STUDIES IN ECO-PHYSIOLOGY OF DACTYLOCTENIUM AEGYPTIUM L., ORYZA SATIVA L. AND PORTERESIA COARCTATA (TATEOKA)

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (BOTANY) OF THE UNIVERSITY OF LAGOS.

JULY 1990

SCHOOL OF POSTGRADUATE STUDIES

UNIVERSITY OF LAGOS

CERTIFICATION

THIS IS TO CERTIFY THAT THE THESIS-

STUDIES IN ECO-PHYSIOLOGY OF DACTYLOCTENIUM AEGYPTIUM L., ORYZA SATIVA L. AND PORTERESIA COARCTATA (TATIOKA).

SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES UNIVERSITY OF LAGOS FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY (BOTANY)

IS A RECORD OF ORIGINAL RESEARCH CARRIED OUT BY

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IN THE DEPARTMENT OF

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ACKNOWLEDGEMENT

Firstly, I am grateful to Almighty God for enabling me complete this work, which despite occasional and inevitable frustrations, I found rewarding to carry out.

I wish to express my gratitude to Prof. O.T. Okusanya, my supervisor, for suggesting the topic of this research, for his thorough supervision and constructive criticism during the preparation of this thesis.

Part of the work reported in this thesis was carried out in the Population and Plant Biology laboratory at the School of Biological Sciences of the University of Sussex, Brighton, England. This was made possible by the British Caledonian Airways that awarded me a Sir Adam Thomson scholarship for one year. For this I am grateful.

This acknowledgement would be incomplete without mentioning the help, guidance and details of techniques given to me during the one year stay at the University of Sussex by Dr. A.R. Yeo.

I wish to express my heartfelt gratitude to my parents, my sisters; Biola, Dupe and Morenike, my brother Dayo and most especially my husband, Kunle Adu who in their own ways contributed to making this project a reality.

I am grateful to all the people I have worked with and shared laboratory with, and I would like to thank Linda Burnharm

of the University of Sussex, for the many things necessitated by the running of the laboratory.

My final thanks to Mrs Funke Kuyoro and Mr. Chris Offor of R & D Holdings, Lagos for jointly typing the thesis.

D E D I C A T I O N

TO DAMILOLA

ABSTRACT

The salinity tolerance of the grass *Dactyloctenium aegyptium* was compared with that of two tropical rice cultivars (*Oryza sativa*) KAU 2 and HG 2153 and a closely related temperate member of the gramineae, *Porteresia coarctata*. Experiments were also carried out to determine the tolerance mechanism of *D. aegyptium* with a view to making recommendations as to its use as a biological soil desalinizer and reclamation species in Nigeria.

The effects of varying the levels of potassium, nitrate and sulphate in the culture solution on the growth, mineral composition and ion relations at 10 and 25% sea water concentrations were investigated in *D. aegyptium* to determine how the supply of these nutrients to this species in their natural habitats might help them overcome high salinity problems.

The photosynthetic ability, stomatal conductance to water and carbon dioxide and transpiration at different salinity regimes were also investigated in *D. aegyptium* to find out the physiological basis for the effect of salinity on its growth.

The germination ecology of *D. aegyptium* was further investigated to find out the response of the plant to such ecological factors like light and dark regimes, pH, salinity, soil types, soil moisture and temperature.

Based on the response of the four species tested to salinity, the species can be divided into two main groups namely,

those that have their growth suppressed as sea water concentration increased, that is, the two rice varieties, and those that are either not affected in growth or have insignificant stimulation of growth (dry weight) at low salinity that is *P. coarctata* and *D. aegyptium* respectively.

P. coarctata appears to be the most tolerant species followed by *D. aegyptium*, the rice cultivar, KAU 2, and the least tolerant being the other rice cultivar HG 2153. *P. coarctata* accumulated relatively less Na^+ and Cl^- in their shoots and maintained the lowest Na:K ratio at 30% sea water concentration compared with the other species.

