 150 and 200 mM NaCl was observed. In Sammy there was actually an increase in the mean number of fruits per plant from 2.5 to 5.5 in 12.5mM and almost 4 in 25 and 50 mM. In higher salinities the number of fruits per plant seemed to be not affected as it was comparable to the NaCl free treatment. (Fig. 4.11)

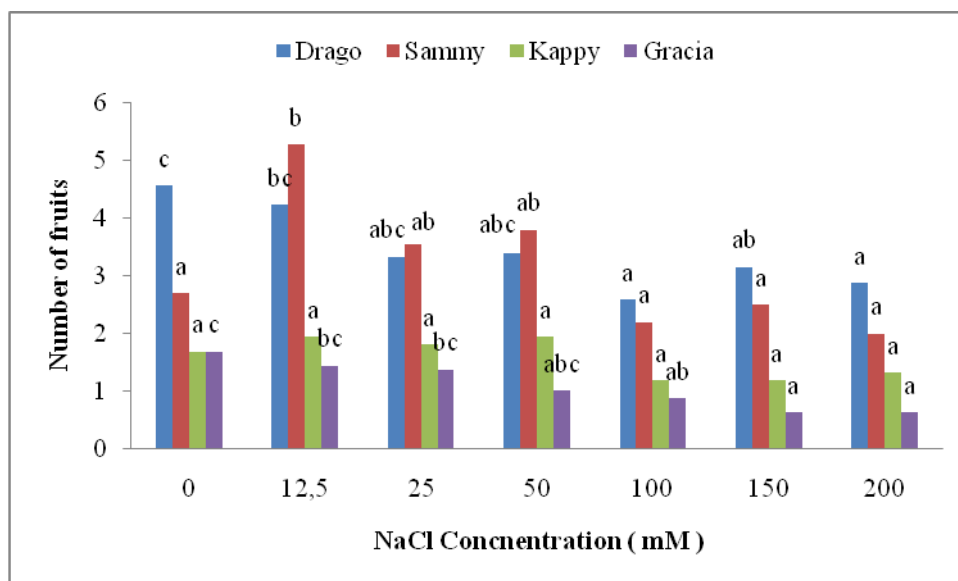


Figure 4.11: Effect of NaCl on the number of fruits per plant of all pepper hybrids cultured in perlite, for 12 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

d. Weight of plants

1. Fresh weight of above ground plant part

Regarding the weight of plants, in all cases the mean fresh weight per plant was reduced as the concentrations of NaCl increased, especially at the higher concentrations (150 and 200 mM) (Fig. 4.12).

In Drago, mean fresh weight decreased significantly in all NaCl concentrations. The plants grown at the concentrations of 12.5, 25, and 50 mM, weighted about 71% of the weight of the plants grown without NaCl. The weight was less than 45% in the concentrations of 100 and 150 and about 25% in the concentration of 200 mM NaCl (Fig. 4.12, Table 4.1)

In Sammy, the fresh weight per plant did not change significantly up to 50 mM NaCl while in 100, 150 and 200 mM NaCl the fresh weight was significantly reduced to 54 to 42% of the initial weight (Fig. 4.12, Table 4.1)

In Kappy the fresh weight per plant did not change significantly up to 50 mM NaCl while in 100, 150 and 200 mM NaCl the fresh weight was significantly reduced gradually from less than 54% of the initial weight to 19% of the initial weight (Fig. 4.12, Table 4.1)

In Gracia, mean fresh weight decreased significantly in all NaCl concentrations, especially and sharply in 100, 150 and 200 mM, where the weight was as little as 8% of the weight of the plants cultured on 0 mM NaCl (Fig. 4.12, Table 4.1).

Furthermore the fresh weight was about 8 times higher than the dry weight in all cases and Drago and Sammy plants were about 3 to 4 times heavier than the Kappy and Gracia plants.

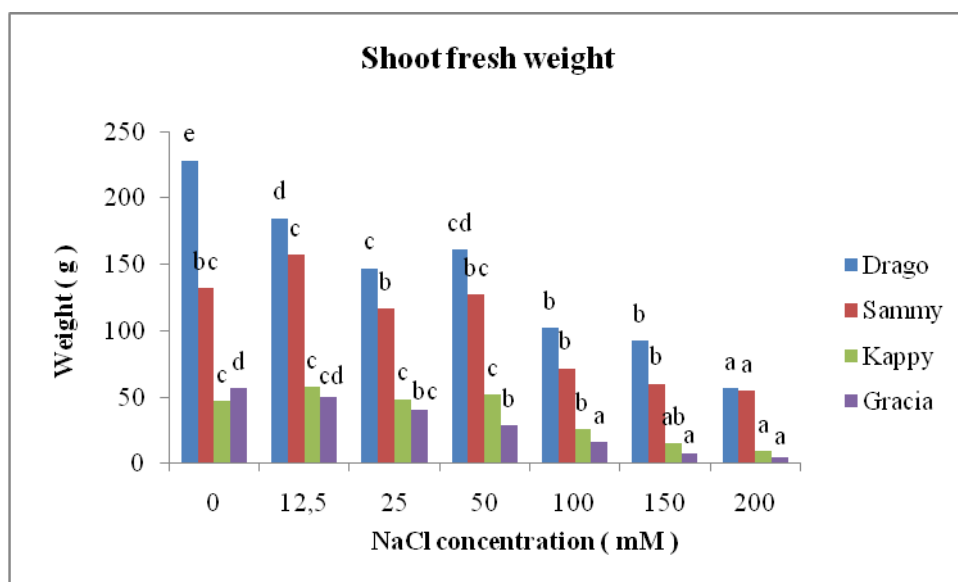


Figure 4.12: Effect of NaCl on the mean fresh weight of shoot (above ground part) of Drago, Sammy, Kappy and Gracia after culture in perlite for 12 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

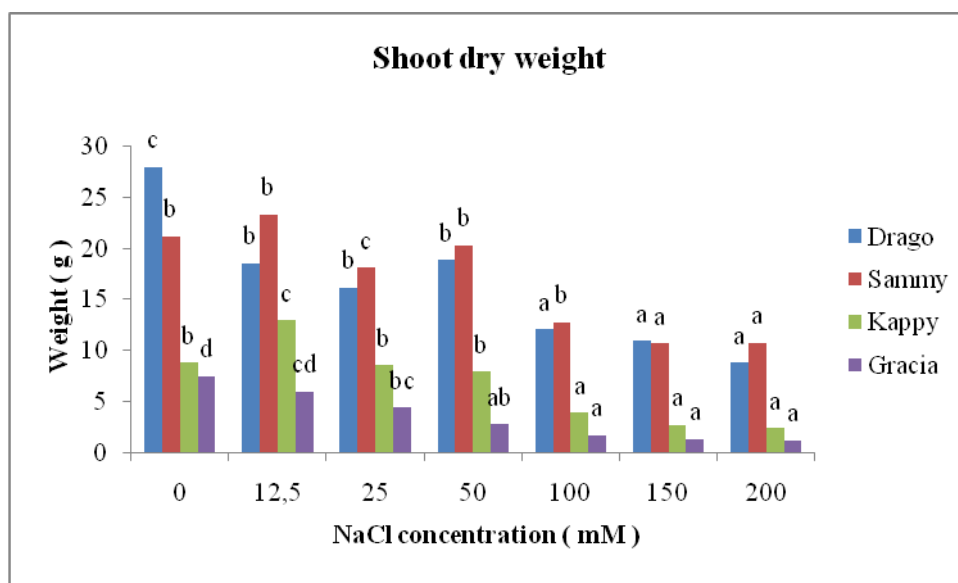


Figure 4.13: Effect of NaCl on the mean dry weight of shoot (above ground part) of Drago, Sammy, Kappy and Gracia after cultured in perlite for 12 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

Table 4.1 : % of shoot fresh and dry weight of all hybrids and all NaCl treatments.

	Drago	Sammy	Kappy	Gracia
NaCl (mM)	% shoot fresh weight compared to 0 NaCl			
0	100	100	100	100
12,5	81	119	122	89
25	64	88	103	72
50	71	96	109	51
100	45	54	54	28
150	41	45	32	13
200	25	42	19	8
NaCl (mM)	% shoot dry weight compared to 0 NaCl			
0	100	100	100	100
12.5	66	110	147	80
25	58	85	97	59
50	67	96	90	37
100	43	60	45	23
150	39	50	31	18
200	31	51	27	16

2. Dry weight of above ground plant part

In Drago the dry weight was significantly reduced in 12.5, 25, and 50 mM to 67 to 58% of the weight in 0 NaCl, while in higher concentrations the weight was reduced even more to 43 to 31 % of the weight in 0 NaCl (Fig. 4.13, Table 4.1)

In Sammy the dry weight was significantly affected in 150 and 200 mM NaCl where it was about half of the weight in NaCl free culture (Fig. 4.13 Table 4.1).

In Kappy the mean dry weight of the aerial part of the plant significantly increased in 12,5 mM NaCl, but in 25 and 50 mM was comparable to that of the plants cultured

without NaCl. In 100, 150 and 200 mM NaCl the dry weight was significantly and dramatically reduced to 45 to 27% (Fig. 4.13, Table 4.1).

In Gracia the dry weight was reduced significantly in all concentration of NaCl, the reduction being more pronounced in the concentrations of 100, 150 and 200 mM NaCl where the weight was reduced to 23, 18 and 16% of the initial weight (Fig. 4.13, Table 4.1) .

3. Root fresh weight

The fresh weight of roots was not affected in the same manner for all hybrids (Fig. 4.14).

In Drago there was a small but not significant decrease following the increase of NaCl concentrations. In Sammy there was not a significant change up to 100 mM, but further increase of NaCl concentration negatively affected the root fresh weight significantly. In Kappy and Gracia there was a small decrease in the root fresh weight especially in high concentrations of NaCl. This decrease was not significant in Kappy but it was for Gracia. In all cases the fresh weight is about 8 times higher from the dry weight in all.

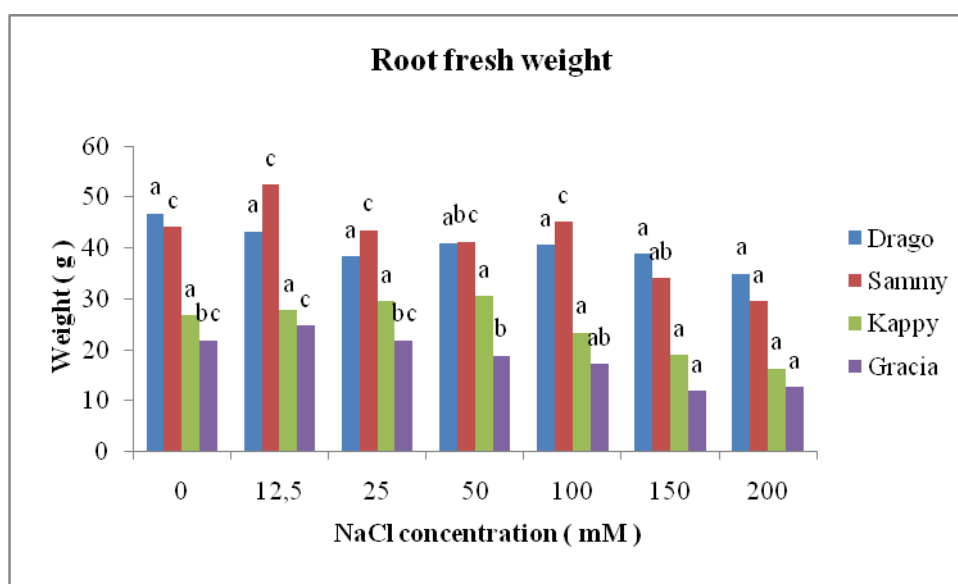


Figure 4.14: Effect of NaCl on the mean fresh weight of roots per plant of Drago, Sammy, Kappy and Gracia after cultured in perlite for 12 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

4. Root dry weight

Root dry weight in Drago was significantly reduced by NaCl only in the 200mM concentration. In Sammy it was reduced in 150, and 200 mM NaCl. In Kappy and Gracia it was significantly reduced in lower concentrations of NaCl (50 to 200 mM) (Fig. 4.15)

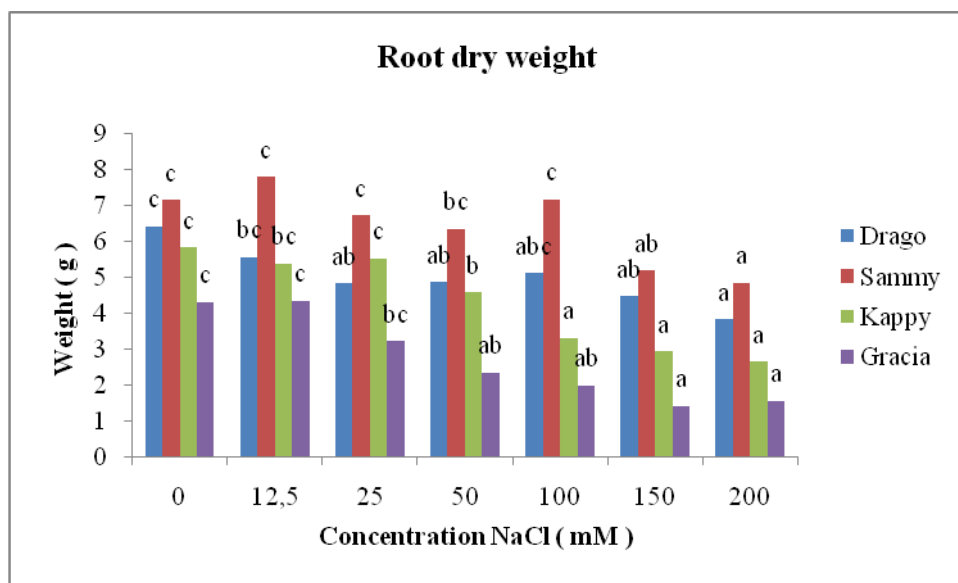


Figure 4.15: Effect of NaCl on the mean dry weight of roots per plant of Drago, Sammy, Kappy and Gracia after cultured in perlite for 12 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

5. Leaf fresh weight

The leaf fresh and dry weight decreased significantly at the concentrations of 100, 150 and 200 mM NaCl, as shown in Fig. 4.16. and 4.17.

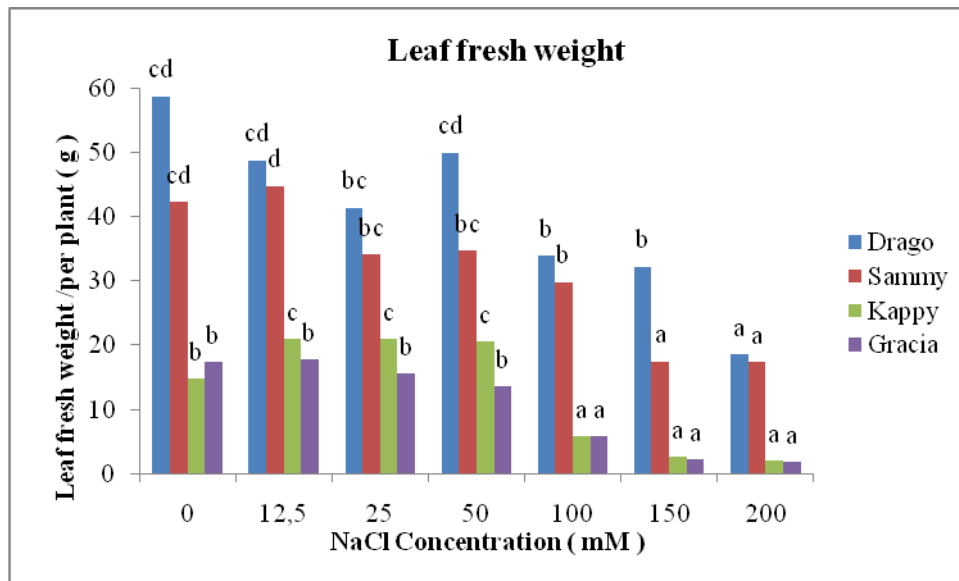


Figure 4.16: Effect of NaCl on the mean fresh weight of leaves per plant of Drago, Sammy, Kappy and Gracia after culture in perlite for 12 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

In Drago and Sammy the leaf fresh weight was gradually decreased following the increase in NaCl concentrations, to only 32 and 41% respectively (Fig. 4.16, Table 4.2).

In Kappy, leaf fresh weight increased significantly in the concentrations of 12.5, 25 and 50 compared to 0 mM NaCl. In the high concentrations of 100, 150 and 200 mM the leaf fresh weight was significantly lower than in all low concentrations (Fig. 4.16).

In Gracia leaf fresh weight did not significantly change in the concentrations of 12.5, 25 and 50 compared to 0 mM NaCl while in 100, 150 and 200 mM NaCl, the leaf fresh weight was significantly lower than all the low concentrations (Fig. 4.16).

The fresh weight / dry weight of leaves is presented in Table 4.2. At low concentrations and about 2 times higher at concentrations of 100, 150 and 200 mM NaCl (Fig. 4.16).

Table 4.2: % of leaves fresh and dry weight of all hybrids and all NaCl treatments.

	Drago	Sammy	Kappy	Gracia
NaCl (mM)	% leaf fresh weight compared to 0 NaCl			
0	100	100	100	100
12,5	83	106	142	102
25	70	81	142	90
50	85	82	139	78
100	58	70	40	33
150	55	41	18	12
200	32	41	13	10
NaCl (mM)	% leaf dry weight compared to 0 NaCl			
0	100	100	100	100
12,5	68	97	142	82
25	57	79	144	65
50	68	80	134	49
100	53	73	47	25
150	52	49	35	18
200	45	46	30	23

6. Leaf Dry weight

In Drago, leaf dry weight was significantly reduced compared to 0 NaCl, the reduction gradually increasing in all NaCl concentrations from 12.5 up to 200 mM NaCl. Leaf dry weight in the 200 mM NaCl treatment was 45% of the control treatment (Fig. 4.17, Table 4.2).

In Sammy although there was a similar trend, significant differences existed only from 100 mM onwards. Leaf dry weight in 200 mM was less than half (46%) of the weight in the control. (Fig. 4.17, Table 4.2).

In Kappy there was a significant increase in dry weight of leaves in the concentrations of 12.5, 25 and 50 mM NaCl, about 40% more than -the control,

followed by a significant decrease to only 47, 35, 31% of the dry weight in the control in the concentrations of 100, 150 and 200 mM NaCl respectively (Fig. 4.17, Table 4.2). In Gracia leaf dry weight decreased following the increase in NaCl. At 100 mM the leaf DW was less than 25% of that in the control (Fig. 4.17, Table 4.2).

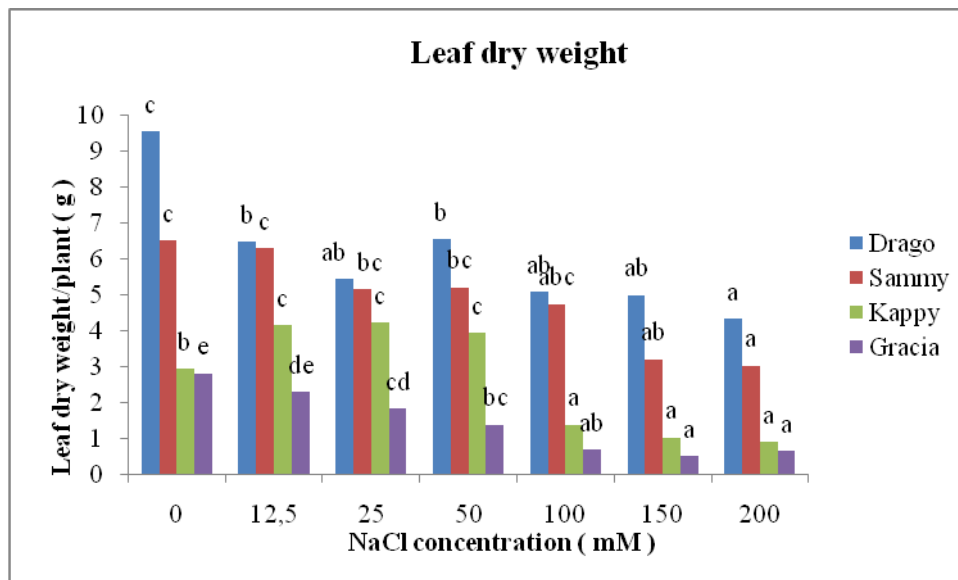


Figure 4.17: Effect of NaCl on the mean dry weight of leaves per plant of Drago, Sammy, Kappy and Gracia after culture in perlite for 12 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

7. Fruit Fresh Weight

Drago is the most productive hybrid but regarding fruit weight is negatively affected by the salt concentration as early as 12.5 mM NaCl, where there was a significant reduction of 17.5%. In concentrations 25 and 50 the reduction was even higher (about 35 %). In 100 and 150 mM NaCl the reduction is more than 60% and in 200 mM more than 80 % (Fig 4.18, Table 4.3).

Sammy's fruit fresh weight seems to be positively affected at 12.5 mM where there was a significant increase of 31%. It is unaffected at 25 and 50 mM. At 100 the reduction is about 35 % while at 150 and 200 mM NaCl, a little more than 50 %. The reduction is less than that in Drago (Fig 4.18, Table 4.3).

In Kappy there was a small and not significant increase of fruit fresh weight at 12.5 mM but from 25 mM there was a gradual decrease of fruit fresh weight until 200

mM where the fresh weight was only 12% of the weight at 0 mM NaCl (Fig 4.18, Table 4.3).

In Gracia there was a sharp and significant decrease of fruit FW at 12.5 mM NaCl which gradually increased up to 200 mM NaCl, where the FW was only 4,7% of the FW at 0 mM NaCl (Fig 4.18, Table 4.3).

Furthermore in all hybrids the fresh weight of fruit was about 8 times higher than the dry weight at low concentrations of NaCl, and 4 about times higher at high concentrations of NaCl.

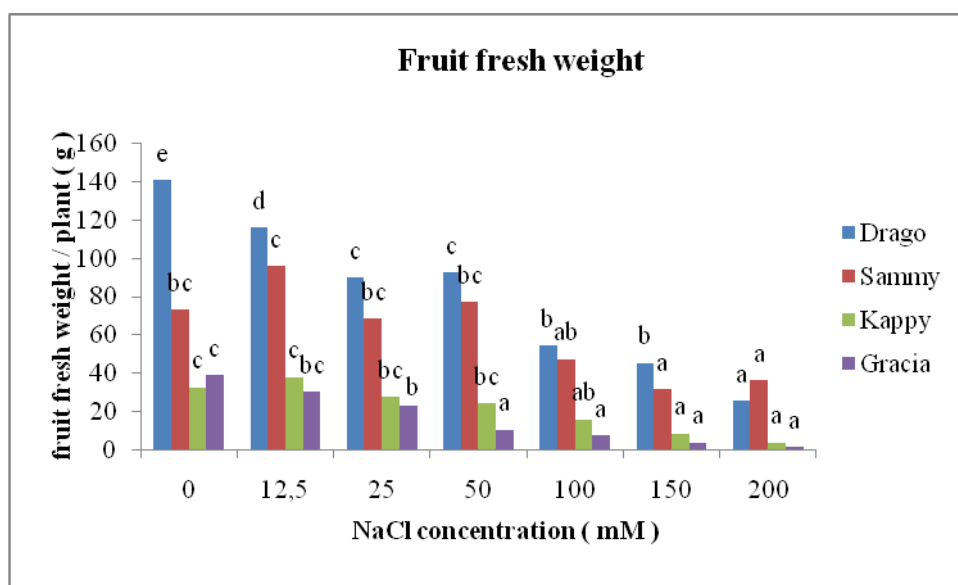


Figure 4.18: Effect of NaCl on the mean fresh weight of fruits per plant of Drago, Sammy, Kappy and Gracia after culture in perlite for 12 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

8. Fruit Dry Weight

The mean fruit dry weight per plant was affected by NaCl treatments in the same manner as for the fruit fresh weight. In Drago at low concentrations (12.5, 25 and 50) there was a significant reduction in the dry weight to 62 to 72 % of the dry weight of the control. At high concentrations the reduction was much higher (Fig. 4.19, Table 4.3). In Sammy there was an increase in dry weight at the low concentrations and reduction in high concentrations though not as dramatic as in the other hybrids (approx 50%). In Kappy there was a significant increase in fruit dry weight at the lowest concentration and a sharp reduction in all higher concentrations to only 14% in 200

mM NaCl, compared to the control. In Gracia the reduction was significant even at 12.5 mM. At 50 mM the fruit dry weight was less than 30% of the initial weight (Fig. 4.19, Table 4.3).

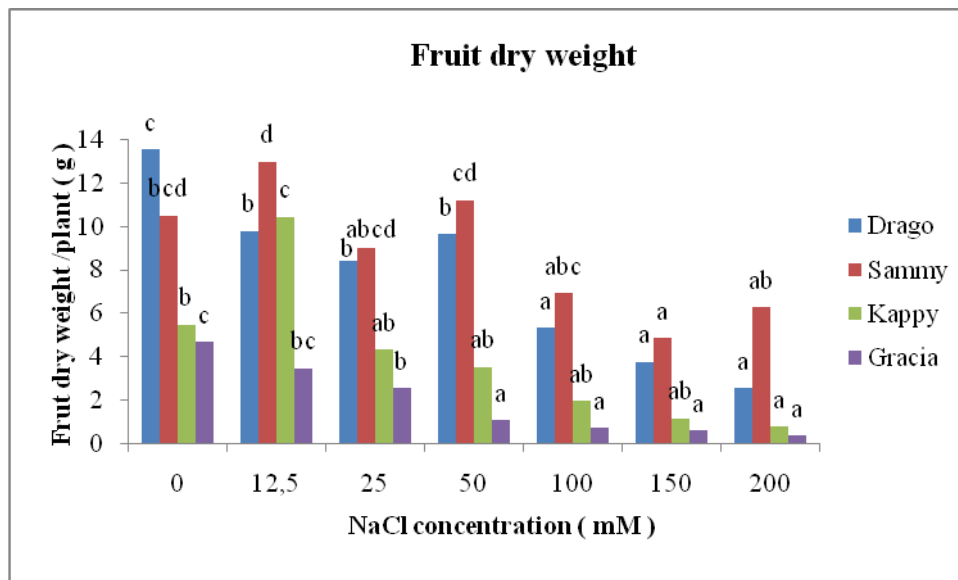


Figure 4.19: Effect of NaCl on the mean dry weight of fruits per plant of Drago, Sammy, Kappy and Gracia after culture in perlite for 12 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

Table 4.3: Mean % fruit fresh weight production of all hybrids after 12 weeks culture on perlite and treatment with NaCl.

	Drago	Sammy	Kappy	Gracia
NaCl (mM)	% fruit fresh weight compared to 0 mM NaCl			
0	100	100	100	100
12,5	82,5	131,1	116,4	78,6
25	64,0	93,8	84,5	60,3
50	65,8	105,9	75,0	27,8
100	38,6	65,0	49,2	20,1
150	32,4	43,6	26,6	10,4
200	18,2	49,8	12,1	4,7
NaCl (mM)	% fruit dry weight compared to 0 mM NaCl			
0	100	100	100	100
12,5	72,4	123,6	191,0	74,2
25	62,0	85,9	78,8	54,7
50	71,3	106,7	64,5	22,7
100	39,5	66,0	35,7	15,7
150	27,8	46,4	21,4	12,5
200	19,0	59,9	14,1	8,2

Table 4.4: Shoot/ Root ratio of fresh and dry weight.

	Shoot / Root Fresh weight				Shoot / Root Dry weight			
mM NaCl	Drago	Sammy	Kappy	Gracia	Drago	Sammy	Kappy	Gracia
0,0	4,9	3,0	1,8	2,6	4,4	3,0	1,5	1,7
12,5	4,3	3,0	2,1	2,0	3,3	3,0	2,4	1,4
25,0	3,8	2,7	1,6	1,9	3,3	2,7	1,5	1,4
50,0	3,9	3,1	1,7	1,5	3,9	3,2	1,7	1,2
100,0	2,5	1,6	1,1	0,9	2,4	1,8	1,2	0,8
150,0	2,4	1,8	0,8	0,6	2,4	2,1	0,9	0,9
200,0	1,6	1,9	0,6	0,4	2,3	2,2	0,9	0,8

The Shoot /root (Table 4.4) was decreased in all hybrids due to salt. The reduction was similar in fresh and dry weight ratio and was more in Gracia and Kappy and less in Sammy and Drago. In Sammy the root to shoot ratio remained unchanged up to 50 mM NaCl. Drago and Gracia were affected from 12.5 mM NaCl and Kappy at 25mM NaCl. In Kappy and Gracia from 150 mM and 100 mM NaCl respectively the root system was heavier than the above ground part.

Table 4.5: Mean % water content of root, shoot, leaf and fruit of all hybrids after 12 weeks culture on perlite and treatment with NaCl calculated after subtracting the dry weight from the fresh weight of the mentioned plant organs.

	% water content							
	Root	Shoot	Leaf	Fruit	Root	Shoot	Leaf	Fruit
mM NaCl	Drago				Sammy			
0	86,3	87,7	83,7	90,4	83,8	84,0	84,6	85,6
12,5	87,1	90,0	86,7	91,6	85,1	85,2	85,9	86,5
25	87,3	89,0	86,9	90,7	84,5	84,5	84,9	86,9
50	88,1	88,3	86,9	89,6	84,6	84,0	85,0	85,5
100	87,4	88,2	85,0	90,2	84,1	82,2	84,1	85,4
150	88,4	88,2	84,5	91,7	84,7	82,2	81,7	84,7
200	89,0	84,5	76,7	90,0	83,6	80,4	82,6	82,7
mM NaCl	Kappy				Gracia			
0	78,1	81,4	80,1	83,3	80,3	86,7	83,8	88,0
12,5	80,6	77,6	80,1	72,6	82,4	88,0	87,1	88,7
25	81,3	82,4	79,9	84,4	85,3	89,2	88,3	89,1
50	85,0	84,7	80,9	85,6	87,5	90,3	89,8	90,2
100	85,8	84,7	76,4	87,9	88,4	89,3	88,1	90,6
150	84,5	81,9	61,2	86,5	88,1	82,2	76,2	85,6
200	83,7	74,3	54,3	80,6	87,6	73,7	63,0	79,1

The succulence of roots increased in all hybrids. In Sammy, the increase was very small -1%- and so in Drago –less than 3%. In Kappy and Gracia was higher namely from 7 to 8%, especially in 50 to 150 mM NaCl.

The shoot and leaf succulence, in all hybrids, increased slightly at medium NaCl concentrations and decreased at high concentrations, especially at 200 mM. In Kappy and Gracia the decrease of water content was high and sharp, especially in the leaves and less in the shoot or fruit (Table 4.5).

4.1.2. Physiological parameters

a. Chlorophyll content and Chlorophyll Fluorescence of the Leaves

The chlorophyll content and the chlorophyll fluorescence in the leaves was measured during the first, fourth, seventh and tenth week of NaCl treatment. Until the fourth week there were no significant differences among these parameters in any hybrid. In the seventh week there were small reductions due to the negative effect of salinity, significant only at the highest concentrations of some hybrids. In the tenth week the reductions were bigger and significant, mainly at the concentrations of 150 and 200 mM NaCl. In Drago, chlorophyll content did not change until the fourth week, (data not shown) The tenth week (Fig.4.20), chlorophyll content slightly increased until 25 mM NaCl, and then it decreased especially in 150 and 200 mM NaCl. In the seventh week the trend was similar to that on the 10th week but the differences were not statistically significant (data not shown).

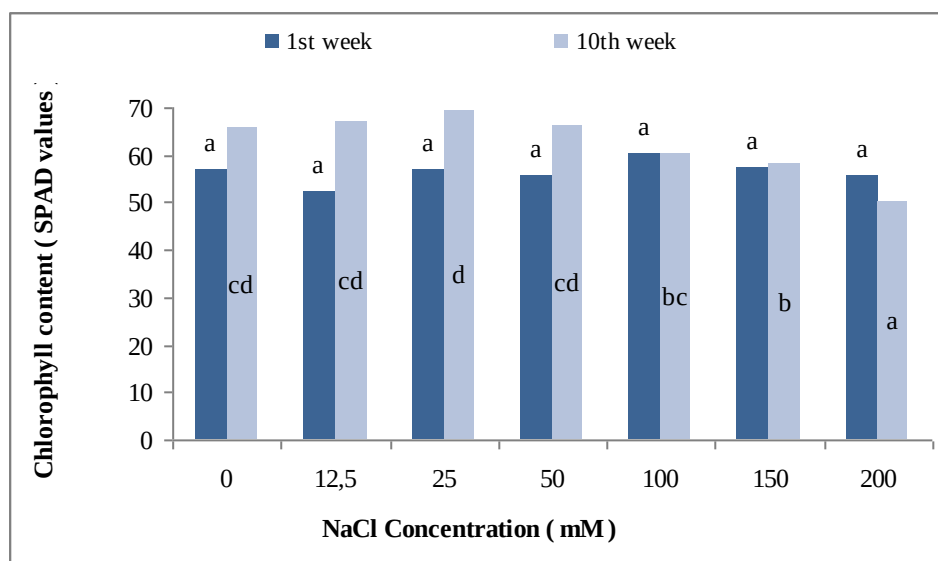


Figure 4.20: Effect of NaCl on the chlorophyll content of Drago pepper plants cultured in perlite. Measurements taken at the first, and tenth week of culture.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

In Drago, chlorophyll fluorescence did not significantly change until the seventh week, (data not shown) In the tenth week (Fig.4.21), it decreased significantly in 100, 150 and 200 mM NaCl.

