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Salt Sensitivity of Rice, Maize, Sugar Beet, and Cotton During Germination and Early Vegetative Growth

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To my Family,
especially my wife Angela
and my sons Daniel and Alexander.

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1 General Introduction

1.1 Introduction

Throughout the history of mankind, salinity has been a relevant factor in crop and food production. Ancient civilizations such as that of Mesopotamia were devastated due to a buildup of salinity in the cultivated soils. This ultimately resulted in drastic decreases in crop yields, which contributed to the decline and downfall of these civilizations. The remnants of these civilizations are evidence of how salinity can affect nations (Hale & Orcutt, 1987).

In arid and semi-arid regions, soil water may be a limiting factor for successful seed germination and plant establishment. Also, salt stress adversely affects plants at all stages of their life cycle, but many plants are most sensitive to salt during seed germination. However, not all salinity problems are confined to the semi-arid regions of the world. Some 20% of the potentially exploitable saline soils of the world are in the humid regions of south and southeast Asia and about half of these (30 X 10^6 ha) are coastal saline soils (Ponnamperuma and Bandyopadhya, 1980).

Research on salinity and its related problems is of vital importance because it seems that the mistakes made by the Sumerians in the Tigris and Euphrate Basin of Mesopotamia over 4,000 years ago are being repeated today in almost every major irrigation development in the world. It is claimed that mismanaged irrigation systems and the resulting salinity to varying degrees is undermining the productivity of at least one-third of 230×10^6 ha of the world's irrigated land (Ramagopal, 1993). Salinity currently affects one-third of all irrigated cropped land from which one-fourth of the agricultural crops are produced. It is estimated that irrigated agriculture in the world has increased approximately 300% during the last 35 years (Boyer, 1982). Second, with the steady growth of population, especially in the developing world, and the demand for agricultural products, the need to confront these problems is urgent. Also the utilization of more land area for housing and industrial activities forces agriculture onto marginally productive areas which are characterized often by salinity and shortage of water.

Scientists have dealt with the salinity problem by improving the environment of the plant through reclamation procedures. These

procedures have included land leveling, surface and subsurface drainage, application of soil amendments, and improved irrigation practices to provide leaching. However, reclamation procedures are not universally effective as they are expensive and require continuous management control. A good example is the Colorado River system in the USA, where in 1982, the annual bill amounted to $133 million and this is expected to increase to over $250 million by the year 2000 (Holburt, 1984). As a result, reclamation is not complete. This realization logically leads to the idea of providing more saltresistant crops which will improve yields on saline soils not fully reclaimed and provide farmers with another management option to use in conjunction with reclamation programs.

Although some progress has been made in selection for salt resistance, there still is a lack of knowledge about the mechanisms of resistance available for plant breeders in the selection processes. An understanding of the molecular basis of resistance mechanisms and the effect of soil salinity upon crop yields can be better interpreted with the advent of increasing information concerning the effect of saline substrates on

specific metabolic processes within the plant, and consequently to all programs attempting to develop salt-resistant crop cultivars. The identification of specific characteristics related to salt resistance such as proteins, amino acids, and specific carbohydrates will provide potential biological markers useful in the identification and genetic manipulation of salt-resistant plants and plant cells.

In order to face the increasing salinity problem, researchers should emphasize the important crops on which humans and their animals depend. Maize (*Zea mays L.*), rice (*Oryza sativa L.*), sugar beet (*Beta vulgaris L.*), and cotton (*Gossypium hirsutum L.*) are considered important crop species in the world. These plant species were also chosen for their contrasting resistance for salinity in this investigation.

1.2 Characterization of the seeds

Seeds represent the means for survival and spread for most species of higher plants. One way or the other, seeds are the source of life-sustaining nutrients for men and animals.

Symbolically, they represent renewal and illustrate the tenacity of life in the face of a hostile world. Seeds are highly organized packets of energy that provide for the complete development of the primary plant body. They contain low amounts of free water (5 - 10%). When seeds are placed in water to initiate germination, rapid uptake of water into the tissue occurs. Concomitant with water uptake, there is a large and rapid leakage of solutes: ion, amino acids, sugar, and organic acids into the medium.

The process of germination occurs in four stages:
I.   Imbibition. The uptake of water, largely by protein components of the seed. II. Hydration and activation of informational mechanisms: nucleic acids and enzymes. III. Cell enlargement and cell division. IV. Emergence of root and shoot through the coat (Berlyn, 1972).

There is an opposition between the growth (turgor) force exerted by the embryo and the restraint imposed by the coat. The balanced condition is altered in favor of germination by stratification, light, or some other treatment that increases

turgor potential of the embryo. For populations of seeds, the cumulative germination over time is characterized by: (a) duration of time to the start of germination, (b) maximum rate of germination, and (c) total germination in percent.

Germination is reactivation of growth triggered by environmental stimuli as simple as availability of water and oxygen, or as complex as temperature, light, endogenous inhibitor and promoter interactions.

The reason why we chose to study seed germination is because: (1) seed germination is the most sensitive stage in the life cycle of the plant, (2) imbibition and following germination is characterized as a major structural and functional change in seed development, (3) because seeds are normally dormant, the event of germination must happen in a particular time, that is with imbibition. Thus seed germination offers an excellent system to study its development and concomitant effect by salt.

1.3 Description of plant species involved in the experiment

1.3.1 Maize

Maize (*Zea mays L.*) is one of the three major cereal crops in the world. Although little agreement exists as to the origin and early evolution of maize, there is general agreement that maize (corn) was first domesticated 7,000 to 10,000 years ago in tropical Central America. Many tropical races of corn are short-day plants, whereas modern temperate-zone cultivars appear to be almost wholly indifferent to day length (Stevenson and Goodman, 1972). The area of maize production in the world was 130 million ha and total world production was 507 million tons in 1995 (Pomeranz, 1987). The corn kernel is the largest of all cereals. It is flattened and is wedge-shaped. The endosperm contributes about 80-85% of the kernel weight (Salunkhe, et al., 1985).

The major component of the grain is starch. Starch is the basis for almost all industrial uses of the maize grain. Maize is an annual plant belonging to the grass family (Gramineae or Poaceae). *Zea mays* is monoecious, with staminate flowers on the terminal branched end of a tall, erect stem. Maize is a tall

plant with a fibrous root system. The plant height tends to increase with increasing relative maturity. A long photoperiod also increases plant height, whereas drought stress may reduce plant height. The plants exist in various sizes and shapes depending on their origin, relative maturity, specific end use, or the environmental conditions under which they grow. Corn has a remarkable diversity of vegetative types and is grown in a wide range of environmental conditions. As a result of genetic improvement, the potential yield of corn has been increased by approximately 50% during this century in the United States alone (Frey, 1984).

The importance of cereal crops, in particular maize, cannot be overemphasized. These earliest domesticated plant sources still play the most vital and pivotal role in sustaining our daily lives and our very existence on this planet. In fact, if any of the other food groups were to become unavailable, humankind still could survive and remain tolerably healthy. A failure of cereal crops, however, would bring starvation and malnutrition to most parts of the world.

1.3.2 Rice

Rice (*Oryza sativa L.*) is the world's most important cereal, occupying a land area of nearly 150 million ha with an average productivity of 3.5 t/ha. It is grown largely in Asia (>90% of the total area) with smaller areas in America, Africa, and parts of Europe. The reason why rice has been able to support so many people for so long is due to the physical environment in which rice is grown. The cultivation of rice extends over a wide range of climatic, soil and hydrological conditions; from wet tropical to semi-arid and warm temperate regions, and from heavy clay to poor sandy soils and from dryland to swampland in fresh or brackish water. Topographical conditions vary from uplands (drylands) in plateau regions, with problems of deficit soil moisture, to medium lands with efficient water management and lowlands with excess water up to a depth of 5 m (Yoshida, 1977).

Rice belongs to the family of the Gramineae and probably originated in southeast Asia, particularly in India and Indo-China, from where the richest diversity of cultivated forms has been reported (Vishnu-Mittre, 1974). *Oryza sativa* is divided into two main subspecies, *indica* and *japonica*, which have some

distinct morphological, physiological, and genetic characteristics. Rice is a medium salt-resistant crop and varieties of rice exhibit variability in sensitivity to salinity conditions. Rice-growing ecosystems are broadly classified into three major categories: irrigated, rain-fed uplands, and rain-fed lowlands, including deep water and wetlands, occupying an area of 55% to 32% respectively (IRRI, 1994). The green revolution of the mid-1960s brought about large increases in the yield of irrigated rice, primarily due to the introduction of modern high-yielding varieties and increased use of chemical fertilizers. The growth duration of rice varies from three to eight months, depending on the variety and environmental conditions. Development of the plant from germination to maturity is characterized by a series of separate periods. Such periods are those required for the germination of seeds and the emergence of seedlings; the initiation of the root, leaf, tiller, growth of different vegetative organs, and finally flowering and filling of spikelets. A 120-day variety, when grown in a tropical environment, spends about 60 days in the vegetative phase and 30 days each in the reproductive and ripening phases.

1.3.3 Sugar beet

Sugar beet (*Beta vulgaris L.*), a member of the Chenopodiaceae, is a long-day biennial plant grown almost exclusively as a source of sucrose. However, following sucrose extraction, the pulp often enriched with molasses provides a high energy animal feed. Historically, the crop was developed in central Europe and was grown throughout that continent, but it has now been introduced into all of the populated continents. The success of sugar beet as a crop depends on predictable seed germination, early seedling establishment, and the rapid development of a leaf canopy which is able to utilize the available solar radiation efficiently. Leaf production continues throughout the season, while the tap root enlarges and accumulates sucrose as its main storage product. Generally, sucrose contents are lower and α-amino nitrogen compounds higher in the crown region than in the remainder of the root. The fresh weight content of sucrose in the roots of well-grown varieties is about 18%. According to Dutton et al. (1961), both the root and the leaf of sugar beet play significant roles in the synthesis of sucrose. There is considerable evidence that crop yield and sugar production are directly related to the amount of radiation

intercepted by sugar beet foliage between sowing and harvest: the greater the incident of radiation, the higher the yields that may be expected (Blackburn, 1984).

Rapid establishment of uniform and vigorous sugar beet plants can only be achieved with good quality seed, which germinate quickly and synchronously. These good germination and emergence values are a consequence of successful breeding and innovations in seed production and processing. Variations in seedling growth rate can be due to several interacting factors such as initial seed weight, seed placement, and seed bed structure, and various environmental influences including temperature and water availability. The time taken from germination to emergence of the seedling from the soil can have persistent effects on crop development. Sugar beet can be grown in soils varying in texture from light sands to heavy clays that supply adequate amounts of plant nutrients as well as water.

1.3.4 Cotton

Cotton (*Gossypium hirsutum L.*) despite being cultured like annual crops, differs from annual crops because it exhibits much

of the xerophytic, woody perennial characteristics with a natural mechanism for shedding its mature leaves as found in some of its ancestors. As the harvestable parts of the crop (lint and seed located in bolls) mature, a considerable portion of the plant's assimilates can be partitioned to non-productive plant parts rather than to yield (Hearn, 1979). Typically, cotton has a crop growth rate during bloom of 17 to 19 g day^{-1} (Kerby and Buxton, 1978). To achieve this yield, nearly 240 bolls m^{-2} must mature. Unfortunately, this yield cannot be realized because, in general, only one sympodial branch develops at each main stem node with three to five fruiting sites per branch and only one fruiting form develops per fruiting site. Additional fruiting sites would require formation of additional monopodial branches or more main stem nodes. The time required for young floral buds to develop into mature fruit is longer for cotton than for most crops. Fruiting forms can be susceptible to stress for up to 40 days. Early season fruit loss, regardless of the cause, not only delays maturity (Guinn and Mauney, 1984) but can also lead to excess dry matter partitioning to nonreproductive (vegetative) plant parts and to reduction in yield.

Lint, rather than seed, is the primary harvested product for cotton. Genetic increases in lint yield have resulted in increased total seed biomass per unit area. The close association of lint and seed biomass is expected, in part, because the seed coat epidermis physically supports fiber growth. Lint, seed, and seed cotton biomass are closely related to the number of balls per unit area (Verhalen et al., 1975).

Lint yield can be defined as the product of the total above-ground dry matter per unit area and the percentage of that biomass that is lint. Like most crops, the genetic gains in cotton lint yield achieved by modern cultivars have occurred because of increase in the partitioning of above-ground biomass to fruit (Verhalen and Murray, 1970). These edaphic factors have a controlling effect on the entire vegetation of a given region, and in turn, the associated vegetation is itself a significant ecological factor for this species.

1.4 Effect of salinity on seedling and plant growth

1.4.1 Morphological effect

Besides general stunting of plant growth, salinity causes several specific structural changes that disturb plant water balance or status. The shape and size of plant organs and cells may change in response to salt stress. This includes increased leaf succulence, decreased leaf size and leaf number, reduced numbers of stomata, thickening of the leaf cuticle, and deteriorated or undeveloped xylem (Shannon et al., 1981). Since roots are directly exposed to the saline environment, it seems remarkable that root growth is usually affected less than vegetative shoot growth. The resultant decrease in shoot-root ratio presumably improves water balance by maintaining the potential for water absorption while reducing transpiration. Visual symptoms (leaf burn) may be evident. Symptomology, however, is apparently not as definitive in diagnosing salt stress as in diagnosing iron chlorosis deficiency stress. Kramer et al., (1977) reported that in *Phaseolus* species xylem parenchyma cells differentiated as transfer cells with well-developed wall protuberances adjacent to the half-bordered pits of the vessels. Further, they found that the cytoplasm of these

transfer cells contains cisternae of rough endoplasmic reticulum, the number of which increased greatly when grown in saline culture. Leopold and Willing (1984) proposed that salt-induced lesions in membranes and subsequent leakage of cell contents could be a distinct effect of ion toxicity. Salt also effects the cellular and nuclear volume and inhibits or stimulates nucleic acid and protein synthesis.

1.4.2 Osmotic effect

Many different salts at equivalent osmotic potentials often produce equivalent growth depression (Hayward and Long, 1941). Reduction in growth under saline conditions apparently occurs as a result of a very negative solute potential in the soil solution, which causes the overall water potential to also be quite negative, thus resulting in a decrease in the water uptake by the plant (Hayward and Spurr, 1944). When the salt concentration of the soil solution increases, water potential decreases, the turgor potential of plant cells declines, and cells ultimately cease to grow. Under these water stress conditions, in general, stomata close resulting in the reduction of photosynthesis. Protein breakdown is enhanced and plants show poor growth.

The low osmotic potential of saline soils makes it necessary for plants growing on them to maintain a lower intracellular osmotic potential; otherwise, they would experience water stress due to the movement of water osmotically from the plant tissue into the soil. In order to achieve a lower osmotic potential, osmotic adjustment under saline conditions can occur in plants due to

uptake of inorganic ions from the saline growth medium (Amzallag, 1994), or by internal synthesis of osmotically active organic solutes (Weinberg et al., 1984). Halophytes usually maintain high turgor potential due to accumulation of ions (Wyn Jones, 1981), whereas some glycophytes are generally unable to adapt osmotically, because of reduced uptake and accumulation of ions from the growth medium.

1.4.3 Specific ion effect

An element present in the soil in excess may cause metabolic disorders. It competes for entry with other elements present at smaller concentrations and once absorbed may inhibit enzymes, displace other essential elements from their normal functional sites, precipitate other essential elements, disrupt the structure of water, and otherwise disturb plant metabolism (Epstein, 1972). Actual concentrations need not be very high to produce some of these effects.

The effects of salinity on photosynthesis can be both stomatal (decreased intracellular CO_2 due to stomatal closure, lowering of stomatal conductance (Brugnoli and Lauteri, 1991) or nonstomatal

(decrease in Rubisco activity) and decrease in quantum efficiency of CO_2 uptake (Seemann and Chritchley, 1985), and change in the ionic relations of the chloroplast (Long and Baker, 1986) or change in photochemical reactions (Reddy et al., 1992). The resistance of photosynthetic systems to salinity is associated with the capacity of the plant species to effectively compartmentalize the ions in the vacuole, cytoplasm and chloroplast (Reddy et al., 1992). Reddy et al. (1992) showed lesser ion accumulation in the chloroplast than whole plant grown under saline conditions, suggesting that osmotic adjustment in chloroplast somehow prevents the ion entry into the chloroplasts. Hoffman and Phene (1971) in cotton, and Lapina and Bikmukhametova (1972) in corn, reported increased respiration with increase in salinity. Elevated Ca^{2+} supply has a protective effect for root growth (Cramer et al., 1986). It has been reported that a higher resistance potential of *Citrus sinensis* cell lines is dependent on Ca^{2+} supply (Ben-Hayyim et al., 1987) which establishes a quantitative relationship between the nutritional Ca^{2+} requirement and the degree of salt stress.

Evidence indicates that membrane potentials are rapidly

depolarized by salinity (Schubert and Läuchli, 1988; Läuchli and Schubert, 1989). The negative potential will passively attract cations, conversely it will repel anions. Thus, cations can be readily transported across the plasma membrane and anions are readily transported across the tonoplast. There appear to be two important effects of salinity on proton transport. One is to increase the overall rate of proton transport (Schubert and Läuchli, 1986), and the second is to increase the abundance of H^+ (Perez-Prat et al., 1994).

Very few ionic species in soil solution or adsorbed on soil particles contribute to salinity in a given saline soil. The predominant cations and anions in soils are Ca^{++}, Na^+, Mg^{++}, Cl^- ,SO_4^-, HCO_3^-, and CO_3^-. Saline soils contain Na^+, Ca^{++}, Mg^{++}, but of these cations, Na^+ cannot exceed a given concentration if deterioration of soil structure is to be avoided (Richards, 1954). High concentrations of Mg^{++}, for example, can be harmful to the plant, not only because they are toxic to the plant tissue, but also because they can greatly reduce the absorption of Ca^{++} and K^+ (Hayward and Wadleigh, 1949).

Plant response to excess sodium may be complicated by effects
such as structural deterioration of sodic soil with consequent
poor germination of the plant because of restricted moisture
transmission and seedling emergence. Direct effects of Na$^+$ are
its toxicity to sodium-sensitive crops and the challenge imposed
on the balance of nutrients in the relatively resistant plants
(Mozafar, 1969).

Electrical conductivity (ds/m)
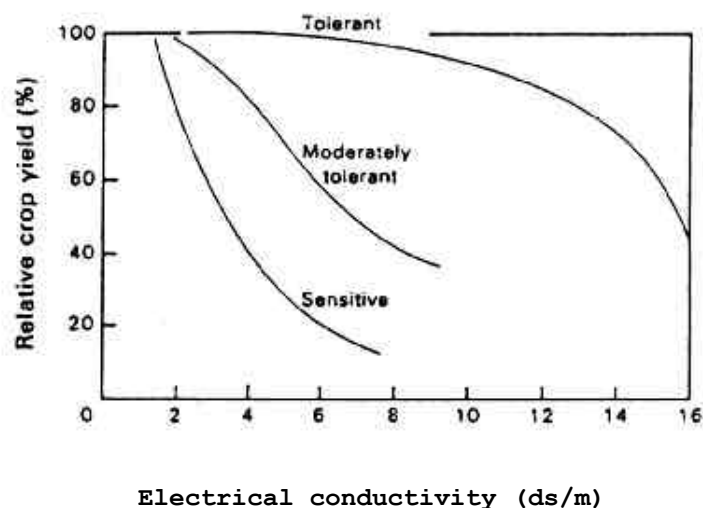
Fig.1.   Salt resistance curves for a range of crop plants
varying in sensitivity to salinity, as measured by electrical
conductivity. (after Reeve and Fireman, 1967).

Because crop plants differ quite markedly in their level of salt
resistance, the effect of salinity on yield is a function of the
threshold salinity above which yield declines and the percentage

yield decrease (see Fig. 1) per unit of salinity increase above the threshold.

Data for a range of major crops varying in salt resistance is also given (see Table 1).

Table 1. Salt resistance of a range of selected agricultural crops. Yield decreases are percentage values per unit increase in salinity above the threshold electrical conductivity (EC). Ratings are: R, resistant; MR, moderately resistant; MS, moderately sensitive; S, sensitive (after Maas and Hoffman, 1977).

Crop	Threshold EC (dS m^{-1})	Yield decrease (%)	Rating
Barley	8.0	5.0	R
Cotton	7.7	5.2	R
Sugar Beet	7.0	5.9	R
Wheat	6.0	7.1	MR
Soybean	5.0	20.0	MR
Rice	3.0	12.0	MS
Tomato	2.5	9.9	MS
Lucerne	2.0	7.3	MS
Maize	1.7	12.0	MS
Peach	1.7	21.0	S
Apricot	1.6	24.0	S

Although the level of salt in most irrigation water would be below the threshold for the more sensitive crops, salt accumulation in irrigated soils from both irrigation and groundwater sources can increase salinity to levels which can reduce the yield of even the more resistant crops.

Based on this information, it is clear that the demands for the use of salinized land for agricultural purpose and the reinstatement of these lands for agricultural use in the future will require a better understanding of the nature of salt resistance during seed germination, especially since a relatively small number of researchers have studied this problem.

Salinity stress may have a greater effect during certain phases of plant's life cycle than others. Seedling establishment and floral development are often thought to be the most sensitive stages (Jones and Jones, 1989).

Some studies indicated that rice is resistant during germination, very sensitive during seedling stage, then gains

resistance again during vegetative growth (Akbar, 1986). At pollination and fertilization, it becomes sensitive and then becomes more resistant at maturity. For some plant species, salinity at the reproductive stage depresses the grain yield much more than at the vegetative growth stage. On the other hand, the most sensitive stage to salinity in cotton plants was the flower bud formation (El-Saidi et al., 1992). During this critical stage, salinity affects the growth of cotton plants by completely stopping the growth and higher rate of shedding takes place. Consequently, reduction in seed cotton yield was very severe. The cotton fibers become shorter and its fineness will also be reduced. On the other hand, during flowering and ball formation stage, there is no severe affect.

The present study was, therefore, conducted to deal with the following objectives.

1.5 Objective of the work

Major objectives:

1   To determine the effect of different levels of salinity (NaCl) on germinating seeds (of sugar beet, cotton, maize, and rice).

2   To investigate the extent of osmotic and specific ion effects on germination by using different types of inorganic salts: NaCl, $CaCl_2$, and Na_2SO_4 on the above mentioned plant species.

3   To evaluate the effect of water potential (drought simulation) using osmotic substances such as PEG and mannitol and comparing these with NaCl during germination on these plant species.

Secondary objectives:

I.   Identify salt-resistant and salt-sensitive plants during germination.

II.   To assess the exogenous application of GA_3 and Kinetin on germination and early seedlings growth under salt-stressed conditions.

III. If differences in salt resistance among species can be identified during germination the objective will be to identify the mechanisms that are responsible for these differences.

1.6 Statistical analysis:

Statistical analysis was performed with Excel spreadsheet from Jandel Scientific. For all the probes and measurement, the mean ($\bar{x}$) and the standard error ($\pm$ SE: $S/\sqrt{n}$) were calculated according to Köhler et al. (1984). The error bar represents the statistical variation in germination in the replicates. The entire experiment was a randomized complete-block design with each block containing 50 seedlings of each salinity combination. Each tray contained all three salinity combinations and controls sown in two adjacent rows whose location was randomized as was the placement of trays in the growth chamber. The trays were rotated every day in the chamber.

2 Effect of Salinity on Germination and Early Vegetative growth

2.1 Introduction

Seed germination and seedling growth are critical life stages often subject to high mortality rates. Seeds and seedlings may be less stress-resistant than adults or may be exposed to the more extreme environmental fluctuations at or near the soil surface. In either case, the ability to successfully negotiate this *regeneration niche* can be a strong determinant of species distributions in harsh environments.

In arid and semi-arid regions, soil water may be a limiting factor for successful seed germination and plant establishment. Germination in saline seed beds may be restricted by low soil moisture and osmotic potential or by toxic concentrations of specific ions (Roundy, 1987).

Soils are regarded as saline, if they contain soluble salts in such quantities that they interfere with the growth of most crop plants. Chloride, sulfate, and bicarbonate salts of sodium, calcium, and magnesium contribute to varying degrees to soil and

water salinity.

As the soil dries or as salt levels build up, both water potential and osmotic potential in the soil decline, thus affecting seed growth and development. On the other hand, salinity stress involves excess of ions, whereas water stress is due primarily to a deficit of water without a direct role of ions. Nevertheless, from a practical viewpoint of crop yields, early approaches have assumed that the effect of salt in the soil (osmotic-potential effect) and the effect of soil drying (matrix-potential effect) are additive.