In *D. aegyptium* the use of half of the level of potassium, twice the level of nitrate and half of the level of nitrate in the culture solution resulted in significant stimulation of dry weight at 10% sea water and amelioration of poor growth at 25% sea water concentration. However the use of double the level of potassium or half or double the level of sulphate in the culture solution depressed growth significantly at both concentrations of sea water. At half the level of potassium or nitrate or double the level of nitrate there was increased uptake of potassium and nitrate, reduced uptake of sodium and chloride ions, increased water uptake and plant succulence, increased sugar concentration and increase osmolarity.

In terms of ion relations *D. aegyptium* was found to be a cumulative halophyte as there was daily accumulation of ions in

the shoot and root. The rate of accumulation of the K^+, Na^+ and Cl^- varied in the seedlings that had enhanced growth and those in which there was poor yield. At half the level of potassium and the two levels of nitrate tested, the rate of accumulation of K, (Jk), was significantly higher than the control and other treatments while the rate of accumulation of Na^+ and Cl^- were significantly lower. However, at both levels of sulphate and double the level of K^+, Na^+ and Cl^- accumulations were significantly higher than the control, while the K^+ accumulation was significantly lower than the control. In all the treatments, the ion content of the shoots were significantly higher than those of the roots and there was a stronger detrimental effect of salinity on root than shoot growth.

Increased salinity brought about a 75% reduction in photosynthetic carbondioxide fixation, 73% reduction in stomatal conductance to water and carbondioxide and 64% reduction in transpiration.

There was 100% germination of D. aegyptium in both light and dark, but dark had a slightly lower rate of germination.

There was 100% germination at the range of pH 5.0 to 7.0 and in distilled water of pH 7.0. Only 40% germination was observed at pH 3.5.

There was 100% germination in up to 50% sea water and above this concentration there was a rapid decrease in the final germination and a noticeable delay in the rate of germination,

but there was 8% germination at 100% sea water.

The seeds of D. aegyptium germinated in the three soil types used. Humic soil and red earth had the highest percentage germination followed by sand. However humic soil recorded a higher rate of germination compared to that in red earth.

There was an increase in percentage germination as soil moisture decreased, but there was no significant difference between germination percentage in wet and dry treatments and their final percentage germination was significantly higher than that in the waterlogged condition.

The response of the species to temperature follows the usual minimum, optimum and maximum pattern with minimum at 5 ^{o}C, optimum at between 15 and 31 ^{o}C and maximum at 44 ^{o}C.

The results are discussed in relation to the habitat and ecology of the species as well as the use to which the species could be put as biological desalinizer.

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CHAPTER 1

GENERAL INTRODUCTION

Salinity is the occurrence of a high concentration of soluble salts in the soil or solution in which plants grow (Flowers and Yeo, 1986). To plant life, salinity is just one adverse factor of the environment. To man, salinity creates a problem due to its effect on his crop. Species which are predominantly sensitive to the presence of high concentration of salts in the soil will die. Difficulties arise because of the widespread of saline soils and are compounded by the geographical distribution of man's population and by agricultural practice. These has largely succeeded in increasing salinization in arid and semi-arid land (Raheja, 1966; Flowers, Troke and Yeo, 1977).

The main source of primary salinity can be traced to an oceanic influence, be it past or present, but apart from this, major geological features and soil conditions, resulting from climatic regimes are also associated with salinity (Levitt, 1980).

Secondary salinity is found in arid and semi-arid climates (Epstein, 1972) where rain rarely falls so that the soil profile remains unleached and retains its salts. Such moisture as is present is rapidly removed by evapotranspiration but leaves the salts, so that the upper layers of the soil tend to become increasingly saline. There are also large tracts of potentially fertile land which lie in river deltas which are saline due to the influence of the sea (Chapman, 1968).