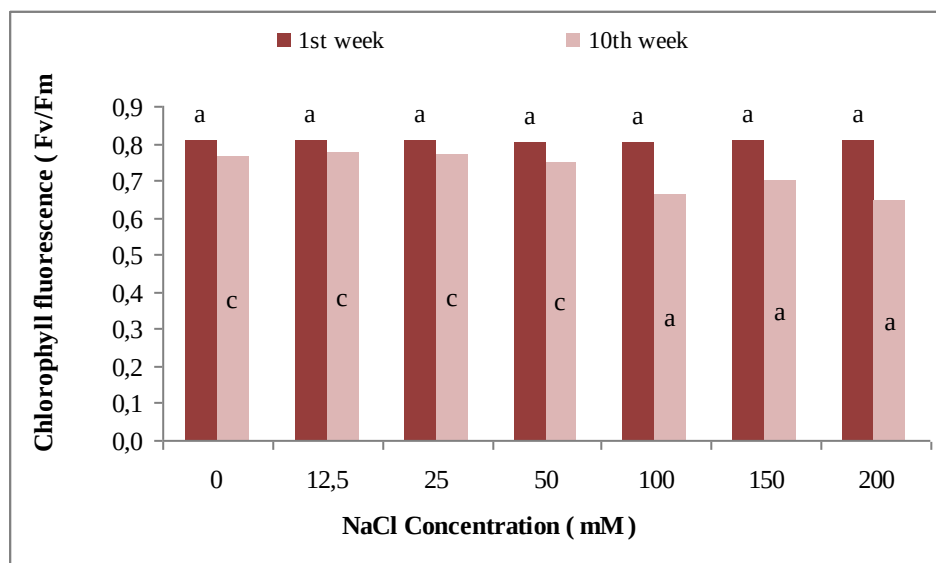


Figure 4.21: Effect of NaCl on the chlorophyll fluorescence of Drago pepper plants cultured in perlite. Measurements taken at the first, and tenth week of culture.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

In case of Sammy as show in Fig. 4.22 and 4.23, the chlorophyll content and the chlorophyll fluorescence decreased at concentrations of 150 and 200 mM NaCl significantly only in the last measurement at the tenth week of culture.

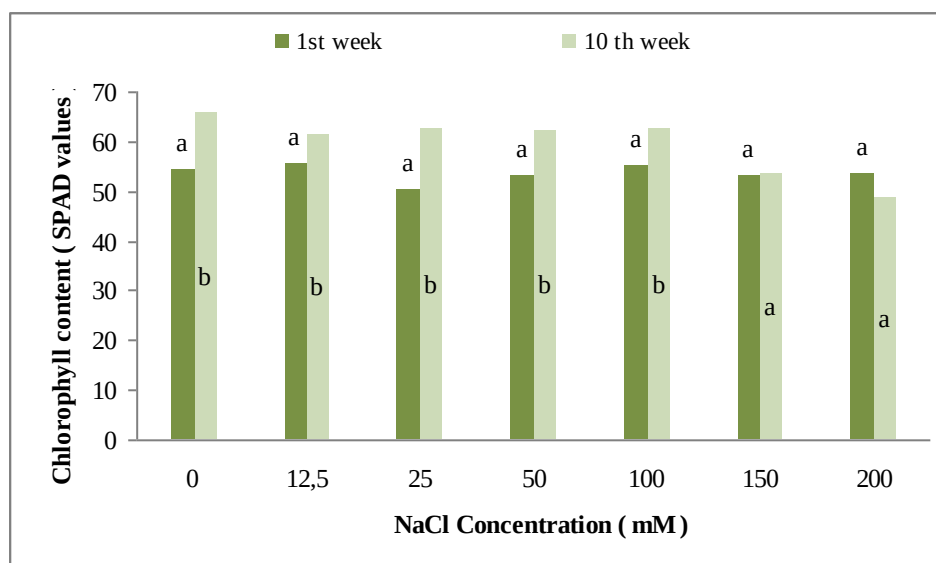


Figure 4.22: Effect of NaCl on the chlorophyll content of Sammy pepper plants cultured in perlite. Measurements taken at the first and tenth week of culture.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

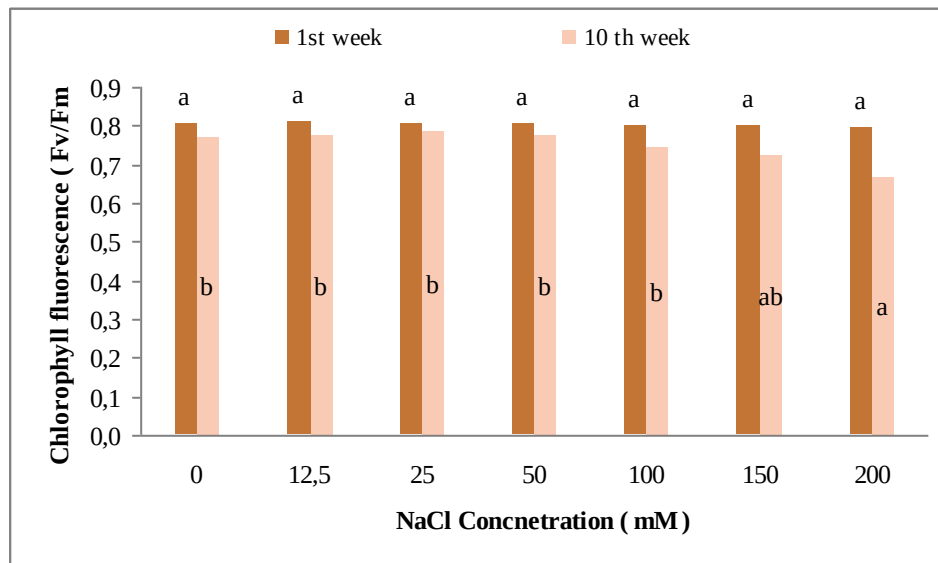


Figure 4.23: Effect of NaCl on the chlorophyll fluorescence of Sammy pepper plants cultured in perlite. Measurements taken at the first and tenth week of culture.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

In Kappy chlorophyll content (Fig. 4.24) as well as the chlorophyll fluorescence (Fig.4.25) were affected by the presence of salt. In the seventh week there was a small but significant increase in chlorophyll fluorescence up to 50 mM NaCl and a small decrease at 100 to 200 mM NaCl. In the tenth week the decrease was obvious at concentration of 25 mM, but the decrease was dramatic at concentrations of 100, 150 and 200 mM NaCl. Chlorophyll fluorescence (Fig.4.25), in the seventh week, was slightly affected by salt, significantly only at concentrations of 150 and 200 mM NaCl. In the tenth week the negative effect of NaCl was obvious and very sharp but only in the 150 and 200 mM NaCl treatments.

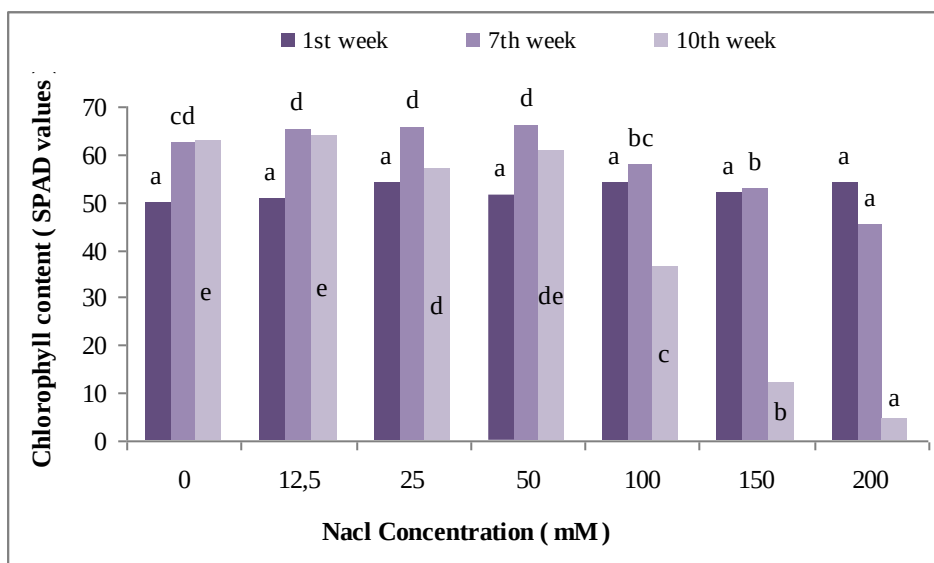


Figure 4.24: Effect of NaCl on the chlorophyll content of Kappy pepper plants cultured in perlite. Measurements taken at the first, seventh and tenth week of culture.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

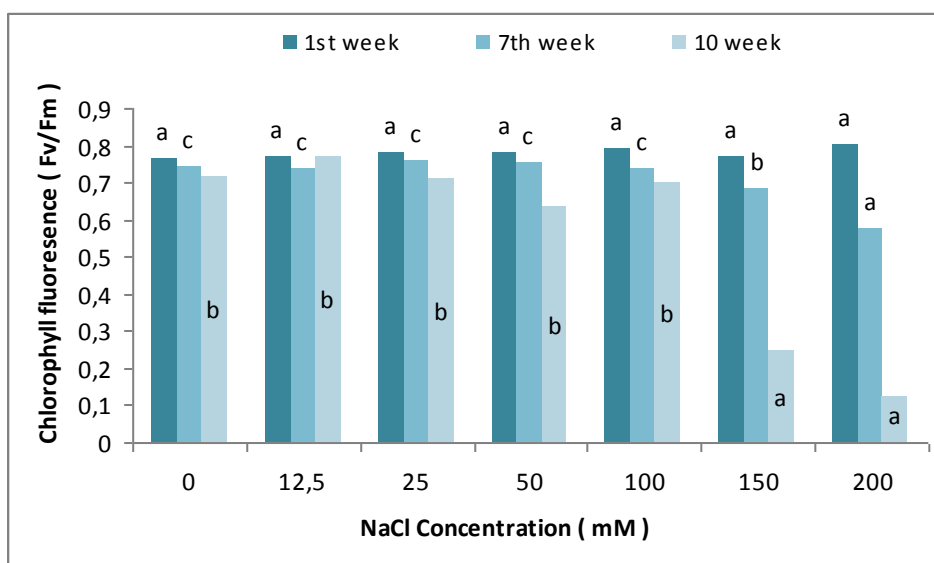


Figure 4.25: Effect of NaCl on the chlorophyll fluorescence of Kappy pepper plants cultured in perlite. Measurements taken at the first, seventh and tenth week of culture.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

In Gracia, in the seventh week there was a small but significant decrease at 150 and 200 mM NaCl in both chlorophyll content and chlorophyll fluorescence (data not shown). In the tenth week there was a small but significant increase in chlorophyll content up to 50 mM and a sharp decrease at 100 to 200 mM NaCl (Fig.4.26). Chlorophyll fluorescence in the seventh week was slightly affected by salt, significantly only at concentrations of 150 and 200 mM NaCl (data not shown) while in the tenth week this parameter was positively affected at 12,5 and 25 mM NaCl, unaffected at 50 and 100 mM NaCl and sharply decreased at 150 and 200 mM NaCl (Fig.4.27).

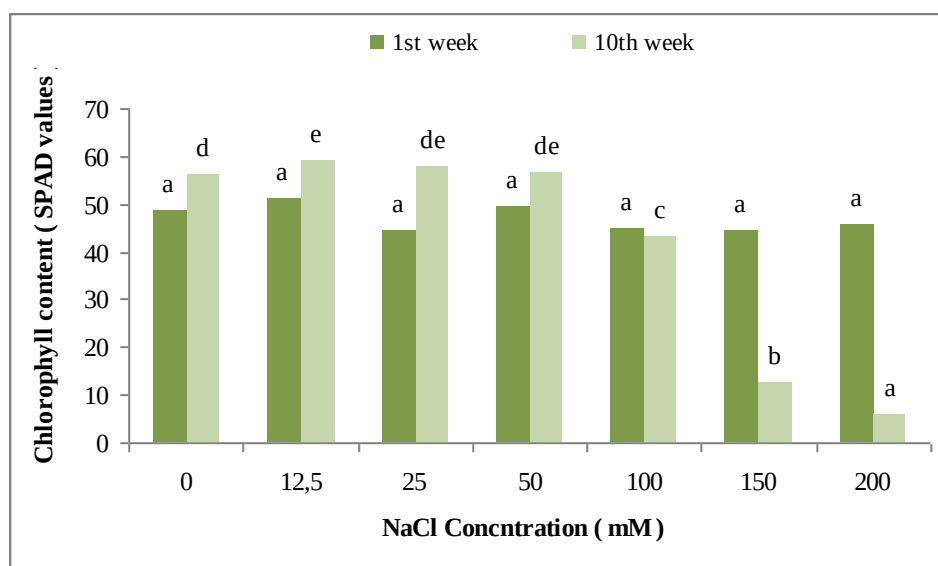


Figure 4.26: Effect of NaCl on the chlorophyll content of Gracia pepper plants cultured in perlite. Measurements taken at the first and tenth week of culture.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

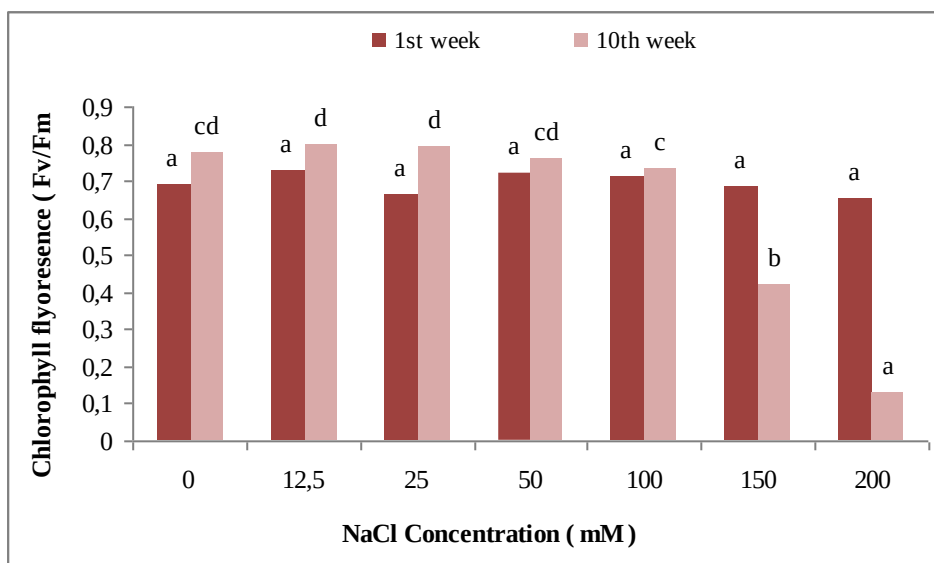


Figure 4.27: Effect of NaCl on the chlorophyll fluorescence of Gracia pepper plants cultured in perlite. Measurements taken at the first and tenth week of culture.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

b. Photosynthetic Rate

According to Fig.4.28 photosynthetic rate significantly decreased as the concentrations of NaCl increased in the case of Drago. In the cases of Sammy, Kappy and Gracia there were no significant changes across the entire range of salt concentrations. In the case of Gracia the levels of the photosynthesis were low compared to the other hybrids.

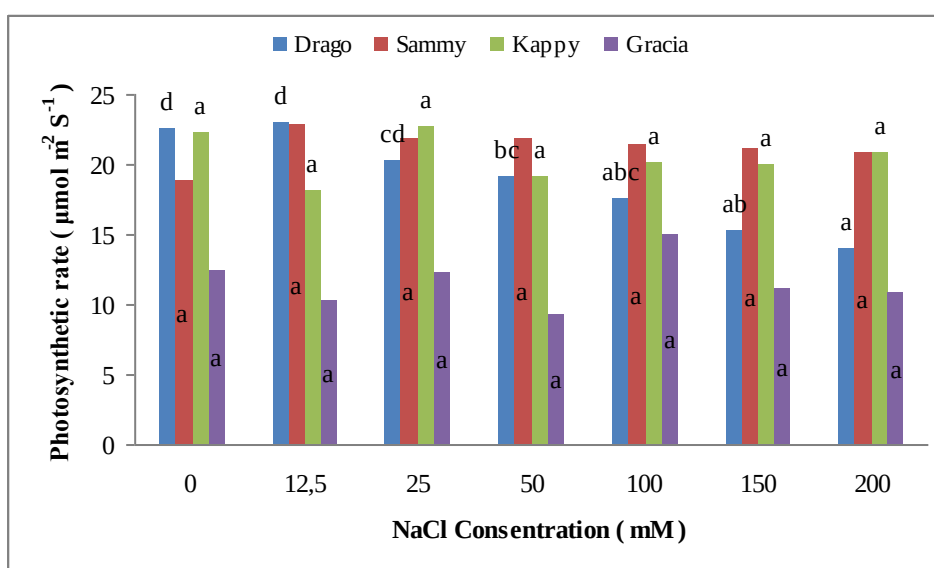


Figure 4.28: Effect of NaCl on the photosynthetic rate of pepper plants cultured in perlite at the 10th week of culture.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

c. Stomatal Conductance

Stomatal conductance (Fig. 4.29), was affected by salt only in the Sammy hybrid in concentrations more than 50 mM NaCl. Drago, Kappy and Gracia hybrids were not affected by salt in terms of this parameter. In Gracia the stomatal conductance just like photosynthesis was very low compared to the other hybrids.

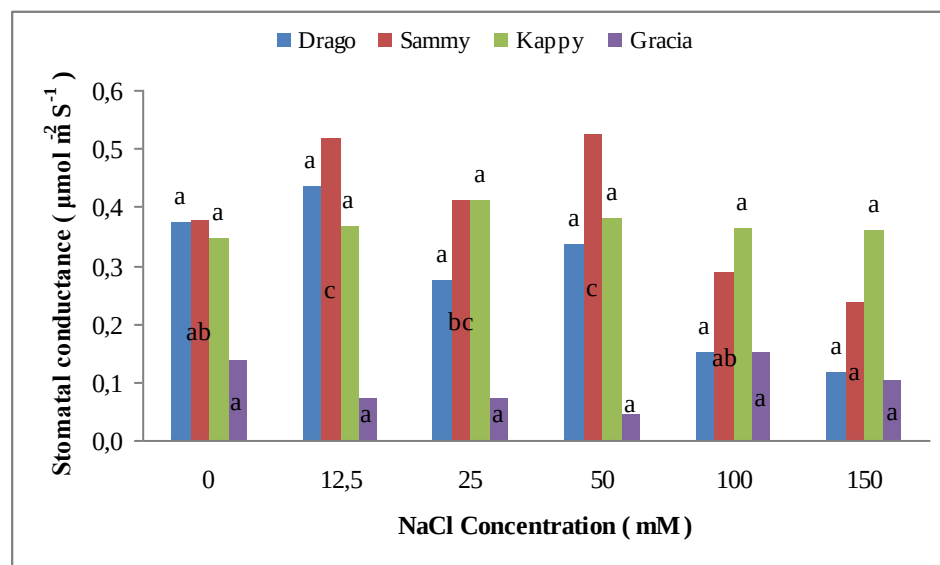


Figure 4.29: Effect of NaCl on the stomatal conductance of pepper plants cultured in perlite at the 10th week of culture.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

d. Intercellular CO₂

The intercellular CO₂ concentration (C_i) decreased with high NaCl concentrations in Drago and Sammy (in 100 and 150 mM NaCl, Fig. 4.30). In Kappy and Gracia there were not significant changes across NaCl concentrations. The level of intercellular CO₂ concentration (C_i) in Gracia hybrid is rather low compared to the other hybrids .

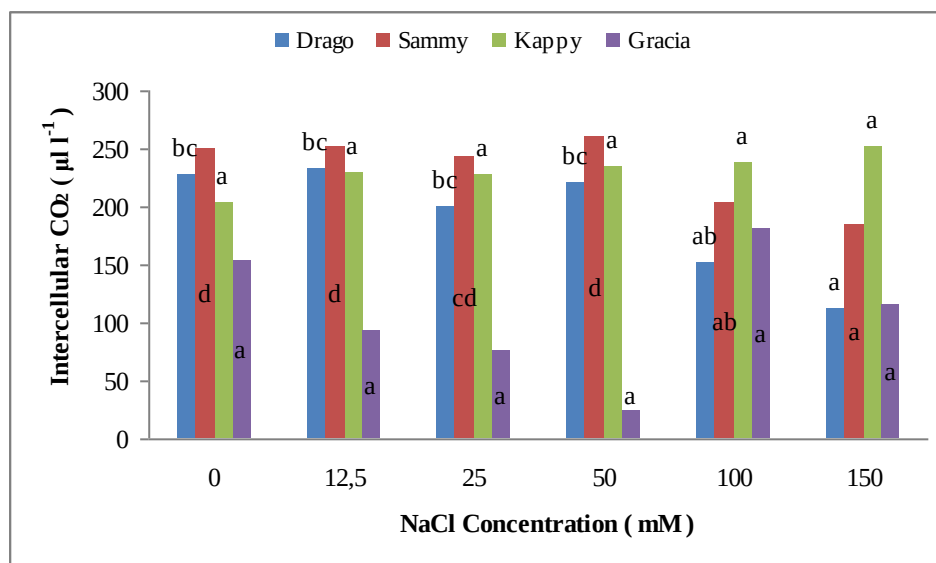


Figure 4.30: Effect of NaCl on the intercellular CO₂ concentration (C_i) of pepper plants cultured in perlite at the 10th week of culture.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test

e. Transpiration rate

Transpiration rate decreased at concentrations of 100 and 150 mM NaCl in Drago and Sammy hybrids (Fig. 4.31). In the cases of Kappy and Gracia the results are similar to intercellular CO₂ concentration (C_i); there are no significant differences between the transpiration rates.

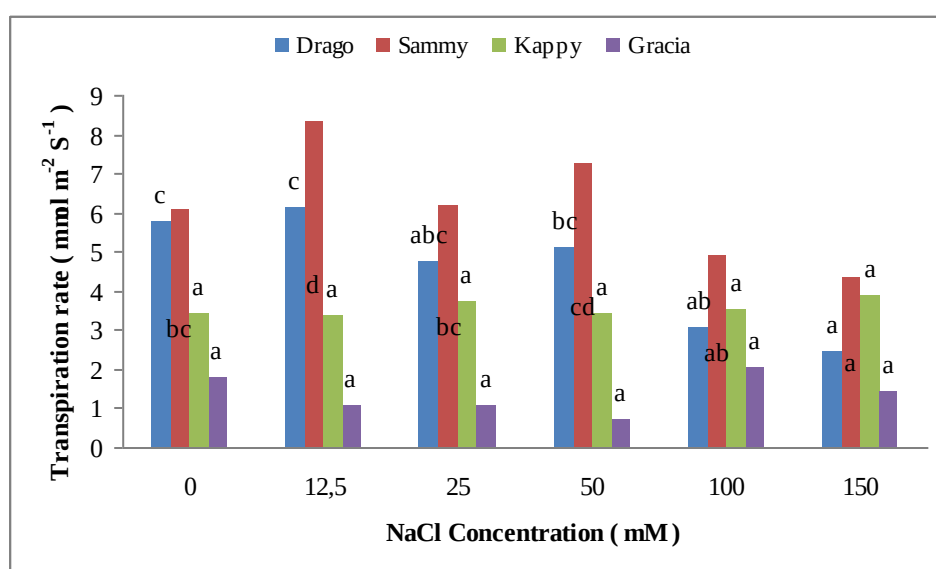


Figure 4.31: Effect of NaCl on the transpiration rate of pepper plants cultured in perlite at the 10th week of culture.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test

4.1.3 Biochemical Parameters

a. Protein Extraction and Protein Determination by Using the Modified Lowry Method.

As shown in Table 4.6 in most of the cases the leaf protein content was affected by the presence of salt. In Drago the protein content increased at the lowest concentration of salt 12.5 and 25 mM. In the medium concentrations of NaCl, the protein content was comparable to that of the 0 mM NaCl, while in the highest concentrations the protein content was reduced 15%, at the most. In the case of Sammy the protein content increased in all NaCl concentrations though this was not statistically significant. In Kappy and Gracia hybrids there was a decrease in the protein content even at the concentration of 12.5 mM, statistically significant in Gracia but not in Kappy. The differences in Kappy were significant only at the highest concentrations of 100, 150 and 200 mM NaCl. The protein content at 200 mM NaCl was 53% for Kappy and 34% for Gracia respectively, as compared to the protein content of leaves 0mM NaCl.

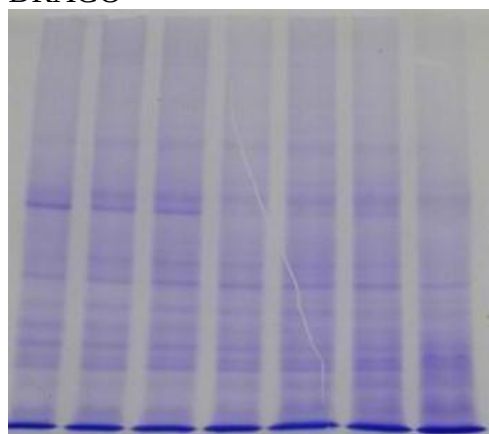
Table 4.6: Effect of NaCl on the protein content of leaves of pepper plants cultured in perlite for 12 weeks.

NaCl stress	Soluble protein content ($\mu\text{g/g}$ fresh leaf)							
	Drago		Sammy		Kappy		Gracia	
		%		%		%		%
0 mM	9435 b	100	5600 a	100	9179 b	100	12112 e	100
12,5 mM	10464 c	111	5995 a	107	7832 b	85	9627 d	79
25 mM	10848 c	115	7157 a	128	8555 b	93	7989 cd	66
50 mM	9451 b	100	6224 a	111	7872 b	86	7072 bc	58
100 mM	8971 b	95	6661 a	119	5765 a	63	8613 cd	71
150 mM	7888 a	84	7179 a	128	4832 a	53	5568 ab	46
200 mM	8096 a	86	6288 a	112	4840 a	53	4133 a	34

b. Protein electrophoresis

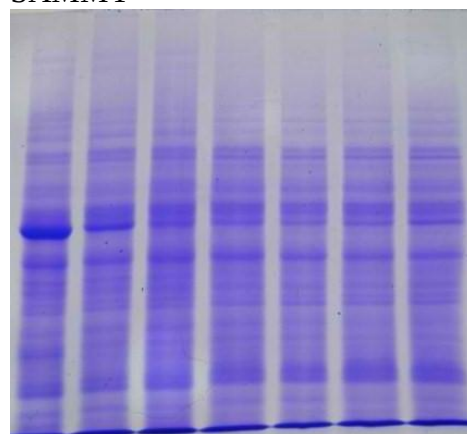
The protein electrophoresis was conducted on four gels, one for each hybrid. In Plate 4.3 are presented the protein electrophoresis gels of leaf extracts of all pepper hybrids after culture in perlite at concentrations of 0, 12.5, 25, 50, 100, 150 and 200 mM NaCl, for 12 weeks.. In each of the seven “wells” of each gel were placed leaf extract of plants grown at different NaCl concentrations.(Plate 4.3). The bands were formed by different proteins. The effect of NaCl on soluble proteins can be judged by the color intensity of the bands. In general, certain protein bands at the higher concentrations of NaCl seem to be lighter than those at the lower concentrations in the cases of Drago and Sammy. In Kappy at concentrations 50 and 100 mM NaCl some protein bands seem to be darker compared to those at the concentrations of 0 mM and 12.5 mM NaCl. In Gracia at concentrations 50 and 100 mM NaCl some protein bands seem to be darker compared to those at the concentrations of 0 mM and 12.5 mM NaCl.

DRAGO



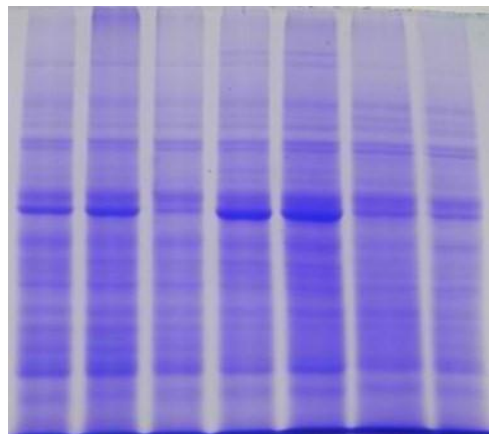
0 12.5 25 50 100 150 200
mM NaCl

SAMMY



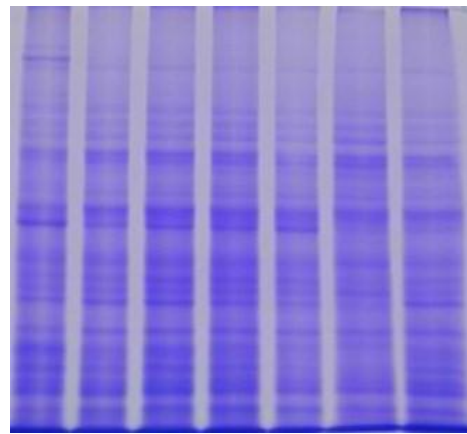
0 12.5 25 50 100 150 200
mM NaCl

KAPPY



0 12.5 25 50 100 150 200
mM NaCl

GRACIA



0 12.5 25 50 100 150 200
mM NaCl

Plate 4.3: Protein electrophoresis gels of leaf extracts of all pepper hybrids after culture in perlite at concentrations of 0, 12.5, 25, 50, 100, 150 and 200 mM NaCl, for 12 weeks.

c. Polyacrylamide Gel Electrophoresis and Immunoblot Analysis

Plate 4.4 presents the results of the polyacrylamide gel electrophoresis and immunoblot analysis. In the case of Sammy the level of GDH protein remained stable in all concentrations of NaCl. But in cases of Kappy and Gracia at the concentrations of 150 and 200 mM NaCl there was an increase, especially at concentration of 150 mM NaCl.

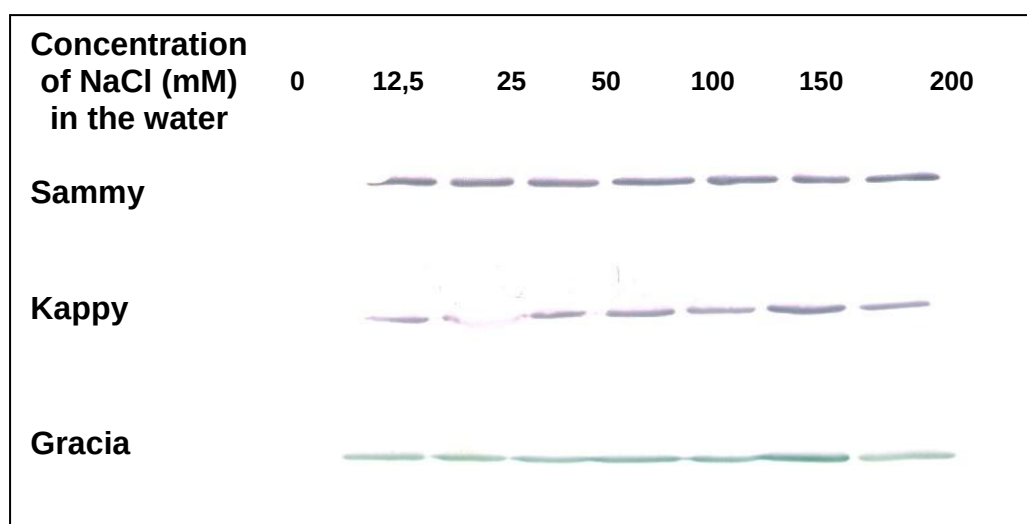


Plate 4.4: Western blot analysis of GDH in Sammy, Kappy and Gracia leaf extracts obtained from plants grown in perlite at different concentrations of NaCl for 12 weeks. For immunoblot analysis proteins were resolved in 10% SDS polyacrilamide gels, transferred onto nitrocellulose membranes and probed with anti-GDH serum, as described in section “Materials and Methods”

d. Activity of Antioxidant Enzymes

Results of antioxidant enzyme activities are shown in Tables 4.7 and 4.8. In Drago hybrid there were no significant differences in the activity of GPX, POD and APX enzymes across the concentrations, while in SOD the activity increased at 150 and 200 mM NaCl. In Sammy all enzymes increased, especially at the high concentrations of 150 and 200 mM NaCl however the increase was statistically significant only in the cases of the activity of GPX and POD. In Kappy the activity of APX, POD and GPX

decreased but apart from GPX the differences were not statistically significant. SOD enzyme activity increased though not significantly. In Gracia hybrid the activity of APX, and GPX activity decreased while POD and SOD were not affected by salt in this hybrid.