Saline irrigation water is also a problem and has become increasingly more serious as water of less and less desirable quality is exploited for irrigation and as greater intensity of water use leads to degradation. Similarly, river waters have become highly regulated. Salts are concentrated when water evaporates from reservoirs and new irrigation projects aggravate the salinity problem for downstream users.

Although there are many parameters involved with seeds

germinating in a dry soil or in salt water, they are all stressed by a common factor: negative water potentials. One of the important and commonly damaging effects in deserts is the presence of high salt concentrations in the soil. A seed faces two problems in such areas: one problem is that of obtaining water from a soil of negative osmotic potential and the other problem are the high concentrations of potentially toxic ions. Because of experimental accommodation, restriction of water uptake followed by inhibition of germination, osmotic solutions have been widely used to evaluate drought resistance of seedling growth under stress conditions. While studying the effect of drought on seed germination, water potential is usually created by addition of various osmotic substances to water.

There are at least three components of salt stress, caused primarily by NaCl, which affect seeds: osmotic stress, specific ion toxicity, and induced nutrient deficiency (Greenway and Munns, 1980; Kingsbury and Epstein, 1986). The specific ions likely to be most abundant and to cause the greatest problems are sodium (Na^+) and chloride (Cl^-). Mayer and Poljakoff-Mayber (1963) reported that *Atriplex halimus L.* responded differently

to salinity at germination than during vegetative growth, with salt stimulation occurring in the latter. They indicated that salinity resistance for vegetatively growing plants is 10-100 times greater than at the germination stage.

Total germination of many species may be more affected by low osmotic potential than by specific ion effects (Rauser and Crowle, 1963). However, radical growth may be strongly inhibited by specific ions. Successful seedling establishment depends on the frequency and the amount of precipitation as well as on the ability of the seed species to germinate and grow while soil moisture and osmotic potentials decrease (Roundy, 1985). The changes induced by salinity in any one of the particular physiological or anatomical parameters vary considerably. This depends on plant species, stage of development, and external factors such as edaphic conditions, salt regime, and climatic conditions. Therefore, it is difficult to quantify plant responses to salinity in a way which could be meaningful for extrapolation from species to species, or from one set of environmental conditions to another (Poljakoff-Mayber and Gale, 1975).

Despite the voluminous literature in the field of salinity, a clear picture of the role of salinity in seedling growth has not yet emerged. The present study was therefore initiated to investigate first the extent of salinity and second osmotic and specific ion effects of a number of substrates on the germination and early vegetative growth of maize, rice, sugar beet, and cotton and to find out whether or not specific ion effects can be identified. The technique used was to compare the germination response of seeds under isotonic conditions in different types of inorganic salt solutions (Hyder and Yasmin, 1972).

The following study was designed to compare the effect of water potential, initiated by the three most commonly used osmotic substances, sodium chloride, mannitol, and polyethylene glycol (mol. wt. 6000), on the germination and early vegetative growth of four plant species, namely *Zea mays L.*, *Beta vulgaris L.*, *Oryza sativa L.*, and *Gossypium hirsutum L.*

2.2 Materials and methods

Seeds of four plant species, rice (*Oryza sativa* L. cv. AI-NAN-TSAO), sugar beet (*Beta vulgaris* L. cv. Evita), maize (*Zea mays* L. cv. Pioneer 3906), and cotton (*Gossypium hirsutum* L. cv. Aleppo 33), differing in salt sensitivity, were used in the experiments. The rice, sugar beet, maize, and cotton cultivars were obtained from China, Germany (KWS Kleinwanzlebener Saatzucht AG), U.S.A. (Chicago), and Syria, respectively. The sugar beet was monogerm.

For the germination experiments plastic petri dishes (94 mm diameter, 16 mm height) with a tight-fitting lid were used (C.A. Greiner u. Söhne Kunststoffwerke, Germany). Fine sand was washed thoroughly with deionized water, dried completely at 125°C, and 20 g were filled into each petri dish.

In this experiment, the effect of different NaCl concentrations on germination and on root and shoot length of the seedlings was tested. The nutrient solution consisted of 1 mM Na_2SO_4, 1 mM K_2SO_4, 1 μM H_3BO_3, 1 mM $CaSO_4$ with varying concentrations of NaCl (0, 50, 100, 200 mM). The pH of all solutions was adjusted to

6.0   (± 0.2).

For each plant species three independent germination experiments were conducted, comprising 100 seeds for each of the four NaCl treatments (total of 1200). Seeds were hand-sorted to eliminate broken and small seeds. They were weighed initially. Seeds were stored in cloth bags at 5°C until experiments were carried out. In one petri dish 50 seeds were germinated with 20 ml solution (4 ml for sugar beet seeds). The experiments were carried out in an instrumentation specialties model growth chamber (Memmert ICE 400-800, Germany) at 25°C in the dark and at high humidity (greater than 80%) to help prevent evaporation of the germinating solution.

Seeds were incubated for 5 d and germination was evaluated every 24 h. After 48 h seeds had started to germinate (seeds were considered to be germinated with the emergence of the radicle). The germinating seeds were counted at daily intervals, and the germination percentage was expressed as the percentage of the non-NaCl treatment (control). The lengths of roots and shoots of the germinated seeds which were more than 2 mm in length were

measured and recorded. In all treatments a continuous increase in the number of germinating seeds as well as in the lengths of roots and shoots was observed during the subsequent days of germination. Depleted solution was replenished to each petri dish.

In the following experiment, plant seedlings were exposed to different salt solutions, comparing NaCl with $CaCl_2$ and Na_2SO_4 with an osmotic potential equivalent to 100 mM and 200 mM NaCl and a control (no salt addition). The osmotic potentials were determined by using the freezing point technique by means of an osmometer model (Roebling, Mikro-Osmometter Typ 4B, Germany) which was calibrated with KCl standard solutions. This experiment was conducted with the same plant species and cultivars as used previously. Germination of plants, experimental design and timing of treatments in relation to growth stage were identical to the conditions outlined for the previous experiments. The measured osmotic potential at the two salt levels in equal osmoticum was -0.79 and -1.22 MPa respectively.

In the follow-up experiment it was investigated whether effects

of NaCl on germination resulted from osmotic stress. For this purpose germination tests were conducted comparing NaCl with polyethylene glycol (PEG) and mannitol. Solutions of PEG (mol.wt.6000) and mannitol were prepared with osmotic potentials equivalent to 100 mM and 200 mM NaCl. The osmotic potentials of all solutions were adjusted according to calibration curves obtained by plotting a graph of concentration versus osmotic potential (Fig. 6). The values for the osmotic potentials at the equal osmoticum for the three solutions were -0.80 and -1.24 MPa respectively. The osmotic potentials were determined by using the freezing point technique which is described above.
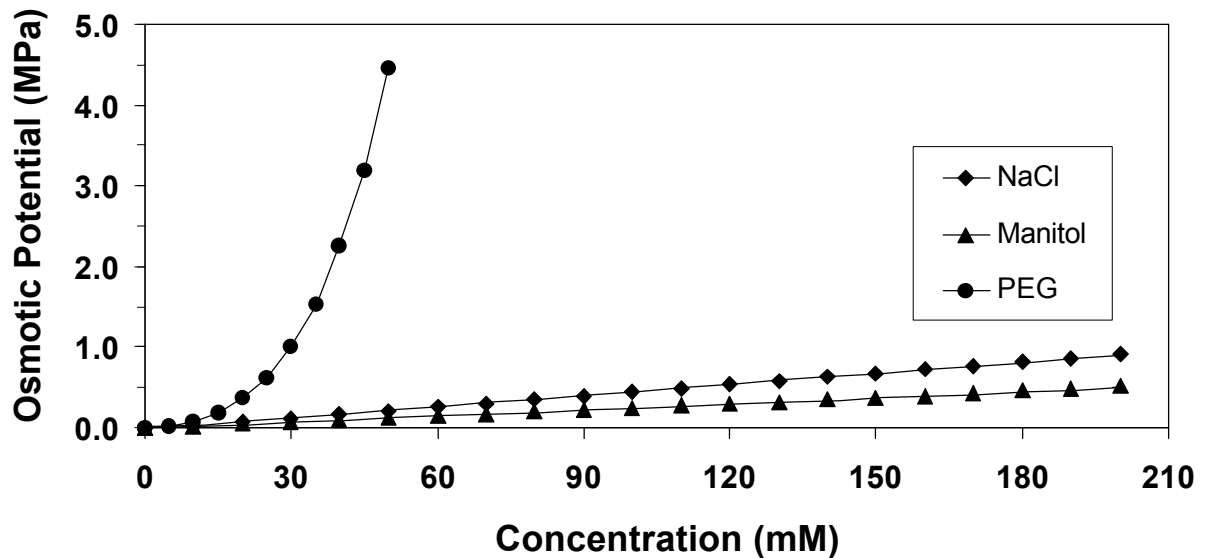
Fig. 6: The osmotic potentials of NaCl, Mannitol, and PEG solutions differing in concentration. Calibration curves obtained by plotting a graph of concentration versus osmotic potential.

Seeds were treated as described in the previous experiment, except using PEG and mannitol to induce osmotic stress in comparison with NaCl. For each treatment three replicate incubations of 100 seeds were performed, and germinated seeds were counted daily.

2.3 Results

2.3.1 Effect of NaCl on germination and early vegetative growth

Studies carried out to evaluate the influence of salinity (NaCl) on seedling vigor of germinating seeds indicated that an

increased salinity level caused delayed emergence of root and shoot in germinating seeds compared to controls (Table 2).

A continuous increase in length of root and shoot was observed in subsequent hours of germination in all four plant species in the control as well as salt treatments. Increased salt concentration caused a decrease in germination. The decrease was more in sugar beet (Fig. 2) and cotton (Table 2). The germination of sugar beet and cotton was strongly inhibited by both 100 and 200 mM NaCl applications. The percentage of germination was less than 10% after application of 200 mM NaCl for both of these plant species (Table 2).

Table 2: Effect of NaCl stress on seed germination and growth parameters (root and shoot elongation) of various plant species; measurements after 5 d of incubation. Means ± standard error of 3 replicate incubations with 100 seeds each.

|	Treatment	Seed Germination (%)	Root Length (% of control)	Shoot Length (% of control)
Maize	0 mM NaCl	100.0±0.7	100.0±1.8	100.0±6.1
	50 mM NaCl	99.7±0.7	101.8±1.7	94.2±3.8
	100 mM NaCl	98.7±0.6	72.7±2.0	67.4±4.1
	200 mM NaCl	87.6±1.5	35.7±2.4	33.4±2.6
Sugar beet	0 mM NaCl	100.0±1.1	100.0±7.2	100.0±10.3
	50 mM NaCl	95.7±2.2	82.9±5.0	84.0±8.7
	100 mM NaCl	80.5±1.9	48.8±6.8	61.7±6.1
	200 mM NaCl	9.6±1.1	15.7±0.5	13.7±13.7
Rice	0 mM NaCl	100.0±0.3	100.0±2.4	100.0±7.7
	50 mM NaCl	99.3±0.3	57.8±2.4	62.1±2.5
	100 mM NaCl	98.7±1.9	53.3±3.0	39.5±1.6
	200 mM NaCl	87.1±2.6	16.0±1.8	0.0±0.0
Cotton	0 mM NaCl	100.0±1.4	100.0±4.2	100.0±5.4
	50 mM NaCl	84.2±5.4	86.7±3.4	70.4±5.0
	100 mM NaCl	59.7±1.7	42.9±3.0	42.8±3.4
	200 mM NaCl	6.3±1.4	0.0±0.0	0.0±0.0
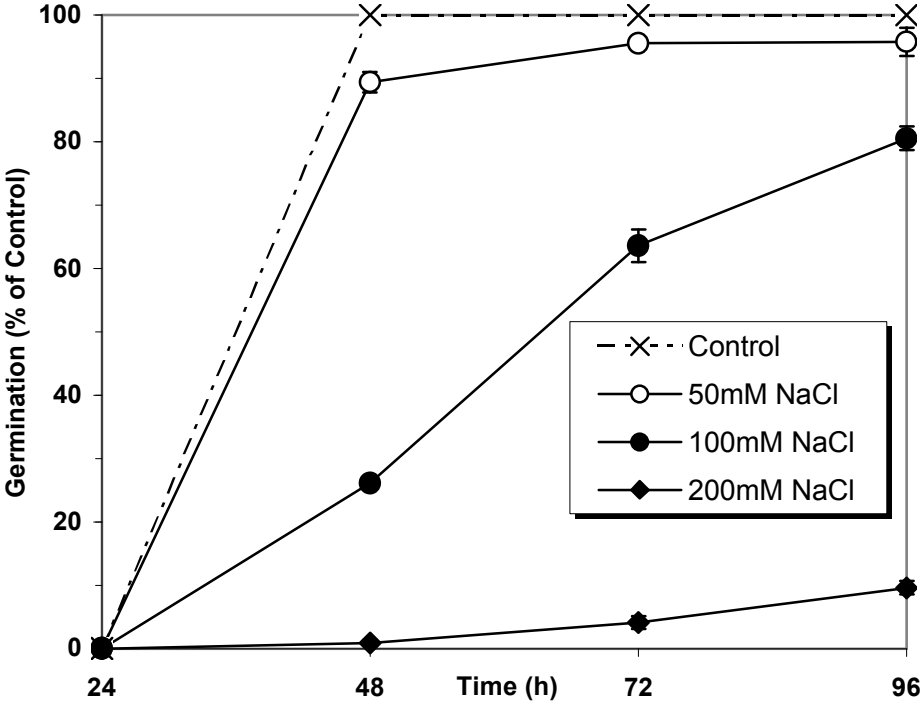
Figure 2: Influence of NaCl concentration on sugar beet germination. Error bar mean ± standard error of 3 replicate incubations with 100 seeds each.
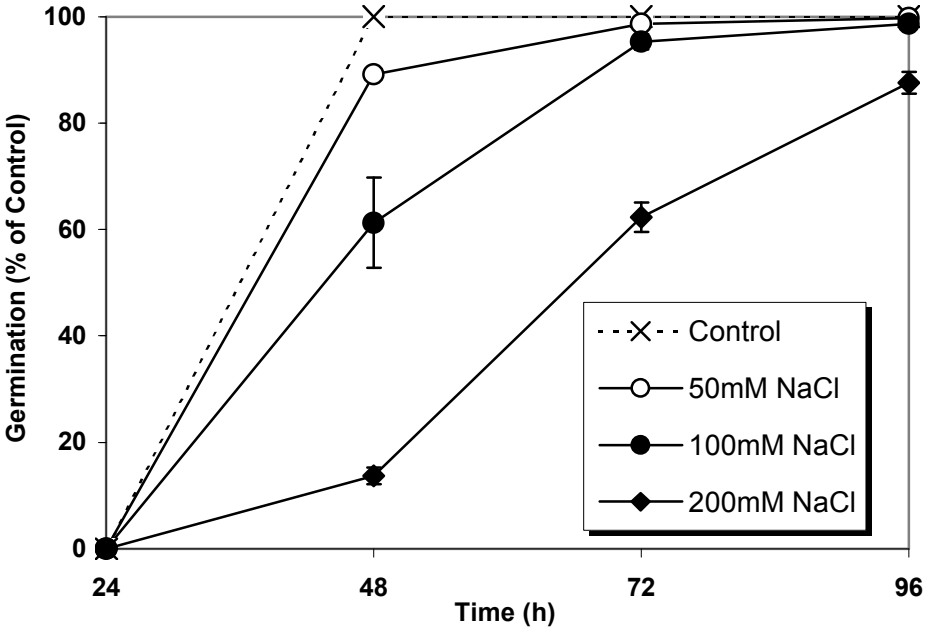
Fig. 3: Influence of NaCl concentration on maize germination. Error bar means ± standard error of 3 replicate incubations with 100 seeds each.

Great inhibition of root, and in particular shoot growth, occurred with NaCl treatments for sugar beet, rice, and cotton seedlings (Table 2). Decrease in length of shoot was more pronounced compared to roots in particular in higher salt treatments (200 mM). In contrast, the lowest inhibition of germination as well as root and shoot growth was observed for maize. The highest reduction after salt treatment occurred with rice and cotton; sugar beet being intermediate (Table 2).

2.3.2 Effect of NaCl, CaCl$_2$, and Na$_2$SO$_4$ on germination and early vegetative growth

Table 3 shows the effect of NaCl, CaCl$_2$, and Na$_2$SO$_4$ concentrations under equal osmotic potential on growth parameters.

Germination percentage of sugar beet and cotton was strongly affected by all salt treatments. The reduction being strongest particularly at the higher level of salt treatment compared to control. No germination was recorded with higher concentrations of CaCl$_2$ and Na$_2$SO$_4$ for cotton and only a few seeds germinated at 200 mM NaCl. Percent germination of maize (Fig. 4, a,b) and

rice remained relatively high in most treatments compared to the control group. However, rice germination was reduced to 13% under the highest Na_2SO_4 condition (Table 3).

Data on the average length (Table 3) of root and shoot of the seedlings of the four plant species raised in increasing levels of salt solutions shows that sugar beet, cotton, and rice showed a strong inhibition. There was no measurable length of roots of this plant species, particularly at the highest level of $CaCl_2$ and Na_2SO_4 for cotton and sugar beet; similarly rice showed no measurable root length at the highest level of Na_2SO_4 (Table 3).

Table 3: Effect of NaCl, CaCl$_2$, and Na$_2$SO$_4$ stress at equiosmotic potentials on seed germination and growth parameters (root and shoot elongation) of various plant species; measurements after 5 d of incubation. Means ± standard error of 3 replicate incubations with 100 seeds each.

	Treatment	Seed Germi-nation (%)	Root Length (% of control)	Shoot Length (% of control)
Maize	0 mM (control)	100.0±0.3	100.0±1.4	100.0±1.8
	100 mM NaCl	84.0±0.9	81.1±1.7	82.7±3.2
	65 mM CaCl$_2$	71.2±1.5	64.7±1.9	76.7±1.5
	54 mM Na$_2$SO$_4$	73.2±1.5	80.1±1.6	66.9±1.4
	200 mM NaCl	68.2±2.1	41.4±2.4	40.3±1.1
	133 mM CaCl$_2$	61.2±2.0	33.0±0.8	33.7±0.7
	109 mM Na$_2$SO$_4$	59.2±1.7	25.9±0.2	29.7±0.1
Sugar beet	0 mM (control)	100.0±0.6	100.0±1.0	100.0±3.9
	100 mM NaCl	59.7±2.1	53.4±1.7	69.1±0.9
	65 mM CaCl$_2$	47.0±3.5	41.8±3.9	62.2±1.8
	54 mM Na$_2$SO$_4$	54.7±1.8	48.2±2.8	57.6±3.7
	200 mM NaCl	5.6±0.9	14.2±1.4	0.0±0.0
	133 mM CaCl$_2$	3.2±0.6	0.0±0.0	0.0±0.0
	109 mM Na$_2$SO$_4$	1.1±0.6	0.0±0.0	0.0±0.0
Rice	0 mM (control)	100.0±1.2	100.0±1.3	100.0±0.9
	100 mM NaCl	94.4±1.8	62.2±0.3	50.6±2.4
	65 mM CaCl$_2$	92.4±4.2	52.7±0.7	54.0±1.3
	54 mM Na$_2$SO$_4$	91.7±1.2	28.5±0.8	45.7±2.0
	200 mM NaCl	29.2±3.6	27.6±0.9	0.0±0.0
	133 mM CaCl$_2$	38.9±1.8	19.9±2.3	0.0±0.0
	109 mM Na$_2$SO$_4$	13.2±1.8	0.0±0.0	0.0±0.0
Cotton	0 mM (control)	100.0±1.7	100.0±1.3	100.0±2.4
	100 mM NaCl	51.5±3.5	46.4±1.5	58.9±0.7
	65 mM CaCl$_2$	31.2±1.3	59.2±3.6	53.7±7.3
	54 mM Na$_2$SO$_4$	28.6±2.6	41.2±2.2	41.9±0.6
	200 mM NaCl	6.9±0.7	22.5±0.3	0.0±0.0
	133 mM CaCl$_2$	0.0±0.0	0.0±0.0	0.0±0.0
	109 mM Na$_2$SO$_4$	0.0±0.0	0.0±0.0	0.0±0.0
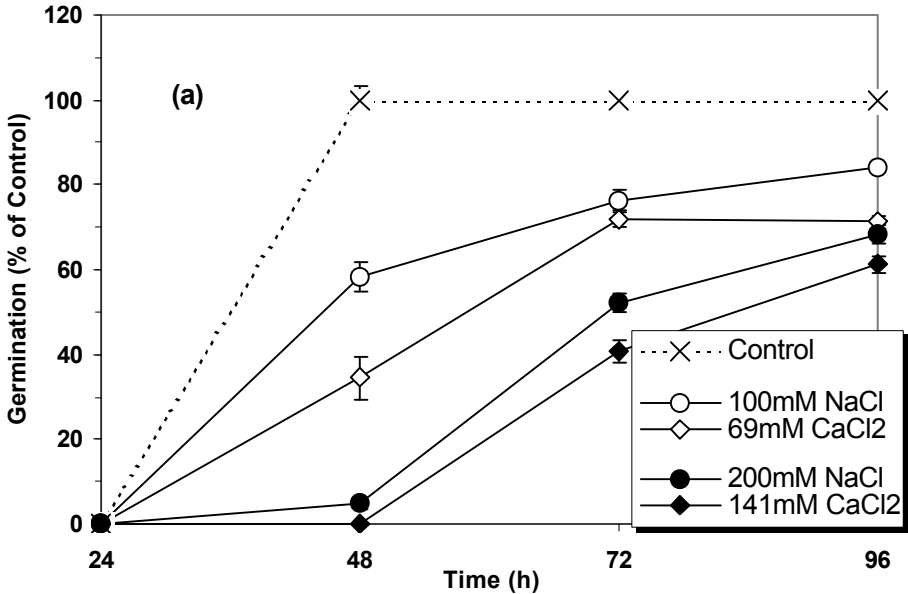
Fig. 4a: Influence of NaCl and CaCl$_2$ concentration on maize germination. Error bar means ± standard error of 3 replicate incubations with 100 seeds each.
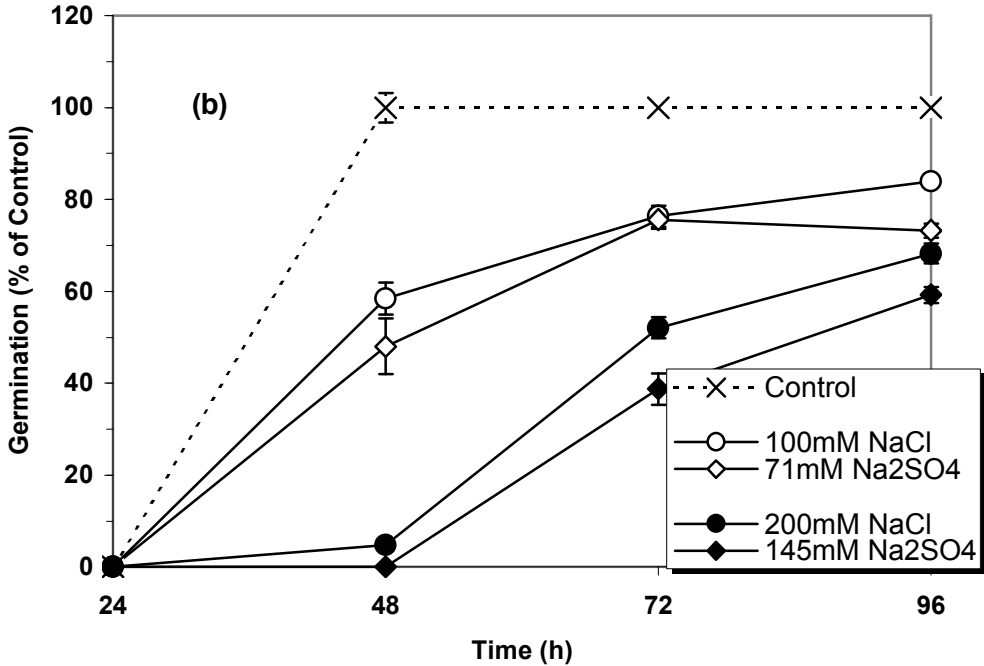
Fig. 4b: Influence of NaCl and Na$_2$SO$_4$ concentration on maize germination. Error bar means ± standard error of 3 replicate incubations with 100 seeds each.
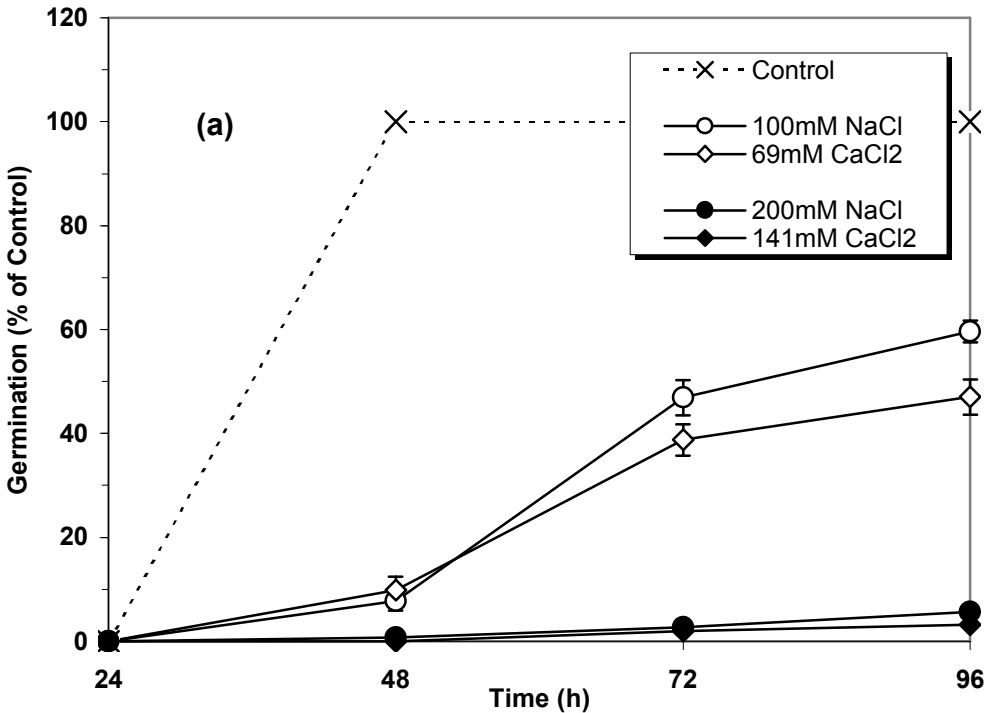
Fig. 5a: Influence of NaCl and CaCl$_2$ concentration on germination of sugar beet seedlings. Error bar means ± standard error of 3 replicate incubations with 100 seeds each.
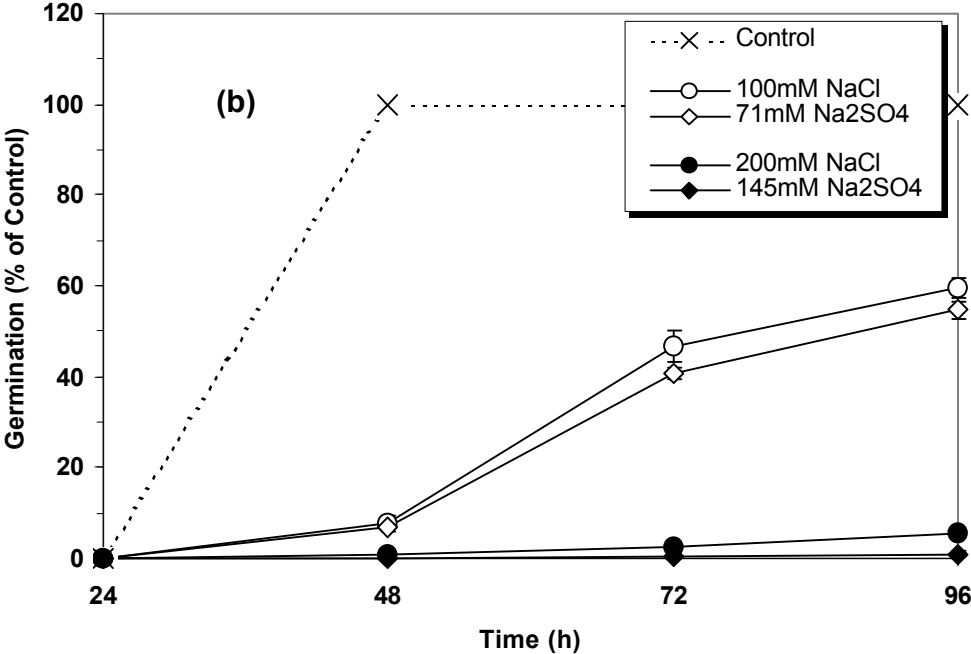
Fig. 5b: Influence of NaCl and Na$_2$SO$_4$ concentration on germination of sugar beet seedlings. Error bar means ± standard error of 3 replicate incubations with 100 seeds each.