The problems of secondary salinization are more serious, since they usually represent losses of once productive agricultural land (Zahran and Wahid, 1982). The distribution of saline soil is extensive, increasing and poorly defined. The uncertainties arise from the problems of soil mapping, the approaches to which can in some cases best be described as semi-quantitative. The soil affected areas occur mainly in the Western United States of America, Mexico, Canada, the pacific slopes of South America, North and South West Africa, parts of Russia, China, India, Pakistan, the Middle East and Australia (Szabolcs, 1979). Some of this saline soils are due to irrigation the scale of which can be judged from its current consumption of the world's total water usage during the farming season. Unfortunately, water supply are often of poor quality, so that evapotranspiration leads to the concentration in the soil of salts added during irrigation (Raheja, 1966; Kingsbury, Epstein and Pearce, 1984; Flowers et al.,1977).

The main elements, combinations of which give rise to the formation of saline soils are calcium, magnesium, sodium, potassium, sulphur, chloride, carbon and nitrogen while copper, zinc and barium often accumulates in minor quantities (Levitt, 1980). Of these elements, the most important are sodium, because of its toxicity and relative abundance in saline soils and chloride which is the most commonly associated anions. Other important ones are calcium and sulphate.

Plants are known to survive over a wide range of salts

concentration. Natraria schoberi L. may grow in soil which contains up to 30% salts and whose leaves may have total salt contents as high as 57% of their total dry weights (Kovda, Van den berg and Hagan, 1973). These plants are called halophytes. Plants that cannot grow in the presence of high concentration of sodium salts are called glycophytes. Many halophytes are able to grow perfectly in low or non- saline environments (Ungar, Hogan and McClelland, 1969) and are therefore facultative halophytes. Others cannot grow in low or non- saline environments and are therefore obligate halophytes (Levitt, 1980). Seed germination is much more resistant than later growth of the seedlings (Ungar, 1974, 1982, 1987).

Halophytes are native flora of saline environment (Shomen-Ilan, Moualem-Beno and Waisel 1985). For terrestrial halophytes, this means a minimum salt concentration of about 100mM in the soil solution (Flowers, Hajibagheri and Clipson, 1986). Under many conditions the salt concentration is about that of sea water or about 500mM and it commonly rises to concentration of the order of 1M in maritime marshes (Flowers, 1985). The consensus of evidence both direct and circumstantial is that similar metabolic requirements have to be met by terrestrial halophytes. In a saline environment all plants face qualitatively similar problems namely:-

1) high external concentration of certain ions like sodium and chloride ions interract with the membrane potential and reduces uptake of water.
2) it also causes nutrient deficiency which occurs as a result of inability of the plants to take up nutrients. This inability

may result in unbalanced ratio of one ion to another in the plant.

3) lowering of the water potential of the medium.

The problems are clearly not independent because one can affect the other, so much so, in fact that to most terrestrial plants the solution to each is mutually exclusive (Prisco and O'Leary, 1972; Bernstein, 1975; Yeo, 1983).

Glycophytes, which include almost all field crops are plants of non-saline habitat having a relatively limited capacity to adapt to salinity. Generally, the growth of most of these plants are inhibited above 0.1M salts and in a lot of cases below this concentration (Nieman, 1962).

Plants growing in excess salts may be characterised by dwarfism, dull foliage, often waxy and bluish in tinge or by an actual killing of the tissues in the form of necrosis or a marginal burn, followed by a loss of turgor, falling of leaves and finally death of the plant (Levitt, 1980; Ansari, 1982). In saline soils, some cereals may produce an abundance of green growth but no grain yield. With sugar beet, the sugar contents of root is low and difficult to extract, and forage may contain salt in such quantities as to make them inedible or dangerous to livestock (Russell, 1968).

As far as obligate halophytes are concerned, survival is generally accompanied by a high ion content in their tissues while glycophytes have a general tendency to exclude ions (Greenway, 1973). In terms of growth rate, a salt accumulating plant will

perform better than a salt excluding one for equivalent degree of osmotic adjustment (Greenway, 1968).