Table 4.7: Effect of NaCl on total activity of APX and GPX in leaves of pepper plants cultured in perlite for 12 weeks.

NaCl	Total activity of APX (U mg FW)				Total activity of GPX (U mg FW)			
Stress	Drago	Sammy	Kappy	Gracia	Drago	Sammy	Kappy	Gracia
0 Mm	0,017a	0,022a	0,065a	0,085a	1,508a	0,757ab	1,480d	1,375c
12,5 mM	0,027a	0,031a	0,066a	0,066a	1,325a	0,757ab	1,080cd	1,320c
25 mM	0,026a	0,033a	0,042a	0,042a	1,187a	0,520a	0,935c	0,915abc
50 mM	0,046a	0,030a	0,019a	0,050a	1,078a	0,490a	1,155cd	1,017bc
100 mM	0,016a	0,035a	0,045a	0,065a	1,178a	1,077bc	0,830bc	0,807ab
150 mM	0,017a	0,044a	0,056a	0,046a	1,053a	1,313b	0,170a	0,680ab
200 Mm	0,025a	0,032a	0,015a	0,044a	1,277a	1,143bc	0,425ab	0,483a

Table 4.8: Effect of NaCl on total activity of POD and SOD in leaves of pepper plants cultured in perlite for 12 weeks.

NaCl	Total activity of POD (U mg FW)				Total activity of SOD (U mg FW)			
Stress	Drago	Sammy	Kappy	Gracia	Drago	Sammy	Kappy	Gracia
0 mM	0,014a	0,004a	0,020d	0,012a	2,003a	2,120a	5,760a	7,575a
12,5 mM	0,014a	0,004a	0,022d	0,016a	2,047a	2,395a	5,673a	7,810a
25 mM	0,014a	0,004a	0,019cd	0,010a	1,965a	2,190a	7,170a	8,085a
50 mM	0,010a	0,005a	0,017bcd	0,010a	2,150ab	2,445a	7,060a	7,963a
100 mM	0,012a	0,005a	0,009ab	0,012a	2,337ab	2,368a	7,210a	8,867a
150 mM	0,012a	0,009b	0,004a	0,014a	2,730b	2,473a	8,060a	8,303a
200 Mm	0,015a	0,009b	0,011abc	0,011a	2,797b	2,915a	8,103a	8,170a

4.2 Culture In Soil

The greenhouse experiments in pots filled with soil lasted approximately three months. Below, the parameters that were measured during the experiments are presented. The height of plants, the number of leaves, and fruits were measured weekly. Chlorophyll content, chlorophyll fluorescence, photosynthesis, conductivity, transpiration and intercellular CO₂ concentration (Ci) were measured every 3 weeks, if the environmental conditions were favourable. At the end of the experiments, the fresh and dry weight of the roots, the fresh and dry weight of the above ground part of the plants and then separately the fresh and dry weight of leaves and fruits were measured. The results are presented below.

4.2.1 Morphological Parameters

Up to the fourth week there were not visible symptoms on the plants. Similar to the experiments in perlite the symptoms appeared at the same period (4th week) and started at the same way. The toxicity and drought symptoms appeared first on the lower leaves and later to the upper leaves, to blossoms and fruits. The older leaves abscised earlier in the salinity stressed plants.

a. Height of Plants

The highest of the plants, in the case of Drago, was in NaCl free culture. In all NaCl concentrations the height increased but the increase was progressively lower as concentrations of NaCl increased. The height increased almost till the 43^d day of the experiment at concentrations 0, 12.5, 25 and 50 mM while at concentrations 100, 150 and 200 mM the increase stopped at 32^d day (see Fig. 4.32). It is noticeable that for the first 18th days the increase was sharp in all concentrations but after this it slowed down sooner in the high concentrations.

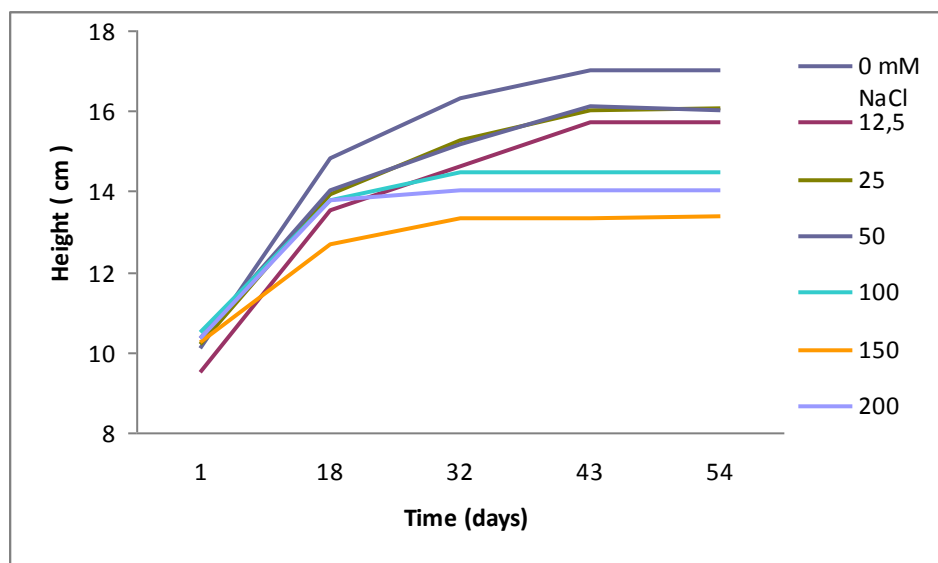


Figure 4.32: Effect of NaCl on the height of Drago pepper plants during culture in soil.

In the case of Sammy (Fig. 4.33) the height increased in all salt concentrations, the biggest increase being in 0 mM NaCl. The increase in 12.5 and 25 mM was smaller while from 50 to 200 mM NaCl, was even smaller, compared to the control. The increase up to the 18th day was more marked at concentrations 0, 12.5 and 25 mM while in the other concentrations it was slower and stopped after a few weeks.

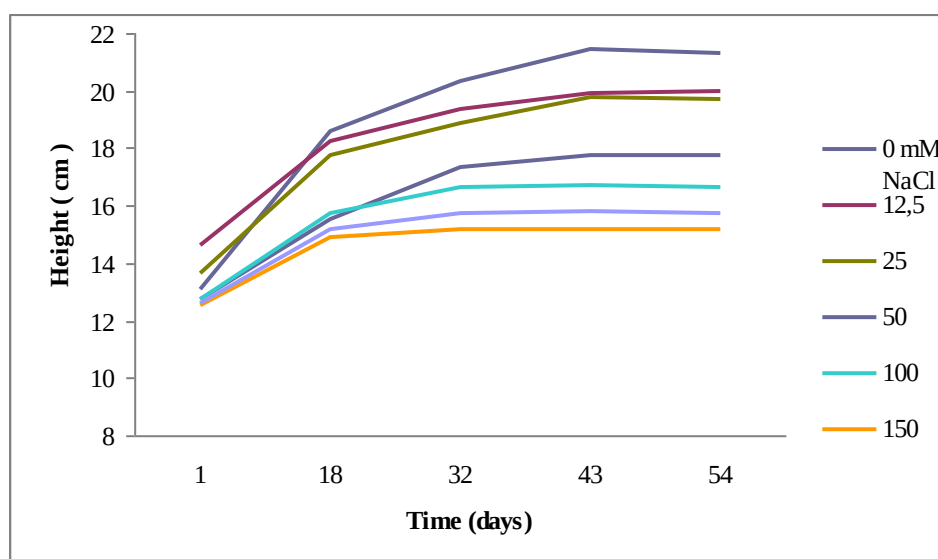


Figure 4.33: Effect of NaCl on the height of Sammy pepper plants during culture in soil.

In the case of Kappy as shown in Fig. 4.34 the results are similar to the other hybrids. The height increased in all salt concentrations, the biggest increase being in 0

mM NaCl. The increase in 12.5, 25 and 50 was smaller while from 100 to 200 was even smaller and stopped at the third week of culture.

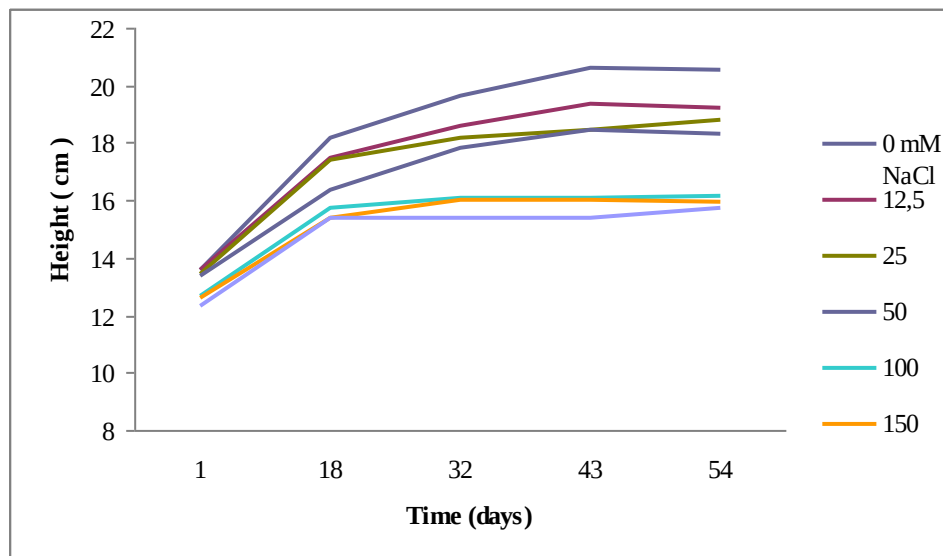


Figure 4.34: Effect of NaCl on the height of Kappy pepper plants during culture in soil.

At the end of the experiment (Fig. 4.35) in all three hybrids the height of the plants was lower compared to 0 mM NaCl, the reduction being higher as the concentrations of NaCl increased. In Drago and Kappy the reduction was more pronounced (and statistically significant) at concentrations of 100, 150 and 200 mM, while in Sammy at concentrations of 50mM NaCl and higher. Drago plants were the smallest followed by Sammy and Kappy plants but there were not big differences among the hybrids.

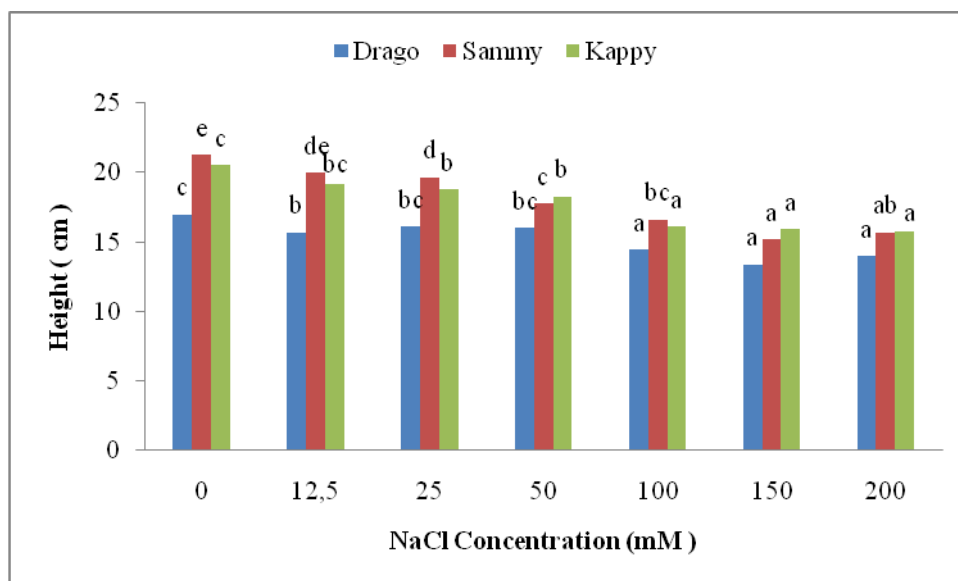


Figure 4.35: Effect of NaCl on the height of pepper plants after cultured in soil for 8 weeks.

Bars with the same colour, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

b. Number of Leaves per Plant

The leaves per plant in the case of Drago are shown in Fig. 4.36. In all concentrations there was an increase until the end of the first month. Then there was a decrease which in some concentrations was followed by an increase. This did not apply for plants at 0 mM NaCl, where no decrease was shown.

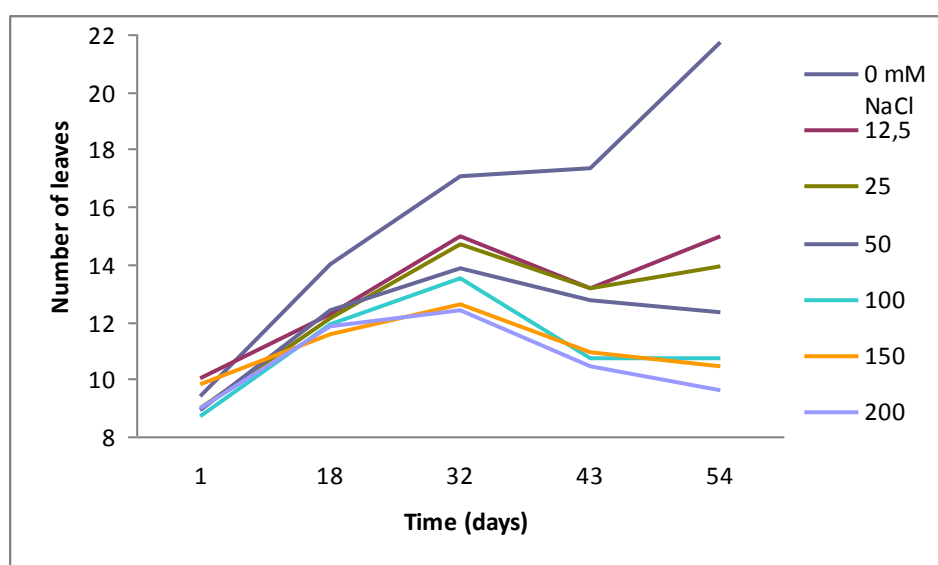


Figure 4.36: Effect of NaCl on the number of leaves of Drago pepper plants during culture in soil.

In Sammy (Fig. 4.37) and Kappy (Fig. 4.38) the results are similar to Drago. At 0 mM NaCl the number of leaves increased during the experiment, while in all other concentrations of NaCl increased only in the first month of the experiment and then decreased significantly, the decrease being bigger in the higher concentrations.

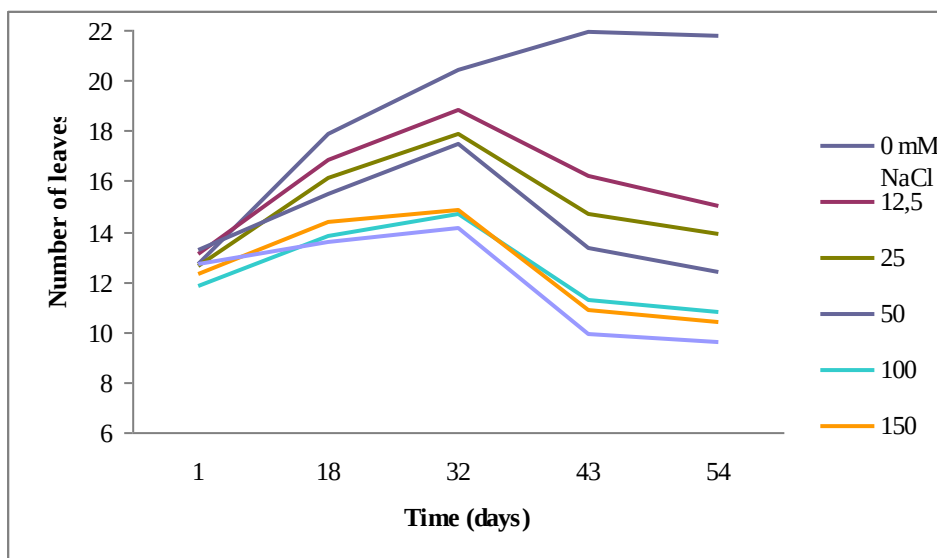


Figure 4.37: Effect of NaCl on the number of leaves of Sammy pepper plants during culture in soil.

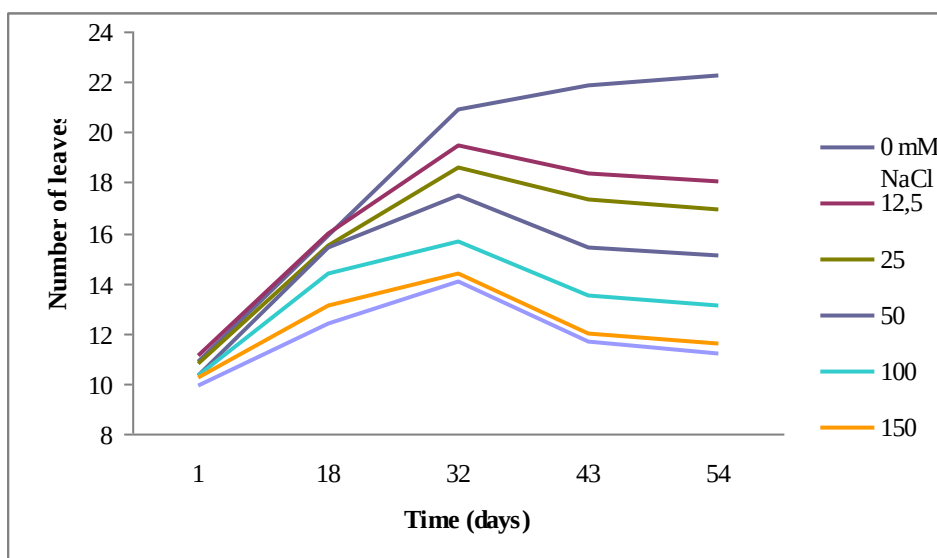


Figure 4.38: Effect of NaCl on the number of leaves of Kappy pepper plants during culture in soil.

The number of leaves per plant at the end of the experiment is presented in Fig. 4.39. As the concentrations of NaCl increased the number of the leaves decreased in all three hybrids. In the case of Drago the decrease was significant from 12.5 mM NaCl while in 100, 150 and 200 mM NaCl the decrease was even bigger compared to 0mM NaCl. Similarly In the case of Sammy and Kappy the number of the leaves per plant decreased sharply at 12.5 mM NaCl and kept decreasing as the NaCl increased.

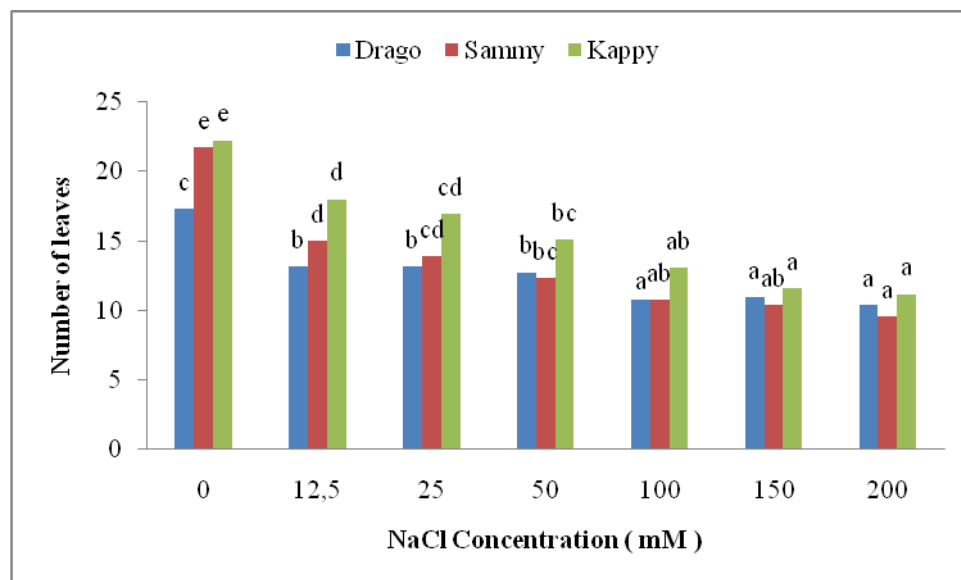


Figure 4.39: Effect of NaCl on the number of leaves of pepper hybrids after culture in soil for 9 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

c. Number of Flowers per Plant

As shown in Fig. 4.40, the number of flowers per plant was affected by salt in all of the hybrids. In the case of Drago the decrease was significant from 12.5 mM while in 100, 150 and 200 mM NaCl the decrease was even bigger compared to 0mM NaCl. At the higher concentrations the number of the flowers was about half of those in 0 mM NaCl. Generally the number of flowers per plant in Drago was much less than in the other hybrids. In the case of Sammy and Kappy there was also a decrease from 25 mM NaCl. At the higher concentrations the number of the flowers was about 2/3 less than those in 0 mM NaCl.

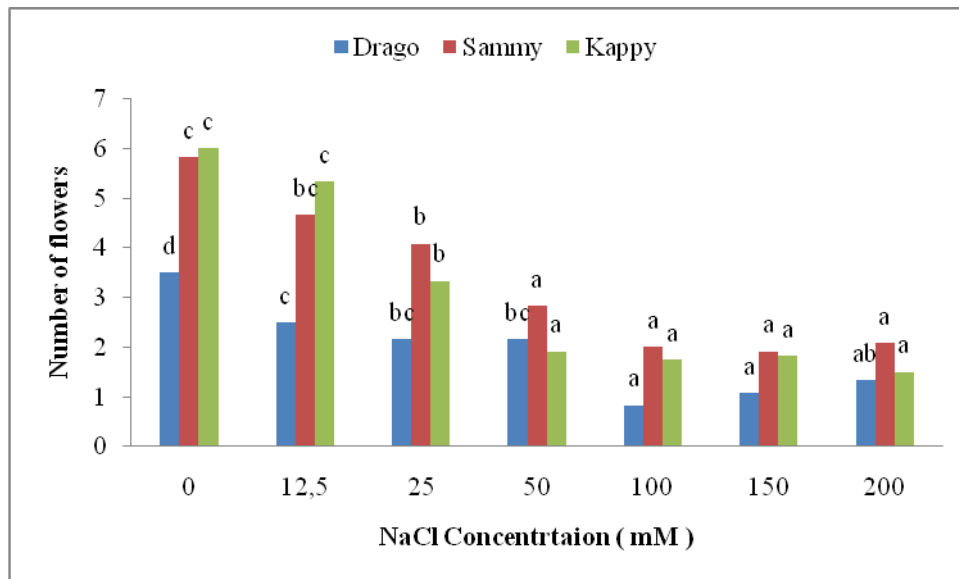


Figure 4.40: Effect of NaCl on the number of flowers of pepper hybrids after culture in soil for 8 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

d. Number of Fruits per Plant

The number of the fruits per plant is shown in Fig. 4.41. In Drago and Kappy there was a negative effect of salt. In Drago a decrease is apparent at all concentrations but statistically significant only at 100, 150 and 200 mM NaCl where there is a 2-3 fold decrease in the number of fruits compared to 0 mM NaCl. The number of the fruits was lower in Kappy and even lower in Drago compared to Sammy. In Sammy there was a significant increase in the number of fruits in all concentrations of NaCl up to 50 mM NaCl, while at 100 mM NaCl the number of fruits per plant did not differ significantly compared to 0 mM NaCl. In higher concentrations there was a sharp decrease in the number of fruits.

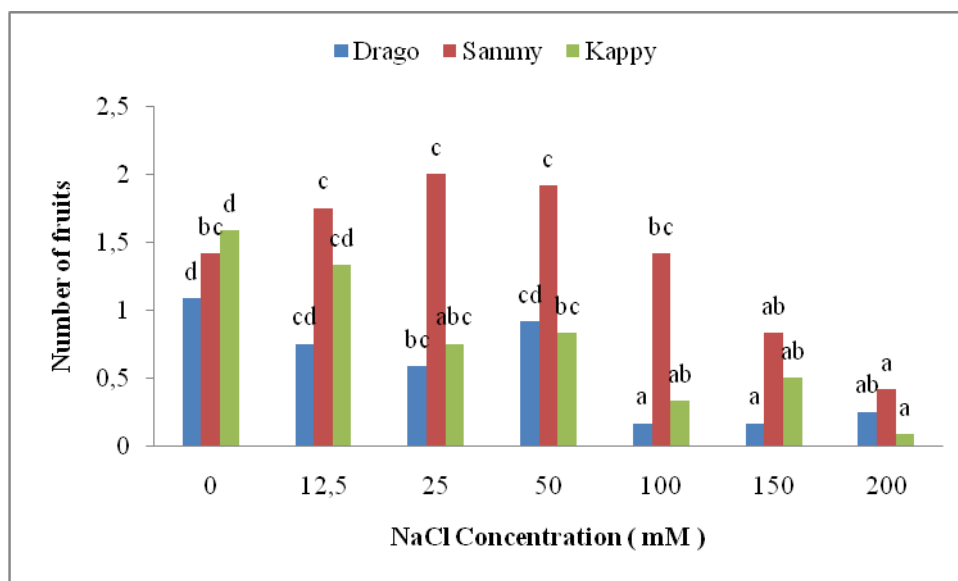


Figure 4.41: Effect of NaCl on the number of fruits of pepper hybrids after culture in soil for 8 weeks.

Bars with the same colour, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

In Table 4.9 is presented the percent of height, leaves, flowers and fruits per plant of all hybrids cultured in NaCl irrigated soil, compared to those cultured in NaCl free soil. In general all parameters, namely height, number of leaves, flowers and fruits per plant were all negatively affected by salt although to a different extent in each hybrid. The only exception was the number of fruit in Sammy where in 12.5, 25, 50, mM NaCl, the number of fruits increased. This was followed by a decrease at 150 and 200 mM NaCl, but the decrease was not as much as in the other hybrids.

Table 4.9: Percent height ,and number of leaves, flowers and fruits per plant of all hybrids cultured in NaCl irrigated soil, compared to those cultured in NaCl free soil

Concentration mM NaCl	Height			Number of Leaves		
	Drago	Sammy	Kappy	Drago	Sammy	Kappy
0	100	100	100	100	100	100
12,5	92	94	94	71	80	89
25	95	92	91	62	70	56
50	94	83	89	62	49	32
100	85	78	79	24	34	29
150	79	71	77	31	33	31
200	82	74	77	38	36	25
mM NaCl	Number of flowers			Number of fruits		
0	100	100	100	100	100	100
12,5	76	69	81	69	124	84
25	76	64	76	54	141	47
50	74	57	68	85	135	53
100	62	49	59	15	100	21
150	63	48	52	15	59	32
200	60	44	50	23	29	5

e. Weight of Plant

1. Shoot Fresh Weight (above ground plant part)

The fresh weight of the pepper plants was affected by salt in all hybrids (Fig. 4.42). In the cases of Drago and Kappy there was a strong decrease in the above ground weight at the higher concentrations which was progressively stronger as the concentrations of NaCl increased. In 150 and 200 mM NaCl this was almost 2 to 3 times lower than the weight at the concentration of 0 mM NaCl. In the case of Sammy there was also a sharp, statistically significant decrease only in the concentrations of 100 to 200 mM NaCl. In all concentrations, the Drago plants were the lightest and Sammy the heaviest.

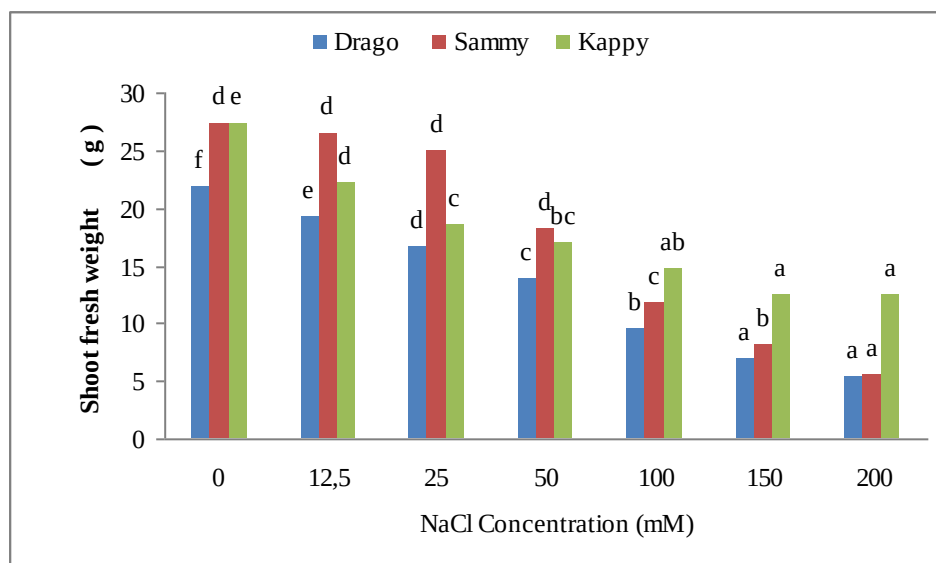


Figure 4.42: Effect of NaCl on the fresh weight of shoot of pepper hybrids after culture in soil for 8 weeks.

Bars with the same colour, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

2. Shoot Dry Weight (above ground plant part)

The dry weight (Fig.4.43) in all hybrids was negatively affected by salt, starting at the 12.5 mM NaCl level except in Sammy, where the decrease was significant at 25mM NaCl and more.

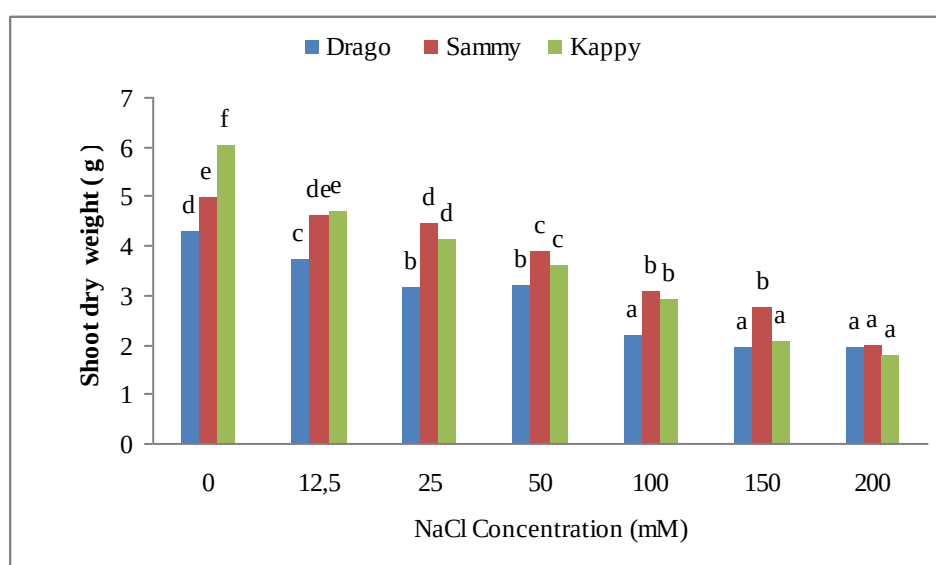


Figure 4.43: Effect of NaCl on the dry weight of above ground part of pepper hybrids after culture in soil for 8 weeks.

Bars with the same colour, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

3. Fresh Weight of Leaves

The fresh weight of leaves in Drago (Fig. 4.44) increased significantly in 12.5 and 25 mM NaCl and then decreased significantly and progressively at 100, 150 and 200 mM NaCl. In the case of Sammy there is a small but not statistically significant increase of the fresh weight of the leaves in 12.5 and 25 mM NaCl and then a significant gradual decrease in all concentrations of NaCl. In Kappy there was a significant decrease only in 50 to 200 mM NaCl, without significant differences as the concentration increased across this range.

4. Dry Weight of Leaves

The dry weight of the leaves was not significantly affected up to 25 to 50 mM NaCl in all three hybrids (Fig. 4.45). In higher concentrations the dry weight decreased significantly in all hybrids (Table 4.11).