Germination percentage of sugar beet (Fig. 5, a,b) and shoot elongation of sugar beet, rice, and cotton showed a strong inhibition, particularly at higher salt concentration; maize was the most resistant of the four species tested.

2.3.3 Effect of NaCl, mannitol and PEG on germination and early vegetative growth

The germination response of the four plant species under investigation showed marked differences in the timing of initiation and completion of germination. Germination started within 48 h and was complete by the 5th d (100%). The final germination of seeds of these seed species under various conditions of salinity or water stress was expressed as a percentage of the germination of seeds of the same population in control (in deionized water). (See Table 4.)

Table 4: Effect of NaCl, PEG, and mannitol stress on seed germination and growth parameters (root and shoot elongation) of various plant species; measurements after 5 d of incubation. Means ± standard error of 3 replicate incubations with 100 seeds each. The osmotic potentials for NaCl, PEG and mannitol at equal osmolarity were -0.80 and -1.24 MPa respectively.

|	Treatment	Seed Germination (%)	Root Length (% of control)	Shoot Length (% of control)
Maize	0.0 mM (control)	100.0±1.2	100.0±3.4	100.0±3.6
	100.0 mM NaCl	88.3±2.5	78.5±2.7	74.7±3.9
	22.5 mM PEG	88.7±1.2	62.8±1.2	49.5±4.4
	191.0 mM Mannitol	93.5±5.2	66.8±7.4	62.3±2.7
	200.0 mM NaCl	65.3±0.9	37.0±2.8	41.6±2.1
	43.5 mM PEG	65.6±3.6	43.5±5.3	32.3±2.7
	368.0 mM Mannitol	74.2±2.2	43.4±5.6	38.0±2.3
Sugar beet	0.0 mM (control)	100.0±1.3	100.0±5.3	100.0±4.8
	100.0 mM NaCl	60.9±3.9	46.6±3.2	61.2±3.1
	22.5 mM PEG	33.1±2.7	29.7±3.3	54.5±1.7
	191.0 mM Mannitol	62.6±4.0	39.8±4.5	55.9±3.1
	200.0 mM NaCl	4.3±1.2	15.6±7.8	36.6±18.
	43.5 mM PEG	9.6±1.2	21.1±3.1	16.3±16.
	368.0 mM Mannitol	18.5±1.4	23.8±3.9	48.7±0.0
Rice	0.0 mM (control)	100.0±1.2	100.0±5.3	100.0±3.4
	100.0 mM NaCl	99.0±0.3	60.6±2.2	42.2±1.5
	22.5 mM PEG	95.9±0.9	83.6±2.8	61.7±3.3
	191.0 mM Mannitol	100.0±0.9	70.9±4.5	49.2±2.5
	200.0 mM NaCl	96.9±0.9	31.6±5.8	29.6±0.0
	43.5 mM PEG	92.1±3.6	66.4±2.9	42.6±2.0
	368.0 mM Mannitol	96.6±0.9	39.2±2.9	33.3±2.1
Cotton	0.0 mM (control)	100.0±1.7	100.0±2.1	100.0±15.
	100.0 mM NaCl	55.0±1.8	56.7±4.4	57.8±7.6
	22.5 mM PEG	51.8±1.1	59.0±3.6	50.8±4.8
	191.0 mM Mannitol	41.0±2.8	48.1±3.0	52.4±3.6
	200.0 mM NaCl	6.4±1.4	35.9±3.8	10.1±10.
	43.5 mM PEG	19.9±1.1	32.4±3.3	32.7±2.5
	368.0 mM Mannitol	6.4±0.4	34.6±4.5	30.2±0.0
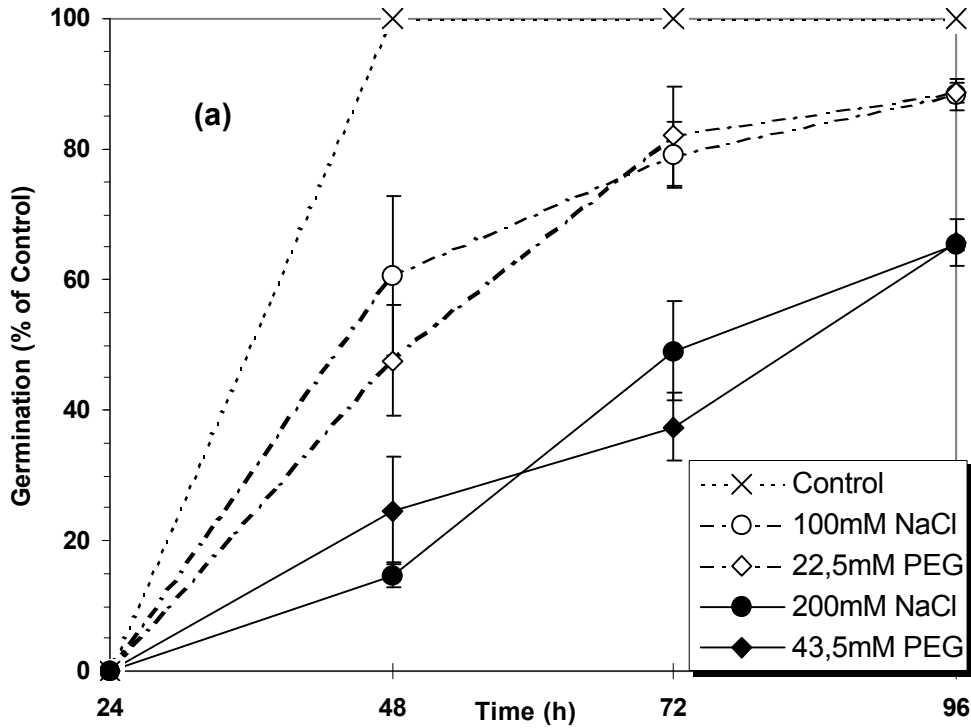
Fig. 7a: Influence of NaCl and PEG concentration on maize germination. Error bar
means ± standard error of 3 replicate incubations with 100 seeds each.
Equal isotonic quantities for NaCl, PEG and mannitol was -0.80 and -1.24 MPa.
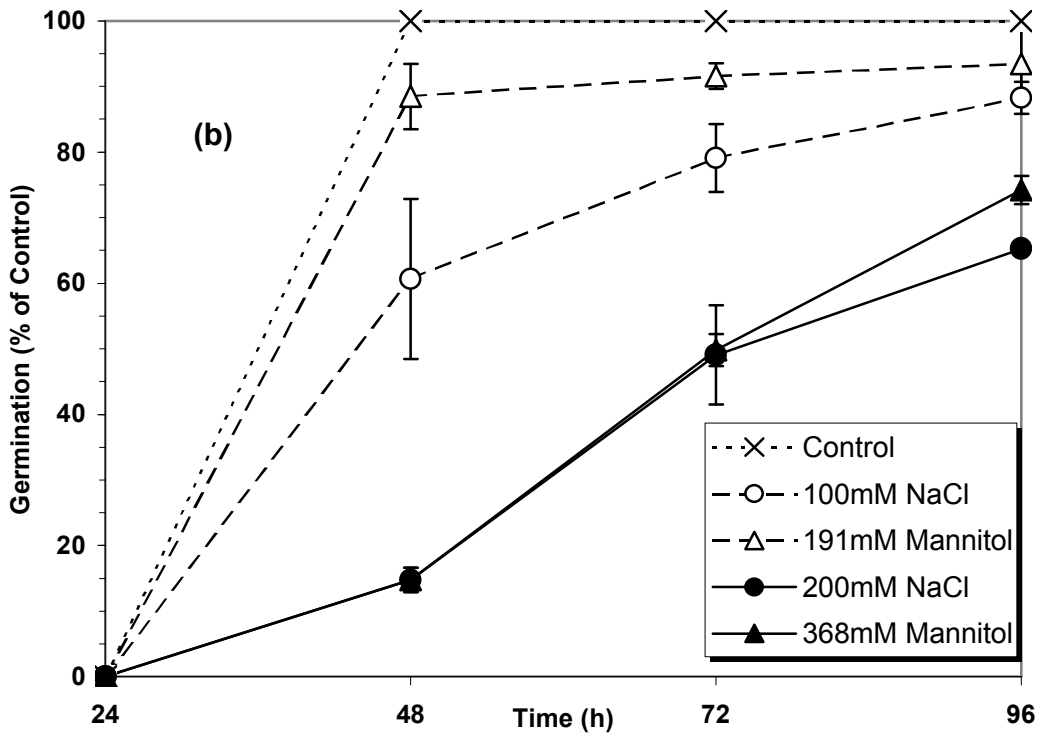
Fig. 7b: Influence of NaCl and Mannitol concentration on maize germination. Error bar
means ± standard error of 3 replicate incubations with 100 seeds each.
Equal isotonic quantities for NaCl, PEG and mannitol was -0.80 and -1.24 MPa.
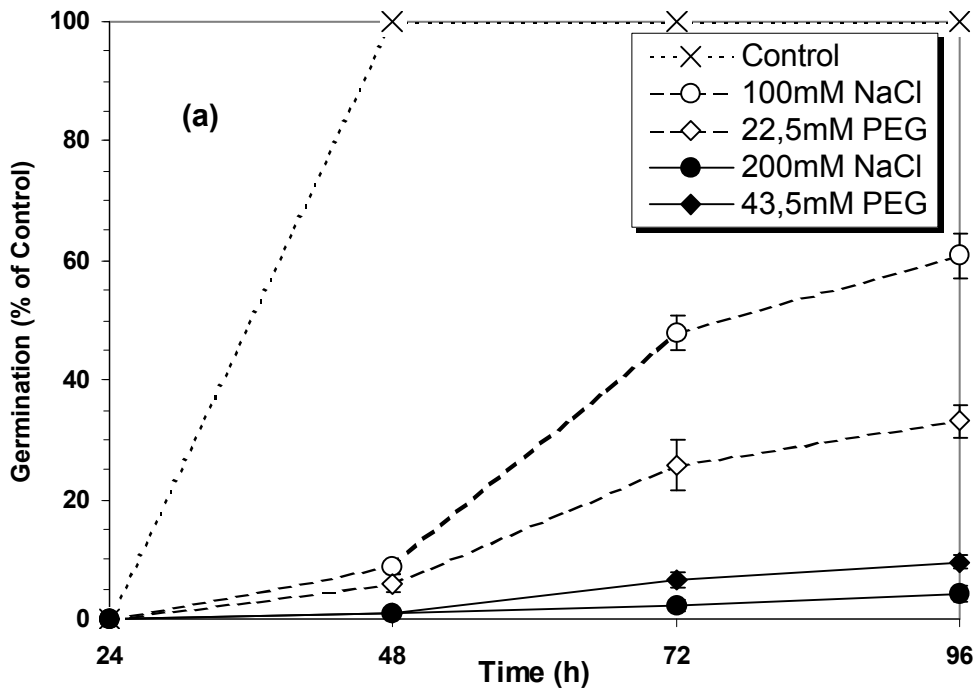
Fig. 8a: Influence of NaCl and PEG concentration on sugarbeet germination. Error bar
means ± standard error of 3 replicate incubations with 100 seeds each.
Equal isatonic quantities for NaCl, PEG and mannitol was -0.80 and -1.24 MPa.
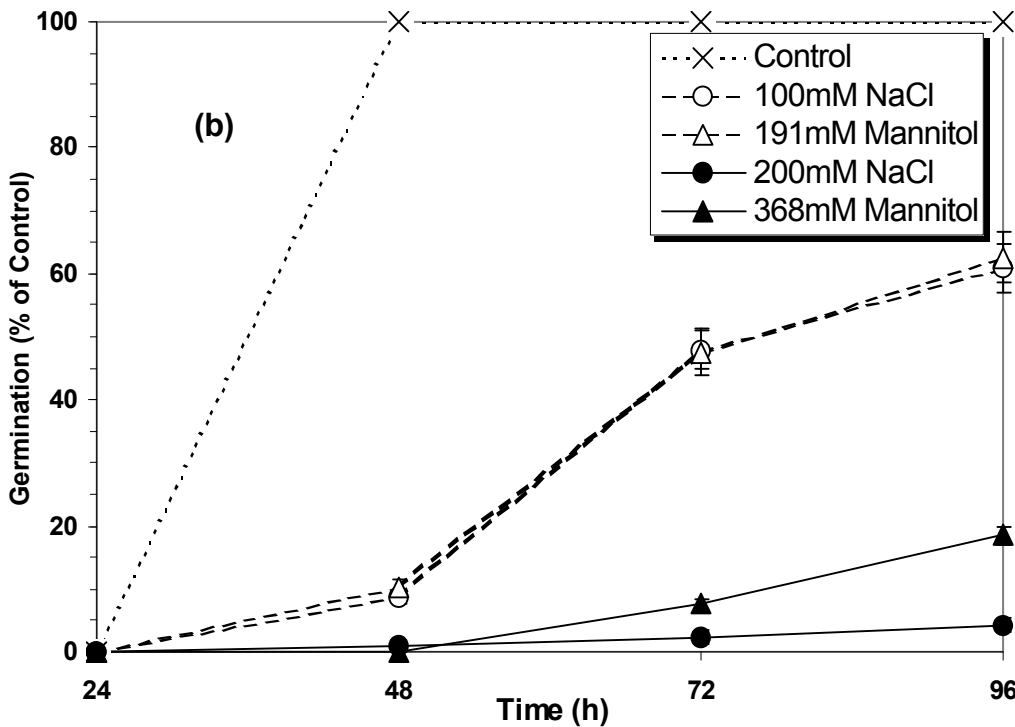
Fig. 8b: Influence of NaCl and Mannitol concentration on sugarbeet germination. Error bar
means ± standard error of 3 replicate incubations with 100 seeds each.
Equal isatonic quantities for NaCl, PEG and mannitol was -0.80 and -1.24 MPa.

Maize and rice showed a high germination number, giving relative germination values close to 100% under moderate treatments for maize (Fig. 7a, b) and over all treatments for rice (Table 4).

Sugar beet (Fig. 8, a, b) and cotton (Table 4) showed a strong reduction of germination at all levels of treatments, the reduction being strongest at higher concentrations of solutes (Table 4). These plant species showed sensitivity to water stress during germination. Water stress beyond -0.45 MPa had a pronounced inhibitory effect on the germination of these plants. Root and shoot growth of all of the plants under investigation suffered most severely when compared to the no-salt control. The reduction was strongest at higher levels of osmotica for root elongation of sugar beet (Table 4).

2.4 Discussion

In general, researchers have concluded that salinity is inhibitory to the germination of seedlings in two ways: (1) causing a complete inhibition of the germination process at salinities beyond the resistance limits of a species, and (2) delaying the germination of seeds at salinities that cause some

stress to seeds, but do not prevent germination.

In our study all species had a reduction in their rate and total germination with increase in salinity compared to no salt controls.

(Table 2) Optimum conditions for germination of the four species studied here had high interspecific variability.   The germination percentage of maize and rice showed a higher level of resistance at various salt concentrations.   These plant species were able to maintain the highest germination percentage with increasing salinity.   It seems that the resistance limits of maize and rice during germination are closer to those of the moderately salt-resistance halophyte *Hordeum jubatum* (Ungar, 1974).   That could be due to their low threshold water potential at which their germination processes operate.   This is probably the main reason why maize and rice germinate at low osmotic and matrix potentials.

Contrarily, sugar beet and cotton did not maintain a high germination rate and percentage in all treatments.   Myers and Morgan (1989) determined that the salt-resistant grass *Diplachne*

fusca had reduced seed germination from 70% in distilled water to 50% in 150 mM NaCl, and down to 7% in 300 mM NaCl. Francois et al. (1984) found that soil salinity up to 50 mM did not significantly inhibit germination of _Sorghum bicolor_ seeds, but salt levels greater than 100 mM delayed germination. The general consequence of increasing the concentration of salts in the medium is growth suppression more or less proportional to the solute concentration, but the degree of suppression varies with different species and even with different biotypes. This reduction in growth may result from salt effects on biomass allocation, ion relations, water status, physiological processes, biochemical reactions, or a combination of such factors (Flowers, et al., 1977; Greenway and Munns, 1980). As salt concentrations increase above a threshold level, both the growth and size of most seedling growth progressively decreases.

In this study, the diminution of shoot growth was more accentuated than root growth for all seed species. This inhibitory affect was demonstrated by the reduction of seedling shoot length for seeds collected at the 50, 100, 200 mM salinity level (Table 2). Increasing salinity caused a decrease in the

height of seedlings from all sources. Shoot and root growth decreased rapidly at 100 mM for cotton and sugar beet. These species showed a greater sensitivity towards root and shoot growth in the early vegetative period and continued to decrease with each increment of salinity.

In general, most of the parameters measured decreased by 50% at 100 and 200 mM level of salinity. Since the resistance of a plant to salinity is measured in terms of biomass decrease relative to biomass obtained under non-saline conditions, the threshold for cotton and sugar beet during germination would be 100 mM salinity level, respectively, and that for maize and rice would be about 200 mM salinity level. These data indicate that cotton and sugar beet in the germination stage were more sensitive to salinity stress than rice and maize. However, there is some indication that salt resistance in some halophytic plant species increases with development, as it was determined by Chapman (1960).

The second experiment was designed to compare the germination and early vegetative growth of seeds under isotonic conditions

in different types of inorganic salt solutions. For an easy comparison between the effects of the different salt species and their osmotic activities on the germination of maize, rice, sugar beet, and cotton a summary of the main results of experiment is presented in Table 3.

It is evident that germination decreased with increasing salt concentration in all of the three different salt species. However, there are marked specific differences associated with salt effects. In general, the data presented in Table 3 agrees with the fact that the salt effects on germination are rather specific to salt or ion species on one hand and to plant species on the other hand (Brown, 1965). If one considers a 10% suppression of germination as a significant affect of salt action, and fifty percent suppression as rather serious action, then by reference to Table 3 it is easy to compare between the effects of the different salt concentrations. The seed germination data of maize, sugar beet, and rice suggest that the seeds of these plant species may adjust osmotically to the salt solutions. However, at higher concentrations, rice shows a strong inhibition by Na_2SO_4, which may indicate ion-specific

effects.   Similarly in cotton, a reduction of germination was obtained specifically by $CaCl_2$ and Na_2SO_4.   In this respect, it may be concluded, depending on their sulphate or chloride form, sodium is more toxic than calcium of equivalent concentration and that the toxicity is due to a specific ion effect.   In the review of the works of Stroganov (1964), in this field it was shown that for the germination of seeds not only the concentration, but also the ionic composition of solution is important.   However, due to the law mobility of calcium and sulphate, the strong inhibition of germination may have been caused by limited osmotic adjustments by means of ion-accumulation in the seeds (Magistad, 1945; Harris and Pittman, 1919; Stroganov, 1964).

The non-ionic, water-soluble polymer PEG with a molecular weight of 6,000 and mannitol are not expected to be rapidly metabolized by the plant seedlings nor penetrate cells (Lawlor, 1970).   The results obtained from this experiment indicated that there was a major difference between the responses of these plant species during the germination and postgermination with respect to applied solutions.   The higher reduction of sugar beet and

cotton germination may be associated with lower initial imbibition of these seeds compared to favorable internal seed structure by maize and rice (see Fig. 16 a,b). In addition, the seed's ability to germinate in an osmotic medium depends mainly on whether the solute can permeate through the seed coat or whether this solute has any toxic effects. The entry of solute reduces true drought effects while the toxicity aggravates. The observed effects may have been the net result of these two processes.

Reduction of root and shoot growth under high salinity and isotonicity is also well documented (Bazzaz, 1973). Elevated salinity may inhibit root and shoot elongation due to slowing down the water uptake by seeds (Werner and Finkelstein, 1995). A possible reason for that could be the restriction of liquid flow of these solutions, due to the differences in the viscosities of NaCl, compared to mannitol and in particular to PEG.