When halophytes and glycophytes are compared, ion accumulation and therefore tolerance, appears to be a superior mechanism for growth in a saline habitat. Tolerance can be of two types:-

1) dehydration avoidance and
2) dehydration tolerance

All salt resistant plants must possess the adaptation by dehydration avoidance, which is the basis of their tolerance of the secondary, salt-induced osmotic stress. Dehydration avoidance permits rehydration of the cell, return of the cell turgor, and recommencement of cell growth. This is possible only as a result of an increase in contents of cell solutes. The increase must be sufficient to lower their osmotic potential below that of their aqueous environment. The maintenance of cell turgor by a sufficient increase in cell solutes to compensate for the external osmotic stress is called osmoregulation. Osmoregulation when exposed to a salt stress may therefore be due to:

1) active uptake of salt or salt ions or
2) synthesis of organic solutes.

If the osmoregulation is due solely to the accumulation of organic solutes it must be linked to stress aviodance by exclusion or extrusion of the salt. If it is due to accumulation of salts, it must be linked to tolerance of the primary toxicity of the accumulated salt ions (Greenway and Munns, 1980; Levitt, 1980).

There could be three approaches to deal with the problem of salinity namely Physical, chemical and biological. Physically, the soil condition may be improved by sub-surface loosening, and the high water table associated with saline soils could be lowered through drainage. A mixed physico- chemical approach make use of gypsum to replace sodium, followed by leaching. The chemical method involves improvement in plant growth regulators with the help of fertilizers, salts or growth hormones such as indole acetic acid, gibberellic acid and cytokinin to protect the plant against salt injury. Synthetic growth regulators and amino acids have also been found to decrease salt injury. Phosphon D has been found to reduce salt injury in leaves and stem of pea plants (Uprety and Sarin, 1975). Also proline counteracted the inhibiting effect of sodium chloride on pea seed germination and root growth (Barnun and Poljakoff-Mayber, 1977) and it increased rice germination under saline conditions (Bal,1976). The biological method involves desalinization and reclamation of the soil by the use of halophytic plants which absorb the salt from the soil and then accumulate it in the leaves which are then shed. Usually the older leaves are shed and replaced by new, low salt leaves (Albert, 1975). When the shed leaves are removed, this results in the loss of salt to the environment. Salt accumulating species planted in saline soil can be harvested from time to time to remove salt from the environment. Also biological desalinization may involve breeding and use of salt resistant varieties of existing crop species. Germplasm can be selected and superior

cultivars developed on the basis of their adaptation to saline soils. A rapid screening method then evaluates promising strains and subsequently transfers their desirable characters to susceptible counterparts (Yeo, 1974; Flowers and Yeo, 1986; Marschner, 1986).

In Nigeria, large scale irrigation is practised in the northern part of the country where most of the food come from. The water used is often of poor quality leading to concentration in the soil of salts after evapotranspiration. This further compounds the problems of these arid and semi-arid parts of the country. The increasing population in Nigeria and the need for increasing crop production must mean that the non-productive lands many of them salt affected, may have to be used to produce salt tolerant crops of economic value, as the frequency of salt affected soils make their utilization necessary. It was in search of plants which can be used as possible biological desalinizers that an attempt was made to identify halophytic species in Nigeria. It was during the course of this adventure that the grass Dactyloctenium aegyptium L. Beauv, growing in the premises of the National Salt Company of Nigeria, Ijoko via Otta (Ogun State), Nigeria was discovered. The species became a resource material and favourite not only because it has its origin in folklore, but it also grows quite easily and fruits for a long time. It was thus selected for screening as a possible biological desalinizer. This is to find out if D. aegyptium is a cumulative halophyte that is, if it can accumulate excess salts which they

absorb from the saline soil in their shoots. The results of the chemical analysis of the plant at harvest when grown in the saline soil will indicate if the plant may play a considerable role in diminishing the salt content of the soil. The cultivation of the saline soils with the grass will be considered as a biological way for soil desalinization and reclamation.

The first step is the proper understanding of the behaviour of plants to the presence of excess salts in their growth medium. One way in which the mechanism of salt tolerance may be investigated is to seek knowledge of the mechanisms used by plants which are truly tolerant, in that they have adapted to the presence of the ion in question within their tissues and then compare with that of the plant under study (Strogonov, 1964; Turner, 1969).