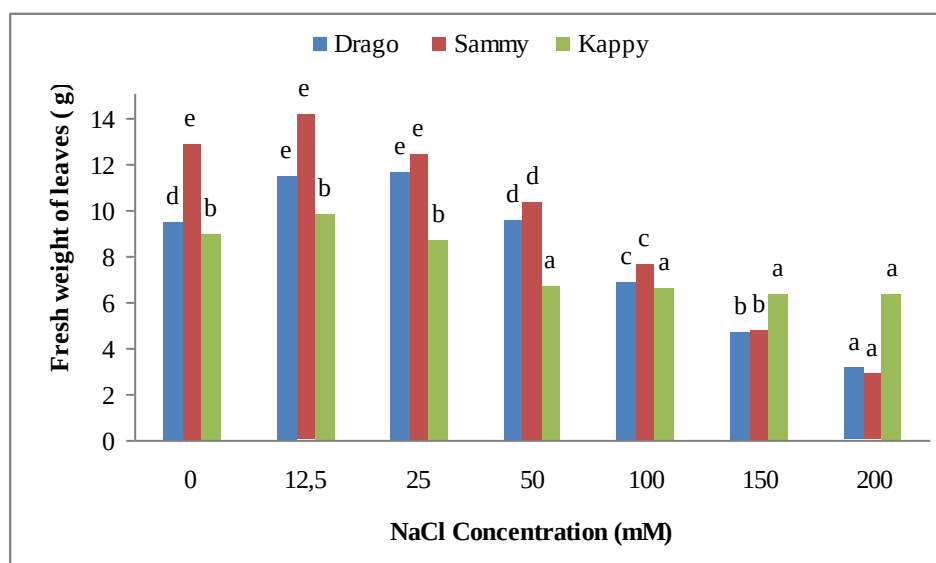


Figure 4.44: Effect of NaCl on the fresh weight of leaves of pepper hybrids after culture in soil for 8 weeks.

Bars with the same colour, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

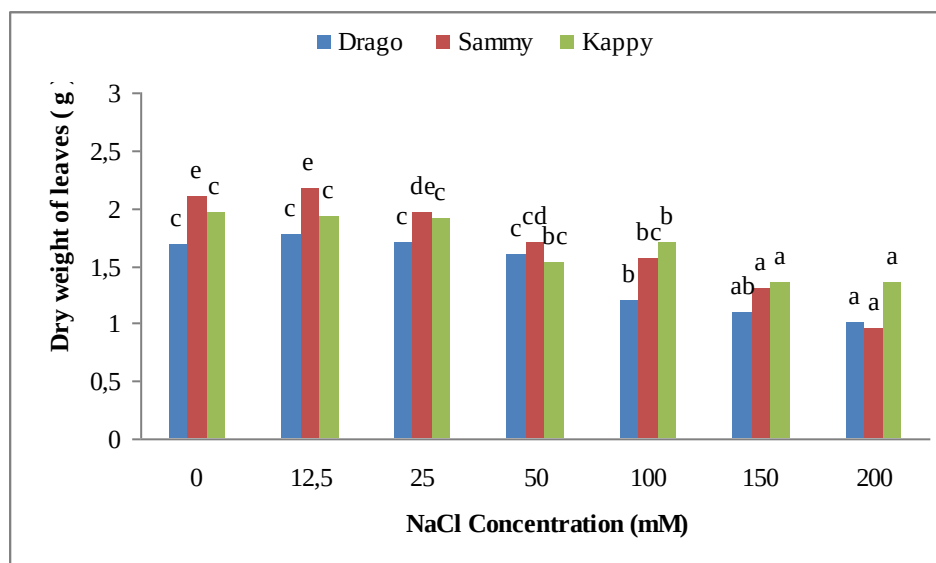


Figure 4.45: Effect of NaCl on the dry weight of leaves of pepper hybrids after culture in soil for 8 weeks.

Bars with the same colour, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

5. Fresh Weight of Fruit

As shown in Fig. 4.46 the fresh weight of the fruits was reduced in all NaCl concentrations. The reduction was sharp and statistically significant in Drago and Sammy in all concentrations starting from 12.5 mM NaCl. In Sammy the reduction was significant at 50 mM NaCl and more. At 0 mM NaCl the weight of the fruits of the Kappy plants was much higher than those of Drago and Sammy plants.

6. Dry Weight of Fruit

The dry weight of the fruits, Fig. 4.47 followed the same trend as did the fruit fresh weight. It seemed that Drago and Sammy fruits were much more succulent compared to Kappy.

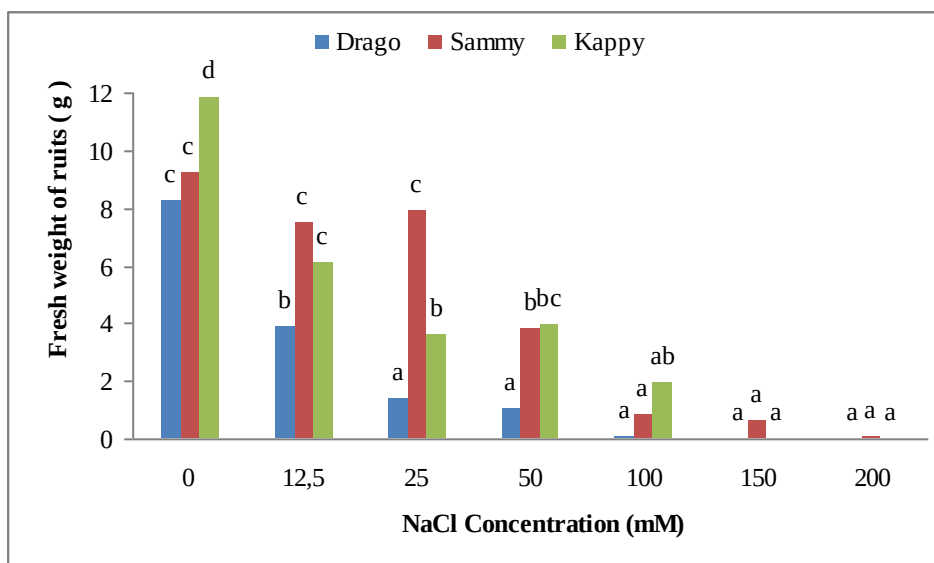


Figure 4.46: Effect of NaCl on the fruitfresh weight of pepper hybrids after culture in soil for 8 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

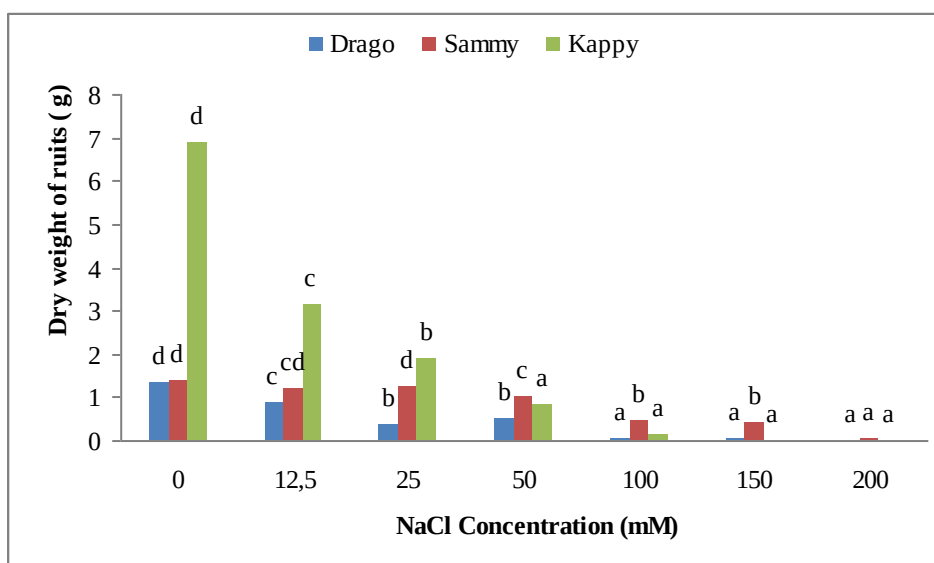


Figure 4.47: Figure 4.2.12: Effect of NaCl on the fruit dry weight of pepper hybrids after culture in soil for 8 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

The fresh and dry weight was reduced to the percentages presented in Table 4.10. It seems that shoot and leaf fresh weight in Kappy was reduced less than it was in the other hybrids, while its shoot dry weight was reduced more than the other hybrids in high concentrations. Fruit fresh and dry weight was less reduced in all concentrations in Sammy.

Table 4.10: % of fresh and dry weight of shoot, leaves, and fruits per plant of all hybrids cultured in NaCl irrigated soil, compared to those cultured in NaCl free soil.

Concentration mM NaCl	Shoot Fresh Weight				Shoot dry Weight		
	Drago	Sammy	Kappy		Drago	Sammy	Kappy
0	100	100	100		100	100	100
12,5	88	97	81		87	93	78
25	77	91	68		73	89	68
50	64	66	62		74	78	59
100	44	43	54		51	62	48
150	32	30	46		46	55	34
200	25	20	46		45	40	30
mM NaCl	Leaves Fresh Weight				Leaves dry Weight		
0	100	100	100		100	100	100
12,5	121	111	109		105	104	97
25	123	97	97		100	93	97
50	102	81	74		95	81	72
100	73	60	73		71	75	86
150	50	37	71		64	62	69
200	33	22	70		60	46	69
mM NaCl	Fruit Fresh Weight				Fruit dry Weight		
0	100	100	100		100	100	100
12,5	47	81	52		63	88	46
25	17	86	30		28	92	27
50	12	41	34		36	74	12
100	1	9	16		3	33	2
150	0	6	0		2	29	0
200	0	1	0		0	2	0

The water content (fresh weight – dry weight) is presented in Table 4.11 as a percentage of the fresh weight of shoot leaf and fruit. Water content is reduced in shoot and leaf in all concentrations for Drago and Sammy from about 80 to 65 %, while in

fruits the reduction is sharper, at about 30%. In Kappy the water content increased in the higher concentrations of salt in the shoots and fruits but not in the leaves where the water content did not change.

Table 4.11 : Percent of water content of shoot, leaves, and fruits per plant of all hybrids cultured in NaCl irrigated soil, compared to those cultured in NaCl free soil.

Concentration mM NaCl	Drago	Sammy	Kappy
	% shoot water content		
0	80	82	34
12,5	81	83	37
25	81	82	33
50	77	79	37
100	77	74	40
150	72	66	51
200	64	64	57
	% leaf water content		
0	82	84	78
12,5	85	85	80
25	85	84	78
50	83	84	79
100	83	79	74
150	77	73	79
200	67	66	78
	% fruit water content		
0	84	85	42
12,5	78	84	49
25	73	84	47
50	51	73	79
100	21	45	93
150	28	47	—
200	—	33	—

4.2.2. Physiological parameters

a. Chlorophyll Content and Chlorophyll fluorescence of the Leaves

Chlorophyll content and chlorophyll fluorescence was measured in the leaves every two weeks. Until the fourth week of culture with NaCl, there were no significant differences in both chlorophyll content and chlorophyll fluorescence which

therefore seemed unaffected at this stage (data not shown). Significant differences were recorded only at the fifth week and only at the highest concentrations (data not shown).

In Drago (Fig.4.48) the chlorophyll content in the leaves at the beginning of the experiment was at the same level in all concentrations, but after 8 weeks of culture it was affected negatively. The chlorophyll content decreased following the increase of the concentrations of salt. In plants cultured at 12.5, 25, 50 mM NaCl the chlorophyll content of the leaves did not differ, but they had significantly lower chlorophyll content compared to 0 mM NaCl. At the higher concentrations the chlorophyll content was even lower compared to 0 mM NaCl. The chlorophyll fluorescence, Fig.4.49, decreased only at 100, 150 and 200 mM NaCl,. At 150 and 200 mM NaCl the level of the chlorophyll fluorescence was about 1/3 lower than it was at the same concentrations at the beginning of the experiment.

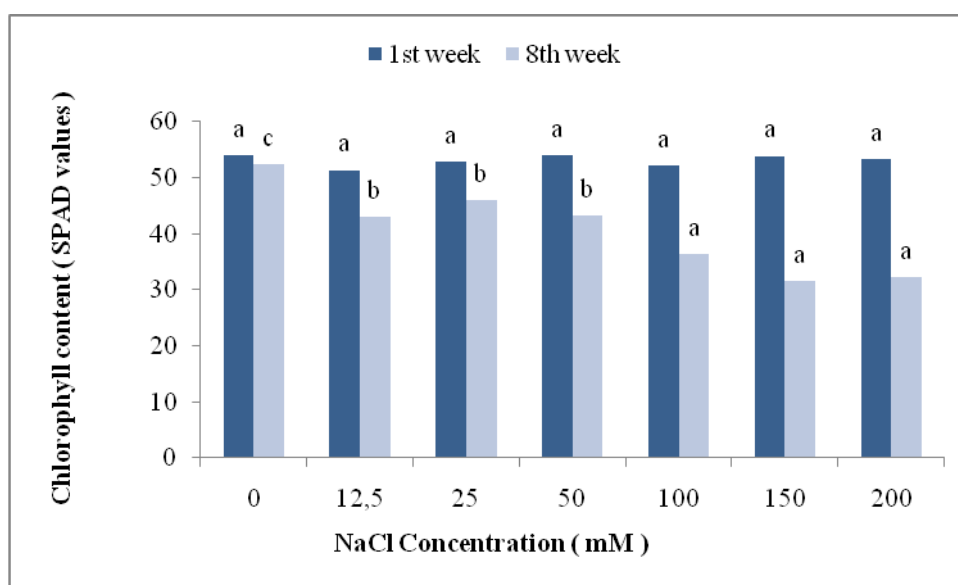


Figure 4.48: Effect of NaCl on the chlorophyll content of Drago pepper plants cultured in soil for 8 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

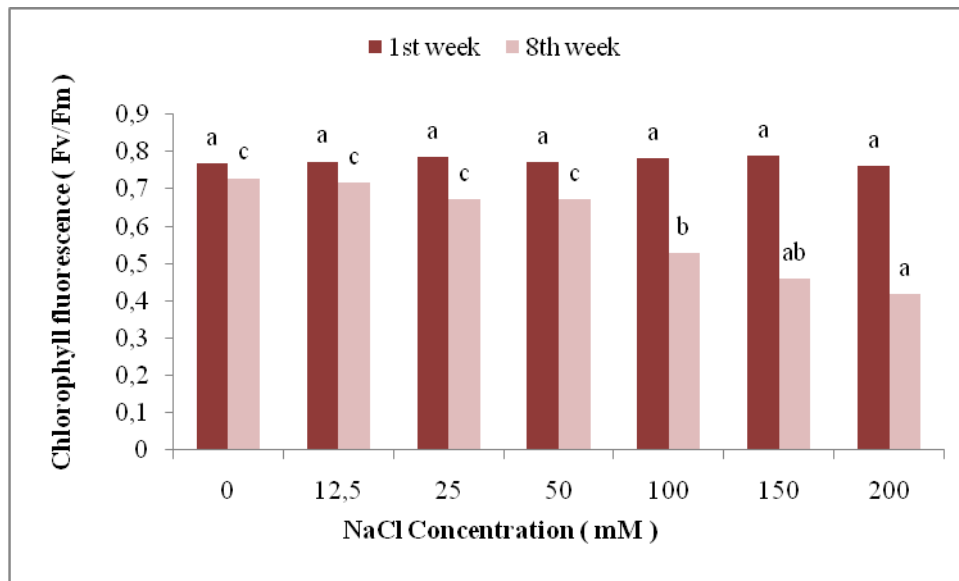


Figure 4.49: Effect of NaCl on the chlorophyll content of Drago pepper plants cultured in soil for 8 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

In Sammy (Fig. 4.50) the chlorophyll content decreased dramatically at the end of the experiment. Chlorophyll content started being affected by salt on the fourth week of culture. These differences became more pronounced on the 6th and 8th week of culture. At the concentrations of 12.5, 25 and 50 mM the chlorophyll contents of the leaves were equally lower compared to those of the 0mM NaCl treatment. At the 100,150 and 200 mM NaCl concentrations there was a decrease in the content of about 50 % lower than at the beginning of the experiment. The chlorophyll fluorescence of the leaves of Sammy , as shown in Fig. 4.51, was not affected by concentrations up to 50 mM NaCl even at the end of the 8th week of culture. Only at the highest concentrations -was there a significant decrease on the 6th but mostly on the 8th week .

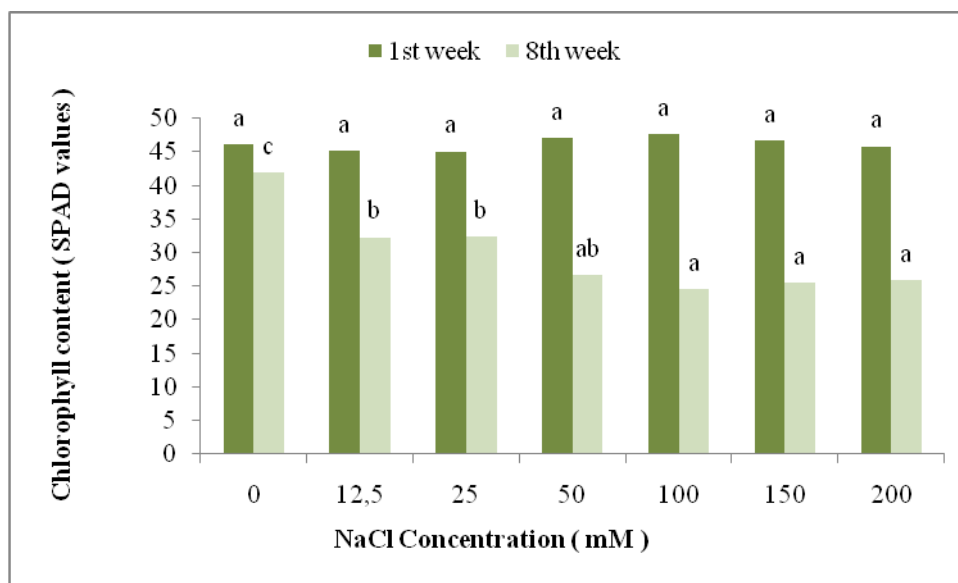


Figure 4.50: Effect of NaCl on the chlorophyll content of Sammy pepper plants cultured in soil for 8 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

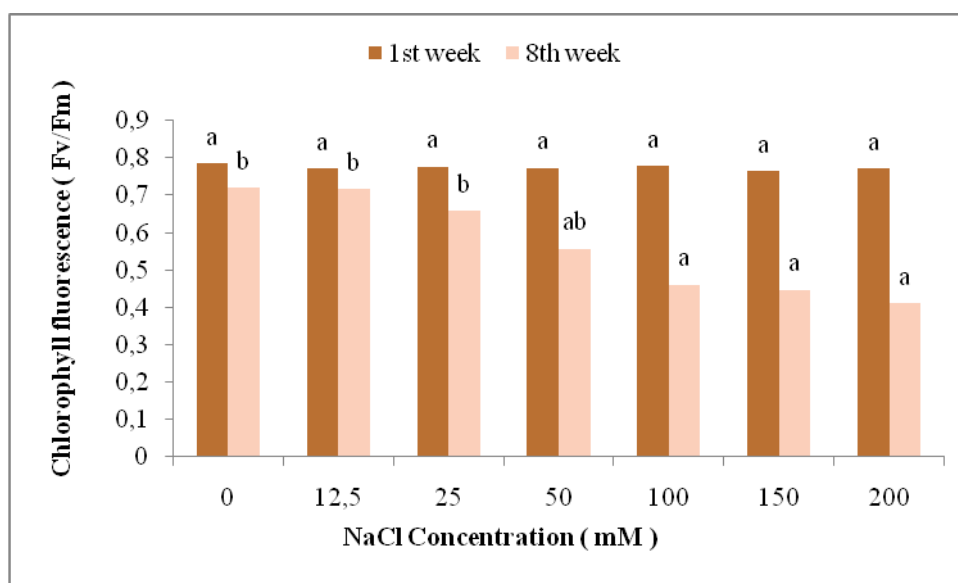


Figure 4.51: Effect of NaCl on the chlorophyll fluorescence of Sammy pepper plants cultured in soil for 8 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

In Kappy (Fig. 4.52) the chlorophyll content was affected only at the 6 and 8th week. The negative effect of salt was significant at 12.5 mM, but in higher

concentrations the chlorophyll content was even lower. The chlorophyll fluorescence, Fig. 4.53, was affected only at the 8th week at 100, 150 and 200 mM NaCl, without significant differences among these concentrations.

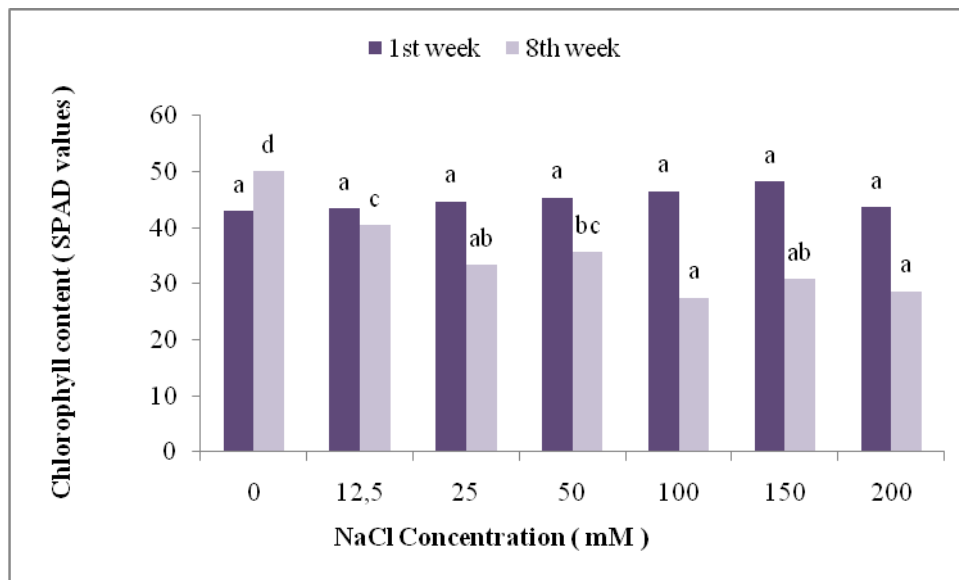


Figure 4.52: Effect of NaCl on the chlorophyll content of Kappy pepper plants cultured in soil for 8 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

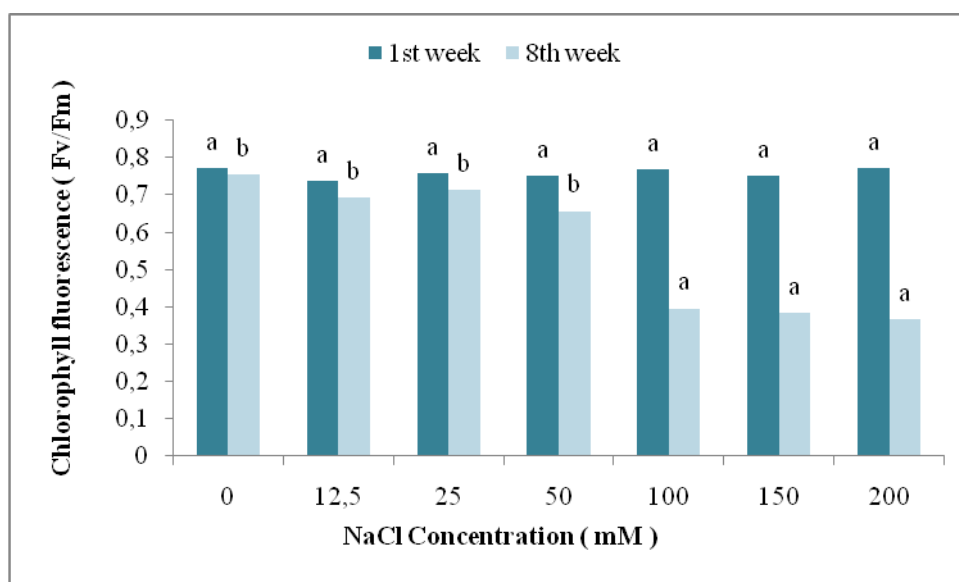


Figure 4.53: Effect of NaCl on the chlorophyll fluorescence of Kappy pepper plants cultured in soil for 8 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

b. Photosynthetic Rate

The photosynthetic rate was measured every two weeks. It was found that it was affected by NaCl only after the 4th week but the differences were not significant for all hybrids and all concentrations (data not shown). On the 8th week the differences were more pronounced (Fig.4.54) In the Drago hybrid the photosynthetic rate appeared to decrease at concentrations of 25 mM NaCl and higher but significantly only at 150 and 200 mM NaCl. In these concentrations the photosynthetic rate was reduced to just 25 % of the photosynthetic rate at 0 and 12.5 mM NaCl Sammy's photosynthetic rate was reduced at concentrations of 100mM NaCl and more, while Kappy's from 50 mM NaCl and more.

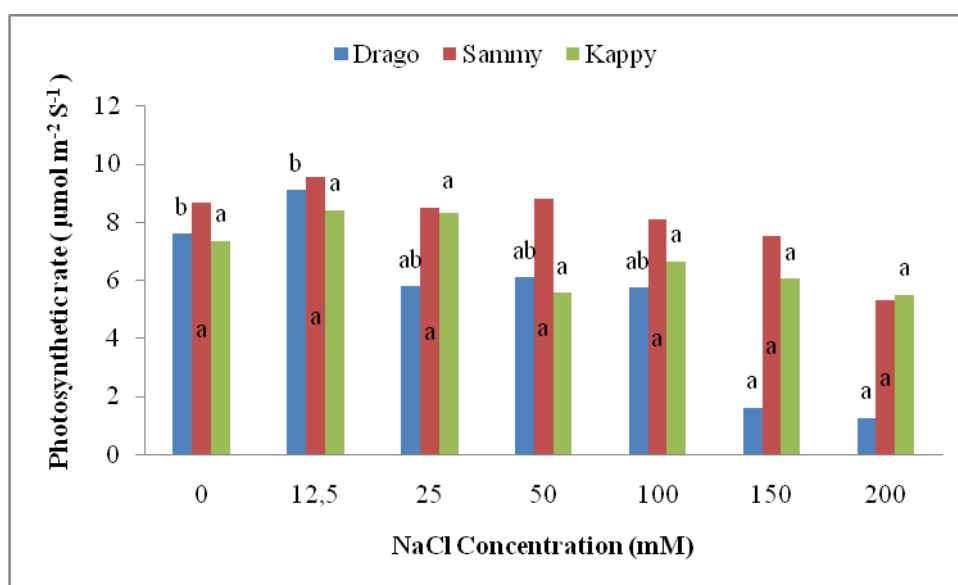


Figure 4.54: Effect of NaCl on the photosynthetic rate of leaves of Drago, Sammy and Kappy cultured in soil for 8 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

c. Stomatal Conductance

Stomatal conductance appeared to be affected by NaCl - after the 6th week but the differences were not significant (data not shown). On the 8th week stomatal conductance was negatively affected by the increased NaCl. (Fig.4.55) In Drago the stomatal conductance decreased at concentrations of 25 mM NaCl and higher. The

biggest reduction was at 150 and 200 mM NaCl. In these concentrations the stomatal conductance was reduced to only 20% of the stomatal conductance at 0 and 12.5 mM NaCl. In Sammy and Kappy it was reduced at concentrations of 100mM and more, but the effect was not as sharp as it was in Drago.

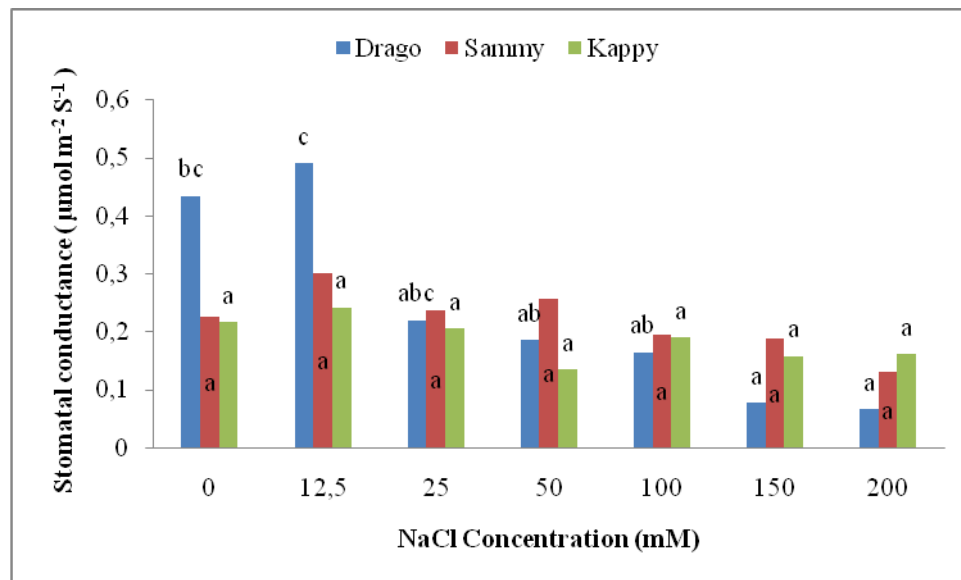


Figure 4.55: Effect of NaCl on the stomatal conductance of leaves of Drago, Sammy and Kappy cultured in soil for 8 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

d. Intercellular CO₂ Concentration (C_i)

The intercellular CO₂ concentration (C_i) of the leaves (Fig. 4.56) was negatively affected by salt only in Drago at concentrations of 150 and 200 mM NaCl. In Sammy and Kappy C_i was not significantly affected.

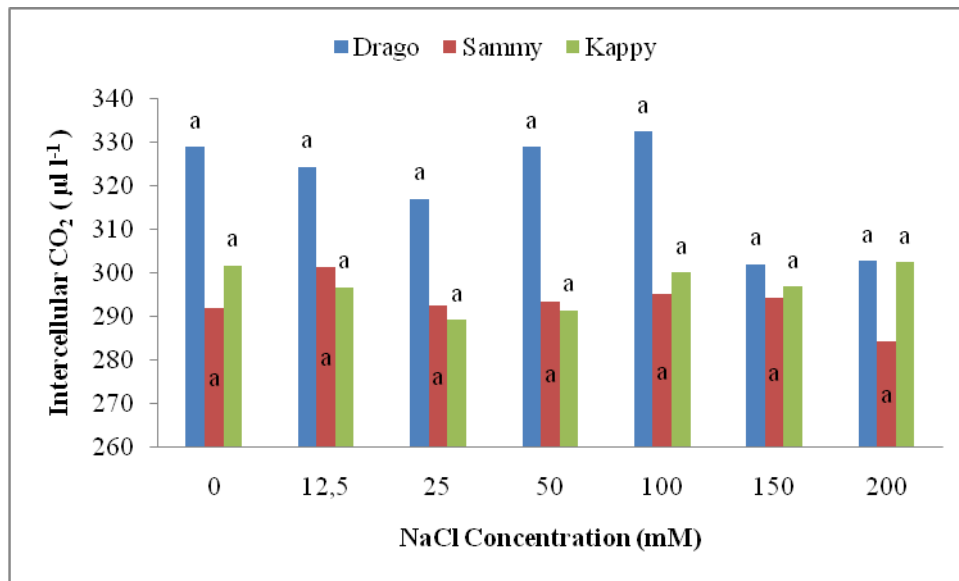


Figure 4.56: Effect of NaCl on the intercellular CO₂ concentration (C_i) of leaves of Drago, Sammy and Kappy cultured in soil for 8 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

e. Transpiration Rate

The transpiration rate of the leaves was negatively affected by salt only in Drago. The reduction in transpiration rate occurred at 25 mM NaCl and more, but it was bigger at concentrations of 150 and 200 mM NaCl – approx 33% of the transpiration at 0 mM NaCl. In Sammy and Kappy transpiration rate was not significantly affected(Fig. 4.57).

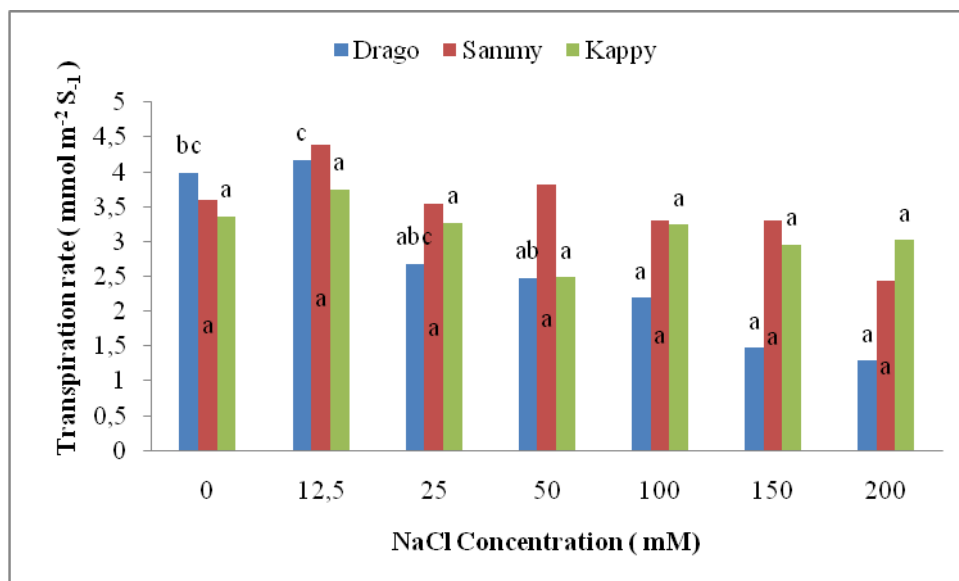


Figure 4.57: Effect of NaCl on the transpiration rate of leaves of Drago, Sammy and Kappy cultured in soil for 8 weeks.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

4.2.3 Biochemical parameters

a. Protein Extraction and Protein Determination by the Modified Lowry Method.