According to Michel and Kaufmann (1973), the viscosity of aqueous solutions increases rapidly with molecular weight and

increasing concentration of PEG. In contrast, NaCl has comparatively marginal effects on solution viscosity. For example, the reported viscosity of PEG 6000 solutions at -2.0 MPa was approximately four centipoise, compared with about one centipoise for iso-osmotic NaCl. A four time greater solution viscosity of PEG 6000, as compared with iso-osmotic NaCl, could, therefore, account for a four times greater reduction in flow rates. Higher viscosity may also explain the inhibitory affect of PEG, but not NaCl.

The data suggested that germination percentage, root length, and shoot length compared to control were not affected by specific ion toxicity. This suggests that the affect of these stress agents on the seeds under investigation was mainly an osmotic affect. It is clear that in all four plant species, the greatest reduction in germination and early vegetative growth in relation to decreasing osmotic potential was in sugar beet and cotton, followed by maize and rice. This investigation also showed that at the low osmotic potential, all solutions of NaCl, PEG and mannitol inhibited the processes of germination and root and shoot elongation.

3 Effect of Phytohormones under Salt Stress Conditions

3.1 Effect of exogenous applications of GA$_3$ and kinetin on germination

3.1.1 Introduction

Plant hormones are a group of naturally occurring organic substances which influence physiological processes at low concentrations. The processes influenced involve mainly growth, differentiation, and development, though other processes such as stomatal movement may also be affected. Phytohormones are a unique set of compounds, with unique metabolism and properties. Their universal characteristics are that they are natural compounds in plants with an ability to affect physiological processes at very low concentrations.

The concept of phytohormones derives from Darwin's experiments on the phototropism of coleoptiles, which indicated the presence of a transported signal (Jacobs, 1979). Application of abiotic stresses during germination and early cycle of plant species results in altered levels of plant hormones and decreased plant growth (Morgan, 1990). The decreased cytokinin and gibberellic acid and increased abscisic acid contents observed in salt-

stressed plants (Boucard and Unger, 1976) has led to the suggestion that salt stress-induced changes in membrane permeability and water relations are related to changes in hormone balance. The subsequent growth reduction could be attributed to altered endogenous hormonal levels, as hormonal regulation is involved in membrane permeability and water relations (Ilan, 1971). Exogenously applied gibberellins and cytokinins probably compensate for a natural or environmentally induced deficiency (Wareing, 1982). Treatments generally stimulate, directly or indirectly, the natural conditions that initiate germination.

In order to study deleterious effects many methods have been used. One method is exogenous seed treatment with phytohormones. Kahn et al. (1957) reported that an osmotic inhibition of the germination of lettuce seed by mannitol may be overcome by treatment of the seeds with gibberellin. Chaudhuri and Wiebe (1968) found that GA and Kinetin increased salt resistance in wheat by increasing percentage of germination.

The exogenous application of GA_3 and kinetin on germination and early seedling growth under salt stress conditions provides an

attractive approach to encounter the effects of salinity. The present study was, therefore, undertaken to explore the possibilities of using phytohormones to counteract the salt effects on germination and early seedling growth.

3.1.2 Materials and methods

Sugarbeet (*Beta vulgaris* L. cv. Evita) and maize (*Zea mays* L. cv. Pioneer 3906) seeds were used throughout this study. The concentration of the salt (NaCl) solution was 100 mM and 200 mM. GA_3 and kinetin were prepared in distilled water and salt solution to prepare different substrates in which the seeds were sown. In order to study the effect of GA_3 and kinetin on germination and early seedling growth, GA_3 and kinetin were added to each culture solution at concentrations of 20 μM and 5 μM, respectively. These concentrations were selected according to experience from a number of pre-experiments on both of these plant species. GA_3 solutions were freshly prepared on the day of the experiment.

The germination percentage and length of roots and coleoptile in control, stressed and phytohormone-treated stressed seedlings

were measured. The experimental preparations and procedure were the same as in previously designed experiments (See page 32).

3.1.3 Results

GA_3 and kinetin at 20 μM and 5 μM respectively, when added to a medium containing 100 and 200 mM NaCl, were found to be effective in promoting germination and seedling growth in these plant species. However, the degree of the effectiveness varied with the medium applied, the plant species and the length of germination period. Both kinetin and GA_3 substantially accelerated the germination of maize inhibited by salinity (Fig. 9 a,b). The GA_3 was more effective, especially at 200 mM NaCl concentration, in comparison to kinetin, in particular after 48 h. After 96 h, however, there was no affect of either hormone on the percent germination under salt stress conditions.

Fig. 9a: Influence of NaCl and NaCl + kinetin on the germination of maize seedlings. Error bar means ± standard error of 3 replicate incubations with 100 seeds each.

Fig. 9b: Influence of NaCl and NaCl + GA$_3$ on the germination of maize seedlings. Error bar means ± standard error of 3 replicate incubations with 100 seeds each.

Fig. 10a: Influence of NaCl and NaCl + kinetin on the germination of sugarbeet seedlings. Error bar means ± standard error of 3 replicate incubations with 100 seeds each.
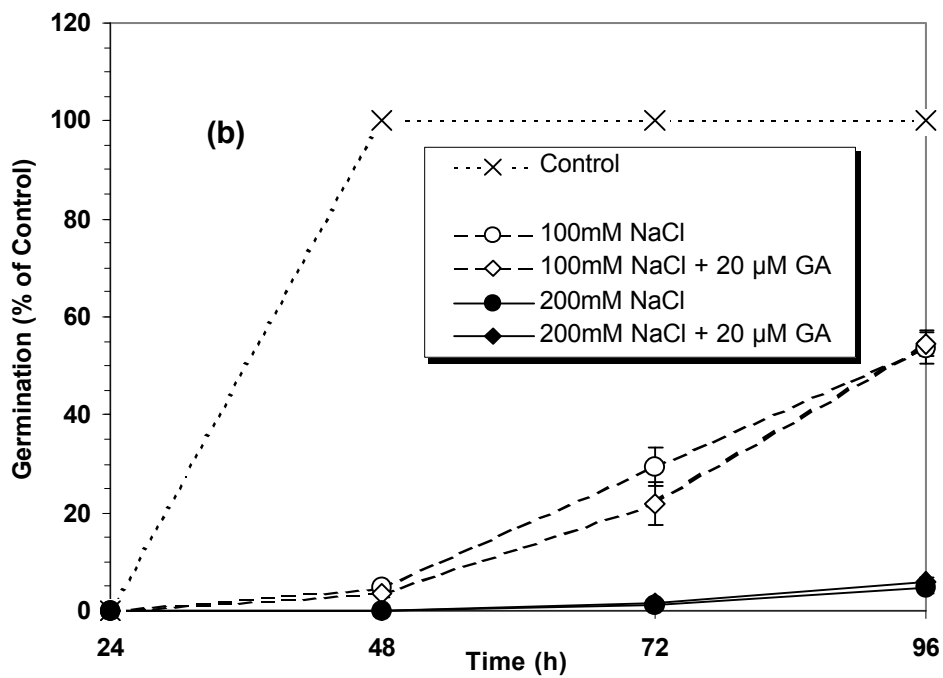
Fig. 10b: Influence of NaCl and NaCl + GA$_3$ on the germination of sugarbeet seedlings. Error bar means ± standard error of 3 replicate incubations with 100 seeds each.
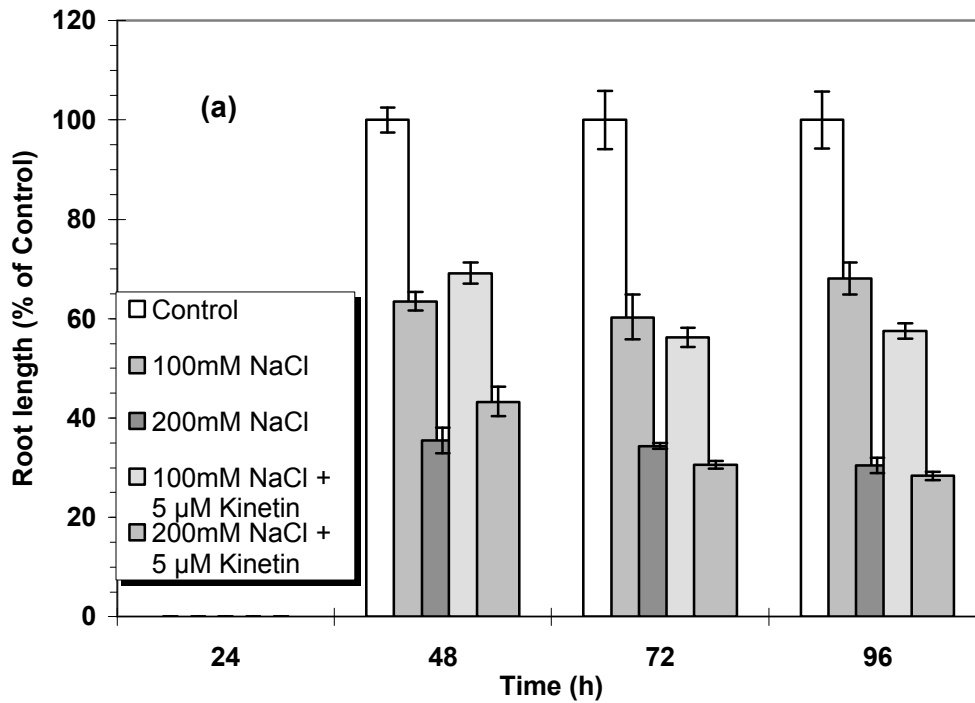
Fig. 11a: Influence of NaCl and NaCl + kinetin on the root length of maize seedlings.
Error bar means ± standard error of 3 replicate incubations with 100 seeds each.
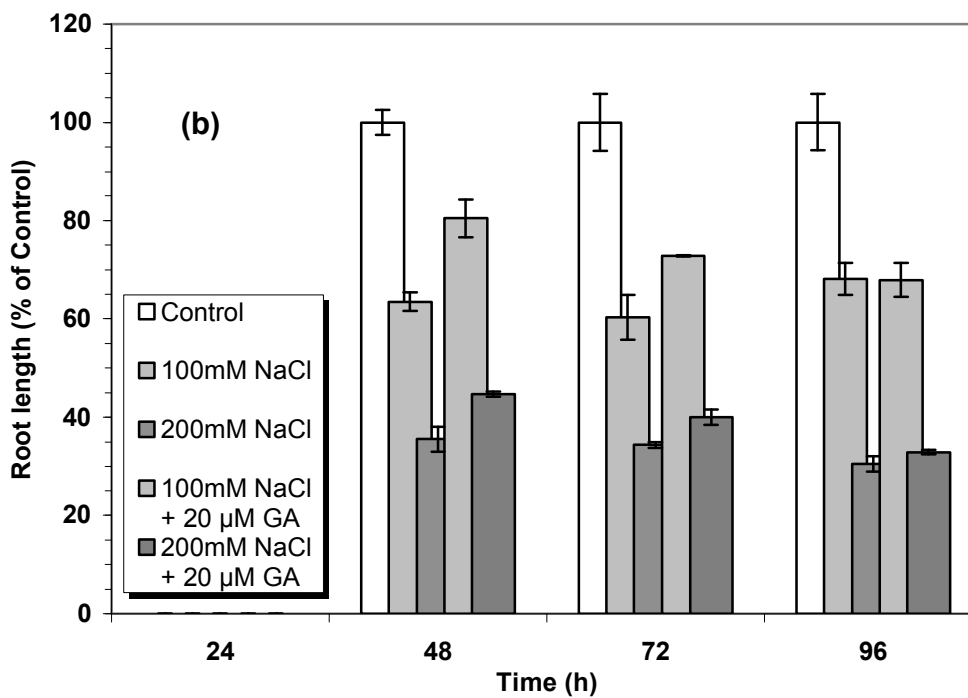
Fig. 11b: Influence of NaCl and NaCl + GA$_3$ on the root length of maize seedlings.
Error bar means ± standard error of 3 replicate incubations with 100 seeds each.
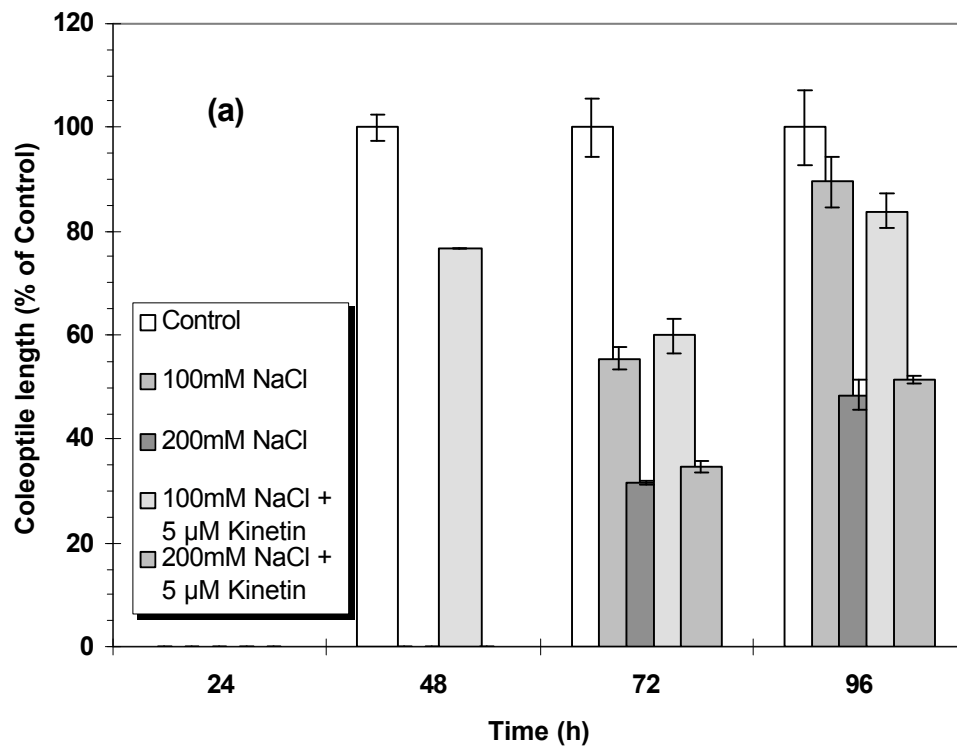
Fig. 12a: Influence of NaCl and NaCl + kinetin on the coleoptile length of maize seedlings. Error bar means ± standard error of 3 replicate incubations with 100 seeds each.
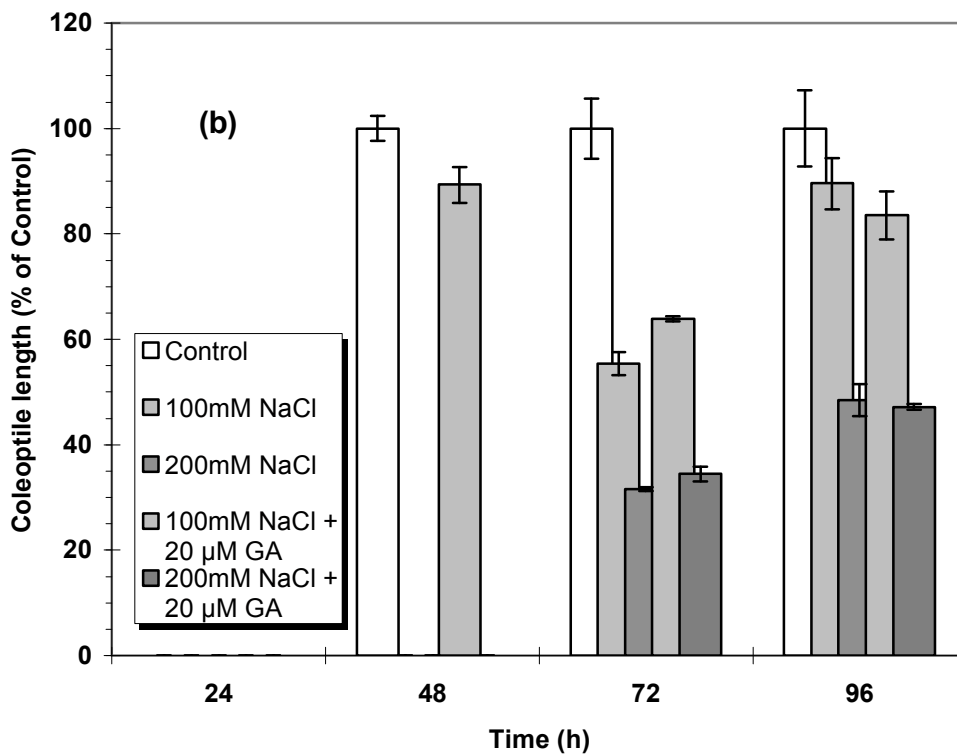
Fig. 12b: Influence of NaCl and NaCl + GA₃ on the coleoptile length of maize seedlings. Error bar means ± standard error of 3 replicate incubations with 100 seeds each.

Sugarbeet responded somewhat differently to the addition of GA_3 and kinetin to NaCl-containing medium. Kinetin increased the percentage of germination after 48 h. In a later time period it was able to maintain a gradual increase in germination percentage compared to non-treated salt solutions (Fig. 10a). *Contrarily*, GA_3 had no significant affect on promoting germination in sugar beet seed.

A stimulation of root growth was observed after both kinetin and GA_3 treatment of maize after 48 h. However, GA_3 was found to be more effective in increasing the root growth of stressed seedlings as compared with kinetin in this plant species (Fig.11, a, b).

Both GA_3 and kinetin had no promoting effect on root growth and coleoptile elongation of sugar beet after 96 h (Fig. 13, a, b).
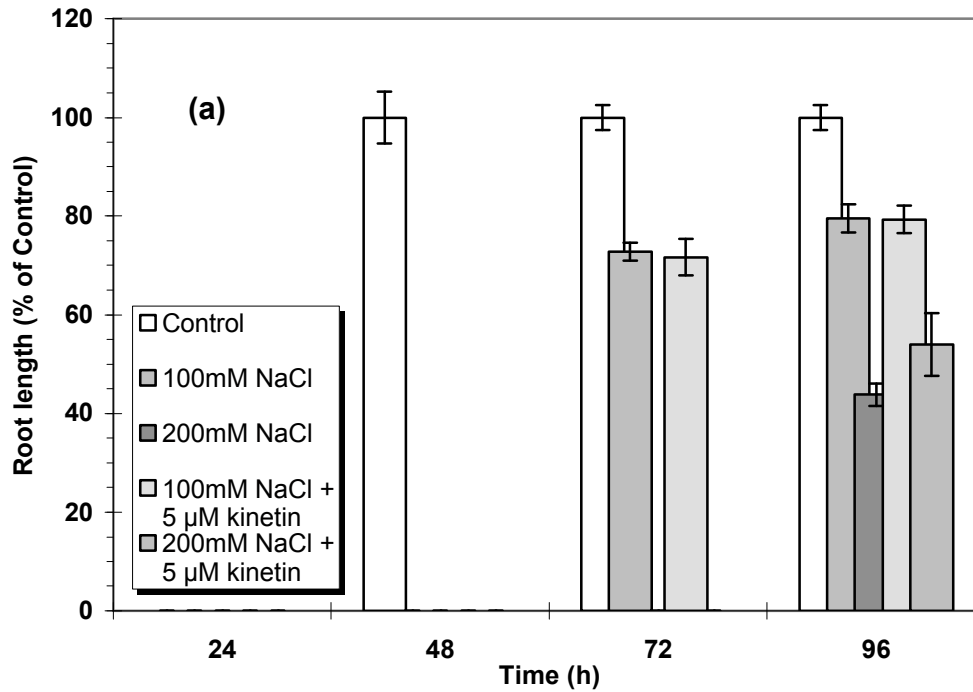
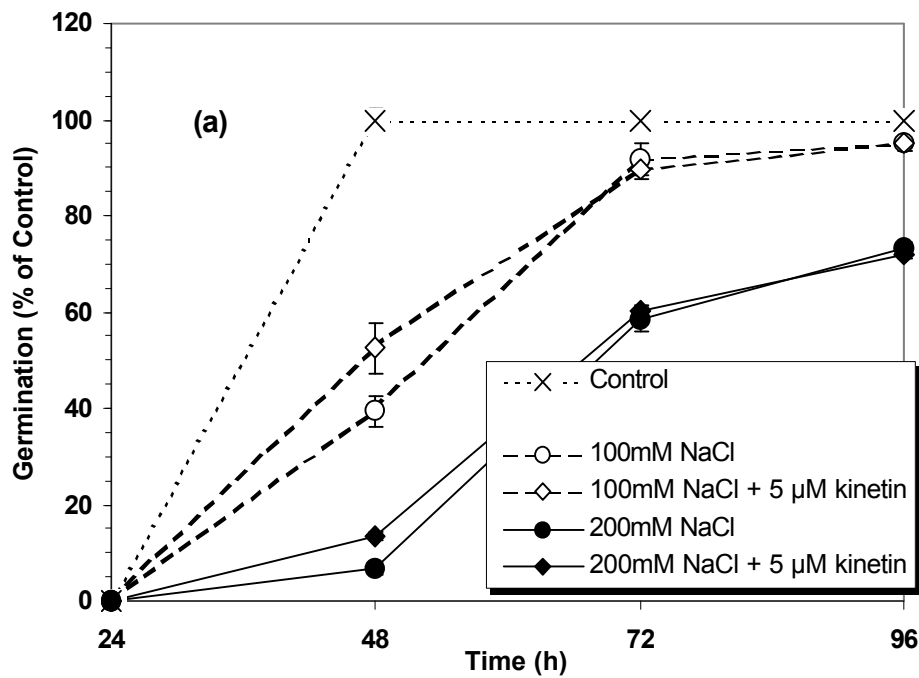**Fig. 13a**: Influence of NaCl and NaCl + kinetin on the root length of sugarbeet seedlings. Error bar means ± standard error of 3 replicate incubations with 100 seeds each.
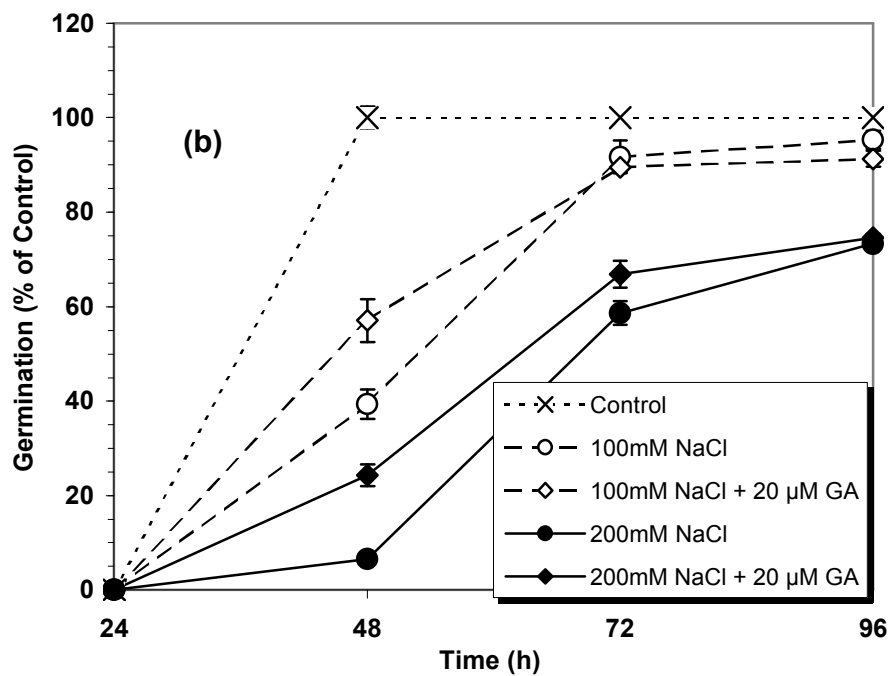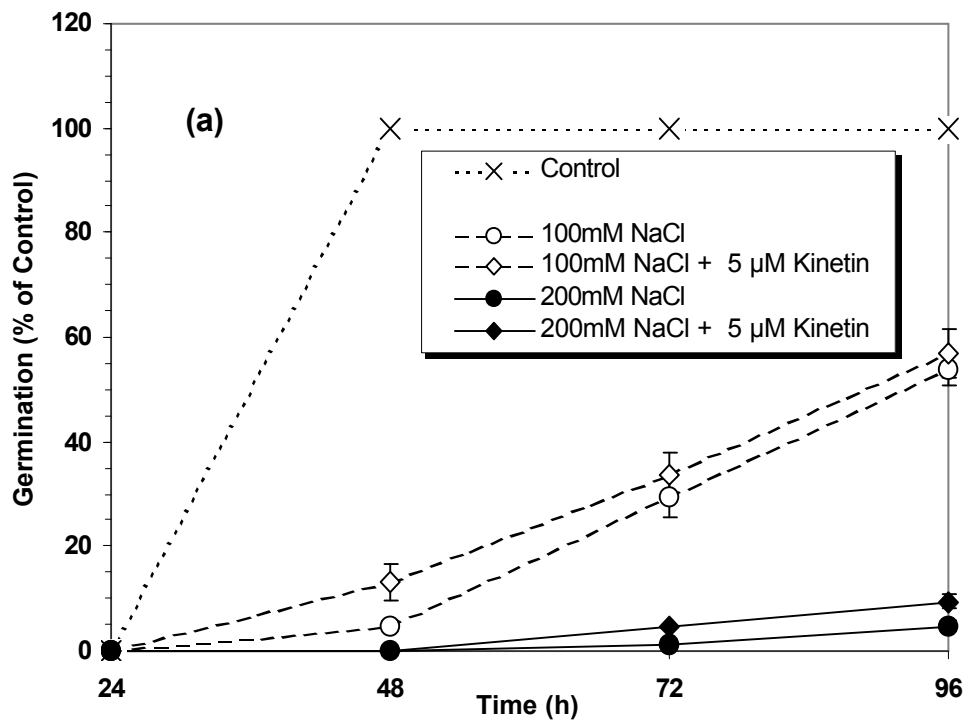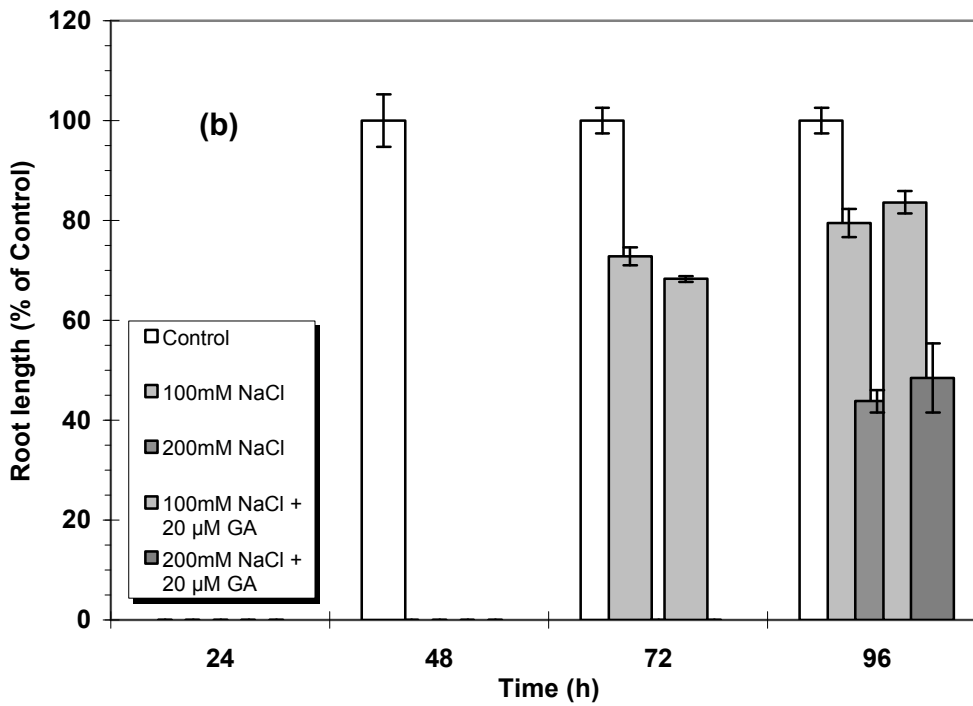