In this study, the salinity tolerance of the tropical grass, D. aegyptium is compared where possible with two salt sensitive tropical rice cultivars (Oryza sativa L.) KAU 2, HG 2153 and a closely related salinity resistant temperate member of the gramineae Porteresia coarctata (Tateoka)

D. aegyptium is a more or less prostrate spreading annual herb, 15-60 cm high, rooting at the lower nodes, with an inflorescence of 2-5 digitately arranged terminal spikes. The leaves are broadely linear, 5-20cm long 2-7mm wide, tappering to a fine point. They are hairy especially on the margins with 1mm long ligule which is membranous. The inflorescence has spikes which are 3-5 cm long, and 5-8 mm wide terminating in a short

point. The spikelets are 3-5 mm long with 3-5 flowers set at right angles to the axis and densely crowded in two overlapping rows along its lower side, one of the scales at the base of the floret has a short oblique awn. The seed (grain) is about 0.8 mm long, light brown in colour, rounded in shape and with a hard coat. D. aegyptium is the only Nigerian species in the genus. It is widespread and abundant in farmland and waste spaces throughout most of Nigeria and often one of the first colonizers of bare ground giving an impression that it can adapt to harsh environmental conditions. It is sometimes a perrenial grass with much branched running shoots at the nodes and upright stems in small tufts (Stanfield, 1970). Apart from the work of Sharma (1982) no report appears to be in the literature concerning this species.

Rice (O. Sativa) is a freely tillering annual grass, 50-150 cm tall. On germination the primary root emerges, followed by two additional roots. Adventitous roots are then produced from the basal nodes of the primary stem and tillers. The culm is more or less erect, although lodging may occur. It is cylindrical, smooth 35-150 cm long, 6-10 mm in diameter, with solid nodes and hollow internodes. Above each node is a pronounced pulvinus with an intercalary meristem. The leaves alternate in two ranks with a single leaf at each node. The number of nodes and leaves are greatest on the main culm and the number declines progressively with the rise in tiller order. The leaf blade is long and narrow, 30-50 cm x 1.2-2.5 cm, some what pubescent, often with spiny hairs on margins. The

inflorescence has a terminal panicle, 14-42 cm long, each with 50-500 spikelets but usually about 100. The inflorescence may be open or compact, exact or dropping, The main axis or rachis bear a variable number of primary branches, one to several at each node. Each secondary branch bears one or more spikelets. Each spikelets is laterally compressed, containing a single hermaphrodite flower, borne on a short pedicel, which is enlarged at the top with two oblique sides. The pedicel is normally firm and only breaks on threshing. There are six stamens in two alternating whorls, the filaments are slender and the anthers versatile. The pistil has broad smooth ovary and single anatropous ovule. The style has two plumose stigmas, which may be white or purplish. The grain is white or translucent and the size varies from 5-14.5 mm long and 1.9-3.7 mm broad. The period from flowering to ripening of the grain is usually about 30 days (Purseglove, 1976).

Rice is a grain eaten by one third of the world's population. It is a species whose recent evolutionary history has been in fresh water marshes. It is adapted to waterlogged conditions possessing well developed aerenchyma and root oxidation properties (Yoshida, 1981). Rice as a crop is rather sensitive to salinity and it exhibits high uptake of sodium and chloride from quite moderate external concentrations (Flowers and Yeo, 1981). There is however considerable variation in salinity resistance both between and within cultivars (Akbar, 1972; Flowers and Yeo, 1981). Investigations of these differences are being conducted with the aim of identifying physiological criteria which may be used for

the improvement of breeding programmes aimed at producing varieties able to alleviate the substantial effects of soil and ground water salinity on tropical rice production (IRRI, 1974, 1981; Ponnamperuma, 1984).