The protein content of the leaves of all hybrids is presented in Table 4.12. In the case of Drago, the protein content was negatively affected from the presence of salt. At the concentration of 0 mM NaCl there was the highest content and this was followed by the other concentrations with no big differences between them. In the case of Sammy there was a decrease in the protein content which first appeared at 12.5 mM NaCl and was further reduced in the highest concentrations. In Kappy the protein content was not affected by salt at the low concentration but was affected at 150 and 200 mM NaCl.

Table 4.12: Effect of NaCl on the protein content of leaves of pepper plants cultured in soil for 8 weeks.

NaCl stress	Soluble protein content ($\mu\text{g/g}$ fresh leaf)		
	Drago	Sammy	Kappy
0 mM	8827 c	9888 d	8176 a
12,5 mM	7611 b	8581 c	8560 a
25 mM	6891 ab	7835 bc	6357 a
50 mM	7424 ab	7440 ab	9307 a
100 mM	6517 a	7397 ab	9296 a
150 mM	6709 ab	7312 ab	6952 a
200 mM	7440 ab	6533 a	7172 a

b. Protein Electrophoresis

The protein electrophoresis was conducted on three gels, one for each hybrid. In Plate 4.5 are presented the protein electrophoresis gels of leaf extracts of all pepper hybrids after culture in perlite at concentrations of 0, 12.5, 25, 50, 100, 150 and 200 mM NaCl, for 12 weeks. In each of the seven "wells" of each gel were placed leaf extract of plants grown at different NaCl concentrations. (Plate 4.5). The bands were formed by different proteins. The effect of NaCl on soluble proteins can be judged by the color intensity of the bands. In general, certain protein bands at the higher

concentrations of NaCl seem to be lighter than those at the lower concentrations in all hybrids.

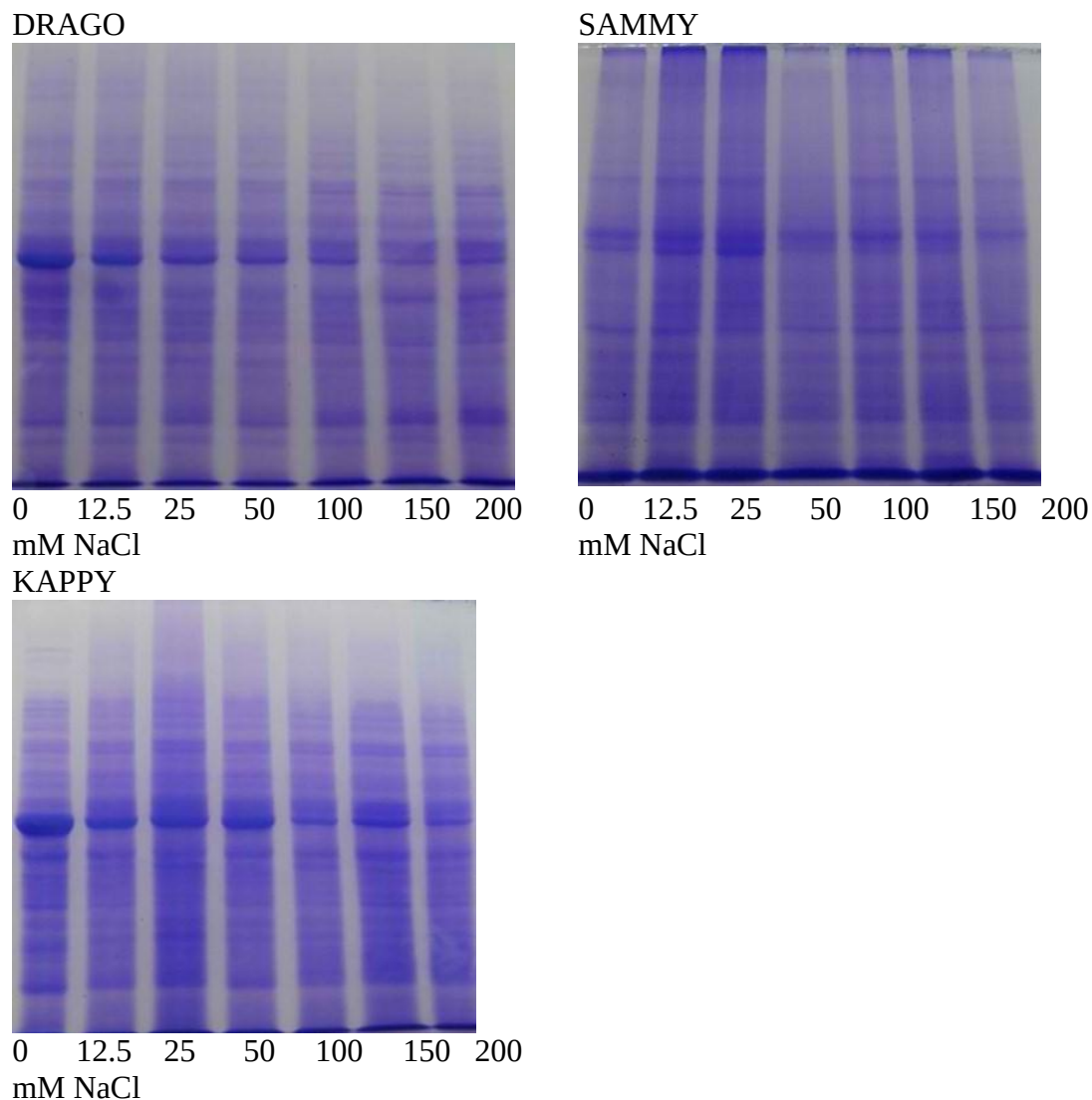


Plate 4.5: Protein electrophoresis gels of leaf extracts of all pepper hybrids after culture in perlite at concentrations of 0, 12.5, 25, 50, 100, 150 and 200 mM NaCl, for 12 weeks.

c. Polyacrylamide Gel Electrophoresis and Immunoblot Analysis

In case of Sammy and Kappy (Plate 4.6) there was an increase of the level of GDH protein following the increase in NaCl concentrations. In Sammy the increase was more obvious at concentrations of 100, 150 and 200 mM NaCl. In the case of Kappy the increase at the concentrations of 150 and 200 mM NaCl was very obvious . In both hybrids the concentration of GDH was very low – in the case of Kappy in

particular, at the concentration of 0 mM NaCl the level of GDH protein content is very low.

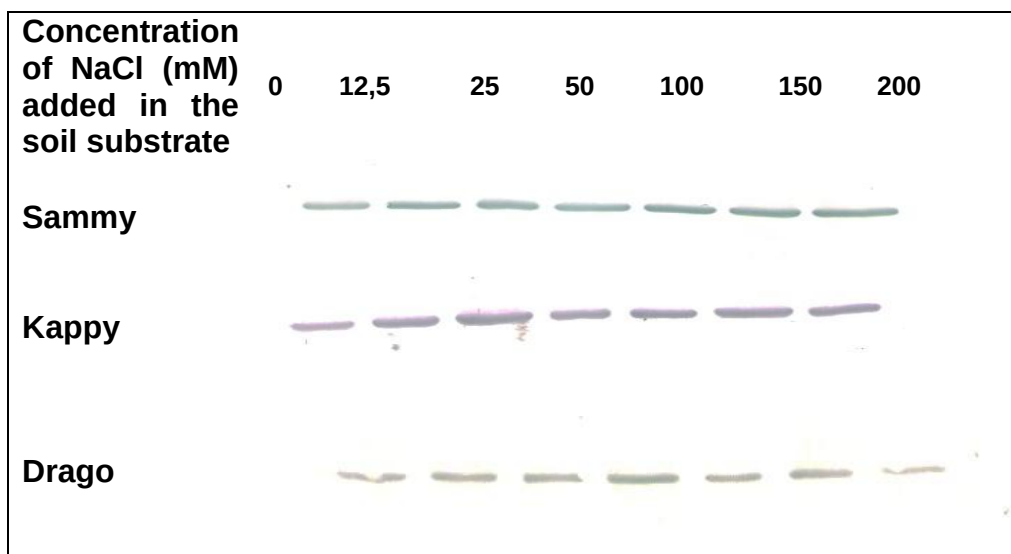


Plate 4.6: Effect of NaCl on the isoenzymic profile of GDH in leaves of pepper hybrids cultured in soil for 8 weeks. Samples were resolved in 10% acrylamide SDS-PAGE, transferred to nitrocellulose membranes, and probed with ant-GDH serum.

d. Activity of antioxidant enzymes

In Tables 4.13, 4.14 the activity of the antioxidant enzymes in young fully developed leaves is presented. In Drago and Sammy hybrid there were no significant differences in the activity of GPX, POD, and APX enzyme. In Kappy there was a small significant increase of APX activity at 50 and 100 mM NaCl

Table 4.13: Effect of NaCl on total activity of APX and GPX in leaves of pepper hybrids cultured in soil for 8 weeks.

NaCl stress	Total activity of APX (U mg FW)			Total activity of GPX (U mg FW)		
	Drago	Sammy	Kappy	Drago	Sammy	Kappy
0 mM	5,52 a	0,01 a	0,01 a	0,29 a	0,13 a	0,27 a
12,5 mM	5,49 a	0,02 a	0,02 a	0,33 a	0,07 a	0,41 a
25 mM	6,05 a	0,01 a	0,02 a	0,34 a	0,04 a	0,43 a
50 mM	4,03 a	0,02 a	0,06 b	0,27 a	0,02 a	0,23 a
100 mM	5,80 a	0,02 a	0,03 ab	0,17 a	0,07 a	0,23 a
150 mM	5,04 a	0,02 a	0,02 a	0,26 a	0,03 a	0,25 a
200 Mm	3,95 a	0,01 a	0,01 a	0,32 a	0,11 a	0,27 a

Table 4.14: Effect of NaCl on total activity of POD in leaves of pepper hybrids cultured in soil for 8 weeks.

NaCl stress	Total activity of POD (U mg FW)		
	Drago	Sammy	Kappy
0 mM	0,005 a	0,005 a	0,005 a
12,5 mM	0,005 a	0,005 a	0,006 a
25 mM	0,005 a	0,004 a	0,006 a
50 mM	0,004 a	0,004 a	0,006 a
100 mM	0,005 a	0,004 a	0,005 a
150 mM	0,004 a	0,005 a	0,005 a
200 Mm	0,005 a	0,006 a	0,007 a

4.3. Experiments in vitro

4.3.1. Effect of NaCl on the pepper hybrids Drago, Sammy, Gracia and Kappy, after in vitro culture in saline substrate with 0, 12.5, 50, 100, 150, 200 mM NaCl

The in vitro experiments lasted 10 weeks. Salinity started to affect the leaves after the 4th week of transplantation or the 3rd after germination. The lower leaves became yellowish, succulent and later fell off. Also there was toxicity in the roots as at the higher concentrations they were brown in contrast to those in low concentrations which were white (Plates 4.7, 4.8, 4.9, 4.10, 4.11, 4.12 and 4.13, Appendix A).

The percentage germination of the seeds was 100% for all hybrids on all NaCl concentrations. There was however a certain delay on reaching the final germination percentages in the 150 and 200 mM treatments. This delay was 7 to 8 days for Drago, Sammy, and Kappy, and 15 days for Gracia. The final percentage germination on NaCl free substrate was reached in 2 days for Sammy, 3 days for Kappy and Drago and 5 days for Gracia. Moreover seeds germinated in a high concentration of salt produced mostly normal looking but also a few extremely stunted seedlings. For Drago, Kappy and Gracia this occurred only at 200 mM NaCl while for Sammy it occurred at 100, 150 and 200 mM NaCl. The percentage of stunted plants was 13, 33 and 20 % for Drago Kappy and Gracia respectively while for Sammy it was 13, 20 and 26 % in 100, 150 and 200 mM NaCl respectively.

The germination experiment was repeated after one year. It was used the same lot of seeds – the remaining from the previous experiments. The seeds were cultured in vitro on a substrate containing NaCl in the following concentrations: 0, 25, 50, 100, 150 and 200 mM. Germination was considered when the root had a length equal to the length of the seed.

Percentage germination was reduced in Gracia by 50 %, in Sammy by 20 %, and in Drago by 12,5% . Only Kappy retained 100% germination. Moreover the highest germination was achieved later than in the first experiment. In Drago the delay was of 3 days, Kappy of 12 days, Sammy of 17 days and Gracia for 30 days. On the NaCl enriched substrates there was reduction of the germination. On the 200 mM, there was not any germination for Drago and Gracia and about 10% for Sammy and Kappy. Apart from that, seed germination of Drago on 150 mM was reduced by 5% compared to

NaCl free substrate, and on lower concentrations even less. Kappy on 150 mM there was 33% reduction of germination and 12% on 100 mM and even less on the lower concentrations. Reduction of germination of Sammy seeds was bigger on 150 mM–25%, on 100 mM 55% compared to 80% on NaCl free substrate. Gracia seeds germination was increasingly reduced in all increasing NaCl substrates.

At the end of the experiments the plantlets were removed from the culture tubes for further measurements. Below are presented the parameters that were measured namely the height of plants, the number of leaves and the weight of roots and of the above-ground part. The results of the second year germination experiment though not shown, were similar and comparable to those of the first year.

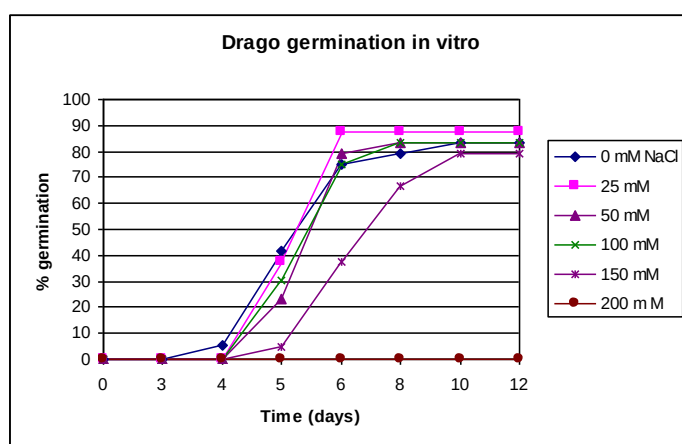


Figure 4.58:
Effect of
NaCl in the
substrate on
the %
germination
of Drago
seeds in
vitro.

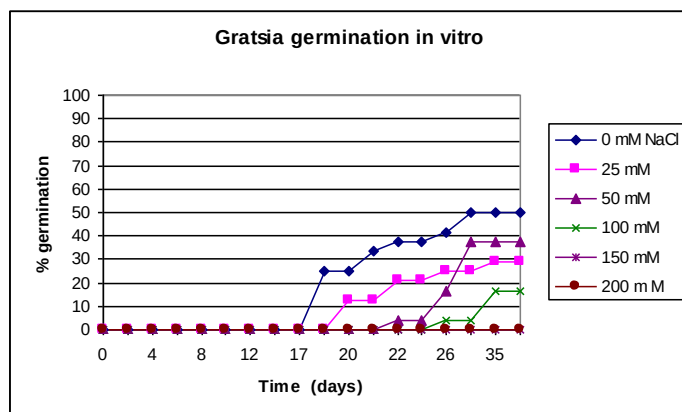


Figure 4.59:
Effect of
NaCl in the
substrate on
the %
germination
of Gracia
seeds in
vitro.

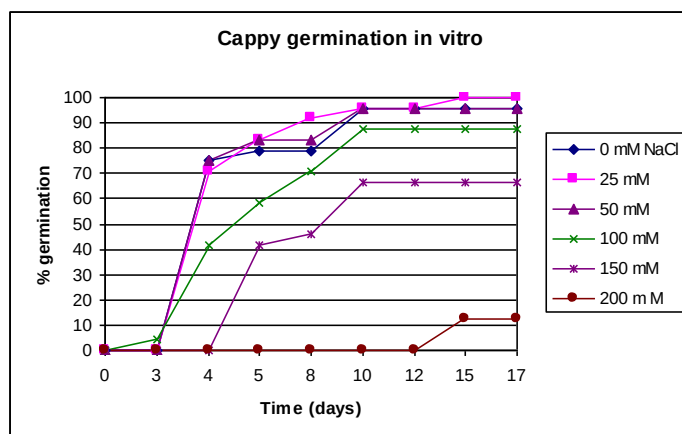


Figure 4.60:
Effect of
NaCl in the
substrate on
the %
germination
of Cappy
seeds in
vitro.

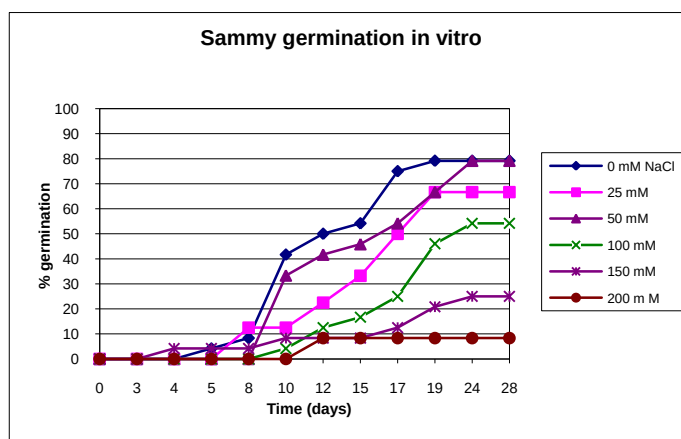


Figure 4.61:
Effect of
NaCl in the
substrate on
the %
germination
of Sammy
seeds in
vitro.

4.3.2. Morphological parameters

a. Height of the plantlets

The height of the transplanted seedlings (presented in Fig. 4.62 as the darker bars) increased at low concentrations of NaCl compared to those cultured on NaCl free substrate. This trait is apparent in all four hybrids although the differences are not always significant. Significantly higher plants were only those of Sammy at concentrations 25 and 50 mM and Kappy at 12,5 mM. Height in all hybrids was significantly reduced at concentrations of 150 and 200 mM (Kappy only at 200) compared to 0 mM NaCl and all other concentrations, the reduction for the 200mM NaCl being significantly larger than in 150 mM NaCl.

The height of the seedlings germinated on NaCl substrates (presented in Fig. 4.62 in lighter color bars) followed a similar trend as for the transplanted seedlings: At low concentrations the plant height of all hybrids appeared slightly bigger than at 0 mM NaCl but this was only significant for Gracia at 50 and 100 mM NaCl.

At high concentrations height in all hybrids was significantly reduced only at the concentration of 200 mM (Drago also at 150) compared to 0 mM NaCl .

The seedlings germinated on substrates with different NaCl levels were higher than those transplanted onto such substrates, in all hybrids except Kappy

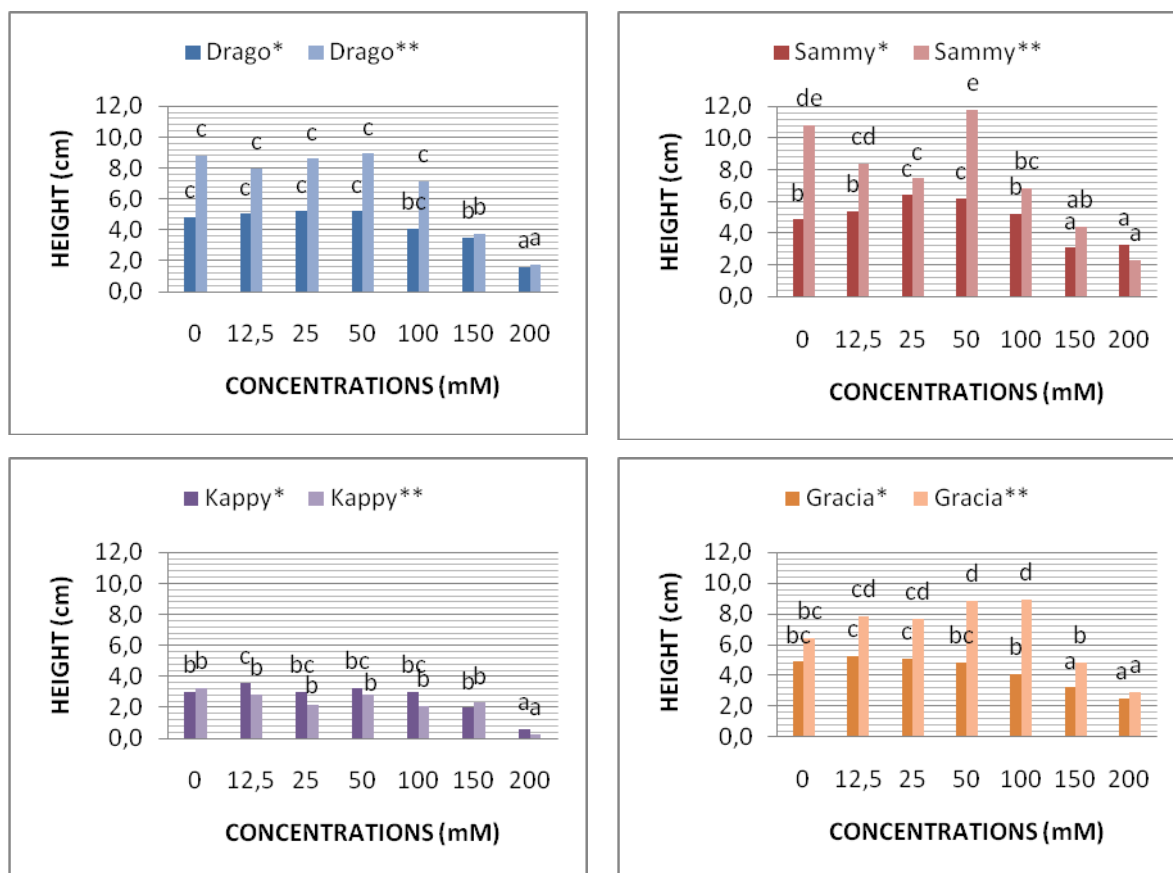


Figure 4.62: Effect of NaCl on the height of pepper hybrids after in vitro culture in saline substrate. * plants transplanted in substrate with NaCl and ** plants germinated into substrate with NaCl.

Bars with the same colour, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

b. Number of leaves of the plantlets

The leaves of the transplanted seedlings (presented in Fig. 4.63 * and 4.64 *) of Sammy* and Gracia* have significantly increased at low concentrations of NaCl compared to those cultured on NaCl free substrate. In Sammy the no of leaves increased by 43 to 60 % on the 12,5 to 25 and 50 mM NaCl treatments while in Gracia the leaves increased by 25% in 12,5 and less in 25 and 50 mM NaCl .

The mean number of leaves of transplanted seedlings of the other two hybrids and also of the seedlings germinated on substrates with NaCl of all hybrids was gradually

reduced following the increase of the NaCl concentration in the substrate. The decrease however, was not significant for substrates with NaCl up to 50 (Drago and Kappy) or up to 100 mM (Sammy and Gracia). For the highest concentrations the decrease was significant in all hybrids. Moreover most of the leaves were yellowish.

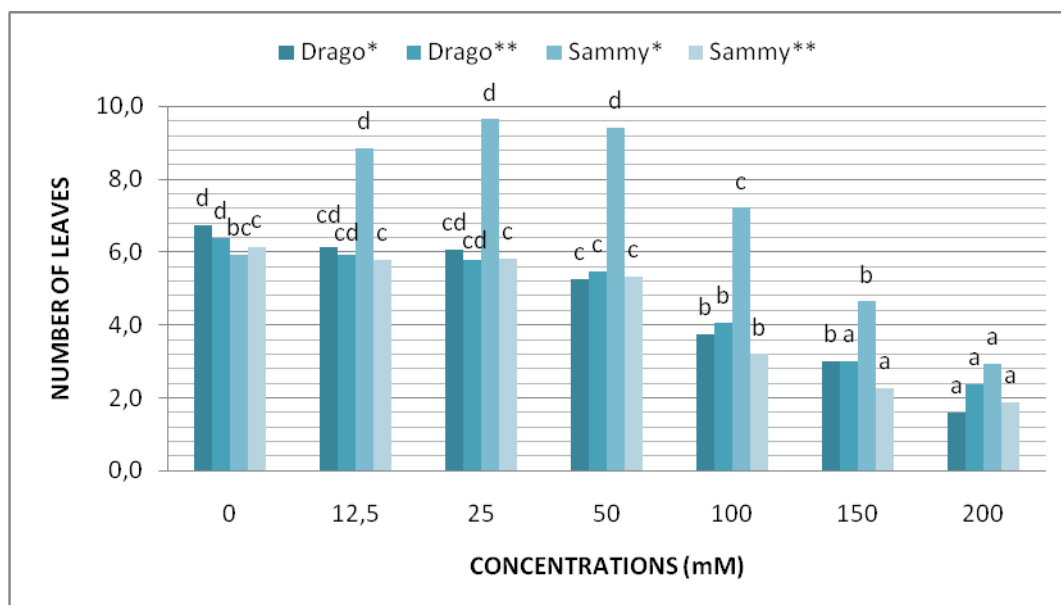


Figure 4.63: Effect of NaCl on the number of leaves of pepper plants after in vitro culture in saline substrate. * plants transplanted in substrate with NaCl and ** plants germinated into substrate with NaCl.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

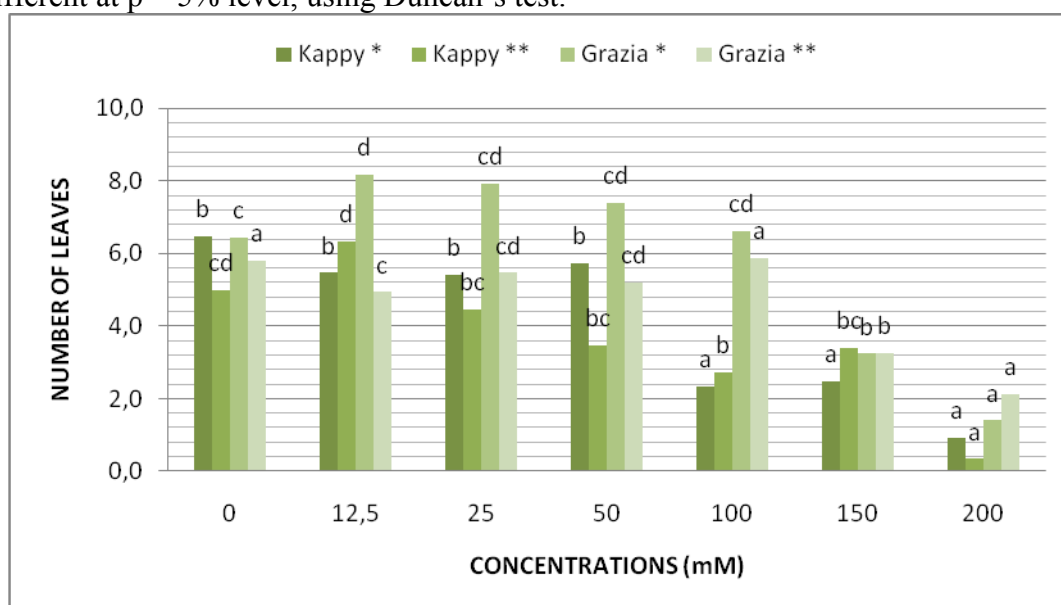


Figure 4.64: Effect of NaCl on the number of leaves of hybrids Kappy and Gracia pepper plants after in vitro culture in saline substrate. * plants transplanted in substrate with NaCl and ** plants germinated in substrate with NaCl.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

c. Plantlet weight

The total weight of the transplanted plantlets on low concentrations of salt (up to 50 mM for Sammy and Kappy and up to 100 mM for Gracia and Drago) slightly increased though not always significantly. In higher concentrations the reduction of the total weight was significantly high. The total plant weight of the germinated plantlets on low concentrations of salt showed a similar trend.

The total weight of the transplanted plantlets was higher than the weight of the germinated plantlets on the saline substrate with the exception of Kappy. For example at 25 mM NaCl Sammy transplanted plantlets were 10.2 times heavier than the germinated plantlets on the saline substrate (Table 4.15), Gracia's 6.2 times, Drago 1.7 times and Kappy 1.02. In 150 mM NaCl Sammy transplanted plantlets were 25 times heavier than the germinated plantlets on the saline substrate (Table 4.20), Gracia's 10.5 times, Drago's 5.8 times and Kappy's 1,4.

Table 4.15: Total plantlet weight of four pepper hybrids cultured in vitro either germinated on, or transplanted onto substrates containing NaCl (0 – 200 mM).

NaCl (mM)	Sammy		Gracia		Drago		Kappy	
	Trans planted	Germin ated	Trans planted	Germin ated	Trans planted	Germin ated	Trans planted	Germin ated
0	3,031c	0,233b	0,387ab	0,178b	0,471b	0,340c	0,368cd	0,343cd
12,5	3,992d	0,233b	2,147e	0,185b	0,569b	0,325bc	0,337bcd	0,408d
25	2,924c	0,287c	1,289cd	0,207bc	0,591b	0,339c	0,368cd	0,361cd
50	3,448cd	0,278bc	2,100e	0,228cd	0,527b	0,340c	0,403d	0,312bcd
100	1,397b	0,237b	1,757de	0,247d	0,466b	0,270b	0,211b	0,217bc
150	1,745b	0,060a	0,822bc	0,078a	0,458b	0,079a	0,241bc	0,174b
200	0,465a	0,078a	0,254a	0,070a	0,208a	0,099a	0,070a	0,009a

Regarding the shoot weight of plants, it can be seen from the Figure.4.65 in the case of Drago that the heaviest plants were observed at the concentrations of 0 - 150 mM NaCl if transplanted, or 0 - 100 mM if germinated on the saline substrate. Significant reduction was observed on 200 mM for the transplanted plants, and on 150 and 200 mM for the germinated plants on the saline substrate.

Moreover the shoot weight of the transplanted plants was 2 times higher than the weight of plants germinated in the substrate with salt, except in concentration of 150 mM NaCl were the first one is about 6 times higher from the second.

Root weight followed a similar trend.

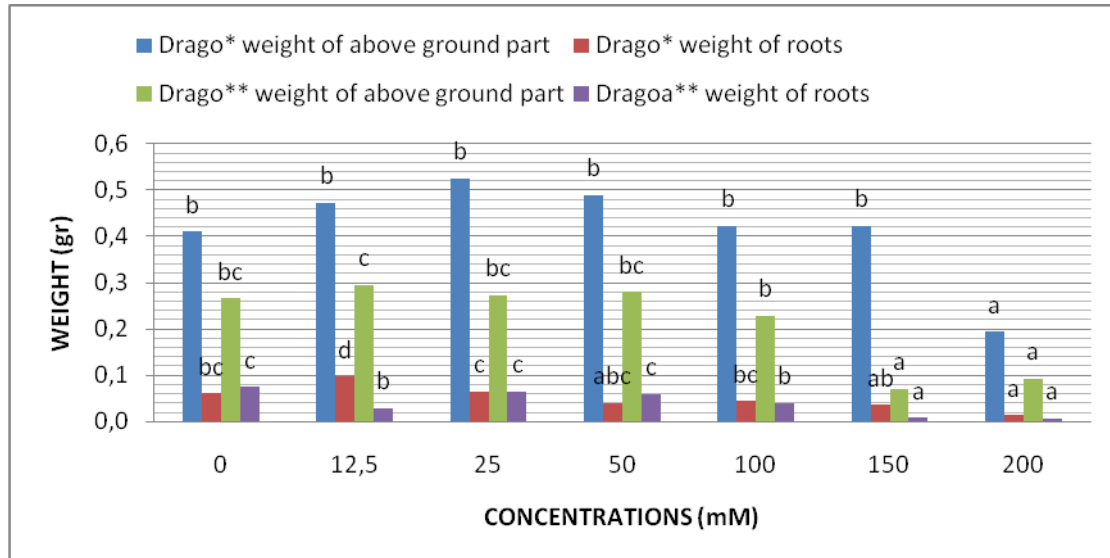


Figure 4.65: Effect of NaCl on mean weight of the roots and the aerial part of Drago plants after in vitro culture in saline substrate. * plants transplanted in substrate with NaCl and ** plants germinated in substrate with NaCl.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

The weight of Sammy plantlets was affected by salt (Fig. 4.66). The significantly lighter plants are in concentrations of 100, 150 and 200 mM NaCl, for the transplanted plantlets and 150 and 200 mM for the germinated plantlets on saline substrate.