Fig. 13b: Influence of NaCl and NaCl + GA$_3$ on the root length of sugarbeet seedlings. Error bar means ± standard error of 3 replicate incubations with 100 seeds each.

3.1.4 Discussion

A widely accepted view is that hormones have specific functions in the various physiological processes necessary for germination and the establishment of a new plant. This is probably the main reason why transient changes in the levels of the different types of hormones have been observed during the process of germination (Khan et al., 1976).

In our study gibberellic acid and kinetin were found to be effective in alleviating salt-induced stress conditions in maize and sugarbeet seeds (Fig. 9a, 10b). It is possible that under saline conditions there is a large decrease in the production of plant hormones in these seeds and exogenous application of phytohormones ameliorates the inhibitory affect of NaCl on germination. Other possible explanations include a changed balance of phytohormones, or a change in the hormone receptors.

Gibberellic acid either reduces moisture requirement or enhances water uptake during germination and early seedling growth. NaCl inhibits growth by reducing both cell division and cell enlargement (Nieman, 1965). The observed increased seedling

growth of stressed maize or sugarbeet seeds with GA$_3$ treatment could be due to an inherent attribute of gibberellic acid in increasing cell division and cell elongation (Scott, 1984).

In dicotyledonous plants there is a large body of literature which indicates that cytokinins stimulate cotyledon expansion (Rijven and Parkash, 1970; Hutton et al. 1982) and that cytokinins are transported between the cotyledons and embryonic axis (Gepstein and Ilan; 1979). Within the cotyledons, the cytokinins are apparently involved in reserve mobilization (Metivier and Paulilo, 1980).

Kinetin has been reported to enhance the uptake of water due to an increased membrance permeability (Kaufmann and Ross, 1970). Kinetin is capable of breaking stress-induced dormancy during germination of tomato, barley, and cotton seeds (Bozcuk, 1981). Moreover, the observed reduction in endogenous cytokinin under stress conditions (Itai et al; 1968) points towards the possibility that cytokinins levels could be a limiting factor under stress conditions and thus explain the fact that an exogenous applicaiton of kinetin resulted in increased growth of sugarbeet seedlings (Fig. 10a).

There is the possibility that cytokinins are primary factors in the initiation of radicle growth. Alternatively, they may play a role in nutrient mobilization, particularly during the germination process and seedling establishment. Recent studies with *Zea mays* have shown that, when zeatin was applied to 24 h imbibed kernels it was rapidly metabolized, and that approximately 99% of the radioactivity remained in the endosperm if applied to this tissue. In view of the rapid rate at which the applied zeatin was metabolized, and the occurrence of enzymes of the cytokinin oxidase type in *Zea mays* kernels, it was concluded that if cytokinins are at all involved in the germination process of maize kernels, it is unlikely that they will exert their effect via derivatives with an unsaturated side chain (Whitty and Hall, 1974; Van Staden, 1981a). Worth mentioning is that dry and imbibing maize kernels contain a number of dihydro cytokinin derivatives (Summons et al, 1980) which are resistant to oxidation.

When radish cotyledons are grown in weak light, cytokinins increase the internal production of reducing sugars (mainly glucose and fructose) and these sugars act osmotically to cause the water uptake that drives growth (Huff and Ross, 1975; Bewli

and Witham, 1976). They then found for both radish and cucumber cotyledons that cytokinin treatment causes increased plasticity (but not elasticity) of the cell walls; that is, the walls become loosened so they will expand faster irreversibly under the existing turgor pressure (Thomas et al., 1981).

GA_3 and kinetin both partially alleviated the affect of salinity on germination and growth of root and coleoptile of maize and sugarbeet, which is supported by the experimental data of many scientists who suggested high salt concentration could be alleviated by GA_3 (Kabar and Baltepe, 1990; Ungar, 1991) and kinetin (Khan and Ungar, 1997b; Khan and Weber 1986; Khan and Rizvi, 1994)

Our data may be interpreted that GA_3 and kinetin counteract the stress conditions by enhancing seed germination and early vegetative growth, which ultimately leads to better seedling growth.

4   Effect of Salt Stress on the Hydrolytic Enzyme Metabolites During Germination of Maize and Sugar Beet

4.1   Introduction

Salinity is a major environmental stress which unfavorably affects germination of seeds (Dubey, 1984). Salt resistance and salt sensitivity are not due to one single factor but result from a number of them (Stewart and Larher, 1980). Dry seed is characterized by a remarkably low rate of metabolism. This is probably a direct result of the very low level of hydration of the seed, whose water content is of the order of 5-10%. Despite this almost complete absence of metabolism in the seed, it cannot be assumed that it lacks the potential for metabolism.

Plants normally encounter salinity for the first time during seed germination. Understanding the responses of plants at this stage is particularly important for elucidating the mechanisms of salt resistance and sensitivity in plants and their survival. Although seed germination is but a single step in the life cycle of a plant, the process is very complex and is influenced by many environmental factors (Mayer and Poljakoff-Mayber, 1963). The metabolic rate begins to rise rapidly when the seeds are

placed into water. The metabolic changes occurring in the early stages of germination are the result of the activity of various enzymes, which are either present in the dry seed or very rapidly become active as the seed imbibes water. Generally, enzymes breaking down starch, protein, lipids, and other storage materials, increase in activity fairly rapidly as germination proceeds. To what extent germination and increased enzyme activity are causally related is at best doubtful as germination proceeds.

Metabolic processes which occur upon imbibition are complex. They can be divided into three major types: 1) breakdown of reserve compounds, 2) transport of breakdown material from one part of the seed to another, 3) synthesis of new compounds during growth. Carbohydrates play an important role in regulating osmotic concentrations of cells during the germination process and could affect resistance to osmotic stress. The large amount of soluble sugars present in germinating seeds are apparently the result of the breakdown of reserve carbohydrates, such as starch and oligosaccharides. The ratio between the various sugars and oligosaccharides changes as a result of the activity of the enzymes discussed above. The

great majority of seed proteins are metabolically inactive and serve merely as food reserves used by the growing embryo during germination. Protein synthesis begins in the various embryonic organs immediately with the beginning of their growth.

Generally, the only substances that have to be taken up by seed in the germination period are water and oxygen. Thus it can be concluded that the dry seed is a well functional unit, which is able to carry out a large number of biochemical reactions if placed into aquatic medium. The purpose of this study was to investigate the changes in the composition of maize and sugar beet seed which occur upon imbibition in salt-treated solutions.

4.2 Materials and methods

The cultivation of sugar beet (*Beta vulgaris* L. cv. Evita) and maize (*Zea mays* L. cv. Pioneer 3906) and experimental procedures were carried out as follows. Briefly, seeds under investigation were grown in plastic petri dishes (94 mm diameter, 16 mm height) with a tight-fitting lid (c.A. Greiner u. Söhne Kunststoffwerke, Germany). Fine sand was washed thoroughly with

deionized water, dried completely at 125°C and 20 g were filled into each petri dish. The nutrient solution consisted of 1 mM Na_2SO_4, 1 mM K_2SO_4, 1 μM H_3BO_3, 1 mM $CaSO_4$ with varying concentrations of NaCl (0, 50, 100, 200 mM). The pH of all solutions was adjusted to 6.0 (±0.2). In one petri dish, 50 seeds were germinated with 20 ml solution (4 ml for sugar beet seeds). The experiments were carried out in an incubation chamber (Memmert ICE 400-800, Germany) at 25° C in the dark. The experiments were conducted in triplicate. For detection of protein and α-amino acid content the extraction technique of Schubert (1986) was used.

4.2.1 Water intake:

The water intake was determined by first weighing the dry seeds, then placing 1 g of germinated and blotted dry seeds of maize and sugar beet, after 48, 72, 95 h respectively, into weighing bottles and placing them into an oven at 105° C for 24 h and reweighing them again in order to determine the amount of water intake per g seedling.

4.2.2 Soluble protein content:

For the quantitative measurement of soluble protein the method of Bradford (1976) using the principle of protein-dye binding with coomassie blue was adapted. For each replicate, three determinations were made. The protein standard was treated in the same way as the homogenate.

4.2.3 α-Amino acid content:

For the analysis of α-amino acids, 200 mg of the whole freshly germinated seeds of each concentration level of NaCl were rapidly frozen in liquid N_2 and stored at -20 °C. The amino acid content in the seedling was determined by spectrophotometry using ninhydrin reagent according to the method of Rosen (1957). Three replicates were analyzed for each treatment (control, 50 mM, 100 mM, 200 mM NaCl) and the average was calculated. Amino acids were measured after 48, 72, and 96 h of germination using the above-outlined procedure. Leucine was used as standard.

4.2.4 Starch content:

For starch extraction and determination, the method of Boehringer was conducted using enzymatic test kits (Boehringer, 1980). For that, 200 mg of the whole freshly germinated seeds, which were frozen in liquid N_2 and stored at -20°C, were used. The principle of starch determination using the Boehringer method:

In the presence of the enzyme amyloglucosidase, starch is hydrolyzed to glucose. The content of glucose is determined with hexokinase and glucose-6-phosphate dehydrogenase.

1. Starch + (n-1)H_2O $\longrightarrow$ n D-glucose
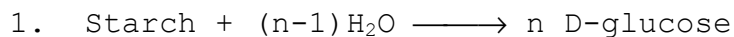
   The glucose is phosphorylated to glucose-6-phosphate by ATP in the presence of hexokinase with formation of ADP.

2. D-glucose + ATP $\longrightarrow$ G-6-P + ADP

   The glucose-6-phosphate is oxidized by (NADP) to gluconate-6-phosphate with formation of (NADPH).

3. G-6-P + NADP$^+$ $\longrightarrow$ D-gluconate-6-phosphate + NADPH + H$^+$

   The amount of which is determined by means of light absorbance by spectrophotometer at absorption maximum of 340 nm.

4.2.5 Soluble sugar content:

For the analysis of various types of soluble sugars, 0.3 g of the whole freshly germinated seeds of each concentration level of NaCl in three replications were frozen with liquid N_2, freeze-dried, and ground with a mortar and pestle. Extraction and the following cleaning were accomplished using a technique described by Mühling (1991). After that the pure liquid extract was diluted at a ratio 1:10. The various types of soluble sugar were determined using the ion exchange column chromatography detection. Identity of the sugars was confirmed by comparing R_F values with those of standards (see Fig. 14).
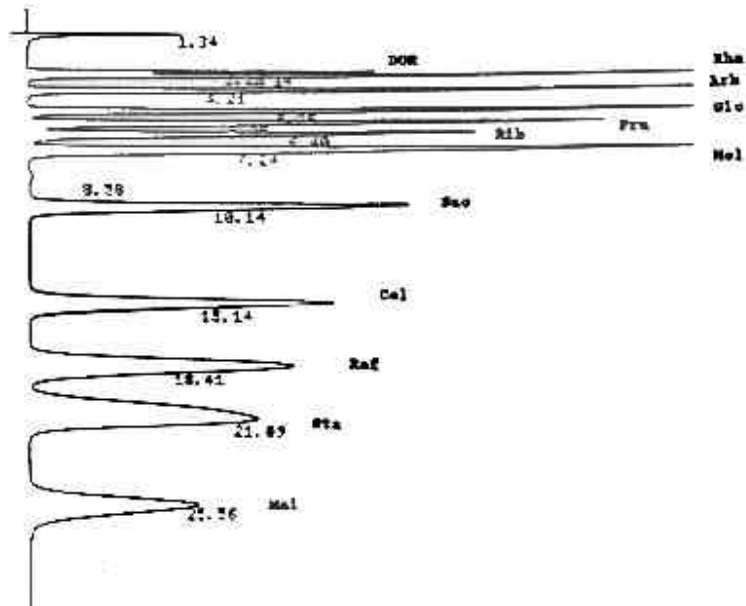
Fig. 14 Chromatogram of sugar standard solution

Elution: 100 mM NaOH Top

Elution: 5 mM NaOH Bottom
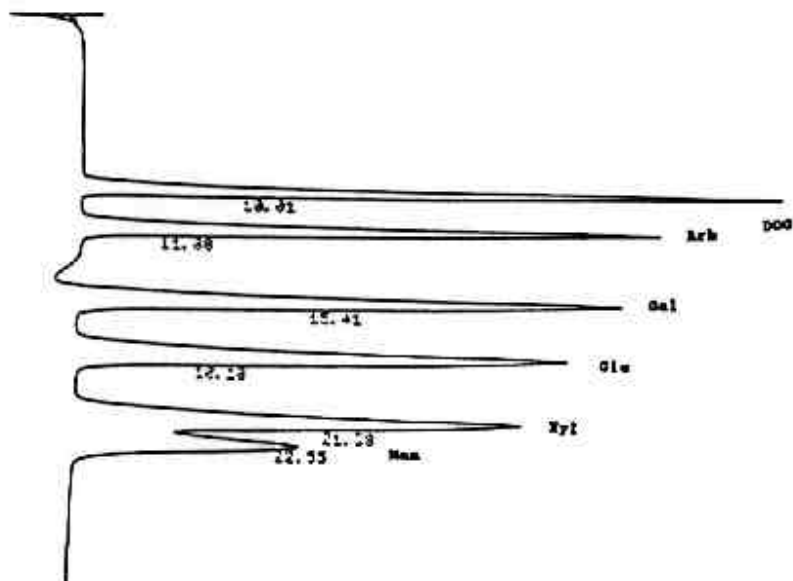
4.3 Results

4.3.1 Water intake

Imbibition of water from NaCl treatments (50, 100, and 200 mM) was decreased because of the low external water potential of the solutions. The larger seeds of maize (Fig. 15a) took up more water and faster than smaller seeds of sugar beet, particularly in the control treament. Higher concentrations of NaCl (200 mM) resulted in less imbibition of water than at lower concentrations of NaCl.
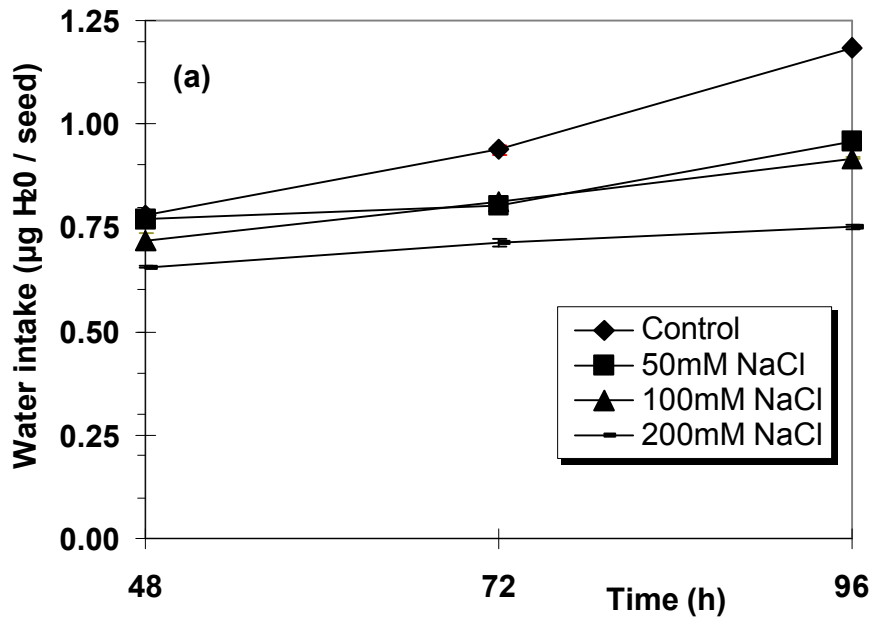
Fig. 15a: Influence of NaCl on water intake during germination of maize seeds.
Error bar means ± standard error of 3 replicate incubations.

Table 5: Influence of NaCl on seed imbibition during germination of maize seeds

(Values represent % decrease in weight due to lower water uptake in seeds)

Hours	Control	50mM NaCl	100mM NaCl	200 mM NaCl
48	100%	99%	92%	84%
72	100%	86%	87%	76%
96	100%	81%	77%	63%
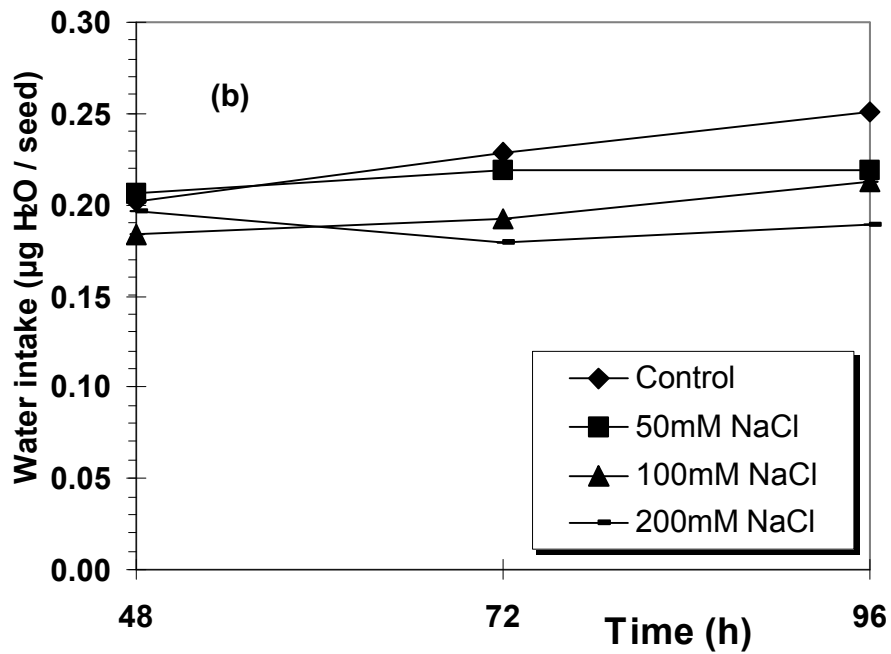
Fig. 15b: Influence of NaCl on w ater intake during germination of sugarbeet seeds. Error bar means ± standard error of 3 replicate incubations.

Table 6: Influence of NaCl on seed imbibition during germination of sugarbeet seeds

(Values represent % decrease in weight due to lower water uptake in seeds)

Hours	Control	50mM NaCl	100mM NaCl	200 mM NaCl
48	100%	102%	91%	97%
72	100%	96%	84%	78%
96	100%	87%	84%	75%

4.3.2 Soluble protein content

Soluble protein contents in seeds changed greatly throughout seed development in salt-treated compared to dry seeds before the start of experiment in maize seedlings. (Fig. 17a). In contrast to maize in sugar beet there were higher protein contents in seeds due to salt treatments relative to the control treatment (Fig. 17b).
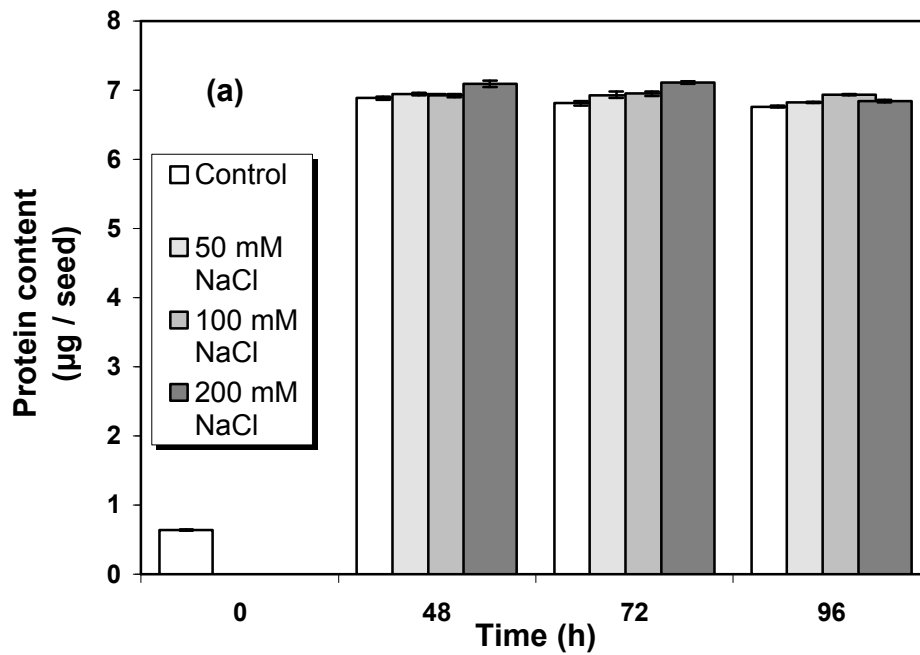
Fig. 17a: Effect of NaCl on protein content during germination in maize seeds. Error bar means ± standard error of 3 replicate incubations. Zero time is protein content in dry seed before germination.
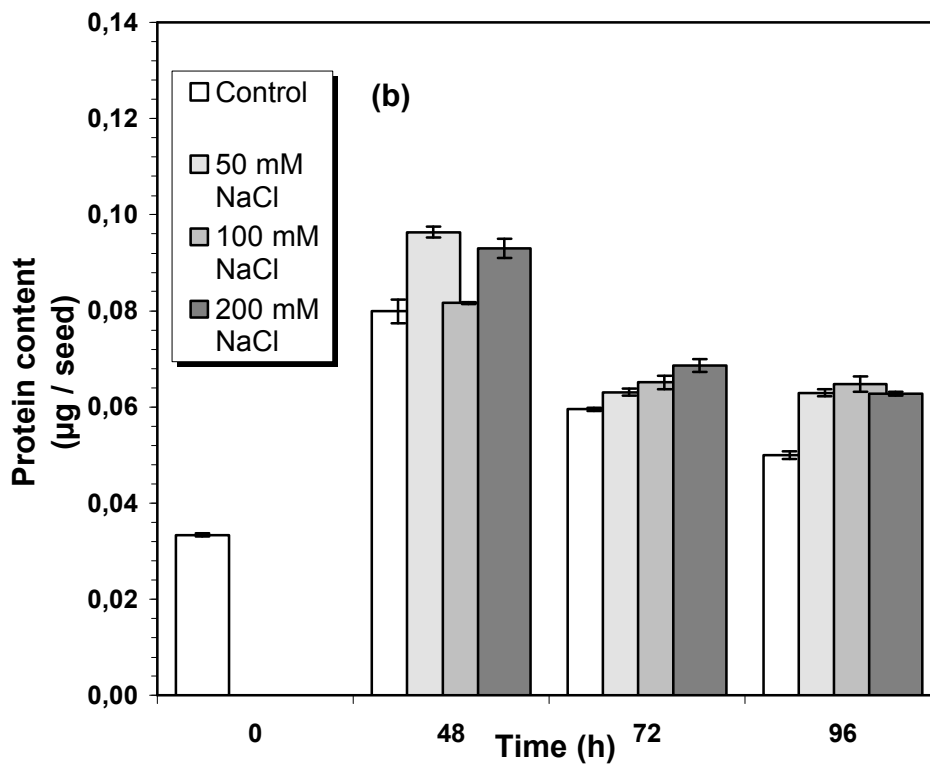
Fig. 17b: Effect of NaCl on protein content in sugarbeet seeds during germination. Error bar means ± standard error of 3 replicate incubations. Zero time is protein content in dry seed before germination.