P. coarctata was named initially as Oryza coarctata (Roxb) and subsequently classified as Sclerophyllum coarctatum (Griff) and it has recently been reclassified again because of its salt glands. The stem is 1.2 - 1.8m tall and erect from a stout creeping rhizome which is smooth, hard and polished with branches. The leaves are 15 - 30 cm by 8.3 - 12.5 mm, unequal sided, rigid smooth or scaberulous and the midrib is obscure. The leaf tip is long and slender and the sheath is long and undulately reticulate. The inflorescence has a terminal panicle 10 - 15 cm long. The main axis or rachis bears a number of branches which are trigonous, stiff and quite smooth. Each branch bears one or more spikelets and each spikelets is laxly imbricate and rigidly chartaceous. The pedicel is clavate at the top and then contracted below the dilated cupular tip. The anthers are very long and slender and the ovary is narrow and elongate (Bor, 1960).

P. coarctata grows in saline marshes and river deltas in South East Asia. Little however is known of its growth rate in saline conditions or the mechanism by which it tolerates salinity. It is known to be a slow growing plant which cannot be propagated by the seeds (Hooker, 1961).

This first part constitutes chapter 1 of the thesis.

Chapter 2 is on the salinity tolerance of *D. aegyptium*, *P. coarctata* and two rice cultivars of KAU 2 and HG 2153. Chapter 3 is on the effect of varying nutrient levels on the growth of *D. aegyptium* at 10 and 25% sea water concentrations. Chapter 4 is on ion relations of salinised *D. aegyptium* plant in varying nutrient levels. Chapter 5 is on the effect of salinity on stomatal conductance, transpiration and photosynthetic capacity of *D. aegyptium*. Lastly Chapter 6 is on the germination studies of *D. aegyptium*.

CHAPTER 2

SALINITY TOLERANCE

INTRODUCTION

The study of the effects of salinity on plant growth is fundamental to any investigation of the physical basis of salt tolerance (Shennan, 1987).

In non-halophytes, resistance to salinity is commonly correlated with the ability to restrict the entry of ions into the shoots. This general principle of avoidance appears essential to glycophytes which as a group, are unable to permit the concentration of inorganic ions necessary for osmotic adjustment to exist in the cells while maintaining normal metabolic and physiological activity (Flowers et al., 1977; Greenway and Munns, 1980). This is widely termed 'salt exclusion' although it is not by any means restricted to glycophytes, but it is practised by all plants which grow in saline conditions (Yeo and Flowers, 1982).

Growth is measured as a progressive change in fresh or dry weight per unit time which is a reasonable and adequate measurement in most glycophytes. However, both basis are of serious limited use for halophytes where comparisons between plants growing in different salinities are made. There are two reasons for this: firstly the growth conditions may dramatically change the water contents per se and secondly enormous accumulation of inorganic ions may occur. Water content may vary twofold and inorganic ions provide half the dry weight (Yeo, 1974). It is not surprising therefore that growth in terms of increase in size, total dry or fresh weight of

halophytes is enhanced on these bases (Yeo and Flowers, 1980)

Halophytic grasses differ from dicotyledonous halophytes in their mechanisms and degree of salt tolerance. The grass Agrostis stolonifera is characterised as sodium excluder and uses organic solutes for osmotic adjustments (Ahmad, Wainright and Stewart, 1981). In contrast, dicotyledonous halophytes from a wide variety of genera accumulate high level of sodium in the shoot tissues (Glenn and O'Leary, 1984). Monocotyledonous halophytes collected from natural population generally have much lower water contents, Na:K ratios and mineral contents than dicotyledonous halophytes collected from the same locations (Albert and Popp, 1977; Gorham, Hughes and Wyn Jones, 1980; Briens and Larher, 1982).