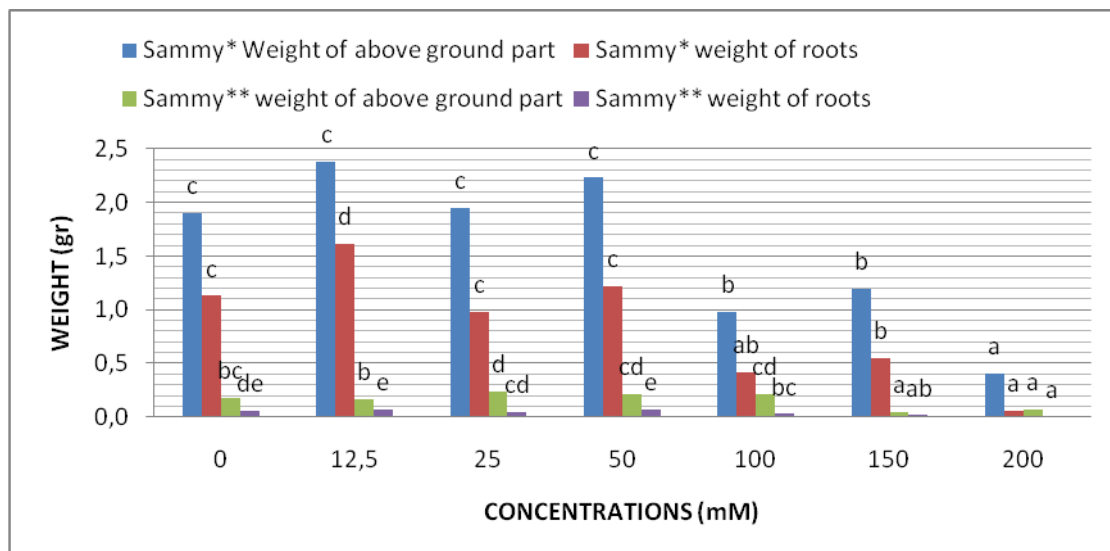


Figure 4.66: Effect of NaCl on mean weight of the roots and the aerial part of Sammy plants after in vitro culture in saline substrate. * plants transplanted in substrate with NaCl and ** plants germinated in substrate with NaCl.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

According to Figure 4.67 shoot weight of transplanted Kappy plantlets are similar to the germinated plantlets, the heaviest plantlets are those in low concentrations of salt.

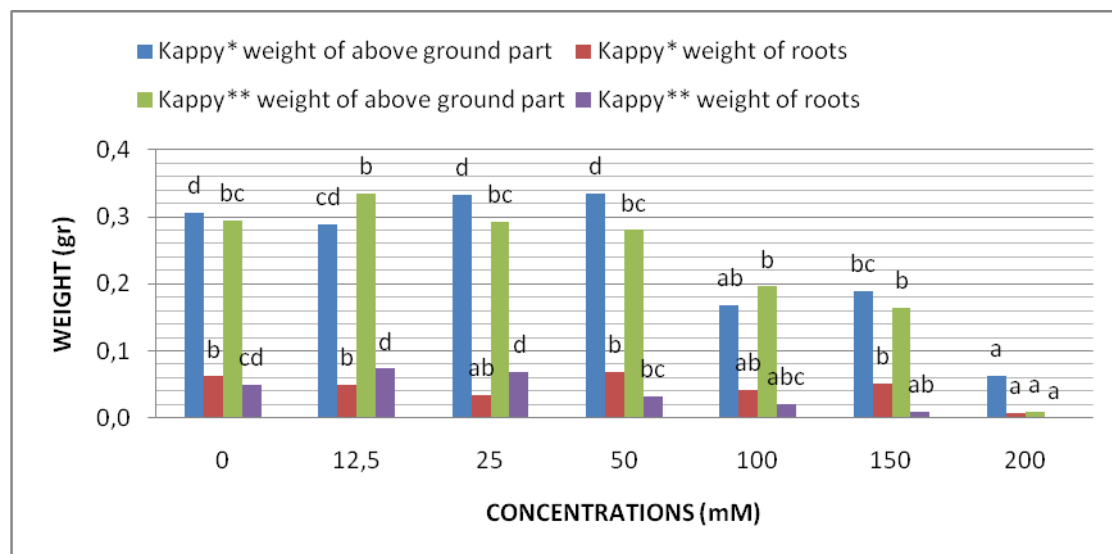


Figure 4.67: Effect of NaCl on mean weight of the roots and the aerial part of Kappy plants after in vitro culture in saline substrate. * plants transplanted in substrate with NaCl and ** plants germinated in substrate with NaCl.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

In both cases of Gracia (Figure 4.68) the weight decreased at the concentrations of 150 and 200 mM NaCl.

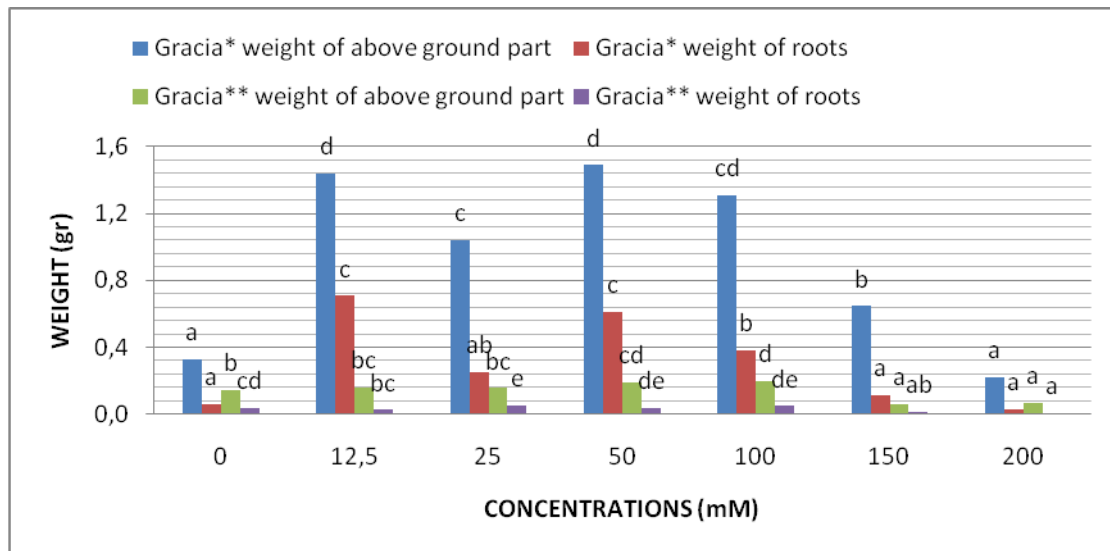


Figure 4.68: Effect of NaCl on mean weight of the roots and the aerial part of Gracia plants after in vitro culture in saline substrate. * plants transplanted in substrate with NaCl and ** plants germinated in substrate with NaCl.

Bars with the same color, followed by the same letter are not significantly different at $p = 5\%$ level, using Duncan's test.

Table 4.16: Shoot to root ratio of the in vitro transplanted or in vitro germinated plantlets on saline substrates of four pepper hybrids.

NaCl (mM)	Sammy		Gracia		Drago		Kappy	
	Trans planted	Germin ated	Trans planted	Germin ated	Trans planted	Germin ated	Trans planted	Germin ated
0	6,7	3,6	1,7	3,2	5,0	6,0	2,3	4,1
12,5	4,9	9,9	1,5	2,5	5,9	4,6	2,0	6,4
25	8,0	4,2	2,0	5,3	7,4	4,3	2,1	3,1
50	12,6	4,6	1,8	3,1	4,9	8,8	2,4	4,7
100	9,6	5,6	2,4	7,6	4,0	9,3	3,4	4,0
150	11,9	9,0	2,2	2,9	3,7	16,7	5,7	3,9
200	14,3	16,7	6,5	19,6	9,6	9,2	6,8	13,0

The root weight followed the trend of the shoots: not affected or slightly positively affected by the low NaCl concentrations, severely reduced by the high concentrations especially at 150 and 200 mM.

On table 4.16 the shoot to root ratios of the plantlets of each hybrid are presented. Although the differences at low concentrations are not significant at 150 and specially at 200 mM the shoot to root ratio increased.

4.3.3. Biochemical parameters

a. Protein Extraction and Protein Determination

As shown in Table 4.17 in most of the cases the protein content was not affected by the increasing concentrations of NaCl. In the case of Sammy germinated on substrate with NaCl there is a significant reduction of the total protein content following the increase in NaCl concentration. In Gracia both germinated and transplanted, the protein content of the plantlets grown in the concentration of 200mM NaCl is statistically different from those on other concentrations.

Table 4.17: Effect of NaCl on the protein content of pepper plantlets under salinity stress in vitro. Plantlets either transplanted or germinated on substrates with NaCl.

NaCl (mM) stress	Sammy		Drago		Kappy		Gracia	
	Trans planted	Germin ated	Trans planted	Germin ated	Trans planted	Germin ated	Trans planted	Germin ated
0	2748a	4472d	2296a	3332a	3068a	3260a	3500b	5332c
12,5	2356a	4156cd	2880a	2688a	3540a	4444a	3584b	5044bc
25	3204a	3656ab	2364a	3448a	3444a	3596a	4796c	5004bc
50	3076a	3792bc	2320a	2744a	4072a	4272a	3152b	4692bc
100	3216a	3328a	1472a	3348a	4364a	3312a	3612b	4580b
150	3152a	3568ab	2104a	2016a	5044a	4284a	3112b	4832bc
200	2048a	3424ab	1400a	2816a	4740a	4792a	1572a	3536a

b. Protein Electrophoresis

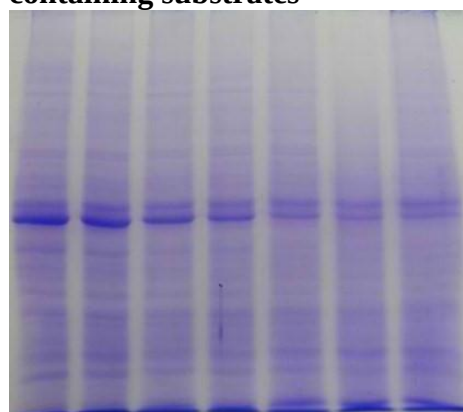
The protein electrophoresis was done on eight gels, one for each hybrid germinated in vitro, on NaCl containing substrates and one for each hybrid transplanted in vitro on on NaCl containing substrates.

In each of the seven “wells” of each gel were placed leaf extract of plants grown at different NaCl concentrations (Plate 4.14). The bands are formed by different protein accumulation.

The effect of NaCl on soluble proteins can be judged by the color intensity of the bands (lighter or darker bands represent lower or higher protein concentration respectively). In Plate 4.15 are presented the protein electrophoresis gels of leaf extracts of all pepper hybrids after culture in perlite at concentrations of 0, 12.5, 25, 50, 100, 150 and 200 mM NaCl, for 12 weeks.

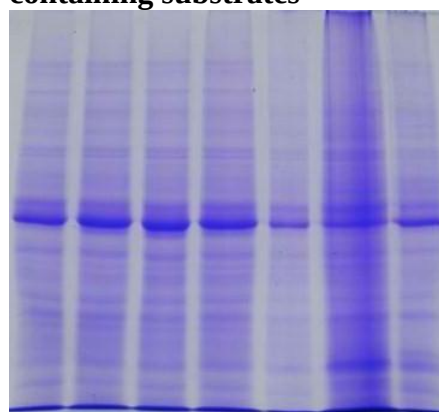
In general, proteins seem less affected in plants germinated onto saline substrates compared to plants transplanted on saline substrates, specially in Drago, Sammy and Gracia. Most proteins seem gradually reduced as the increase at NaCl concentration increases in all cases.

Drago transplanted in vitro onto NaCl containing substrates



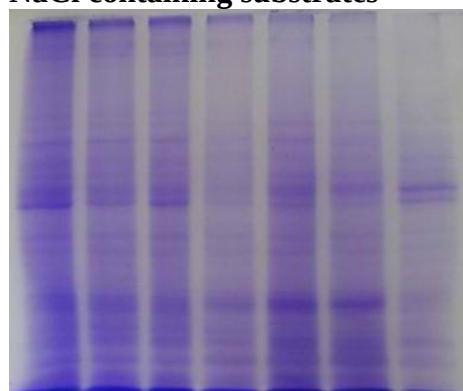
0 12.5 25 50 100 150 200
mM NaCl

Drago germinated in vitro onto NaCl containing substrates



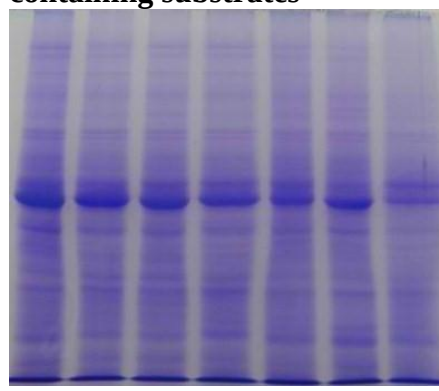
0 12.5 25 50 100 150 200
mM NaCl

Sammy transplanted in vitro onto NaCl containing substrates



0 12.5 25 50 100 150 200
mM NaCl

Sammy germinated in vitro onto NaCl containing substrates



0 12.5 25 50 100 150 200
mM NaCl

Plate 4.14: Protein electrophoresis gels of leaf extracts of Drago and Sammy pepper hybrids cultured in vitro on substrates containing concentrations NaCl, of 0, 12.5, 25, 50, 100, 150 and 200 mM .

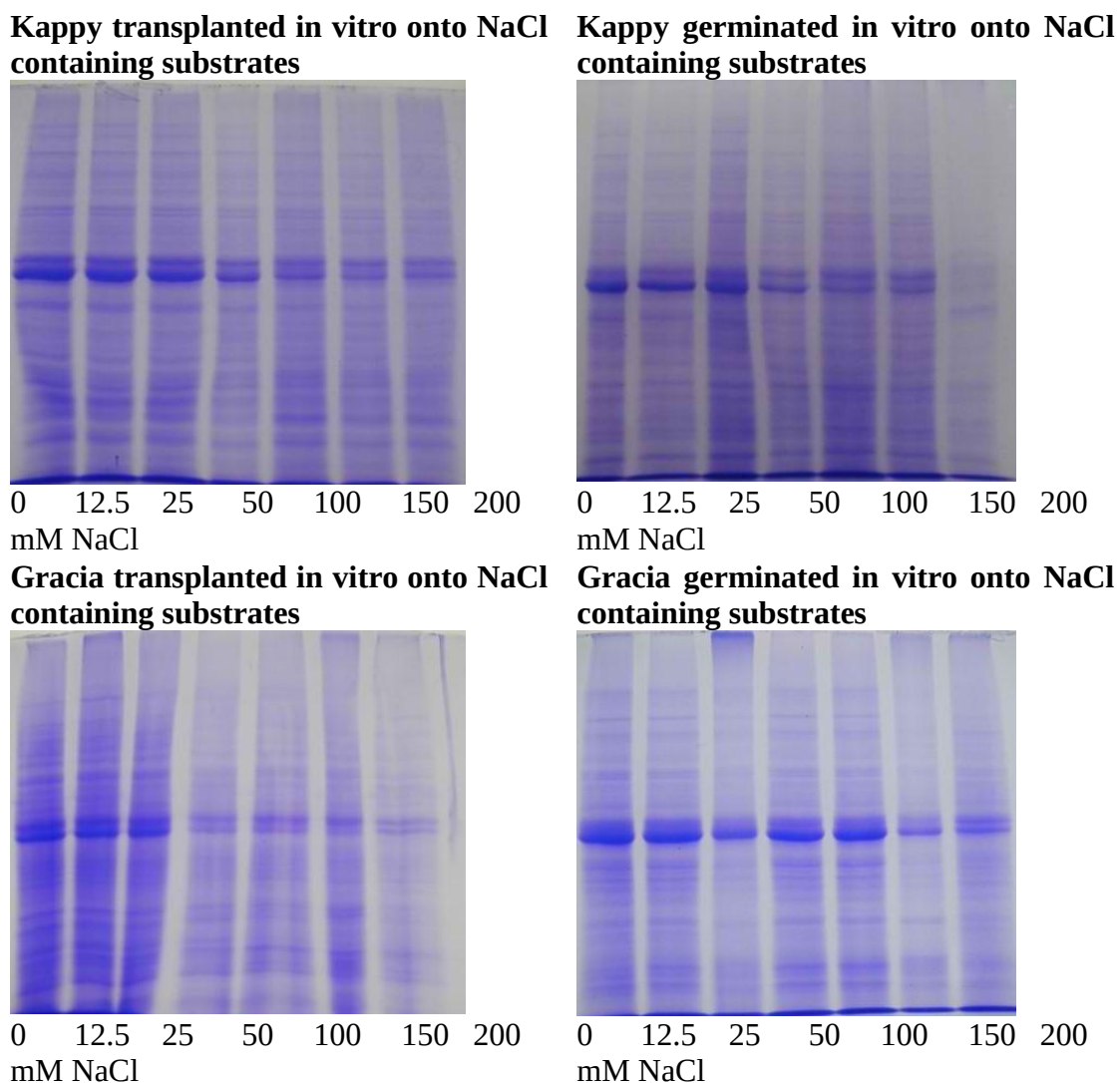


Plate 4.15: Protein gel electrophoresis of leaf extracts of Kappy and Gracia pepper hybrids cultured in vitro on substrates containing concentrations NaCl, of 0, 12.5, 25, 50, 100, 150 and 200 mM.

c. Polyacrylamide Gel Electrophoresis and Immunoblot Analysis

According to Plate 4.16 in most cases the immunoblots of GDH showed that the level of GDH protein remained stable while the concentrations of NaCl increased. Only in Sammy where the plants were put into soil and in Kappy where the plants were put into perlite, a small increase is evident at the concentrations of 150 and 200 mM NaCl.

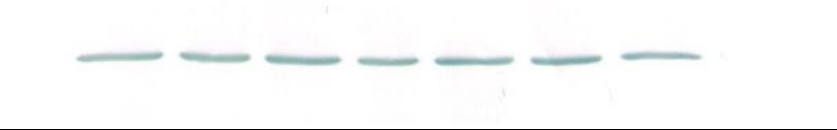
Concentration of NaCl (mM) in the substrate	0 12,5 25 50 100 150 200 mM
Sammy transplanted in vitro	
Sammy germinated in vitro	
Kappy transplanted in vitro	
Kappy germinated in vitro	
Gracia transplanted in vitro	
Gracia germinated in vitro	
Drago germinated in vitro	

Plate 4.16: Western blot analysis of GDH in Sammy, Kappy and Gracia leaf extracts obtained from plants grown in perlite at different concentrations of NaCl for 12 weeks. For immunoblot analysis proteins were resolved in 10% SDS polyacrilamide gels, transferred onto nitrocellulose membranes and probed with anti-GDH serum, as described in section “Materials and Methods”.

Chapter 5. Discussion

Salinity in glycophytes leads to the reduce in the growth and yield of the plants (Mass and Hoffman, 1977). It is proved that the available water in plants which grow in increased salinity is limited due to the high osmotic potential in the root zone (Papadakis *et al.*, 2007). The reduced amount of water entering the plant due to the low osmotic potential of the soil, leads to stomata closure in order to minimize plant transpiration. Stomatal conductance and the consequent restriction of the available CO₂ for carboxylation lead to the reduction of the photosynthetic CO₂ assimilation, and the production of enough assimilates for growth (Brugnoli and Lauteri 1991, Geissler *et al.* 2009). The results of this study show that salt negative affected all pepper hybrids in all cases (hydroponic culture in perlite, culture in soil and in vitro culture). Reductions in plant height, number of leaves per plant, leaf area, root length, and root surface area have been reported in some plants like pepper (Chartzoulakis and Klapaki, 2000), tomato (Mohammad *et al.*, 1998), *Phaseolus vulgaris* (Wignarajah 1990), *Cucumis sativus* (Duan *et al.* 2008), *Solanum melongena* (Savvas and Lenz 2000, Unlukara *et al.* 2008) and other vegetables (Shannon and Grieve 1999), olive cultivars (Chartzoulakis *et al.* 2002), and citrus cultivars (Storey and Walker 1999).

It has been reported that salinity level of 60 mM severely restricted pepper, therefore it is considered salt sensitive plant species (Navarro *et al.*, 2002, Lycoscoufis *et al.*, 2005).

From the experiments conducted in this work, it was found that in different ways of culture the salt stress effects on the pepper hybrids were different. In the in vitro culture the salt effect was better overcome by the plants especially at the lower concentrations. The controlled environment of this culture protects the plants from other biotic or abiotic stresses. When cultured in perlite all hybrids seemed more salt tolerant than when cultured in soil. This could possibly be due to the colder period during which it was conducted the experiment in the soil. Although the greenhouse used was a heated one and the minimum temperature was kept at 14 °C, the mean day temperature during the soil experiment (10 weeks) was 4 °C lower than it was during the same period of the hydroponic experiment. This condition might have combined cold stress to the already imposed salt stress and enhanced the symptoms.

Increased salinity results in a reduction of the germination and / or a delay in many glycophyte seeds but also in many halophytes (Ungar, 1995). Most halophytic species show optimal germination in fresh water although they can germinate in extreme salinities (Khan and Ungar, 1996). It is still a subject of study whether salinity reduces germination by an osmotic effect or because of ion toxicity, or both (Demir and Mavi, 2008). It has been reported that salinity delays germination without affecting percentage germination and that depends on the level of salinity and the plant species or variety ((Bewley and Black, 1994, Kigel, 1995).

In the present work salt had an adverse effect on germination in all four pepper hybrids:

When the used seed was fresh germination was delayed but final percentage germination was not affected even at 200mM NaCl. Salinity at 200mM caused growth arrest on a number of seedlings in all hybrids. Sammy seemed more sensitive as the seedling growth arrest happened in lower concentrations of salt. This is in agreement with the findings of Chartzoulakis and Klapaki, (2000) who reported that salinity affected seedlings growth more than seed germination in the pepper hybrids they investigated.

When the used seed was one year old, germination was not only delayed but also reduced by salinity. At 200mM germination was very low in all hybrids. Drago followed by Kappy were the most tolerant; germination was practically not delayed or reduced when external salinity was up to 100 mM NaCl in Drago and 50 mmol NaCl in Kappy. It seems that NaCl affected the germination of older seed more than the germination of the fresh seed. It has been reported that germination of old seed is more negatively influenced by adverse environmental conditions than the fresh seed (Bewley and Black, 1994).

The height of seedlings was not affected by salinity up to 100 mM NaCl for Drago and 150 mM for Kappy. was negatively affected in almost all salinity levels while in Height in Gracia and Sammy was affected positively up to 100 mM NaCl and negatively in higher salinities.

When salinity was imposed later in the life of the seedlings they remained shorter than those germinated and grown under saline conditions, in all hybrids but Kappy. Otherwise the effect of salt on the height was similar to the height of the not transplanted seedlings. Obviously transplanting was a stressful condition which affected equally the seedlings in all NaCl concentrations but although it affected the

height it did not affect the number of leaves. Number of leaves was negatively affected by salt only at high concentrations. In Gracia (and in a lesser degree in Kappy) the number of leaves actually increased at low concentration of salt specially among the transplanted plants. At high concentrations number of leaves was decreased in Kappy and Drago at 100 mM NaCl and Gracia and Kappy at 150 mM NaCl. The shoot and root weight decreased in Drago at 200 and 150mM NaCl respectively, in Sammy at 100 mM NaCl for shoot and root, in Kappy at 100 and 200 mM NaCl respectively. In Gracia the shoot and root weight increased in lower concentrations of NaCl and was decreased only at 200 mM NaCl.

The seedlings of pepper hybrids were more sensitive to external salinity than seed germination . Germination of fresh seed was not affected, but seedling growth, expressed as height, number of leaves and fresh weight of shoot and root, were reduced when substrate salinity exceeded 100 mM, or 150 mM NaCl . The seedlings which were germinated in vitro and then transplanted onto NaCl substrates performed similarly although they were generally bigger plants.

The reduction in height, number of leaves and fresh weight of shoot and root could be the effect of osmotic stress, as seedlings are more succulent than seeds and therefore more susceptible, combined with higher ion uptake (Demir and Mavi, 2008 Chartzoulakis and Loupassaki, 1997).

The findings of this work partly agree with the results of experiments been conducted in eggplant (Chartzoulakis and Loupassaki, 1997) and pepper hybrids (Chartzoulakis and Klapaki, 2000). In eggplant and pepper hybrids, the percentage germination was affected at salinities 100 mM NaCl and more, while seedling growth parameters were affected at 10 mM NaCl in eggplant and 50 mM NaCl in pepper. In this work the pepper hybrids tested, were more tolerant, cultured in vitro. In vitro the environmental conditions are controlled so the imposed salt stress is not combined with other biotic or abiotic stresses which might enhance the symptoms. Therefore a similar experiment should be conducted in vivo, in the greenhouse, in order to test the results of the present work.

Taking into consideration all the measured parameters, Drago was more salt tolerant at the seed germination and seedling growth in vitro with the rest hybrids following closely.

The height of the plants cultured in perlite Fig. 4.5 was reduced more in Drago specially at 150 and 200 mM NaCl and less in Sammy, Kappy and Gracia while in the soil experiment Fig 4.35 the effect started earlier in Sammy (at concentrations of 50 mM NaCl) while in Drago and Kappy it started at 100 mM NaCl. Previous research reports indicate that the height of the pear (Okubo *et al.*, 2000), apple (Motosugi *et al.*, 1987) and cherry (Papadakis *et al.*, 2007) was reduced by the presence of NaCl in the irrigative solution.

The number of leaves and fruits per plant were also affected from salt in a different way depending on the hybrid and the type of culture . In the hydroponic culture in perlite, the mean number of leaves in Sammy hybrid Fig 4.10 was not affected from salt while in Drago, Kappy and Gracia was decreased at 150 and 200 mM NaCl. In the soil experiment Fig. 4.39 the effect was negative in all hybrids .

In hydroponic culture Fig. 4.11 the effect of salt in the mean number of fruits and leaves per plant was negative in Kappy, Drago and Gracia but positive or neutral in Sammy, depending to NaCl concentration. In the soil culture Fig. 4.41 the number of fruits was negatively affected from salt. The decrease was significant at concentrations of 25 mM NaCl, in Drago and Kappy while in Sammy at 100 mM NaCl. Also the number of the flowers Fig. 4.40 was affected from salt in all cases. The decrease was significant from 12.5 mM in Drago and 25 mM NaCl in Sammy and Kappy while in 100, 150 and 200 the decrease was even bigger compared to 0 mM NaCl.

Salt stress also resulted in a decrease in the fresh and dry weights of leaves, stems, and roots in many plant species like pisum (Hernandez *et al.*, 1995), *Raphanus sativus* (Marcelis and VanHooijdonk, 1999), cotton (Meloni *et al.*, 2001) and pepper (Chartzoulakis and Klapaki, 2000, Navarro *et al.* 2003 and Lycoskoufis *et al.* 2005). In the hydroponic culture in perlite dry weight of shoot, leaves and fruits were increased at the lower concentrations of salt in the case of Kappy but in higher concentration they were decreased. In Drago and Gracia this weights were reduced in all salinities. In Gracia the decrease was sharper as the concentrations of salt increased. The Shoot/root (Table 4.4) was decreased in all hybrids due to salt. In Sammy the root to shoot ratio was not affected up to 50 mMNaCl. Drago and Gracia were affected from 12.5 mM NaCl and Kappy at 25mM NaCl. In Kappy and Gracia from 150 mM and 100 mM NaCl respectively the root system was heavier than the above ground part. The increase

of the root system in the expense of the above ground part or the increased reduction of shoot growth compared to root growth by salinity stress could be explained by the hypothesis that

Under salt stress conditions, the leaves show reduced physiological activity (e.g. photosynthesis) while the root has increased requirements in order to cope with ion disequilibrium and osmotic imbalance caused by salt. Therefore less resources are allocated to the shoot and more to the root. Wignarajah 1990, reported that decreased shoot/root ratios were observed in *Phaseolus* in the early stages of salinity.

The succulence in roots increased in all hybrids and is considered to be a result of ion accumulation which happens when plants' roots are in saline environment specially the 'salt includers'. To maintain osmotic equilibrium water enters the root and thus the root becomes more succulent. In Sammy, the increase was very small -1%- and so in Drago -less than 3%. In Kappy and Gracia was higher namely from 7 to 8%, especially in 50 to 150 mM NaCl.

The shoot and leaf succulence, in all hybrids, increased slightly at medium NaCl concentrations and decreased at high concentrations, especially at 200 mM, due to disrupted osmotic relations. In Kappy and Gracia the decrease of water content was high and sharp, especially in the leaves which were wilting (table 4.5). Those findings show that shoot, leaves and fruits were affected more from salt than roots. Furthermore according to Parida and Das (2005) one of the biochemical ways that plants use to achieve salt tolerance is to control the ion uptake from roots and transport it into leaves. So if there are more Na^+ and Cl^- ions in the shoot and in each part than in the roots it is possible that the effect of salt is significant in the above ground parts.

In soil fresh and dry weight of shoot, leaves and fruits in all three hybrids decreased with Kappy having the smallest decrease at 200 mM compared to 0 mM. Most other parameters changed in the same way as they did in the hydroponic culture. Comparing the hybrids between perlite and soil:

In perlite, Sammy and Kappy were rather tolerant. Kappy was positively affected or not affected at low concentrations but at high concentrations the effects were very pronounced. Sammy was affected negatively only at very high concentrations (100 or 150 mM NaCl and more) and even then the effects were of medium severity. In Drago most parameters were negatively affected by all concentrations of salt but less severely than in Gracia and even in Kappy.

In soil, Sammy and Kappy suffered the negative effects of salt at lower concentrations. In all hybrids the measured parameters were generally more negatively affected by salt.

Chlorophyll content is one of the parameters of tolerance in cultivars in salt (Hernandez *et al.*, 1995; Srivastava *et al.*, 1998). According to Garcia-Sanchez *et al.*, 2002 the decrease in the chlorophyll in citrus cultivars happened due to the high concentrations of Na^+ and Cl^- ions in the leaves. The same was also confirmed by Papadakis *et al.* (2007) in two varieties of cherries. In this study, in all hybrids, there was a decrease in the chlorophyll content as the concentrations of salt increased. At the beginning of the experiments, in both perlite and soil, there were no differences in the chlorophyll content among the concentrations of salt in all hybrids. But according to Figures 4.20, 4.22, 4.24, 4.26 the chlorophyll content at the end of the experiment was decreased from 150 mM in Drago and Sammy and from 100 mM in Kappy and Gracia while in soil (Fig. 4.48, 4.50 and 4.52) the reduction was significant in all salinities. The reduction of chlorophyll content was obvious in the older leaves, but the young fully expanded leaves which were measured did not have any visual symptoms. Moreover the results showed that when cultured in soil salinity effects were more intense regarding the chlorophyll content.

The measurement of chlorophyll fluorescence (Fv/Fm ratio) is considered to be a sensitive method of assessing the efficiency of PSII and the changes in photosynthesis caused by environmental effects (Lichtenthaler 1988). According to our results (Fig. 4.21, 4.23, 4.25, 4.27, 4.49, 4.51 and 4.53) at the beginning of the experiments the Fv/Fm ratio stayed stable in all hybrids within the concentration in normal levels (from 0.75-0.85). But at the end of the experiment there was a decrease in the chlorophyll fluorescence which was much more obvious at the concentrations from 100 to 200 mM NaCl especially in Kappy and Grazia hybrids cultured hydroponically where the Fv/Fm ratio was close to 0.150. In all three hybrids cultured in soil the Fv/Fm ratio was from 0.350 to 0.450. The reduction of chlorophyll content was followed by a reduction of chlorophyll fluorescence which was sharper in culture in soil. But it seems that a small decrease in chlorophyll content did not cause analogous reduction in chlorophyll content.

Salinity reduced the photosynthetic rate and related parameters (stomatal conductance, intercellular CO_2 concentration and transpiration) in plants cultured in

perlite (Fig. 4.28, 4.29, 4.30 and 4.31). The decrease was higher in Drago in all parameters, lower in Sammy and ever lower in Kappy and Gracia. In soil according to Figures 4.54, 4.55, 4.56 and 4.57 there was a significant decrease at concentrations of 150 and 200 mM NaCl in all four parameters in Drago while in Sammy and Kappy there was a reduction but with no significant differences. From the results in chlorophyll content and photosynthesis in both perlite and soil we see that there must be a connection since wherever there is a decrease in the chlorophyll there is also a decrease in the photosynthesis. Also there is a connection between the stomatal conductance and photosynthesis. There is often a correlation between the reduction of the photosynthetic rate and the reduction of the stomatal conductance (Banuls and Primo-Millo, 1995). In this study in all hybrids the photosynthetic rate and the stomatal conductance were reduced in the same way. This may indicate that the closure of the stomata was one of the parameters which contributed to the reduction of the photosynthetic rate.