These differences were more accentuated after 48 h than at any other time. The protein content increased substantially during germination, compared to dry seeds before the start of germination in both of these plant species.

4.3.3 **a**-Amino acid content

During germination a gradual increase in amino acid content occurred in both seed species in all salt treatments. The increase was much higher during 48-96 h in sugar beet than in maize during 48-72 h and thereafter a decline up to 96 h in control as well as salt treatments (50 mM) in maize in the whole seedling basis (Fig. 18b).

At all treatment levels, amino acid contents in sugar beet seeds were much more depressed than in maize seeds, particularly at the high NaCl concentrations.
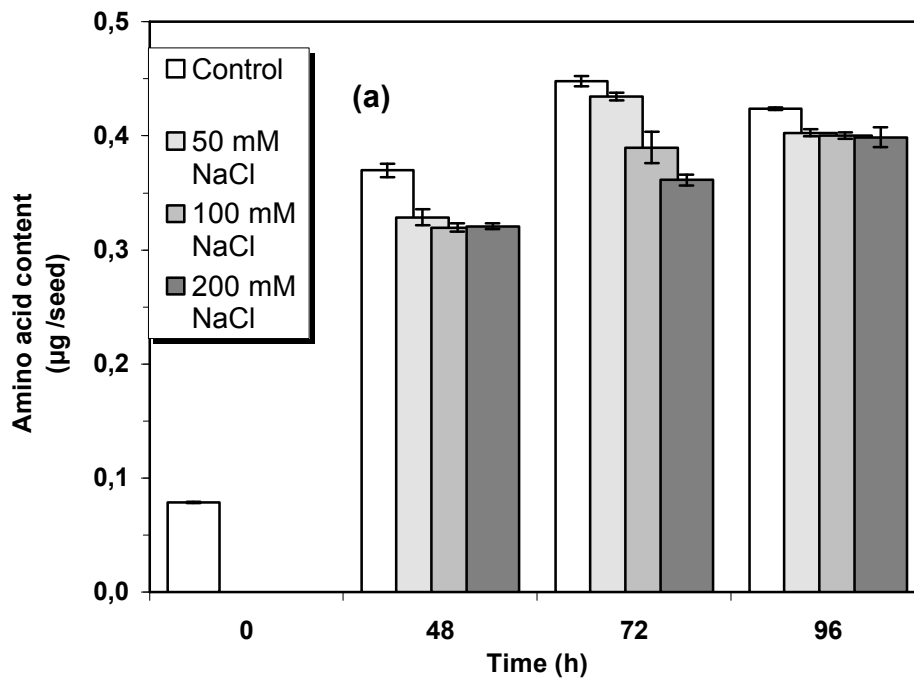
Fig. 18a: Effect of NaCl on amino acid content in maize seeds during germination. Error bar means ± standard error of 3 replicate incubations. Zero time means amino acid content before the start of the experiment in the seeds.
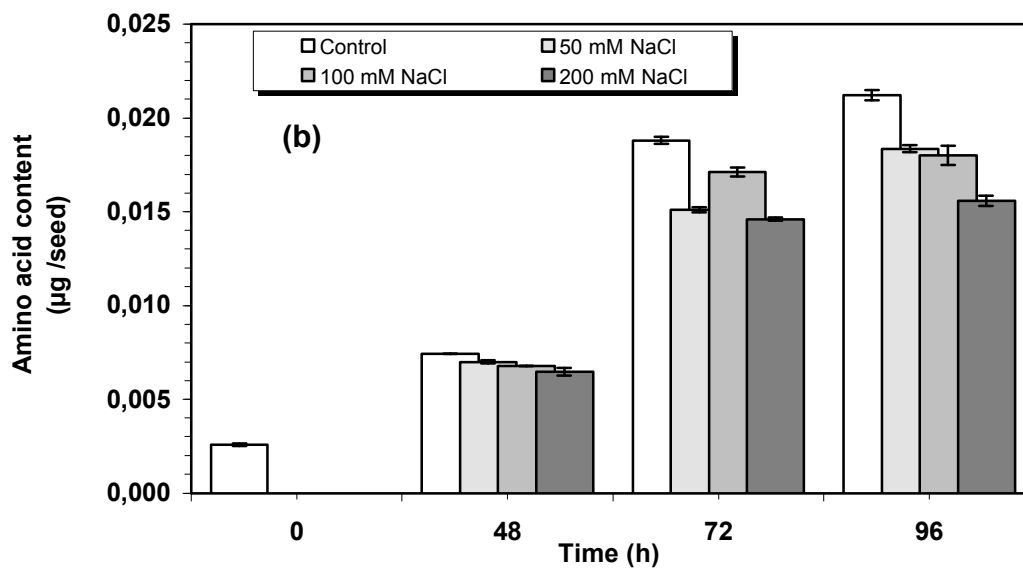
Fig. 18b: Effect of NaCl on amino acid content in sugarbeet seeds during germination. Error bar means ± standard error of 3 replicate incubations. Zero time means amino acid content before the start of the experiment in the seeds.

4.3.4 Starch content

In both populations of seeds (maize and sugar beet), the content of starch in the whole freshly germinated seeds of maize and sugar beet was about 20-50% lower than dry seeds after 48 h.

Interestingly, this decrease in sugar beet seeds was about twice as high as that of maize seeds. There was a dramatic decrease in relative starch content in both seed species as germination progressed compared to its content on day (0) before the start of germination.
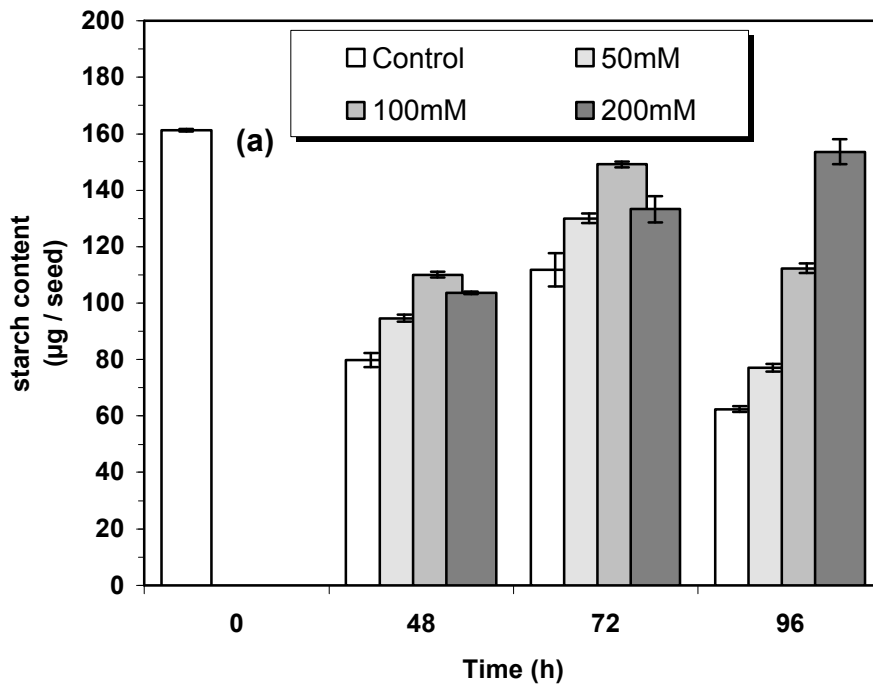
Fig. 19a: Effect of NaCl on starch content in maize seeds during germination.
Error bar means ± standard error of 3 replicate incubations. Zero time means starch
content before the start of the experiment in the seeds.
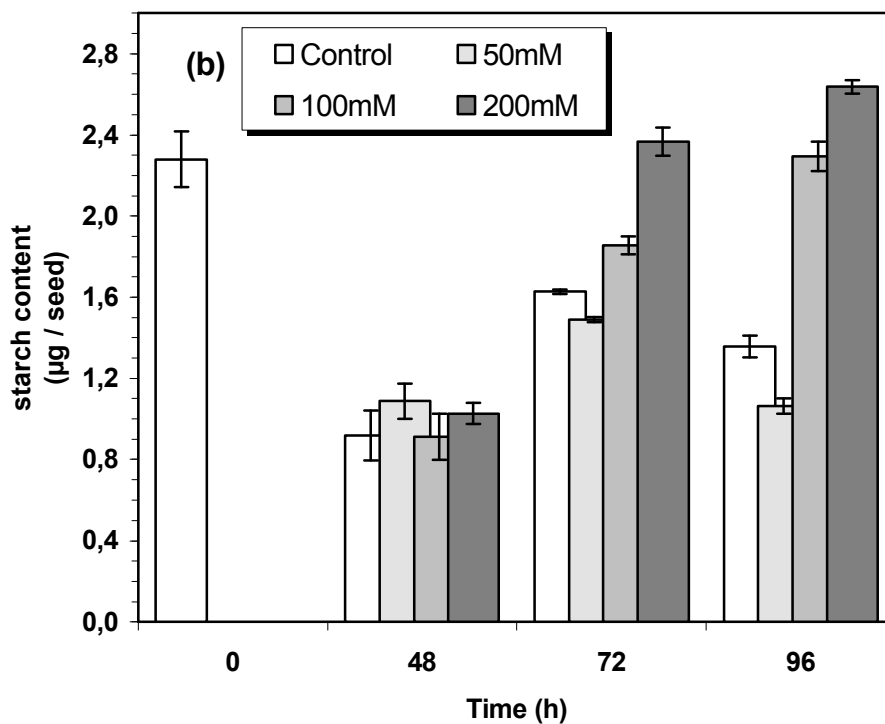
Fig. 19b: Effect of NaCl on starch content in sugarbeet seeds during germination.
Error bar means ± standard error of 3 replicate incubations. Zero time means starch
content before the start of the experiment in the seeds.

4.3.5 Soluble sugar contents

Effects of salinity stress on various types of soluble sugar components of corn and sugar beet seedlings are shown in Fig. 20 and Fig. 21, respectively.

In maize, glucose, fructose and maltose contents were increased in salt-stressed treatments (except sucrose) in all NaCl treatments compared to day (0) dry seed.   Sucrose content decreased in all salt treatments (Fig. 20). The increase in glucose and maltose content was dramatic in maize, both of which were dominant sugars under salt-stressed and non-stressed conditions.

Contents of individual sugars in seeds of the two plant species, differing in their responses to salinity, showed   clear patterns.   Sucrose and maltose levels tended to be lower in sugar beet under salt stress when compared with its content level in dry seeds (Fig. 21).

Maltose level decreased in sugar beet seedlings at all salt levels.   It appears that glucose was the dominant sugar under

both stressed and non-stressed condition in these plant species.
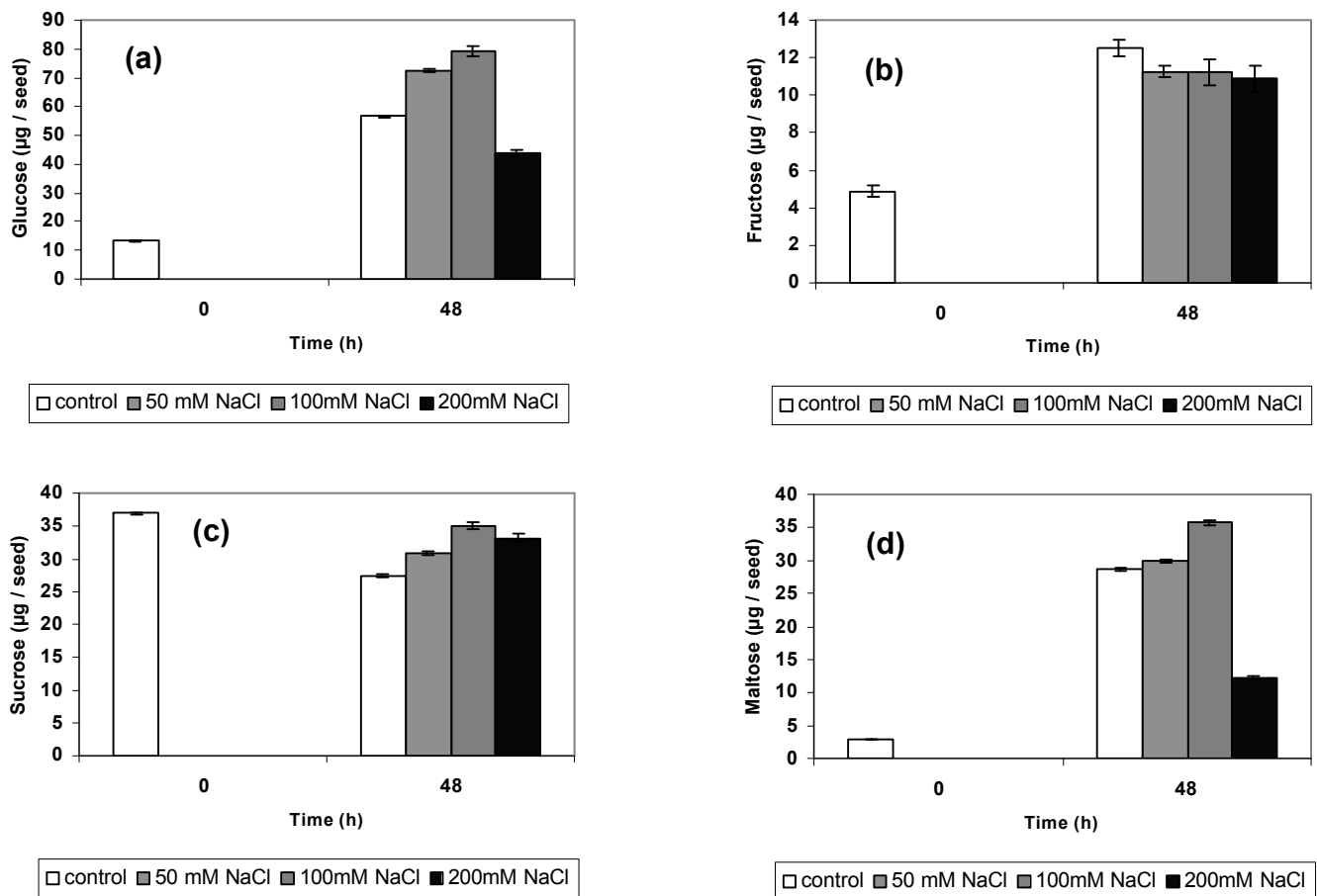
Fig. 20: Effect of NaCl on concentration of glucose (a), fructose (b), sucrose (c) and maltose (d) during germination of maize seeds. Error bar means ± standard error of 3 replicate incubations. Zero time means soluable sugar concentration before the start of the experiment in the seeds.
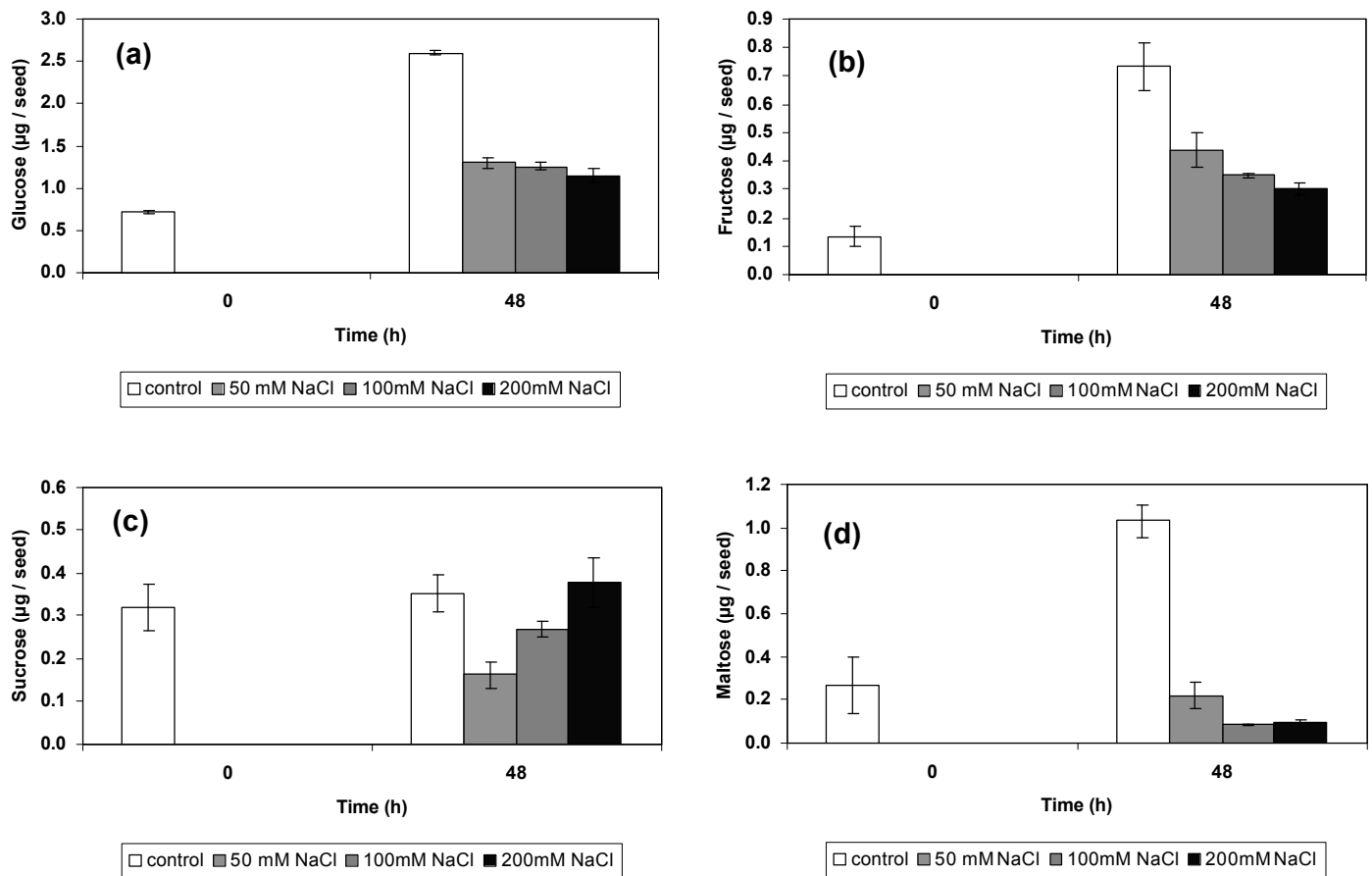
Fig. 21: Effect of NaCl on concentration of glucose (a), fructose (b), sucrose (c) and maltose (d) during germination of sugarbeet seeds. Error bar means ± standard error of 3 replicate incubations. Zero time means soluable sugar concentration before the start of the experiment in the seeds.

4.4 Discussion

The physical process of water uptake leads to the activation of metabolic processes and the dormancy of the seed is broken following hydration.   At the low osmotic potential, NaCl inhibited the processes of imbibition of both maize and sugar beet (Fig. 15 a,b). The drop in the rate of water uptake by the large and small seeds of *Zea mays* L. and *Beta vulgaris* L. when they were soaked in NaCl solutions of increasing concentration is probably caused by the decrease in water potential gradient between the seeds and their surrounding media (Bewley and Black, 1994; Bradford, 1995). This difference in water uptake by maize and sugarbeet when treated with NaCl could be seen in Fig 15 a,b.

The first phase of water uptake by the seeds involves movement of water into the free space (apoplast) and does not depend on the osmotic potential of the surrounding solution (Simon, 1984).
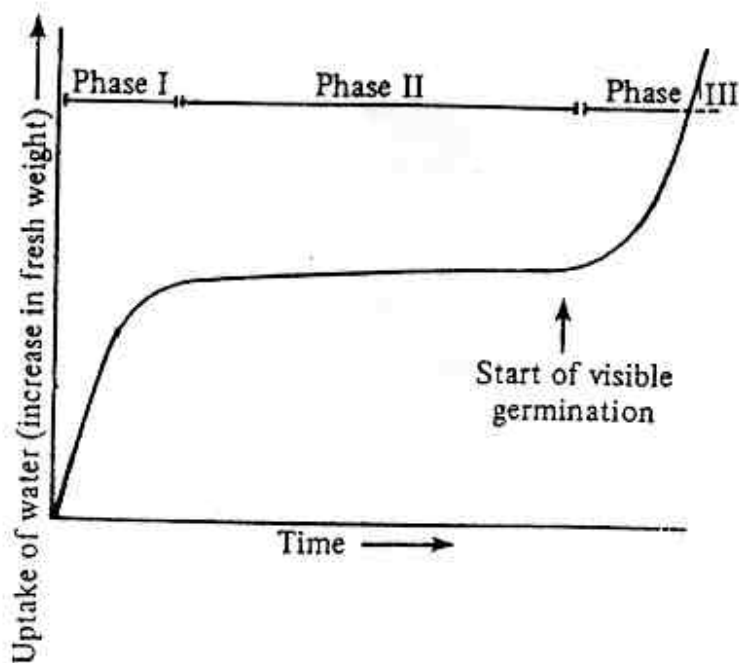
Fig. 22. Triphasic pattern of water uptake by germinating seeds (Bewley and Black, 1978).

The second slower linear phase of water uptake involves the movement of water across cell membranes into the cells of the seeds and is determined by the difference between the osmotic potential of the seed and that of the medium. NaCl may cross the cell membrane into the cytoplasm of the cells unless an active metabolic pump prevents accumulation of the ions. In some cases, NaCl in the cytoplasm can result in toxic accumulation of a particular ion or decreased availability of

some essential nutrients (Levitt, 1972).

The differences observed between species and cultivars with respect to the effects of salinity in germination and enzyme activity could reasonably be due to differences in the seed tissue osmoregulation (Sanchez-Diaz et al., 1982). According to Meyer and Boyer (1972), part of the solutes used for osmotic adjustment in seeds may come from their own reserves (instead of the photosynthates as occurs in plants) (Yeo, 1983). Rush and Epstein (1976) have found that the differences between the resistant against salinity of several species of *Lycopersicum* is due to the capacity of the most resistant ecotype to utilize Na^+ more readily than the others, possibly also substituting Na^+ for K^+ in metabolic functions.