It has being reported that soluble proteins were decreased under salt stress in the leaves of some plants like *Cicer arietinum* (Soussi et al. 1998) and *Vicia faba* (Gadallah, 1999). In this study the soluble protein in the hybrids in perlite (Table 4.6) decreased in Drago, Kappy and Gracia and wasn't affected in Sammy while in soil the presence of salt (Table 4.14) decreased the soluble protein in all three hybrids. These findings agree with the salt tolerance that Sammy showed when cultured hydroponically .

In vitro cultured seedlings germinated or transplanted in salt (Table 4.) in most of the cases the protein content was not affected by the increasing concentrations of NaCl, Only in Sammy (germinated in salt substrate) and Gracia (both transplanted and germinated) there was a significant decrease. Similar results have been found by Kostopoulou (2008) where the content of the soluble protein was not affected by salt in leaves and roots of *Citrus aurantium*. If there is no change in the soluble protein content, this means that there is no change in the protein construction as well (Niknam et al., 2006).

Soil salinity affected the activity of GDH, one of the enzymes which are responsible for ammonia assimilation in plants. In other reports has been observed that the GDH activity was increased under salt stress (Mattioni *et al.*, 1997; Kumar *et al.*, 2000; Sforza *et al.*, 2002). Furthermore it has been reported that increased GDH

activity could be an indicator of salinity tolerance. According to Tables 4.8, 4.15 and 4.16, the activity of GDH was increased at the higher concentration of NaCl more in Kappy and Gracia and less in Sammy in perlite and in both Sammy and Kappy from 100 mM in soil. In the *in vitro* experiments although there is an increase in the higher concentrations in all hybrids the levels of the activity are not much higher than the levels at the lower concentrations. The GDH activity did not follow the indications of growth parameters regarding the salt tolerance.

The plants in order to detoxify the ROS (reactive oxygen species) produced under salt stress, increase the activities of certain antioxidative enzymes such as catalase, peroxidase, glutathione reductase, and superoxide dismutase. Asada, 1999, reported that superoxide dismutase (SOD) plays an important antioxidant role and converts the superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2). The activity of antioxidant enzymes has been reported to increase under salt stress in wheat (Sairam et al 2002) alfalfa (Wang et al., 2009), Cassia, (Agarwal and Pandey 2004) but decreased in wheat roots (Willekens et al., 1997), or was unaffected as in the case of SOD in cucumber (Lechno et al., 1997).

In this study there was a small increase in SOD for all treatments which do not agree with the most reports where the SOD activity decreased as the NaCl increased. GPX and APX activity were affected in different ways from salt. GPX in most of the hybrids (soil and perlite) remained stable while there was a significant decrease in Kappy and Gracia cultured in perlite. Similar to GPX, APX remained stable as the concentrations increased in most hybrids. Sammy in both GPX and APX increased. There were no significant changes in the activity of POD as the concentrations increased.

In most reported experiments concerning antioxidant enzyme activity under salt stress, the stress was imposed to plants for a few days only or a few weeks. For example in alfalfa for 7 days (Wang et al., 2009) and in Cassia for 5 and 7 days, (Agarwal and Pandey 2004). A possibility is that after a long period of salt stress the antioxidant enzyme activity changes.

The results of this work indicate that Sammy was the most tolerant hybrid when grown in hydroponics or soil but not at the seed germination stage. In Sammy and in Kappy, the next tolerant hybrid, the fruit production as number of fruits or fruit weight were even enhanced by the low concentrations of salt. Drago is a very sensitive though very productive hybrid. Although it was negatively affected by salt at all

concentrations, the reduction up to 100 mM NaCl was not very sharp. When salt stress was combined with low temperatures Drago was not able to cope. Gracia was the least tolerant variety.

Chapter 6. Conclusions-Future Work

Conclusions

- The presence of NaCl reduced the height of the plants, the number of leaves, flowers and fruits and the FW and DW of each part and of the roots of the plants in all cases (in vivo and in vitro).
- The presence of NaCl negatively affected the chlorophyll content in the leaves and the chlorophyll fluorescence in all cases and the photosynthetic rate, stomatal conductance, intercellular CO₂ concentration and transpiration mainly in Drago
- NaCl affected the soluble protein content in all cases. and caused an increase in the GDH enzyme
- The presence of NaCl was not affect most of the antioxidant enzymes GPX, APX, SOD and POD.
- According to our results from the in vivo and in vitro experiments the most tolerant hybrid is Sammy, followed by Drago and then comes Kappy and final is Gracia.

Future Work

The investigation on the salinity tolerance or sensitivity of Sammy, Drago, Kappy and Gracia hybrids should continue, in order to gain information on the subject so that growers and researchers could benefit.

The experiments could be conducted for longer period so that the effect of NaCl be investigated in more advanced developmental stages of the plant which are more productive and of greater interest to the growers.

The effect of NaCl on the quantity and the quality of the fruit production is an interesting parameter with great economical interest.

The physiological parameters of the older leaves which were not measured in this work could be of certain interest, as the speed of leaf senescence and abscission is a very important factor of the salt tolerance.

The biochemical parameters could be measured in different stages of the life of the plants at the beginning of the salt stress and later. The results could be more clear and therefore could give scientists tools for salinity tolerance selection.

The tissue analysis of the Na^+ and Cl^- could show how hybrids distribute those ions in the plant parts and draw useful conclusions on the possibility of using grafted plants in order to restrict the ion accumulation in the above ground part of the plant.

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Appendix A

**A****B****C****D****E****F**



Plate 4.1: Effect of NaCl on Drago plants cultured in perlite. A=0 mM, B=12.5 mM, C= 25 mM, D= 50 mM, E= 100 mM, F = 150 mM, G= 200 mM NaCl and H= 0 mM on the left and 200 mM NaCl on the right.



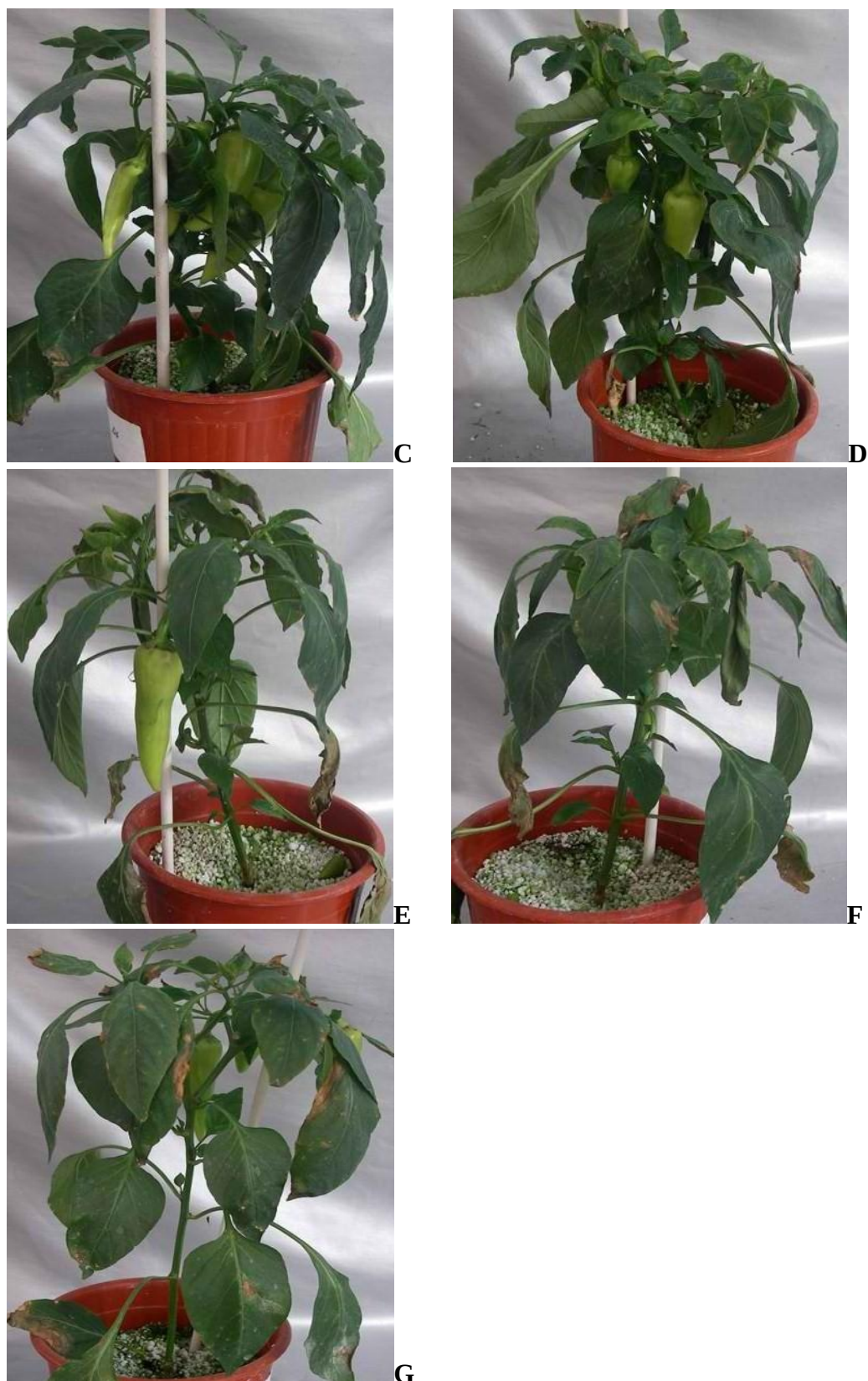


Plate 4.4: Effect of NaCl on Sammy plants cultured in perlite. A=0 mM, B=12.5 mM, C= 25 mM, D= 50 mM, E= 100 mM, F = 150 mM and G= 200 mM NaCl.

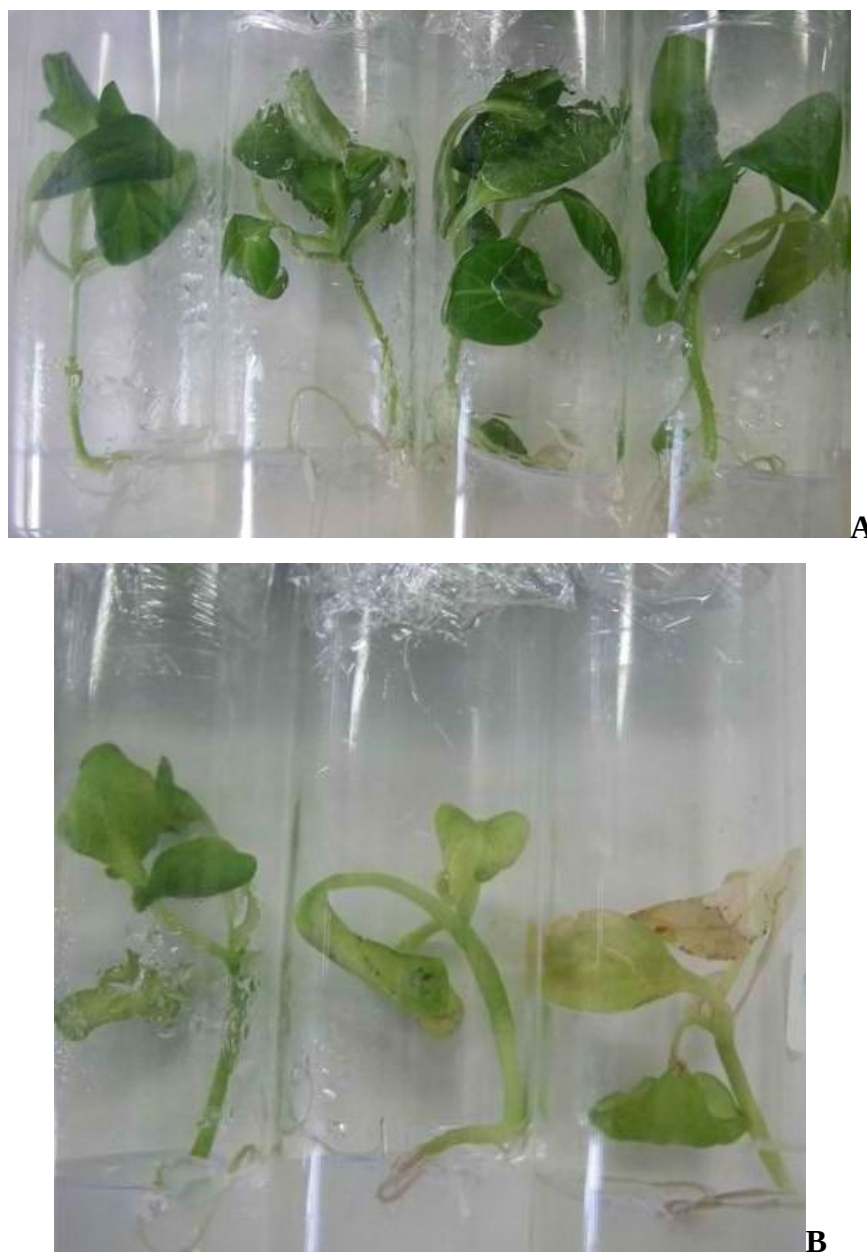


Plate 4.7: Effect of NaCl on Drago plants transplanted. A = 0, 12.5, 25, 50 mM NaCl from the left and B= 100, 150, 200 mM NaCl from the left.

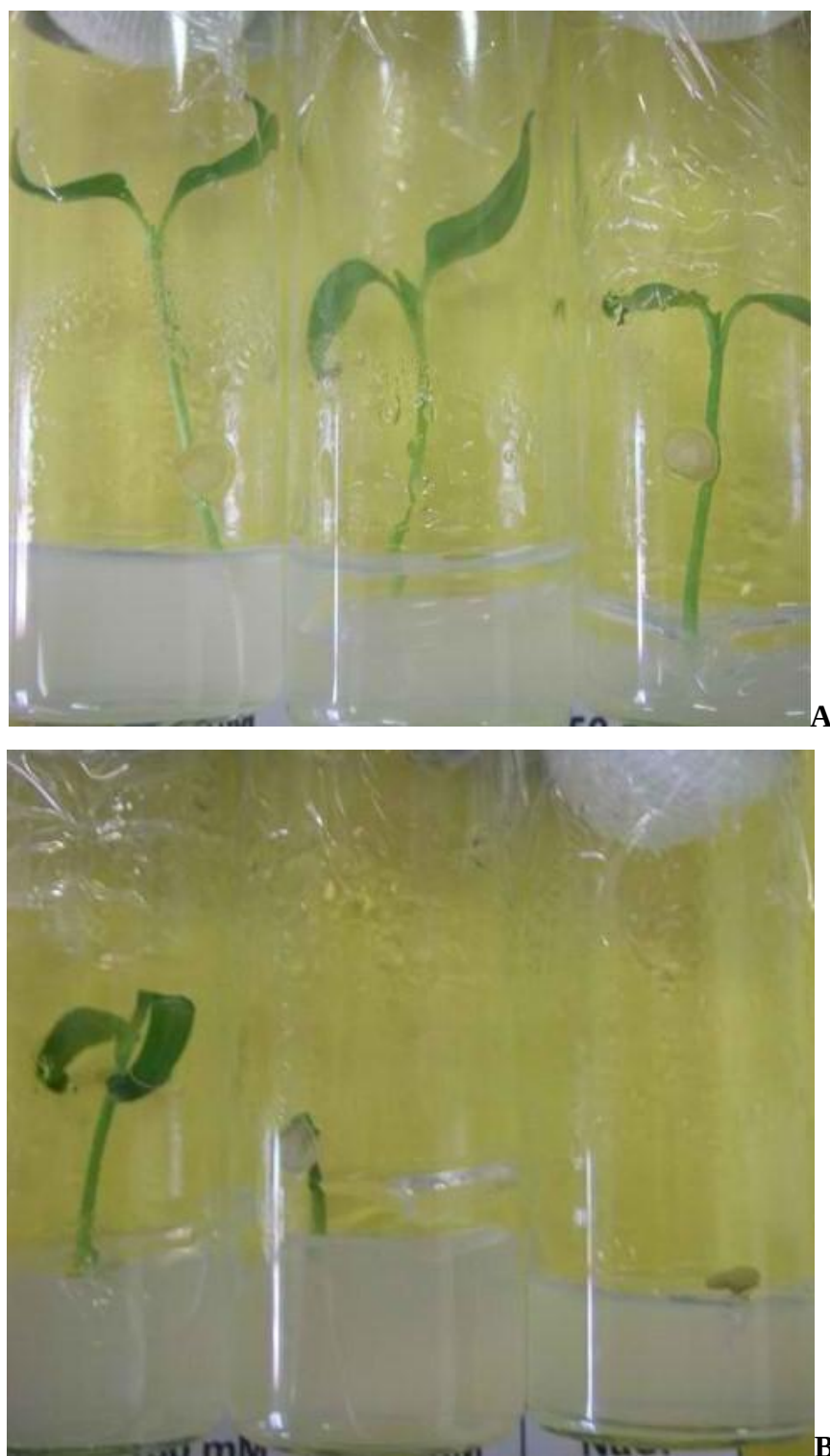


Plate 4.8: Effect of NaCl on Drago plants germinated in substrate with salt. A= 0, 12.5, 25 mM NaCl from the left and B= 100, 150, 200 mM NaCl from the left.



Plate 4.9: Effect of NaCl on Sammy plants transplanted.



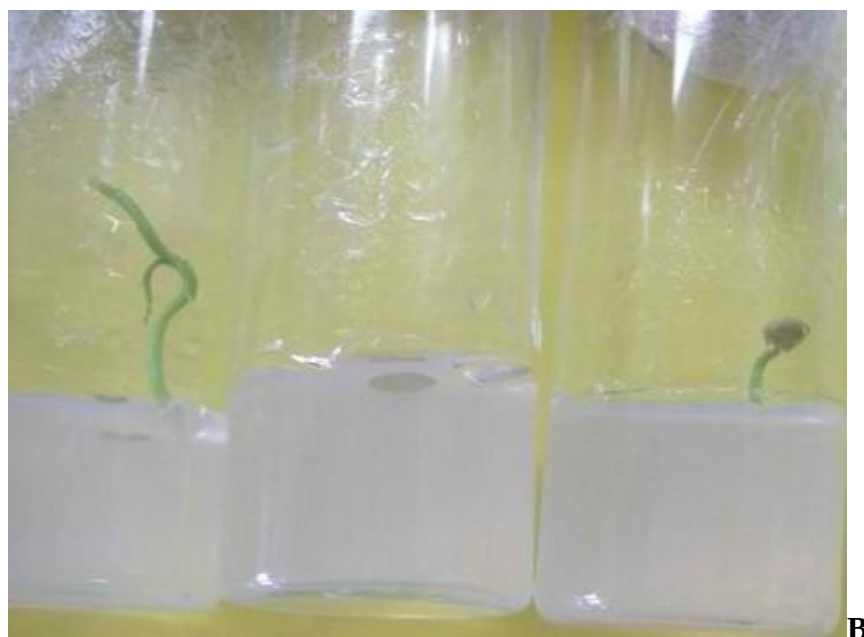
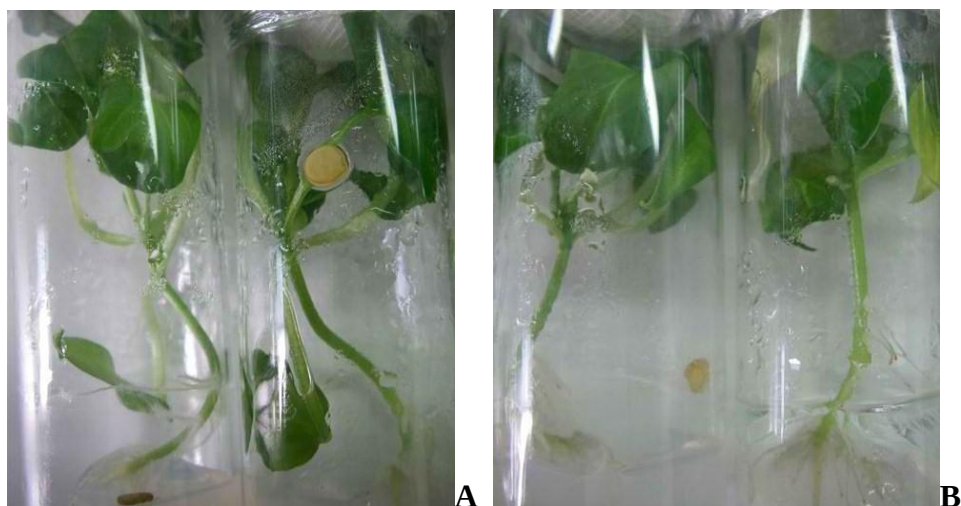


Plate 4.10: Effect of NaCl on Sammy plants germinated in substrate with salt. A= 0, 12.5, 25 mM NaCl from the left and B= 100, 150, 200 mM NaCl from the left.





Plate 4.12: Effect of NaCl in Kappy plants transplanted. A= 0, 12.5, 25, 50, 100 mM NaCl from the left and B= 150, 200 mM NaCl from the left.



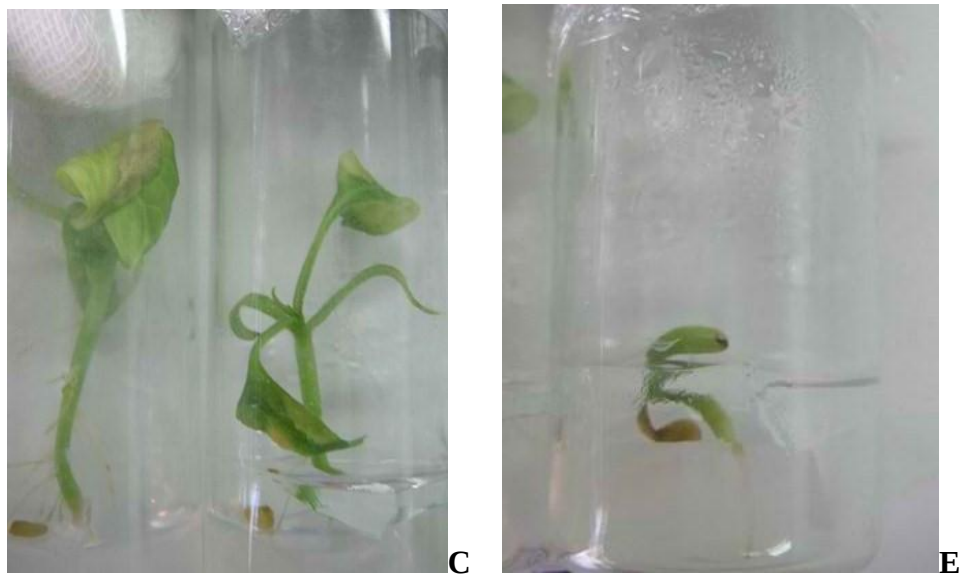


Plate 4.13: Effect of NaCl in Kappy plants germinated in substrate with salt. A= 0, 12.5 mM NaCl from the left, B= 25, 50 mM NaCl from the left, C= 100, 150 mM NaCl from the left and D= 200 mM NaCl from the left.



Plate 4.9: Effect of NaCl on Gracia plants transplanted.

Appendix B

4.1 Hydroponic Culture in Perlite

Figure 4.5: Effect of NaCl on the height of pepper plants after cultured in perlite for 12 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Height (cm) Drago	Between Groups	60,214	6	10,036	3,113	,009
	Within Groups	251,462	78	3,224		
	Total	311,676	84			
Height (cm) Sammy	Between Groups	10,927	6	1,821	,567	,755
	Within Groups	195,837	61	3,210		
	Total	206,765	67			
Height (cm) Kappy	Between Groups	3,366	6	,561	,212	,972
	Within Groups	278,125	105	2,649		
	Total	281,491	111			
Height (cm) Gracia	Between Groups	3,094	6	,516	,129	,993
	Within Groups	420,797	105	4,008		
	Total	423,891	111			

Figure 4.10: Effect of NaCl on the number of leaves per plant of all pepper hybrids cultured in perlite, for 12 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Number of leaves Drago	Between Groups	455,845	6	75,974	3,870	,002
	Within Groups	1531,332	78	19,632		
	Total	1987,176	84			
Number of leaves Sammy	Between Groups	413,775	6	68,963	1,600	,162
	Within Groups	2628,740	61	43,094		
	Total	3042,515	67			
Number of leaves Kappy	Between Groups	205,107	6	34,185	9,463	,000

	Within Groups	379,313	105	3,613		
	Total	584,420	111			
Number of leaves Gracia	Between Groups	72,750	6	12,125	11,647	,000
	Within Groups	109,313	105	1,041		
	Total	182,063	111			

Figure 4.11: Effect of NaCl on the number of fruits per plant of all pepper hybrids cultured in perlite, for 12 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Number of fruits Drago	Between Groups	38,283	6	6,381	2,911	,013
	Within Groups	170,964	78	2,192		
	Total	209,247	84			
Number of fruits Sammy	Between Groups	80,914	6	13,486	2,777	,019
	Within Groups	296,204	61	4,856		
	Total	377,118	67			
Number of fruits Kappy	Between Groups	11,214	6	1,869	,824	,554
	Within Groups	238,063	105	2,267		
	Total	249,277	111			
Number of fruits Gracia	Between Groups	16,732	6	2,789	3,170	,007
	Within Groups	92,375	105	,880		
	Total	109,107	111			

Figure 4.12: Effect of NaCl on the mean fresh weight of shoot (above ground part) of Drago, Sammy, Kappy and Gracia after cultured in perlite for 12 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Fresh weight of above ground part (gr) Drago	Between Groups	241605,758	6	40267,626	46,601	,000
	Within Groups	67398,894	78	864,088		
	Total	309004,652	84			
Fresh weight of above ground (gr)	Between Groups	94771,280	6	15795,213	13,109	,000
	Within Groups	73501,121	61	1204,936		

Sammy part	Total	168272,401	67			
Fresh weight of above ground part (gr) Kappy	Between Groups	36228,247	6	6038,041	17,160	,000
	Within Groups	36946,824	105	351,875		
	Total	73175,071	111			
Fresh weight of above ground part (gr) Gracia	Between Groups	40990,931	6	6831,822	23,058	,000
	Within Groups	31110,958	105	296,295		
	Total	72101,889	111			

Figure 4.13: Effect of NaCl on the mean dry weight of shoot (above ground part) of Drago, Sammy, Kappy and Gracia after cultured in perlite for 12 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Dry weight of above ground part (gr) Drago	Between Groups	3081,017	6	513,503	25,569	,000
	Within Groups	1566,492	78	20,083		
	Total	4647,509	84			
Dry weight of above ground part (gr) Sammy	Between Groups	1628,352	6	271,392	8,901	,000
	Within Groups	1859,904	61	30,490		
	Total	3488,256	67			
Dry weight of above ground part (gr) Kappy	Between Groups	1447,086	6	241,181	8,465	,000
	Within Groups	2991,558	105	28,491		
	Total	4438,643	111			
Dry weight of above ground part (gr) Gracia	Between Groups	585,473	6	97,579	15,569	,000
	Within Groups	658,080	105	6,267		
	Total	1243,553	111			

Figure 4.14: Effect of NaCl on the mean fresh weight of roots per plant of Drago, Sammy, Kappy and Gracia after cultured in perlite for 12 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Fresh weight of roots (gr) Drago	Between Groups	980,116	6	163,353	2,139	,058
	Within Groups	5957,775	78	76,382		
	Total	6937,890	84			

Fresh weight of roots (gr)	Between Groups	3208,023	6	534,670	4,347	,001
Sammy	Within Groups	7502,257	61	122,988		
	Total	10710,280	67			
Fresh weight of roots (gr)	Between Groups	2798,381	6	466,397	15,437	,000
Kappy	Within Groups	3172,394	105	30,213		
	Total	5970,774	111			
Fresh weight of roots (gr)	Between Groups	2245,086	6	374,181	6,432	,000
Gracia	Within Groups	6108,485	105	58,176		
	Total	8353,571	111			

Figure 4.15.: Effect of NaCl on the mean dry weight of roots per plant of Drago, Sammy, Kappy and Gracia after cultured in perlite for 12 weeks..

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Dry weight of roots (gr)	Between Groups	46,469	6	7,745	3,171	,008
Drago	Within Groups	190,493	78	2,442		
	Total	236,962	84			
Dry weight of roots (gr)	Between Groups	66,777	6	11,130	5,141	,000
Sammy	Within Groups	132,069	61	2,165		
	Total	198,846	67			
Dry weight of roots (gr)	Between Groups	171,842	6	28,640	17,895	,000
Kappy	Within Groups	168,051	105	1,600		
	Total	339,893	111			
Dry weight of roots (gr)	Between Groups	146,287	6	24,381	8,637	,000
Gracia	Within Groups	296,414	105	2,823		
	Total	442,701	111			

Figure 4.16: Effect of NaCl on the mean fresh weight of leaves per plant of Drago, Sammy, Kappy and Gracia after cultured in perlite for 12 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Fresh weight	Between	11742,764	6	1957,127	12,397	,000

of leaves (gr)	Groups					
Drago	Within Groups	12314,003	78	157,872		
	Total	24056,767	84			
Fresh weight of leaves (gr)	Between Groups	6868,151	6	1144,692	11,824	,000
Sammy	Within Groups	5905,481	61	96,811		
	Total	12773,632	67			
Fresh weight of leaves (gr)	Between Groups	7436,717	6	1239,453	35,121	,000
Kappy	Within Groups	3705,510	105	35,291		
	Total	11142,227	111			
Fresh weight of leaves (gr)	Between Groups	4860,282	6	810,047	23,950	,000
Gracia	Within Groups	3551,343	105	33,822		
	Total	8411,625	111			

Figure 4.17: Effect of NaCl on the mean dry weight of leaves per plant of Drago, Sammy, Kappy and Gracia after cultured in perlite for 12 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Dry weight of leaves (gr)	Between Groups	229,408	6	38,235	11,087	,000
Drago	Within Groups	268,985	78	3,449		
	Total	498,394	84			
Dry weight of leaves (gr)	Between Groups	106,403	6	17,734	4,032	,002
Sammy	Within Groups	268,306	61	4,398		
	Total	374,710	67			
Dry weight of leaves (gr)	Between Groups	220,946	6	36,824	18,504	,000
Kappy	Within Groups	208,959	105	1,990		
	Total	429,905	111			
Dry weight of leaves (gr)	Between Groups	77,032	6	12,839	10,283	,000
Gracia	Within Groups	131,090	105	1,248		
	Total	208,123	111			

Figure 4.18: Effect of NaCl on the mean fresh weight of fruits per plant of Drago, Sammy, Kappy and Gracia after cultured in perlite for 12 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Fresh weight of fruits (gr) Drago	Between Groups	116963,211	6	19493,869	37,176	,000
	Within Groups	40900,983	78	524,372		
	Total	157864,194	84			
Fresh weight of fruits (gr) Sammy	Between Groups	30462,514	6	5077,086	5,211	,000
	Within Groups	53586,668	55	974,303		
	Total	84049,182	61			
Fresh weight of fruits (gr) Kappy	Between Groups	11554,219	6	1925,703	7,286	,000
	Within Groups	20616,232	78	264,311		
	Total	32170,451	84			
Fresh weight of fruits (gr) Gracia	Between Groups	13811,749	6	2301,958	10,307	,000
	Within Groups	16526,311	74	223,329		
	Total	30338,060	80			

Figure 4.19: Effect of NaCl on the mean dry weight of fruits per plant of Drago, Sammy, Kappy and Gracia after cultured in perlite for 12 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Dry weight of fruits (gr) Drago	Between Groups	1061,740	6	176,957	15,208	,000
	Within Groups	907,564	78	11,635		
	Total	1969,304	84			
Dry weight of fruits (gr) Sammy	Between Groups	484,105	6	80,684	4,128	,002
	Within Groups	1074,931	55	19,544		
	Total	1559,035	61			
Dry weight of fruits (gr) Kappy	Between Groups	778,991	6	129,832	5,319	,000
	Within Groups	1903,790	78	24,408		
	Total	2682,780	84			
Dry weight of fruits (gr) Gracia	Between Groups	192,692	6	32,115	11,971	,000
	Within Groups	198,519	74	2,683		
	Total	391,211	80			

Figure 4.20: Effect of NaCl on the chlorophyll content of Drago pepper plants cultured in perlite. Measurements taken at the first, and tenth week of culture.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Chlorophyll content 1 st week	Between Groups	111,112	6	18,519	2,465	,077
	Within Groups	105,180	14	7,513		
	Total	216,292	20			
Chlorophyll content 10 th week	Between Groups	1065,139	6	177,523	10,033	,000
	Within Groups	371,570	21	17,694		
	Total	1436,709	27			

Figure 4.21: Effect of NaCl on the chlorophyll fluorescence of Drago pepper plants cultured in perlite. Measurements taken at the first, and tenth week of culture.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Chlorophyll fluorescence 1 st week	Between Groups	,000	6	,000	,255	,951
	Within Groups	,002	21	,000		
	Total	,002	27			
Chlorophyll fluorescence 10 th week	Between Groups	,070	6	,012	6,195	,001
	Within Groups	,039	21	,002		
	Total	,109	27			

Figure 4.22: Effect of NaCl on the chlorophyll content of Sammy pepper plants cultured in perlite. Measurements taken at the first and tenth week of culture.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Chlorophyll content 1 st week	Between Groups	52,703	6	8,784	1,764	,179
	Within Groups	69,727	14	4,980		
	Total	122,430	20			

Chlorophyll content 10 th week	Between Groups	823,115	6	137,186	5,517	,003
	Within Groups	397,822	16	24,864		
	Total	1220,937	22			

Figure 4.23: Effect of NaCl on the chlorophyll fluorescence of Sammy pepper plants cultured in perlite. Measurements taken at the first and tenth week of culture.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Chlorophyll fluorescence 1 st week	Between Groups	,000	6	,000	2,325	,101
	Within Groups	,000	12	,000		
	Total	,001	18			
Chlorophyll fluorescence 10 th week	Between Groups	,041	6	,007	5,141	,004
	Within Groups	,022	17	,001		
	Total	,063	23			

Figure 4.24: Effect of NaCl on the chlorophyll content of Kappy pepper plants cultured in perlite. Measurements taken at the first, seventh and tenth week of culture.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Chlorophyll content 1 st week	Between Groups	72,349	6	12,058	,391	,877
	Within Groups	648,358	21	30,874		
	Total	720,707	27			
Chlorophyll content 7 th week	Between Groups	1520,559	6	253,427	21,422	,000
	Within Groups	248,435	21	11,830		
	Total	1768,994	27			
Chlorophyll content 10 th week	Between Groups	15314,164	6	2552,361	192,460	,000
	Within Groups	278,498	21	13,262		
	Total	15592,661	27			

Figure 4.25: Effect of NaCl on the chlorophyll fluorescence of Kappy pepper plants cultured in perlite. Measurements taken at the first, seventh and tenth week of culture.