Unlike distilled water imbibition, salinity modulates the production of proteins (Dell' Aquila and Spada, 1992). Certain aspects of synthesis and metabolism of proteins during salinity stress emerge from analysis of protein. One of the concerns of seed germination is that it involves the synthesis and activity of a number of degradative enzymes. According to Bennett and Chrispeels (1972) during germination of barley seeds, the

activity of hydrolytic enzymes, including proteases, increases. The effects of salinity on such enzymes would, for instance, have a marked influence on protein turnover in germinating seeds. There are some indications that salinity might activate such enzymes (Mercado, 1973), similar to other environmental stresses such as water stress (Wilson, 1975). In maize seeds the percent of protein contents were all identical in the unsalinized and salt treatment suggesting that salinity apparently did not preferentially alter turnover of proteins (Fig 17a). The results presented here indirectly address the nature of such activities during salinity stress. The relative synthesis of proteins of the seeds pre-emergence phase was enhanced by salinity at 48 h as germination began, where proteins associated with the radical emergence phase were produced (Fig 17 a,b).

In addition to the remarkable maintenance of steady-state levels, the data demonstrates that the synthesis of proteins was dynamic during salinity stress. Unlike its effects on maize, salinity induced significant changes in protein content in sugar beet, particularly after 48 h (Fig 17b).

Protein synthesis changes are due to changes in the efficiency of mRNA translation or to regulation of mRNA transcription, transport and stability. As a seed, the embryo passes from a quiescent to a proliferative stage, considerable changes in mRNAs occur, including a rapid loss of mRNAs coding for storage proteins (Cuming, 1984), and the appearance of mRNAs specific for new proteins associated with the germination process (Bewley and Marcus, 1990). Differential mRNA transcription under salt stress has been described in two differently salt-sensitive barley genotypes by Ramagopal (1987).

The accumulation of the proteins, not produced during water imbibition, suggests that the function of "salt stress" protein may be related to the seed adapting to salinity, possibly in a way similar to other kinds of stress responses, for example, to heat shock, in providing a protection function (Abernathy, et al., 1989). The genetic constitution of a selected salt-resistant genotype may better contribute to the regulation of "salt stress" protein synthesis as well as to the maintenance of seed viability under extreme conditions of salinity.

It is not likely that the water limitation impeded the formation

of the protein but rather interferes with its further activation (Mayer, 1977). The biochemical data presented here strengthen that postulate and further suggest that synthesis of the unique proteins of plant life cycle might be crucial for expression of these phenomena.

The results of this study indicate that salinity had a significant effect on protein synthesis during seed germination in sugar beet. While the present study suggests that salinity regulated these processes, it does not identify a specific mechanism and it does not indicate that the same mechanism was responsible for the regulation of protein.

Total α-amino acid analyses were performed to ascertain whether there were differences between these species under salt stress and nonstress conditions, and to note any correlation between the levels of these organic solutes and salt resistance in terms of mechanism for maintaining a low water potential in the seedlings, as is necessary for survival in saline environments (Kramer, 1969).

The rise in amylase activity in the seed during germination is

primarily an α-amylase which, when amylolytic activity is at its peak, accounts for 90% of total amylolytic activity. Starch content of NaCl-treated seeds relative to dry seeds was significantly lower in both plant species (Fig. 19 a,b). This indicates that the hydrolytic enzyme activity was inhibited by salinity. Interestingly, this effect was about twice as high in sugar beet, which in our earlier experiments showed higher salt sensitivity during germination than maize. A similar decrease in the amylase activity under salt stress is widely documented (El-Fouly and Jung, 1972; Sheoran, 1980). A greater proportion of the soluble sugar produced due to amylase and invertase activities from salinized seeds, however, seems to be used up as a respiratory substrate (Dhingra, 1984). This may be one of the factors leading to a poor germination in sugar beet.

During the early period of seedling growth, there is hardly any possibility of appreciable photosynthetic activity and the main source of energy for this growth is derived from the sugars released by enzymatic hydrolysis of starch stored in seeds. It would thus seem logical that the adverse effect of salt on seedling growth is also shared by the sugars as well as the hydrolyzing enzyme amylase.

Sugar analyses were performed in order to find out if there was a similar build-up of sugars under salt stress conditions as another possible means of dealing with low water potentials of saline environments.   Results show that salinity effects germination and, to a greater extent, axis growth, largely due to decreased enzyme hydrolysis with stress and consequently to the reduced sugar input from the endosperm to the embryo. Similarly, the results indicate that sucrose formation is also affected by stress (Fig. 20 and 21).

Our analytical results on the sugar constituents showed that glucose and maltose are the major soluble sugar components in maize at the germination stage, although sucrose comprised the major soluble sugar in the dry seed.

Often speculation has been made concerning the role of scutellum in the embryonic development of Gramineae based on plant anatomical studies.   The scutellum is located between the endosperm and the embryo and its role in the metabolism of germinating cereal seeds is important.   There is also experimental evidence that the scutellum secretes digestive enzymes that hydrolyze the reserve starch in the endosperm, and

eventually the scutellum translocates soluble sugars to the growing parts of the embryo (Dure, 1960). Data of Dowex-I ion exchange column chromatography of the soluble sugar component of the seeds, clearly show that glucose and maltose account for the greatest portion of the soluble sugar in the seeds of maize, while fructose and sucrose are minor components.

Edelman et al. (1959) studied the function of scutellum, they found that glucose is removed from the endosperm, converted to sucrose in the scutellum and transported to the embryo. It will be noted that up to 48 h, the sucrose content remained roughly constant, whereas its content declined compared to dry seed (Fig. 20). Thus our present findings suggest that glucose derived from the endosperm by amylolytic breakdown is mobilized in the scutellum, where it is resynthesized to sucrose. The latter is then transported to the embryonic axis for further metabolic purposes. This mechanism, however, may not be excluded if the sucrose formed is accumulated in the vacuoles for osmotic compensation.

On the other hand, the embryonic axis which is rapidly growing does not accumulate starch but monosaccharides or sucrose which

can be readily used for the various metabolic processes. Contrarily, our finding suggests that even very mild stress greatly affects sugar beet seed germination and development. Most of these effects are very closely related to the inhibition of the seed enzyme activity during stress which in turn seems to be the primary effect of salinity on germination of sugar beet.

Quite likely, osmotic effect of NaCl (water stress) could be aggravated due to energy expenditure requirements for osmoregulation (including accumulation of compatible organic solutes in the cytoplasm). As a consequence of the latter, the transition from the germination stage to young seedlings will be seriously affected. Thus, it appears that a decrease in viability and germination is related to salinity-induced disturbance of metabolic processes leading to the decrease in soluble carbohydrate, α-amino acid and protein. This is also associated with a decrease in the content of starch. Similar depressive effects of salinity of starch content (Downton, 1977, Chandrashekar, and Sandhyarani, 1995) gives further support to the above suggestion.

For all salinity treatments, *Zea mays* seeds showed a greater

enzyme activity than *Beta vulgaris* L. which could be due to a greater amount of carbohydrate in the endosperm in the former. Indeed, the *Zea mays* seeds are much bigger than *Beta vulgaris* L. The great amount of food reserves stored inside, among which minerals and soluble carbohydrates are included (Sömme, 1971) can contribute to fast embryo growth and, consequently, facilitate the radicle emergence when water conditions are favorable. Under salt and water stress these reserves will partially protect the seed from drought and salt toxicity and will thus contribute to osmoregulation (salt stress) and osmotic adjustment (water stress).

5 General discussion

Salinity and drought are the most important devastating problems
in most regions of the world. Seeds are vulnerable to this kind
of stress, especially the osmotic adjustments of the seeds. It
is commonly accepted that most seed species cannot tolerate
salinity higher than 10 to 20 percent sea water, and many do not
grow at even lower concentrations (Ashraf, 1994).

The questions of why some seeds are able to grow well under
saline conditions has been answered in the light of scientific
achievements. In some salt-resistant species, uptake of
excessive sodium is regulated through ion flux mechanisms.
(Jeschke, 1984). In others, if some Na^+ ions manage to reach the
root of growing seed, then upward movement is retarded (Ahmed et
al., 1992), while in still others, if excessive Na^+ ions are
translocated into shoot, the foliage either secretes them out
through glands (Fahn, 1988) or develops succulence to dilute
down their toxic effects.

Change in seed metabolism and accumulation of osmoregulants
could also be responsible for building salt resistant seeds

(Gorham et al., 1985). Growth analysis studies revealed that a salinity level of 50 mM NaCl diminished total germination, root, and shoot growth significantly in sugarbeet and cotton (Fig. 2 and Table 2). Further increase up to 200 mM NaCl diminished seed growth in these species progressively. This inhibitory effect of germination could be due to the water potential gradient between the seed and the external medium (osmotic effect), which leads to a diminished influx of water (See the percent of water influx in maize and sugarbeet seeds Tab. 5 & 6). Maize and rice showed a higher level of germination in various NaCl concentration. They also maintained a higher level of root and shoot growth (Fig. 3 and Table 2).

The results suggest that when seeds are first exposed to salinity (for example, 50 mM NaCl), they appear to osmoregulate rapidly so that turgor pressures are maintained. It is likely that Na^+ and Cl^- accumulated intercellularly in salt-adapted cells are compartmentalized to a greater extent in the vacuoles. The difference between species varieties, the duration of the experiment and growth condition could also explain the responses.

Ashraf et al. (1988) screened a number of lines of blackgram at germination and seedling stages for their resistance ability to NaCl, Na_2SO_4, $CaCl_2$ and $MgSO_4$. A great amount of variation was observed among lines in relation to these salts. NaCl was found to be more toxic at the germination stage than the other salts.

Polyethylene glycols of large molecular weight (6000) are found satisfactory to decrease the osmotic potential of nutrient solutions for studies on the growth of seeds and eventually the whole plant. The permeability of the root system to PEG presumably depends on its physiological condition and on whether it has suffered mechanical damage. Only small amounts of PEG entered the seeds from solution with decreased osmotic potential, presumably because the seeds' permeability changed as the seeds water potential decreased.

On a simple analogy with Ohm's Law an increase in the gradient of water potential between the seeds and the medium would imply a decrease in the permeability of the seeds (Lawlor, 1970). There could be four mechanisms that may be responsible for the effects described: (1) PEG entered the seeds and decreased the osmotic potential of the xylem solution; (2) PEG interfered with

seeds metabolism; (3) PEG lowered the surface tension within the seeds, affecting cell permeability; and (4) PEG blocked the transpiration pathway.

The experiments with mannitol suggest that such a decrease in permeability was not a response to decreased seed water potential. The small quantities of PEG that enter seeds without causing visible damage may not decrease water movement until a minimum concentration is reached. This concentration of PEG would depend on the rate of transpiration, the size of the molecule and the structure of the transpiration pathway. The drop in the rate of water uptake by the large and small seed morphs of maize and sugarbeet when they were soaked in NaCl, of increasing concentration is probably caused by the decrease in water potential gradient between the seeds and their surrounding media (Osmond et al., 1980).

The results obtained are consistent with the view that salinity influences these seeds species during the experiment via an affect on seeds water relations. Since inhibition imposed by NaCl was no more severe than that of PEG or mannitol, it appears that the water stress effect was the principle factor related to

growth inhibition.

The adaptive bioenergetic events which lead to the adaptation of an organism to a change (increase) in salinity are complex. Of major concern is the energy requirement for the removal of the salt. The osmotic imbalance due to the extrusion of salt is overcome by the accumulation of inorganic and organic solutes.

Organic solutes, in addition to inorganic electrolytes, play an important role in osmotic adjustment of seeds under salt stress. The organic cytosolutes generally synthesized in response to low water potential of soil solutions are sugars, organic acids, amino acids and other derivatives (Wyn Jones, 1981). Of these, sugars and organic acids are the major organic solutes in many higher glycophytes (Salisbury and Ross, 1985). Cram (1976) suggested that sugars contribute up to 50 percent of the total osmotic potential in most naturally growing glycophytes.

Little is known about the precise biochemical reactions which govern resistance to salts (Cushman et al., 1990). In view of this, it is highly desirable to (*i*) catalogue various salt stress-responsive proteins/genes and (*ii*) look for utility of

these in enhancing the salt resistance, since the metabolism of seed is affected, causing the inhibition of seed reserve mobilization.

A significant increase in protein kinase-specific activity has been observed in salt-stressed seedling of alfalfa (Yupsanis et al., 1994). Especially since protein kinases and protein phosphatases are important in post-translational modification mechanisms for regulating the function of a number of enzymes as well as structural and modulator proteins through phosphorylation and dephosphorylation mechanisms (Budd and Chollet, 1988). The results obtained in our experiment regarding protein content are in agreement with the finding of Chandrashekar and Sandhyarani (1995), in which they found with increased salinity levels, the protein content increased, while the sugar content decreased in *Crotalaria striata* DC seeds. (Figure 17 a,b)

Our experiment with phytohormones proves that GA_3 and kinetin enhanced germination and seedling growth depending on the species (Fig. 10 a,b). Unfortunately, application of growth-promoting phytohormones was of short duration, with germination

and early vegetative prevailing.

Our suggestion to salinity problems may include modification of the environment (the non-biological approach) and/or of the physiology of the seeds (the biological approach). Pasternak and De Malach (1994) suggested that, given proper soil and drainage conditions, salt stress during sensitive growth stages can be alleviated and consequently the threshold of salinity admissible for irrrigation can be increased, by optimization of saline water irrigation. Seeds for growing on salt-affected soil may be selected on the basis of their salt resistance with reference to the prevailing salinity. Among the conventional crop seeds, one can find salt-resistant species, which can grow under relatively high salinity of soil/irrigation water and still give economically feasible yield. On the other hand, halophytic seeds of commercial importance are capable of growing luxuriantly under very high salinity levels. Advantages could also be taken for growing such salt-resistant seeds with reference to the prevailing salinity of the medium.

However, irrigation/drainage schemes along with chemical soil treatments (non-biological methods) are often slow and costly,

especially for developing countries and therefore alternative solutions are needed (Forster, 1992). Some approach may include the introduction of wild species that have evolved salt resistance in their native habitats, and to develop them into halophytic crops.   Bernstein and Francois (1973) have illustrated directions of build-up profile salinity and its effect on the yield of bell pepper under two salinity regimes.

6   Summary

Salinity is a common problem for agricultural productivity in many parts of the world and in land areas that have become arid. A stage in the plant life-cycle vulnerable to salinity stress is seed germination. Successful seedling establishment depends on the frequency and the amount of precipitation as well as on the ability of seed species to germinate and grow while soil moisture and osmotic potentials decrease. The changes induced by salinity in any one of the particular physiological or anatomical parameters vary considerably. This depends on plant species, stage of development, and external factors such as salt regime. Based on this information, it is clear that the demands for the use of salinized land for agricultural purposes and the reinstatement of these lands for agricultural use in the present time and in the future require a better understanding of the nature of salt resistance and salt sensitivity during seed germination. The present study was, therefore, conducted to deal with the following objectives:

Major objectives:

1. To determine the effect of different levels of salinity (NaCl) on the germination of seeds (of sugar beet, cotton, maize, and rice).

2. To investigate the extent of osmotic and specific ion effects on germination by using different types of inorganic salts: NaCl, $CaCl_2$, and Na_2SO_4 on the above-mentioned plant species.

3. To evaluate the effect of water potential (drought simulation) using osmotic substances such as PEG and mannitol and comparing these with NaCl during germination on these plant species.

Secondary objectives:

I. Identify salt-resistance and salt-sensitive plants during germination.

II. To assess the exogenous application of GA_3 and kinetin

on germination and early seedlings growth under salt-stress conditions.

III. To identify differences in salt resistance among species during germination and to identify the mechanisms that are responsible for these differences.

The results of the series of experiments are summarized as follows:

a. Rice and maize showed high salt resistance during germination. The data also indicates that germination of cotton and sugar beet was more sensitive to the stress caused by NaCl.

b. When substrates of equal osmotic potentials are applied, the overall effect of salts on germination and early seedling growth appears to be as follows: Na_2SO_4 reduces germination much more than $CaCl_2$ followed by NaCl. Any ranking of specific ions seems to be difficult, since particular combinations of cations and anions appear to be more inhibitory than

others.

c. The results indicated, that isotonic solutions of PEG, Mannitol and NaCl yielded similar germination percent and "root/shoot" length, indicating that the decrease in germination and other growth parameters may be due primarily to an osmotic effect.

d. Gibberellic acid and kinetin were found to be effective in alleviating salt-induced stress conditions in maize and sugar beet seeds. Particularly at the high level of salinity treatments, the effect of GA_3 was greater than that of kinetin in maize. However, the case was opposite in sugar beet. In general, seeds may be prevented from germination due to an inhibition of growth regulators or interference of salinity with metabolic activity.

e. The presence of salt in the cells may induce changes in protein activity due to effects of ions on the structure of hydrating water which surrounds the

protein molecule. NaCl may also be inhibitory to the activities of some enzymes that play critical roles in seed germination. The poor germination and higher salt sensitivity of sugar beet (or cotton) may be linked to this and the following sugar input. This implies that transformation into soluble sugar is affected by the imposed stress.

7 Zusammenfassung

Bodenversalzung ist ein häufig vorkommendes Problem für die landwirtschaftliche Produktivität in vielen ariden Teilen der Welt. Die Keimung stellt eine Phase im Lebenszyklus der Pflanzen dar, die empfindlich für Stress durch Versalzung ist. Eine erfolgreiche Keimung hängt sowohl von der Häufigkeit und Menge des Niederschlags ab, als auch von der Fähigkeit einer Saatgutart, zu keimen und zu wachsen, während die Bodenfeuchtigkeit und die osmotischen Potentiale abnehmen. Die durch Versalzung bedingten Veränderungen der einzelnen physiologischen und anatomischen Parameter variieren beträchtlich. Dies hängt von der Planzenart, dem Entwicklungsstadium und externen Faktoren, wie dem Salzregime, ab. Die Nutzung saliner Böden für landwirtschaftliche Zwecke und die Wiederinkulturnahme dieser Böden für die gegenwärtige und zukünftige landwirtschaftliche Nutzung verlangt ein besseres Verständnis der Natur der Salzresistenz und Salzempfindlichkeit während der Saatkeimung. Die vorliegende Studie wurde deshalb durchgeführt, um sich mit den folgenden Zielen auseinanderzusetzen.

Hauptziele:

1. Bestimmung der Wirkung von verschiedenen Graden der Versalzung (NaCl) auf keimendes Saatgut (Zuckerrüben, Baumwolle, Mais und Reis).

2. Untersuchung des Ausmaßes der osmotischen und speziellen Ionenwirkungen auf die Keimung der oben genannten Planzenarten unter Einsatz verschiedener Arten anorganischer Salze (NaCl, $CaCl_2$ und Na_2SO_4).

3. Bewertung der Wirkung des Wasserpotentials (simulierte Trockenheit) unter Einsatz von osmotischen Substanzen wie PEG und Mannitol und der Vergleich dieser Substanzen mit NaCl während der Keimung dieser Pflanzenarten.

Weitere Ziele:

I. Identifikation salzresistanter und salzempfindlicher Pflanzen während der Keimung.

II. Einschätzung der exogenen Anwendung von GA_3 und Kinetin auf die Keimung und das frühe Wachstum der Keimlinge unter Salzstressbedingungen.

III. Falls Unterschiede in der Widerstandsfähigkeit gegenüber Salz unter den Pflanzenarten identifiziert

werden können wäre das Ziel, die Mechanismen zu identifizieren, die für diese Unterschiede verantwortlich sind.

Die Ergebnisse dieser Versuchsreihe lassen sich folgendermaßen zusammenfassen:

a. Die Ergebnisse weisen Reis und Mais während der keimung als resistanter relativ zu Baumwolle und Zuckerrübensamen gegenüber durch NaCl bedingtem Salzstress aus.

b. Bei der Anwendung von Substraten gleicher osmotischer Potentiale erscheint die Gesamtwirkung auf die Keimung und das frühe Setzlingswachstum folgendermaßen: Na_2So_4 reduziert die Keimung weit mehr als CaCl, gefolgt von NaCl. Jegliche Einstufung bestimmter Ionen erweist sich als schwierig, da bestimmte Kombinationen von Kationen und Anionen hemmender erscheinen als andere.

c. Die in diesem Versuch gewonnenen Ergebnisse zeigten, dass isotonische Lösungen von PEG, Mannitol und NaCl vergleichbare Keimprozentsätze und „root/shoot" Längen erbrachten, was darauf hinweist, dass die Verminderung

in der Keimung und anderen Wachstumsparametern in
erster Linie auf einen osmotischen Effekt
zurückzuführen sind.

d.   Gibberellinsäure und Kinetin erwiesen sich als wirksam
in der Linderung von salzinduzierten Stressbedingungen
bei Mais und Zuckerrüben. Besonders auf der hohen
Stufe der Salzbehandlungen war die Wirkung von GA_3
größer als die von Kinetin bei Mais. Bei Zuckerrüben
ist der Fall jedoch umgekehrt. Im allgemeinen kann die
Keimung des Saatguts durch eine Hemmung der
Wachstumsfaktoren und durch die Versalzung mit der
metabolischen Aktivität beeinträchtigt werden.

e.   Die Anwesenheit von salzionen in den Zellen könnte
Veränderungen in der Enzymaktivität bewirken, aufgrund
der Wirkung von Ionen auf die Struktur von
hydratisierendem Wasser, das die Proteinmoleküle
umgibt. NaCl könnte ebenfalls eine hemmende Wirkung
auf die Aktivität einiger Enzyme haben, die eine
entscheidende Rolle in der Saatkeimung spielen. Die
geringe Keimung und höhere Salzempfindlichkeit von
Zuckerrüben (und Baumwolle) könnte damit in Verbindung

stehen. Das weist darauf hin, dass die Umwandlung in lösliche Zucker durch den auferlegten Stress beeinflusst wird.