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
Chlorophyll fluorescence 1 st week	Between Groups	,004	6	,001	,898	,514
	Within Groups	,016	21	,001		
	Total	,020	27			
Chlorophyll fluorescence 7 th week	Between Groups	,106	6	,018	23,920	,000
	Within Groups	,015	21	,001		
	Total	,121	27			
Chlorophyll fluorescence 10 th week	Between Groups	1,519	6	,253	27,277	,000
	Within Groups	,195	21	,009		
	Total	1,714	27			

Figure 4.26: Effect of NaCl on the chlorophyll content of Gracia pepper plants cultured in perlite. Measurements taken at the first and tenth week of culture.

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
Chlorophyll content 1 st week	Between Groups	804,094	6	134,016	10,160	,000
	Within Groups	276,988	21	13,190		
	Total	1081,081	27			
Chlorophyll content 10 th week	Between Groups	12489,634	6	2081,606	697,801	,000
	Within Groups	62,645	21	2,983		
	Total	12552,279	27			

Figure 4.27: Effect of NaCl on the chlorophyll fluorescence of Gracia pepper plants cultured in perlite. Measurements taken at the first and tenth week of culture.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Chlorophyll fluorescence 1 st week	Between Groups	,036	6	,006	11,541	,000
	Within Groups	,011	21	,001		
	Total	,047	27			
Chlorophyll fluorescence 10 th week	Between Groups	1,594	6	,266	376,504	,000
	Within Groups	,015	21	,001		
	Total	1,609	27			

Figure 4.28: Effect of NaCl on the photosynthetic rate of pepper plants cultured in perlite at the 10th week of culture .

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Photosynthetic rate Drago	Between Groups	230,038	6	38,340	8,492	,001
	Within Groups	63,211	14	4,515		
	Total	293,248	20			
Photosynthetic rate Sammy	Between Groups	27,168	6	4,528	2,220	,103
	Within Groups	28,551	14	2,039		
	Total	55,719	20			
Photosynthetic rate Kappy	Between Groups	52,415	6	8,736	,557	,759
	Within Groups	282,503	18	15,695		
	Total	334,918	24			
Photosynthetic rate Gracia	Between Groups	48,223	6	8,037	,725	,638
	Within Groups	144,125	13	11,087		
	Total	192,347	19			

Figure 4.29: Effect of NaCl on the stomatal conductance of pepper plants cultured in perlite at the 10th week of culture.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Stomatal conductance Drago	Between Groups	,263	6	,044	2,329	,090
	Within Groups	,264	14	,019		
	Total	,527	20			
Stomatal conductance Sammy	Between Groups	,229	6	,038	6,374	,002
	Within Groups	,084	14	,006		
	Total	,313	20			
Stomatal conductance Kappy	Between Groups	,055	6	,009	,191	,975
	Within Groups	,868	18	,048		
	Total	,923	24			
Stomatal conductance Gracia	Between Groups	,022	6	,004	1,111	,407
	Within Groups	,043	13	,003		
	Total	,065	19			

Figure 4.30: Effect of NaCl on the intercellular CO₂ concentration (Ci) of pepper plants cultured in perlite at the 10th week of culture.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Intercellular CO ₂ concentration (Ci) Drago	Between Groups	42648,857	6	7108,143	3,633	,022
	Within Groups	27389,608	14	1956,401		
	Total	70038,464	20			
Intercellular CO ₂ concentration (Ci) Sammy	Between Groups	15054,081	6	2509,013	9,504	,000
	Within Groups	3695,799	14	263,986		
	Total	18749,880	20			
Intercellular CO ₂	Between Groups	13704,699	6	2284,116	,880	,529

concentration (Ci) Kappy	Within Groups	46735,409	18	2596,412		
	Total	60440,108	24			
Intercellular CO ₂ concentration (Ci) Gracia	Between Groups	41506,710	6	6917,785	1,013	,458
	Within Groups	88737,878	13	6825,991		
	Total	130244,588	19			

Figure 4.31: Effect of NaCl on the transpiration rate of pepper plants cultured in perlite at the 10th week of culture.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Transpiration Drago	Between Groups	35,374	6	5,896	3,622	,022
	Within Groups	22,791	14	1,628		
	Total	58,166	20			
Transpiration Sammy	Between Groups	35,167	6	5,861	8,072	,001
	Within Groups	10,166	14	,726		
	Total	45,333	20			
Transpiration Kappy	Between Groups	2,201	6	,367	,204	,971
	Within Groups	32,339	18	1,797		
	Total	34,541	24			
Transpiration Gracia	Between Groups	3,106	6	,518	1,061	,433
	Within Groups	6,345	13	,488		
	Total	9,451	19			

Table 4.6: Effect of NaCl on the protein content of leaves of pepper plants cultured in perlite for 12 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Proteins Drago	Between Groups	1376900,571	6	229483,429	16,817	,000
	Within Groups	191040,000	14	13645,714		

	Total	1567940,571	20			
Proteins Sammy	Between Groups	387362,133	6	64560,356	1,010	,460
	Within Groups	830730,667	13	63902,359		
	Total	1218092,800	19			
Proteins Kappy	Between Groups	3147924,800	6	524654,133	40,496	,000
	Within Groups	168424,000	13	12955,692		
	Total	3316348,800	19			
Proteins Gracia	Between Groups	6610910,133	6	1101818,356	19,445	,000
	Within Groups	736618,667	13	56662,974		
	Total	7347528,800	19			

Table 4.7: Effect of NaCl on total activity of APX and GPX in leaves of pepper plants cultured in perlite for 12 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
APX mg (FW) Drago	Between Groups	,002	6	,000	1,462	,247
	Within Groups	,005	18	,000		
	Total	,007	24			
GPX mg (FW) Drago	Between Groups	,553	6	,092	2,156	,097
	Within Groups	,770	18	,043		
	Total	1,323	24			
APX mg (FW) Sammy	Between Groups	,001	6	,000	,706	,650
	Within Groups	,003	15	,000		
	Total	,004	21			
GPX mg (FW) Sammy	Between Groups	2,008	6	,335	4,131	,012
	Within Groups	1,215	15	,081		
	Total	3,223	21			
APX mg (FW) Kappy	Between Groups	,011	6	,002	,984	,461

	Within Groups	,041	21	,002		
	Total	,052	27			
GPX mg (FW) Kappy	Between Groups	3,785	6	,631	8,272	,000
	Within Groups	1,373	18	,076		
	Total	5,158	24			
APX mg (FW) Gracia	Between Groups	,006	6	,001	,764	,606
	Within Groups	,027	21	,001		
	Total	,033	27			
GPX mg (FW) Gracia	Between Groups	2,304	6	,384	5,501	,003
	Within Groups	1,186	17	,070		
	Total	3,490	23			

Table 4.8: Effect of NaCl on total activity of POD and SOD in leaves of pepper plants cultured in perlite for 12 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
POD mg (FW) Drago	Between Groups	,000	6	,000	,351	,901
	Within Groups	,001	21	,000		
	Total	,001	27			
SOD mg (FW) Drago	Between Groups	2,343	6	,390	2,803	,044
	Within Groups	2,368	17	,139		
	Total	4,710	23			
POD mg (FW) Sammy	Between Groups	,000	6	,000	5,973	,003
	Within Groups	,000	14	,000		
	Total	,000	20			
SOD mg (FW) Sammy	Between Groups	1,578	6	,263	,974	,467

	Within Groups	5,670	21	,270		
	Total	7,248	27			
POD mg (FW) Kappy	Between Groups	,001	6	,000	5,597	,002
	Within Groups	,000	17	,000		
	Total	,001	23			
SOD mg (FW) Kappy	Between Groups	39,105	6	6,517	4,559	,006
	Within Groups	25,733	18	1,430		
	Total	64,838	24			
POD mg (FW) Gracia	Between Groups	,000	6	,000	,898	,518
	Within Groups	,000	17	,000		
	Total	,000	23			
SOD mg (FW) Gracia	Between Groups	3,395	6	,566	1,185	,357
	Within Groups	8,592	18	,477		
	Total	11,987	24			

4.2 Culture In Soil

Figure 4.35: Effect of NaCl on the height of pepper plants after cultured in soil for 8 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Height (cm) Drago	Between Groups	122,786	6	20,464	10,545	,000
	Within Groups	149,438	77	1,941		
	Total	272,223	83			
Height (cm) Sammy	Between Groups	394,071	6	65,679	24,070	,000
	Within Groups	210,104	77	2,729		
	Total	604,176	83			
Height (cm)	Between Groups	255,363	6	42,561	13,996	,000

Kappy	Within Groups	234,146	77	3,041		
	Total	489,509	83			

Figure 4.39: Effect of NaCl on the number of leaves of pepper hybrids after cultured in soil for 9 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Number of leaves Drago	Between Groups	408,952	6	68,159	24,486	,000
	Within Groups	214,333	77	2,784		
	Total	623,286	83			
Number of leaves Sammy	Between Groups	1250,119	6	208,353	27,857	,000
	Within Groups	575,917	77	7,479		
	Total	1826,036	83			
Number of leaves Kappy	Between Groups	1127,119	6	187,853	21,411	,000
	Within Groups	675,583	77	8,774		
	Total	1802,702	83			

Figure 4.40: Effect of NaCl on the number of flowers of pepper hybrids after cultured in soil for 8 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Number of flowers Drago	Between Groups	62,119	6	10,353	9,893	,000
	Within Groups	80,583	77	1,047		
	Total	142,702	83			
Number of flowers Sammy	Between Groups	170,238	6	28,373	13,762	,000
	Within Groups	158,750	77	2,062		
	Total	328,988	83			
Number of flowers Kappy	Between Groups	250,071	6	41,679	19,430	,000
	Within Groups	165,167	77	2,145		
	Total	415,238	83			

Figure 4.41: Effect of NaCl on the number of fruits of pepper hybrids after cultured in soil for 8 weeks.

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
Number of fruits Drago	Between Groups	10,119	6	1,687	7,831	,000
	Within Groups	16,583	77	,215		
	Total	26,702	83			
Number of fruits Sammy	Between Groups	24,452	6	4,075	6,083	,000
	Within Groups	51,583	77	,670		
	Total	76,036	83			
Number of fruits Kappy	Between Groups	20,619	6	3,437	5,503	,000
	Within Groups	48,083	77	,624		
	Total	68,702	83			

Figure 4.42: Effect of NaCl on the fresh weight of shoot of pepper hybrids after cultured in soil for 8 weeks.

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
Fresh weight of above ground part (gr) Drago	Between Groups	2826,752	6	471,125	57,828	,000
	Within Groups	627,315	77	8,147		
	Total	3454,067	83			
Fresh weight of above ground part (gr) Sammy	Between Groups	5999,800	6	999,967	77,065	,000
	Within Groups	999,121	77	12,976		
	Total	6998,920	83			
Fresh weight of above ground part (gr) Kappy	Between Groups	2127,701	6	354,617	37,890	,000
	Within Groups	720,657	77	9,359		
	Total	2848,358	83			

Figure 4.43: Effect of NaCl on the dry weight of above ground part of pepper hybrids after cultured in soil for 8 weeks.

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
Dry weight of above ground part (gr) Drago	Between Groups	61,225	6	10,204	55,663	,000
	Within Groups	14,116	77	,183		
	Total	75,341	83			
Dry weight of above ground part (gr) Sammy	Between Groups	87,090	6	14,515	52,927	,000
	Within Groups	21,117	77	,274		
	Total	108,206	83			
Dry weight of above ground part (gr) Kappy	Between Groups	1471,153	6	245,192	71,080	,000
	Within Groups	265,614	77	3,450		
	Total	1736,767	83			

Figure 4.44: Effect of NaCl on the fresh weight of leaves of pepper hybrids after cultured in soil for 8 weeks.

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
Fresh weight of leaves (gr) Drago	Between Groups	787,791	6	131,298	83,261	,000
	Within Groups	121,425	77	1,577		
	Total	909,216	83			
Fresh weight of leaves (gr) Sammy	Between Groups	1342,776	6	223,796	53,144	,000
	Within Groups	324,257	77	4,211		
	Total	1667,032	83			
Fresh weight of leaves (gr) Kappy	Between Groups	153,701	6	25,617	15,058	,000
	Within Groups	130,996	77	1,701		
	Total	284,697	83			

Figure 4.45: Effect of NaCl on the dry weight of leaves of pepper hybrids after cultured in soil for 8 weeks.

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.

Dry weight of leaves (gr) Drago	Between Groups	7,515	6	1,253	34,243	,000
	Within Groups	2,816	77	,037		
	Total	10,332	83			
Dry weight of leaves (gr) Sammy	Between Groups	14,052	6	2,342	17,973	,000
	Within Groups	10,034	77	,130		
	Total	24,086	83			
Dry weight of leaves (gr) Kappy	Between Groups	233,753	6	38,959	22,838	,000
	Within Groups	131,353	77	1,706		
	Total	365,107	83			

Figure 4.46 Effect of NaCl on the fruit fresh weight of pepper hybrids after cultured in soil for 8 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Fresh weight of fruits (gr) Drago	Between Groups	672,328	6	112,055	25,668	,000
	Within Groups	336,152	77	4,366		
	Total	1008,480	83			
Fresh weight of fruits (gr) Sammy	Between Groups	1098,196	6	183,033	27,821	,000
	Within Groups	506,575	77	6,579		
	Total	1604,771	83			
Fresh weight of fruits (gr) Kappy	Between Groups	1228,090	6	204,682	27,892	,000
	Within Groups	565,052	77	7,338		
	Total	1793,141	83			

Figure 4.47: Effect of NaCl on the fruit dry weight of pepper hybrids after cultured in soil for 8 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Dry weight of fruits (gr) Drago	Between Groups	18,699	6	3,116	31,941	,000
	Within Groups	7,513	77	,098		
	Total	26,211	83			
Dry weight of	Between Groups	19,430	6	3,238	49,636	,000

fruits (gr) Sammy	Within Groups	5,024	77	,065		
	Total	24,454	83			
Dry weight of fruits (gr) Kappy	Between Groups	456,642	6	76,107	50,878	,000
	Within Groups	115,183	77	1,496		
	Total	571,824	83			

Figure 4.48: Effect of NaCl on the chlorophyll content of Drago pepper plants cultured in soil for 8 weeks .

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Chlorophyll content 1 st week	Between Groups	37,533	6	6,255	1,001	,440
	Within Groups	218,655	35	6,247		
	Total	256,188	41			
Chlorophyll content 8 th week	Between Groups	2104,733	6	350,789	12,962	,000
	Within Groups	947,220	35	27,063		
	Total	3051,953	41			

Figure 4.49: Effect of NaCl on the chlorophyll content of Drago pepper plants cultured in soil for 8 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Chlorophyll fluorescence 1 st week	Between Groups	,004	6	,001	1,060	,405
	Within Groups	,021	35	,001		
	Total	,025	41			
Chlorophyll fluorescence 8 th week	Between Groups	,597	6	,099	12,361	,000
	Within Groups	,282	35	,008		
	Total	,878	41			

Figure 4.50: Effect of NaCl on the chlorophyll content of Sammy pepper plants cultured in soil for 8 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Chlorophyll content 1 st week	Between Groups	32,472	6	5,412	,459	,833
	Within Groups	412,432	35	11,784		
	Total	444,904	41			
Chlorophyll content 8 th week	Between Groups	1321,980	6	220,330	9,932	,000
	Within Groups	709,856	32	22,183		
	Total	2031,836	38			

Figure 4.51: Effect of NaCl on the chlorophyll fluorescence of Sammy pepper plants cultured in soil for 8 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Chlorophyll fluorescence 1 st week	Between Groups	,001	6	,000	,630	,705
	Within Groups	,013	35	,000		
	Total	,014	41			
Chlorophyll fluorescence 8 th week	Between Groups	,629	6	,105	4,740	,001
	Within Groups	,774	35	,022		
	Total	1,402	41			

Figure 4.52: Effect of NaCl on the chlorophyll content of Kappy pepper plants cultured in soil for 8 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Chlorophyll content 1 st week	Between Groups	132,849	6	22,142	1,025	,426
	Within Groups	756,195	35	21,606		
	Total	889,044	41			
Chlorophyll content	Between Groups	2235,705	6	372,617	14,802	,000

8 th week	Within Groups	881,060	35	25,173		
	Total	3116,765	41			

Figure 4.53: Effect of NaCl on the chlorophyll fluorescence of Kappy pepper plants cultured in soil for 8 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Chlorophyll fluorescence 1 st week	Between Groups	,006	6	,001	1,837	,120
	Within Groups	,018	35	,001		
	Total	,023	41			
Chlorophyll fluorescence 8 th week	Between Groups	1,105	6	,184	15,542	,000
	Within Groups	,415	35	,012		
	Total	1,519	41			

Figure 4.54: Effect of NaCl on the photosynthetic rate of leaves of Drago, Sammy and Kappy cultured in soil for 8 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Photosynthetic rate Drago	Between Groups	164,184	6	27,364	3,478	,018
	Within Groups	141,601	18	7,867		
	Total	305,785	24			
Photosynthetic rate Sammy	Between Groups	30,247	6	5,041	,331	,912
	Within Groups	273,762	18	15,209		
	Total	304,009	24			
Photosynthetic rate Kappy	Between Groups	29,583	6	4,931	,387	,877
	Within Groups	203,836	16	12,740		
	Total	233,420	22			

Figure 4.55: Effect of NaCl on the stomatal conductance of leaves of Drago, Sammy and Kappy cultured in soil for 8 weeks.

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
Stomatal conductance Drago	Between Groups	,636	6	,106	4,485	,006
	Within Groups	,425	18	,024		
	Total	1,061	24			
Stomatal conductance Sammy	Between Groups	,056	6	,009	,734	,629
	Within Groups	,230	18	,013		
	Total	,286	24			
Stomatal conductance Kappy	Between Groups	,029	6	,005	,416	,857
	Within Groups	,184	16	,011		
	Total	,213	22			

Figure 4.56: Effect of NaCl on the intercellular CO₂ concentration (Ci) of leaves of Drago, Sammy and Kappy cultured in soil for 8 weeks.

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
Intercellular CO ₂ concentration (Ci) Drago	Between Groups	3271,614	6	545,269	1,479	,241
	Within Groups	6637,163	18	368,731		
	Total	9908,777	24			
Intercellular CO ₂ concentration (Ci) Sammy	Between Groups	490,116	6	81,686	,217	,966
	Within Groups	6782,681	18	376,816		
	Total	7272,797	24			
Intercellular CO ₂ concentration (Ci) Kappy	Between Groups	440,141	6	73,357	,430	,848
	Within Groups	2729,008	16	170,563		
	Total	3169,148	22			

Figure 4.57: Effect of NaCl on the transpiration rate of leaves of Drago, Sammy and Kappy cultured in soil for 8 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Transpiration Drago	Between Groups	27,396	6	4,566	6,010	,001
	Within Groups	13,676	18	,760		
	Total	41,072	24			
Transpiration Sammy	Between Groups	6,627	6	1,104	,685	,664
	Within Groups	29,012	18	1,612		
	Total	35,639	24			
Transpiration Kappy	Between Groups	2,873	6	,479	,363	,892
	Within Groups	21,094	16	1,318		
	Total	23,966	22			

Table 4.12: Effect of NaCl on the protein content of leaves of pepper plants cultured in soil for 8 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Proteins Drago	Between Groups	670631,619	6	111771,937	6,270	,002
	Within Groups	249589,333	14	17827,810		
	Total	920220,952	20			
Proteins Sammy	Between Groups	1328324,571	6	221387,429	12,699	,000
	Within Groups	244074,667	14	17433,905		
	Total	1572399,238	20			
Proteins Kappy	Between Groups	1335996,444	6	222666,074	5,584	,007
	Within Groups	438632,000	11	39875,636		
	Total	1774628,444	17			

Table 4.13: Effect of NaCl on total activity of APX and GPX in leaves of pepper hybrids cultured in soil for 8 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
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APX mg (FW) Drago	Between Groups	,001	6	,000	,231	,958
	Within Groups	,005	11	,000		
	Total	,006	17			
GPX mg (FW) Drago	Between Groups	,050	6	,008	,988	,486
	Within Groups	,076	9	,008		
	Total	,127	15			
APX mg (FW) Sammy	Between Groups	,000	6	,000	,156	,983
	Within Groups	,002	9	,000		
	Total	,002	15			
GPX mg (FW) Sammy	Between Groups	,021	6	,003	1,977	,163
	Within Groups	,018	10	,002		
	Total	,039	16			
APX mg (FW) Kappy	Between Groups	,007	6	,001	2,533	,071
	Within Groups	,006	14	,000		
	Total	,013	20			
GPX mg (FW) Kappy	Between Groups	,116	6	,019	,536	,772
	Within Groups	,434	12	,036		
	Total	,550	18			

Table 4.14: Effect of NaCl on total activity of POD in leaves of pepper hybrids cultured in soil for 8 weeks.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
POD mg (FW) Drago	Between Groups	,000	6	,000	,451	,832

	Within Groups	,000	13	,000		
	Total	,000	19			
POD mg (FW) Sammy	Between Groups	,000	6	,000	2,015	,142
	Within Groups	,000	12	,000		
	Total	,000	18			
POD mg (FW) Kappy	Between Groups	,000	6	,000	,576	,744
	Within Groups	,000	14	,000		
	Total	,000	20			

4.3. Experiments in vitro

Figure 4.62: Effect of NaCl on the height of pepper hybrids after in vitro culture in saline substrate. * plants transplanted in substrate with NaCl and ** plants germinated in substrate with NaCl.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Height (cm) Drago *	Between Groups	155,324	6	25,887	13,526	,000
	Within Groups	187,567	98	1,914		
	Total	342,890	104			
Height (cm) Drago **	Between Groups	721,114	6	120,186	17,435	,000
	Within Groups	675,560	98	6,893		
	Total	1396,674	104			
Height (cm) Sammy *	Between Groups	145,570	6	24,262	20,043	,000
	Within Groups	112,577	93	1,211		
	Total	258,148	99			
Height (cm) Sammy **	Between Groups	1000,604	6	166,767	14,552	,000
	Within Groups	1077,227	94	11,460		
	Total	2077,832	100			
Height (cm) Kappy *	Between Groups	93,220	6	15,537	6,516	,000
	Within Groups	214,600	90	2,384		

	Total	307,820	96			
Height (cm) Kappy **	Between Groups	86,481	6	14,413	6,100	,000
	Within Groups	231,567	98	2,363		
	Total	318,048	104			
Height (cm) Gracia *	Between Groups	100,769	6	16,795	13,061	,000
	Within Groups	124,729	97	1,286		
	Total	225,498	103			
Height (cm) Gracia **	Between Groups	448,124	6	74,687	15,644	,000
	Within Groups	467,867	98	4,774		
	Total	915,990	104			

Figure 4.63: Effect of NaCl on the number of leaves of pepper plants after in vitro culture in saline substrate. * plants transplanted in substrate with NaCl and ** plants germinated in substrate with NaCl.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Number of leaves Drago *	Between Groups	326,895	6	54,483	35,344	,000
	Within Groups	151,067	98	1,541		
	Total	477,962	104			
Number of leaves Drago **	Between Groups	211,731	6	35,288	36,554	,000
	Within Groups	92,677	96	,965		
	Total	304,408	102			
Number of leaves Sammy *	Between Groups	571,205	6	95,201	19,860	,000
	Within Groups	445,795	93	4,793		
	Total	1017,000	99			
Number of leaves Sammy **	Between Groups	294,504	6	49,084	41,133	,000
	Within Groups	112,170	94	1,193		
	Total	406,673	100			

Figure 4.64: Effect of NaCl on the number of leaves of hybrids Kappy and Gracia pepper plants after in vitro culture in saline substrate. * plants transplanted in substrate with NaCl and ** plants germinated in substrate with NaCl.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Number of leaves Kappy *	Between Groups	414,629	6	69,105	12,730	,000
	Within Groups	532,000	98	5,429		
	Total	946,629	104			
Number of leaves Kappy **	Between Groups	324,324	6	54,054	9,186	,000
	Within Groups	576,667	98	5,884		
	Total	900,990	104			
Number of leaves Gracia *	Between Groups	619,230	6	103,205	27,532	,000
	Within Groups	363,606	97	3,749		
	Total	982,837	103			
Number of leaves Gracia **	Between Groups	181,467	6	30,244	26,030	,000
	Within Groups	113,867	98	1,162		
	Total	295,333	104			

Table 4.15: Total plantlet weight of four pepper hybrids cultured in vitro either transplanted on substrates containing NaCl (0 – 200 mM), or germinated on them.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Total planet weight (gr) Drago transplanted	Between Groups	1,450	6	,242	9,083	,000
	Within Groups	2,608	98	,027		
	Total	4,058	104			
Total planet weight (gr) Drago germinated in salt	Between Groups	1,227	6	,205	34,218	,000
	Within Groups	,586	98	,006		
	Total	1,813	104			
Total planet weight (gr) Sammy transplanted	Between Groups	136.914	6	22.819	17.792	.000
	Within Groups	119.278	93	1.283		
	Total	256.192	99			
Total planet	Between Groups	,742	6	,124	31,403	,000

weight (gr) Sammy germinated in salt	Within Groups	,370	94	,004		
	Total	1,112	100			
Tota lplanet weight (gr) Kappy transplanted	Between Groups	1,262	6	,210	6,022	,000
	Within Groups	3,424	98	,035		
	Total	4,687	104			
Total planet weight (gr) Kappy germinated in salt	Between Groups	1,709	6	,285	7,311	,000
	Within Groups	3,819	98	,039		
	Total	5,528	104			
Total planet weight (gr) Gracia transplanted	Between Groups	52,239	6	8,707	21,033	,000
	Within Groups	40,153	97	,414		
	Total	92,392	103			
Tota lplanet weight (gr) Gracia germinated in salt	Between Groups	,441	6	,073	28,685	,000
	Within Groups	,251	98	,003		
	Total	,692	104			

Figure 4.65: Effect of NaCl on mean weight of the roots and the aerial part of Drago plants after in vitro culture in saline substrate. * plants transplanted in substrate with NaCl and ** plants germinated in substrate with NaCl.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Weight of above ground part (gr) Drago *	Between Groups	1,045	6	,174	8,072	,000
	Within Groups	2,114	98	,022		
	Total	3,159	104			
Weight of roots (gr) Drago *	Between Groups	,063	6	,011	8,082	,000
	Within Groups	,128	98	,001		
	Total	,192	104			
Weight of above ground part (gr)	Between Groups	,784	6	,131	25,406	,000
	Within Groups	,504	98	,005		

) Drago **	Total	1,288	104			
Weight of roots (gr) Drago **	Between Groups	,068	6	,011	21,448	,000
	Within Groups	,052	98	,001		
	Total	,120	104			

Figure 4.66: Effect of NaCl on mean weight of the roots and the aerial part of Sammy plants after in vitro culture in saline substrate. * plants transplanted in substrate with NaCl and ** plants germinated in substrate with NaCl.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Weight of above ground part (gr) Sammy *	Between Groups	46,144	6	7,691	18,458	,000
	Within Groups	38,750	93	,417		
	Total	84,894	99			
Weight of roots (gr) Sammy *	Between Groups	24,654	6	4,109	16,104	,000
	Within Groups	23,729	93	,255		
	Total	48,383	99			
Weight of above ground part (gr) Sammy **	Between Groups	,460	6	,077	27,984	,000
	Within Groups	,258	94	,003		
	Total	,718	100			
Weight of roots (gr) Sammy **	Between Groups	,057	6	,009	14,647	,000
	Within Groups	,060	94	,001		
	Total	,117	100			

Figure 4.67: Effect of NaCl on mean weight of the roots and the aerial part of Kappy plants after in vitro culture in saline substrate. * plants transplanted in substrate with NaCl and ** plants germinated in substrate with NaCl.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Weight of above ground part (gr)	Between Groups	,948	6	,158	6,847	,000
	Within Groups	2,261	98	,023		

Kappy *	Total	3,209	104			
Weight of roots (gr) Kappy *	Between Groups	,037	6	,006	2,638	,021
	Within Groups	,231	98	,002		
	Total	,268	104			
Weight of above ground part (gr) Kappy **	Between Groups	1,134	6	,189	6,639	,000
	Within Groups	2,791	98	,028		
	Total	3,925	104			
Weight of roots (gr) Kappy **	Between Groups	,072	6	,012	7,539	,000
	Within Groups	,157	98	,002		
	Total	,229	104			

Figure 4.68: Effect of NaCl on mean weight of the roots and the aerial part of Gracia plants after in vitro culture in saline substrate. * plants transplanted in substrate with NaCl and ** plants germinated in substrate with NaCl.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Weight of above ground part (gr) Gracia *	Between Groups	23,402	6	3,900	24,670	,000
	Within Groups	15,336	97	,158		
	Total	38,738	103			
Weight of roots (gr) Gracia *	Between Groups	6,386	6	1,064	13,872	,000
	Within Groups	7,443	97	,077		
	Total	13,829	103			
Weight of above ground part (gr) Gracia **	Between Groups	,270	6	,045	20,545	,000
	Within Groups	,215	98	,002		
	Total	,485	104			
Weight of roots (gr) Gracia **	Between Groups	,026	6	,004	12,443	,000
	Within Groups	,034	98	,000		
	Total	,060	104			

Table 4.17: Effect of NaCl on the protein content of pepper plantletss at the end of salinity stress in vitro .Plantlets either transplanted or germinated on substrates with NaCl.

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Proteins Drago transplanted	Between Groups	263006,549	6	43834,425	2,939	,064
	Within Groups	149139,333	10	14913,933		
	Total	412145,882	16			
Proteins Drago germinated in salt	Between Groups	214678,902	6	35779,817	,934	,511
	Within Groups	382949,333	10	38294,933		
	Total	597628,235	16			
Proteins Sammy transplanted	Between Groups	236264,533	6	39377,422	,592	,732
	Within Groups	864394,667	13	66491,897		
	Total	1100659,200	19			
Proteins Sammy germinated in salt	Between Groups	162068,667	6	27011,444	11,283	,001
	Within Groups	23939,333	10	2393,933		
	Total	186008,000	16			
Proteins Kappy transplanted	Between Groups	591936,800	6	98656,133	,733	,632
	Within Groups	1748520,000	13	134501,538		
	Total	2340456,800	19			
Proteins Kappy germinated in salt	Between Groups	363914,133	6	60652,356	,542	,768
	Within Groups	1454866,667	13	111912,821		
	Total	1818780,800	19			
Proteins Gracia transplanted	Between Groups	1024700,533	6	170783,422	17,120	,000
	Within Groups	129682,667	13	9975,590		
	Total	1154383,200	19			
Proteins Gracia	Between Groups	373102,467	6	62183,744	7,596	,001

germinated in salt	Within Groups	106429,333	13	8186,872		
	Total	479531,800	19			