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Tab 7: The effects of NaCl on germination in sugarbeet				
	control	50mM NaCl	100mM NaCl	200mM NaCl
48 hr				
exp.1	72	69	19	1
exp.2	81	65	21	1
exp.3	73	68	19	0
mean	75.333	67.333	19.667	0.667
standard error	±3.781	±1.595	±0.885	±0.442
72 hr				
exp.1	88	84	53	5
exp.2	92	86	61	4
exp.3	89	87	57	2
mean	89.667	85.667	57.000	3.667
standard error	±1.340	±0.984	±2.576	±0.984
96 hr				
exp.1	95	87	73	7
exp.2	95	94	79	10
exp.3	92	89	75	10
mean	94.000	90.000	75.667	9.000
standard error	±1.064	±2.215	±1.876	±1.064

Tab 8: The effects of NaCl on germination in maize				
	control	50mM NaCl	100mM NaCl	200mM NaCl
48 hr				
exp.1	94	82	43	10
exp.2	91	83	70	15
exp.3	91	81	56	13
mean	92.000	82.000	56.333	12.667
standard error	±1.087	±0.628	±8.474	±1.579
72 hr				
exp.1	100	98	97	58
exp.2	98	96	92	60
exp.3	99	99	94	67
mean	99.000	97.667	94.333	61.667
standard error	±0.583	±0.891	±1.468	±2.756
96 hr				
exp.1	100	100	99	85
exp.2	98	98	97	90
exp.3	100	99	98	86
mean	99.333	99.000	98.000	87.000
standard error	±0.671	±0.581	±0.581	±2.083

Tab 9: The effects of NaCl-CaCl2-Na2So4-on germination in maize							
48 hr	control	100mM NaCl	200mM NaCl	69mM CaCl$_2$	141mM CaCl$_2$	71mM Na$_2$SO$_4$	145mM Na$_2$SO$_4$
exp.1	39	24	2	11	0	18	0
exp.2	43	27	1	14	0	17	0
exp.3	43	22	3	18	0	25	0
mean	41.667	24.333	2.000	14.333	0.000	20.000	0.000
standard error	±1.333	±1.453	±0.577	±2.028	±0.000	±2.517	±0.000
72 hr	control	100mM NaCl	200mM NaCl	69mM CaCl$_2$	141mM CaCl$_2$	71mM Na$_2$SO$_4$	145mM Na$_2$SO$_4$
exp.1	91	65	44	66	39	71	39
exp.2	89	72	46	67	32	65	29
exp.3	91	70	51	62	39	69	37
mean	90.333	69.000	47.000	65.000	36.667	68.333	35.000
standard error	±0.667	±2.082	±2.082	±1.528	±2.333	±1.764	±3.055
96 hr	control	100mM NaCl	200mM NaCl	69mM CaCl$_2$	141mM CaCl$_2$	71mM Na$_2$SO$_4$	145mM Na$_2$SO$_4$
exp.1	100	82	69	73	59	70	56
exp.2	99	85	71	72	65	75	59
exp.3	100	84	64	68	59	74	62
mean	99.667	83.667	68.000	71.000	61.000	73.000	59.000
standard error	±0.333	±0.882	±2.082	±1.528	±2.000	±1.528	±1.732

Tab 10: The effects of NaCl-CaCl2-Na2So4-on germination in sugarbeet						
48 hr	control	100mM NaCl	200mM NaCl	69mM $CaCl_2$	141mM $CaCl_2$	71mM Na_2SO_4	145mM Na_2SO_4
exp.1	45	2	0	4	0	4	0
exp.2	48	4	1	3	0	3	0
exp.3	49	5	0	7	0	3	0
mean	47.333	3.667	0.333	4.667	0.000	3.333	0.000
standard error	±1.202	±0.882	±0.333	±1.202	±0.000	±0.333	±0.000
72 hr	control	100mM NaCl	200mM NaCl	69mM $CaCl_2$	141mM $CaCl_2$	71mM Na_2SO_4	145mM Na_2SO_4
exp.1	87	36	2	29	1	33	0
exp.2	82	39	3	33	2	35	0
exp.3	89	46	2	38	2	37	1
mean	86.000	40.333	2.333	33.333	1.667	35.000	0.333
standard error	±2.082	±2.963	±0.333	±2.603	±0.333	±1.155	±0.333
96 hr	control	100mM NaCl	200mM NaCl	69mM $CaCl_2$	141mM $CaCl_2$	71mM Na_2SO_4	145mM Na_2SO_4
exp.1	94	57	5	40	3	49	0
exp.2	95	53	7	43	2	52	1
exp.3	96	60	4	51	4	55	2
mean	95.000	56.667	5.333	44.667	3.000	52.000	1.000
standard error	±0.577	±2.028	±0.882	±3.283	±0.577	±1.732	±0.577

Tab 11: The effects of NaCl,peg,manitol, on germination in maize						
	control	100mM NaCl	200mM NaCl	22,5mM PEG	43,5mM PEG	191mM Mannitol	368mM Mannitol
48 hr						
exp.1	33	19	4	14	10	21	4
exp.2	28	6	3	10	3	17	3
exp.3	31	12	2	5	2	16	2
mean	30.667	12.333	3.000	9.667	5.000	18.000	3.000
standard error	±1.453	±3.756	±0.577	±2.603	±2.517	±1.528	±0.577
72 hr						
exp.1	92	81	58	86	43	86	47
exp.2	90	65	41	76	32	84	48
exp.3	91	70	35	62	27	80	41
mean	91.000	72.000	44.667	74.667	34.000	83.333	45.333
standard error	±0.577	±4.726	±6.888	±6.960	±4.726	±1.764	±2.186
96 hr						
exp.1	99	89	65	88	69	98	68
exp.2	97	87	62	86	65	93	75
exp.3	95	81	63	84	57	81	73
mean	97.000	85.667	63.333	86.000	63.667	90.667	72.000
standard error	±1.155	±2.404	±0.882	±1.155	±3.528	±5.044	±2.082

Tab 12: The effects of NaCl-peg-manitol on germination in sugarbeet							
	control	100mM NaCl	200mM NaCl	22,5mM PEG	43,5mM PEG	191mM Mannitol	368mM Mannitol
48 hr							
exp.1	66	6	1	2	1	6	0
exp.2	63	6	1	4	1	8	0
exp.3	56	4	0	5	0	5	0
mean	61.667	5.333	0.667	3.667	0.667	6.333	0.000
standard error	±2.963	±0.667	±0.333	±0.882	±0.333	±0.882	±0.000
72 hr							
exp.1	91	47	4	19	5	37	8
exp.2	85	40	1	30	4	48	6
exp.3	87	39	1	19	8	40	6
mean	87.667	42.000	2.000	22.667	5.667	41.667	6.667
standard error	±1.764	±2.517	±1.000	±3.667	±1.202	±3.283	±0.667
96 hr							
exp.1	96	64	6	29	9	52	20
exp.2	92	55	2	36	7	59	16
exp.3	93	52	4	28	11	65	16
mean	93.667	57.000	4.000	31.000	9.000	58.667	17.333
standard error	±1.202	±3.606	±1.155	±2.517	±1.155	±3.756	±1.333

Tab 13: The effects of Salinity, Kinetin and GA3 on germination in maize						
48 hr	control	100mM Salinity	200mM Salinity	100mM NaCl + 5 µM kinetin	200mM NaCl + 5 µM kinetin	100mM NaCl + 20 µM GA	200mM NaCl + 20 µM GA
exp.1	63	25	5	34	10	32	14
exp.2	67	30	5	29	8	39	19
exp.3	68	23	3	41	9	42	15
mean	66.000	26.000	4.333	34.667	9.000	37.667	16.000
standard error	±1.528	±2.082	±0.667	±3.480	±0.577	±2.963	±1.528
72 hr	control	100mM Salinity	200mM Salinity	100mM NaCl + 5 µM kinetin	200mM NaCl + 5 µM kinetin	100mM NaCl + 20 µM GA	200mM NaCl + 20 µM GA
exp.1	90	86	52	86	53	83	57
exp.2	94	90	59	80	56	85	66
exp.3	94	79	52	83	58	81	63
mean	92.667	85.000	54.333	83.000	55.667	83.000	62.000
standard error	±1.333	±3.215	±2.333	±1.732	±1.453	±1.155	±2.646
96 hr	control	100mM Salinity	200mM Salinity	100mM NaCl + 5 µM kinetin	200mM NaCl + 5 µM kinetin	100mM NaCl + 20 µM GA	200mM NaCl + 20 µM GA
exp.1	100	94	71	96	70	88	74
exp.2	100	99	74	94	73	93	76
exp.3	100	93	75	95	73	93	74
mean	100.000	95.333	73.333	95.000	72.000	91.333	74.667
standard error	±0.000	±1.856	±1.202	±0.577	±1.000	±1.667	±0.667

Tab 14: The effects of Salinity, Kinetin and GA3 on germination in sugarbeet							
48 hr	control	100mM Salinity	200mM Salinity	100mM NaCl+ 5µM Kinetin	200mM NaCl+ 5µM Kinetin	100mM NaCl+20 µM GA3	200mM NaCl+20 µM GA3
exp.1	35	2	0	3	0	1	0
exp.2	35	2	0	4	0	1	0
exp.3	36	1	0	7	0	2	0
mean	35.333	1.667	0.000	4.667	0.000	1.333	0.000
standard error	±0.333	±0.333	±0.000	±1.202	±0.000	±0.333	±0.000
72 hr	control	100mM Salinity	200mM Salinity	100mM NaCl+ 5µM Kinetin	200mM NaCl+ 5µM Kinetin	100mM NaCl+20 µM GA3	200mM NaCl+20 µM GA3
exp.1	85	21	1	26	4	12	2
exp.2	86	32	0	25	4	19	1
exp.3	87	23	2	36	4	25	1
mean	86.000	25.333	1.000	29.000	4.000	18.667	1.333
standard error	±0.577	±3.383	±0.577	±3.512	±0.000	±3.756	±0.333
96 hr	control	100mM Salinity	200mM Salinity	100mM NaCl+ 5µM Kinetin	200mM NaCl+ 5µM Kinetin	100mM NaCl+20 µM GA3	200mM NaCl+20 µM GA3
exp.1	92	51	6	53	11	55	4
exp.2	93	54	4	45	7	49	5
exp.3	92	44	3	60	8	47	7
mean	92.333	49.667	4.333	52.667	8.667	50.333	5.333
standard error	±0.333	±2.963	±0.882	±4.333	±1.202	±2.404	±0.882

Tab 15: The effects of Salinity, Kinetin and GA3 on root elongation on maize seedlings						
48 hr	control	100mM Salinity	200mM Salinity	100mM NaCl+ 5µM Kinetin	200mM NaCl+ 5µM Kinetin	100mM NaCl+ 20µM GA3	200mM NaCl + 20µM GA3
exp.1	6.03	4.16	2.50	4.42	2.50	4.81	2.91
exp.2	7.02	3.96	2.50	4.39	3.17	5.37	2.90
exp.3	6.65	4.39	2.00	4.82	2.86	5.67	3.00
mean	6.567	4.170	2.333	4.543	2.843	5.283	2.937
standard error	±0.289	±0.124	±0.167	±0.139	±0.194	±0.252	±0.032
72 hr	control	100mM Salinity	200mM Salinity	100mM NaCl+ 5µM Kinetin	200mM NaCl+ 5µM Kinetin	100mM NaCl+ 20µM GA3	200mM NaCl + 20µM GA3
exp.1	15.81	10.13	5.92	9.58	5.19	12.99	6.58
exp.2	19.31	12.37	6.22	9.80	5.65	13.02	7.32
exp.3	18.41	9.79	6.25	10.70	5.56	12.95	7.49
mean	17.843	10.763	6.130	10.027	5.467	12.987	7.130
standard error	±1.049	±0.809	±0.105	±0.343	±0.141	±0.020	±0.279
96 hr	control	100mM Salinity	200mM Salinity	100mM NaCl+ 5µM Kinetin	200mM NaCl+ 5µM Kinetin	100mM NaCl+ 20µM GA3	200mM NaCl + 20µM GA3
exp.1	28.34	19.83	8.59	16.13	7.93	18.08	9.64
exp.2	32.97	21.97	9.97	17.30	8.78	21.22	9.56
exp.3	27.54	18.72	8.51	17.65	8.48	21.04	10.03
mean	29.617	20.173	9.023	17.027	8.397	20.113	9.743
standard error	±1.692	±0.954	±0.474	±0.460	±0.249	±1.018	±0.145

Tab 16: The effects of Salinity, Kinetin and GA3 on coleoptile elongation on maize seedlings							
48 hr	control	100mM Salinity	200mM Salinity	100mM + 5μM Kinetin	200mM + 5μM Kinetin	100mM + 20μM GA3	200mM + 20μM GA3
exp.1	3.79	0.00	0.00	3.00	0.00	3.50	0.00
exp.2	4.09	0.00	0.00	3.00	0.00	3.25	0.00
exp.3	3.83	0.00	0.00	3.00	0.00	3.71	0.00
mean	3.903	0.000	0.000	3.000	0.000	3.487	0.000
standard error	±0.094	±0.000	±0.000	±0.000	±0.000	±0.133	±0.000
72 hr	control	100mM Salinity	200mM Salinity	100mM + 5μM Kinetin	200mM + 5μM Kinetin	100mM + 20μM GA3	200mM + 20μM GA3
exp.1	9.17	5.25	3.25	5.45	3.50	6.52	3.77
exp.2	10.04	6.01	3.12	6.08	3.29	6.37	3.37
exp.3	11.16	5.56	3.23	6.64	3.73	6.52	3.33
mean	10.123	5.607	3.200	6.057	3.507	6.470	3.490
standard error	±0.576	±0.221	±0.040	±0.344	±0.127	±0.050	±0.140
96 hr	control	100mM Salinity	200mM Salinity	100mM + 5μM Kinetin	200mM + 5μM Kinetin	100mM + 20μM GA3	200mM + 20μM GA3
exp.1	11.65	8.88	4.71	8.89	5.50	8.27	5.15
exp.2	11.98	10.01	5.86	8.86	5.82	9.40	5.13
exp.3	9.44	10.73	5.46	9.97	5.71	9.96	5.32
mean	11.023	9.873	5.343	9.240	5.677	9.210	5.200
standard error	±0.797	±0.538	±0.337	±0.365	±0.094	±0.497	±0.060

Tab 17: The effects of Salinity, Kinetin and GA3 on root elongation on sugarbeet seedlings						
48 hr	control	100mM Salinity	200mM Salinity	100mM NaCl+ 5μM kinetin	200mM NaCl+ 5μM kinetin	100mM NaCl+ 20μM GA3	200mM NaCl+ 20μM GA3
exp.1	3.89	0.00	0.00	0.00	0.00	0.00	0.00
exp.2	3.70	0.00	0.00	0.00	0.00	0.00	0.00
exp.3	3.25	0.00	0.00	0.00	0.00	0.00	0.00
mean	3.613	0.000	0.000	0.000	0.000	0.000	0.000
standard error	±0.190	±0.000	±0.000	±0.000	±0.000	±0.000	±0.000
72 hr	control	100mM Salinity	200mM Salinity	100mM NaCl+ 5μM kinetin	200mM NaCl+ 5μM kinetin	100mM NaCl+ 20μM GA3	200mM NaCl+ 20μM GA3
exp.1	4.28	3.08	0.00	2.85	0.00	3.00	0.00
exp.2	4.35	3.36	0.00	3.30	0.00	3.00	0.00
exp.3	4.65	3.23	0.00	3.37	0.00	3.07	0.00
mean	4.427	3.223	0.000	3.173	0.000	3.023	0.000
standard error	±0.113	±0.081	±0.000	±0.163	±0.000	±0.023	±0.000
96 hr	control	100mM Salinity	200mM Salinity	100mM NaCl+ 5μM kinetin	200mM NaCl+ 5μM kinetin	100mM NaCl+ 20μM GA3	200mM NaCl+ 20μM GA3
exp.1	4.82	3.71	2.33	3.87	2.00	4.21	2.00
exp.2	4.60	4.10	2.00	3.57	2.80	3.84	2.00
exp.3	5.03	3.68	2.00	4.02	3.00	4.04	3.00
mean	4.817	3.830	2.110	3.820	2.600	4.030	2.333
standard error	±0.124	±0.135	±0.110	±0.132	±0.306	±0.107	±0.333

Tab 18: Effect of NaCl on protein content during germination in maize seeds			
	Control		
0 hr			
exp.1	0.655		
exp.2	0.629		
exp.3	0.641		
mean	0.642		
standard error	±0.008		
	Control	50 mM NaCl	100 mM NaCl	200 mM NaCl
48 hr			
exp.1	6.852	6.927	6.970	7.177
exp.2	6.932	6.931	6.893	7.078
exp.3	6.873	6.986	6.920	7.026
mean	6.886	6.948	6.928	7.093
standard error	±0.024	±0.019	±0.022	±0.044
72 hr			
exp.1	6.869	6.987	6.981	7.104
exp.2	6.809	6.970	6.987	7.143
exp.3	6.765	6.839	6.889	7.089
mean	6.814	6.932	6.952	7.112
standard error	±0.030	±0.047	±0.032	±0.016
96 hr			
exp.1	6.740	6.830	6.923	6.828
exp.2	6.772	6.809	6.960	6.886
exp.3	6.781	6.838	6.935	6.816
mean	6.776	6.826	6.940	6.843
standard error	±0.004	±0.009	±0.011	±0.022

Tab 19: Effect of NaCl on protein content during germination in sugarbeet seeds			
	Control		
0 hr			
exp.1	0.033		
exp.2	0.034		
exp.3	0.034		
mean	0.033		
standard error	±0.0003		
	Control	50 mM NaCl	100 mM NaCl	200 mM NaCl
48 hr			
exp.1	0.077	0.098	0.081	0.091
exp.2	0.079	0.097	0.082	0.091
exp.3	0.085	0.094	0.082	0.097
mean	0.080	0.096	0.082	0.093
standard error	±0.0025	±0.0011	±0.0002	±0.0020
72 hr			
exp.1	0.060	0.064	0.066	0.069
exp.2	0.059	0.063	0.067	0.071
exp.3	0.060	0.062	0.062	0.066
mean	0.060	0.063	0.065	0.069
standard error	±0.0003	±0.0007	±0.0014	±0.0014
96 hr			
exp.1	0.051	0.064	0.066	0.063
exp.2	0.051	0.062	0.067	0.062
exp.3	0.049	0.063	0.062	0.063
mean	0.050	0.063	0.065	0.063
standard error	±0.0007	±0.0007	±0.0016	±0.0004

Tab 20: Effect of NaCl on amino acid content during germination in maize seeds			
	Control		
0 hr			
exp.1	0.078		
exp.2	0.079		
exp.3	0.079		
mean	0.079		
standard error	±0.0005		
	Control	50 mM NaCl	100 mM NaCl	200 mM NaCl
48 hr			
exp.1	0.358	0.316	0.322	0.320
exp.2	0.376	0.340	0.324	0.325
exp.3	0.376	0.330	0.313	0.317
mean	0.370	0.328	0.320	0.321
standard error	±0.0059	±0.0070	±0.0035	±0.0024
72 hr			
exp.1	0.439	0.428	0.374	0.353
exp.2	0.451	0.435	0.379	0.369
exp.3	0.454	0.440	0.417	0.362
mean	0.448	0.434	0.390	0.361
standard error	±0.0044	±0.0034	±0.0135	±0.0045
96 hr			
exp.1	0.422	0.403	0.396	0.382
exp.2	0.424	0.408	0.404	0.408
exp.3	0.426	0.397	0.401	0.407
mean	0.424	0.403	0.400	0.399
standard error	±0.0012	±0.0030	±0.0025	±0.0086

Tab 21: Effect of NaCl on amino acid content during germination in sugarbeet seeds				
	Control			
0 hr				
exp.1	0.0025			
exp.2	0.0027			
exp.3	0.0027			
mean	0.0026			
standard error	±0.00007			
	Control	50 mM NaCl	100 mM NaCl	200 mM NaCl
48 hr				
exp.1	0.0075	0.0071	0.0068	0.0064
exp.2	0.0074	0.0071	0.0068	0.0062
exp.3	0.0074	0.0068	0.0068	0.0068
mean	0.0074	0.0070	0.0068	0.0065
standard error	±0.00002	±0.00009	±0.00001	±0.00019
72 hr				
exp.1	0.0191	0.0149	0.0167	0.0145
exp.2	0.0184	0.0151	0.0175	0.0146
exp.3	0.0189	0.0153	0.0171	0.0148
mean	0.0188	0.0151	0.0171	0.0146
standard error	±0.00020	±0.00013	±0.00022	±0.00009
96 hr				
exp.1	0.0207	0.0180	0.0172	0.0161
exp.2	0.0213	0.0185	0.0179	0.0155
exp.3	0.0216	0.0186	0.0189	0.0152
mean	0.0212	0.0184	0.0180	0.0156
standard error	±0.00026	±0.00018	±0.00050	±0.00027

Tab 22: Effect of NaCl on starch content during germination in maize seeds			
	Control		
0 hr			
exp.1	162.00		
exp.2	160.75		
exp.3	160.75		
mean	161.17		
standard error	±0.417		
	Control	50 mM NaCl	100 mM NaCl	200 mM NaCl
48 hr			
exp.1	74.81	94.81	109.26	102.96
exp.2	82.22	92.22	112.22	104.44
exp.3	82.59	96.67	108.89	103.70
mean	79.88	94.57	110.12	103.70
standard error	±2.533	±1.289	±1.055	±0.428
72 hr			
exp.1	106.67	132.22	147.41	142.59
exp.2	123.70	131.11	149.26	129.26
exp.3	105.19	126.67	150.74	127.78
mean	111.85	130.00	149.14	133.21
standard error	±5.941	±1.697	±0.964	±4.711
96 hr			
exp.1	63.33	79.63	108.89	158.52
exp.2	60.37	77.04	113.70	144.81
exp.3	63.70	74.81	114.44	157.41
mean	62.47	77.16	112.35	153.58
standard error	±1.055	±1.391	±1.742	±4.394

Tab 23: Effect of NaCl on starch content during germination in sugarbeet seeds			
	Control		
0 hr			
exp.1	2.11		
exp.2	2.55		
exp.3	2.18		
mean	2.28		
standard error	±0.137		
	Control	50 mM NaCl	100 mM NaCl	200 mM NaCl
48 hr			
exp.1	0.74	1.14	0.72	1.03
exp.2	1.15	1.21	1.12	1.12
exp.3	0.86	0.92	0.90	0.94
mean	0.92	1.09	0.91	1.03
standard error	±0.123	±0.087	±0.115	±0.052
72 hr			
exp.1	1.64	1.48	1.77	2.50
exp.2	1.60	1.51	1.89	2.31
exp.3	1.64	1.48	1.91	2.29
mean	1.63	1.49	1.86	2.37
standard error	±0.012	±0.012	±0.045	±0.069
96 hr			
exp.1	1.46	1.05	2.31	2.70
exp.2	1.28	1.14	2.16	2.59
exp.3	1.33	1.01	2.41	2.61
mean	1.36	1.06	2.29	2.64
standard error	±0.053	±0.038	±0.073	±0.033

0 hr (a)	Control	Tab 24: Effect of NaCl on content of glucose (a), fructose (b), sucrose (c), and maltose (d) during germination of maize seeds		
exp.1	13.86			
exp.2	13.84			
exp.3	12.69			
mean	13.47			
standard error	±0.387			
48 hr	Control	50 mM NaCl	100 mM NaCl	200 mM NaCl
exp.1	57.02	73.93	76.04	41.56
exp.2	55.00	70.07	76.05	42.28
exp.3	57.83	73.56	85.29	48.35
mean	56.62	72.52	79.13	44.06
standard error	±0.842	±1.230	±3.084	±2.153
0 hr (b)				
exp.1	4.63			
exp.2	5.60			
exp.3	4.41			
mean	4.88			
standard error	±0.364			
48 hr				
exp.1	13.81	10.91	10.39	9.75
exp.2	12.69	12.42	9.53	9.46
exp.3	11.04	10.50	13.85	13.38
mean	12.51	11.28	11.26	10.86
standard error	±0.806	±0.582	±1.319	±1.261
0 hr (c)				
exp.1	36.67			
exp.2	37.26			
exp.3	37.00			
mean	36.98			
standard error	±0.170			
48 hr				
exp.1	27.36	30.99	33.81	33.52
exp.2	26.63	31.55	36.71	30.85
exp.3	28.00	30.15	34.45	35.11
mean	27.33	30.90	34.99	33.16
standard error	±0.395	±0.408	±0.879	±1.242
0 hr (d)				
exp.1	2.86			
exp.2	2.78			
exp.3	2.98			
mean	2.87			
standard error	±0.059			
48 hr				
exp.1	28.56	30.12	35.51	12.53
exp.2	28.16	30.41	36.89	11.47
exp.3	29.67	29.10	34.82	12.59
mean	28.80	29.88	35.74	12.20
standard error	±0.451	±0.399	±0.609	±0.362

0 hr (a)	Control	Tab 25: Effect of NaCl on content of glucose (a), fructose (b), sucrose (c), and maltose (d) during germination of sugarbeet seeds		
exp.1	0.74			
exp.2	0.74			
exp.3	0.68			
mean	0.72			
standard error	±0.020			
48 hr	Control	50 mM NaCl	100 mM NaCl	200 mM NaCl
exp.1	2.64	1.41	1.17	1.12
exp.2	2.62	1.19	1.31	1.03
exp.3	2.55	1.30	1.30	1.30
mean	2.60	1.30	1.26	1.15
standard error	±0.026	±0.062	±0.046	±0.080
0 hr (b)				
exp.1	0.14			
exp.2	0.13			
exp.3	0.13			
mean	0.13			
standard error	±0.002			
48 hr				
exp.1	0.58	0.52	0.34	0.27
exp.2	0.75	0.33	0.37	0.29
exp.3	0.87	0.47	0.34	0.34
mean	0.73	0.44	0.35	0.30
standard error	±0.086	±0.058	±0.009	±0.022
0 hr (c)				
exp.1	0.32			
exp.2	0.32			
exp.3	0.31			
mean	0.32			
standard error	±0.003			
48 hr				
exp.1	0.29	0.20	0.30	0.49
exp.2	0.34	0.10	0.27	0.28
exp.3	0.44	0.19	0.24	0.37
mean	0.35	0.16	0.27	0.38
standard error	±0.044	±0.031	±0.019	±0.060
0 hr (d)				
exp.1	0.28			
exp.2	0.27			
exp.3	0.25			
mean	0.27			
standard error	±0.008			
48 hr				
exp.1	0.89	0.10	0.10	0.12
exp.2	1.06	0.27	0.08	0.08
exp.3	1.15	0.29	0.08	0.08
mean	1.03	0.22	0.08	0.09
standard error	±0.076	±0.061	±0.007	±0.014

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