The sodium exclusive method of salt tolerance may be less efficient than the sodium accumulation mechanism (Gorham et al,1980). Grasses typically have lower water contents of the shoot tissues when grown on salt solutions compared to controls (Gorham, Mc Donnell and Wyn Jones, 1984). This has been interpreted as a symptom of water stress due to incomplete osmotic adjustment (Ahmad et al.,1981) but Glenn and O'Leary (1984) interpreted a similar phenomenom in dicotyledonous halophytes as a positive adjustment to salinity than to concentrated solutes in the cell sap. These traits vary quantitatively among species and have been used to distinguish 'physiotypes' of halophytes collected from natural population (Albert, 1975; Albert and Popp, 1977). However, the relationship between growth , salt uptake and water content of only a few species have been explored over a wide

range of salinities under controlled growing conditions (Kaplan and Gale, 1972; Storey and Wyn Jones, 1979; Yeo and Flowers, 1980).

The analysis of variance in each plant species was carried out using the shoot dry weight of the treatments. This was to determine whether the treatments had a significant effect on the growth of each species. Where the treatments had a significant effect on the growth, the least significant difference was calculated for comparisons of treatment means.

The main aim of this research is to compare the effects of salinity on the growth of two salt sensitive tropical rice cultivars (Oryza sativa) KAU 2 and HG 2153, a closely related salinity resistant temperate member of gramineae, Porteresia coarctata and a tropical grass Dactyloctenium aegyptium. The basis of comparison being measurement of fresh and dry weight, ion and water contents, sugar contents and water potential measurements.

Materials and Methods

Seeds of the cultivars and breeding lines of the two rice varieties (KAU 2 and HG2153) were obtained from the International Rice Research Institute, Manilla, Philipine, and from Prof P.J. Tomy Kerala, Agricultural University, Kenya.
Cuttings of *P. coarctata* were obtained from the population growing in pots in the greenhouse of the University of Sussex. Cuttings were left to grow in sand which was moistened with Yoshida culture solution (Table 1a) to a uniform size for 3 months in the greenhouse before the salinity trials started.

Seeds of *D. aegyptium* were collected from a population growing in the premises of a salt refinery industry in Nigeria (National Salt Company of Nigeria, Ijoko, Ogun State) (38^{o} 03′ N;6^{o} 44′E) between August and September, 1986. The seeds were planted in sand, in seed trays which were moistened with Stout and Arnon, 1939, solution (Table 2). The seedlings were allowed to grow in sand for two weeks before transplanting.

The seeds of the two cultivars of *O. sativa* were imbibed for 24h in aerated deionized water and then transferred to grids over the surface of Yoshida culture solution. Seven-day old seeedlings were transplanted into 50 cm diameter pots of sand moistened with Yoshida culture soilution. Salinization of the rice seedlings started five days after transplanting. The treatments were 0,5,10,20 and 30% artificial sea water (Table 1b).

After 3 months of establishment and acclimatization, the seedlings of *P. coarctata* were salinized with artificial sea water and the treatments were 0, 10, 20, 30, 40 and 50% sea water.

Table 1 a

Rice culture solution. Yoshida, Forna, cock and Gomez, 1972

Stock	Reagents	gl^{-1}
1	NH_4NO_3	91.4
2	K_2SO_4	71.4
3a	KH_2PO_4	46.2
b	K_2HPO_4	8.6
4	$CACl_2.6H_2O$	175.0
5	$MgSO_4.7H_2O$	324.0
6	Minor nutrients	
a	$MnCl_2.4H_2O$	1.5
b	$(NH_4)_6.Mo_7O_{24}.4H_2O$	0.074
c	H_3BO_3	093
d	$ZnSO_4.7H_2O$	0.035
e	$CuSO_4.7H_2O$	0.03
7	FeNaEDTA	10.5
8	$FeSO_4$	2.5

The Yoshida culture solution contains (in $molm^{-3}$) K (1.5); Ca (0.75); Mg (1.6); S (2.2); P (1.0); Cl (1.5); NH (1.4); NO_3 (1.4); Na (0.4).

Table 1b

Artificial sea water (Instant Ocean).

Stock	Reagents	gl^{-1}
1	NaCl	23.477
2	$MgCl_2$	4981
3	Na_2SO_4	3971
4	$CaCl_2$	1.10

Table 2

Culture solution (Stout and Arnon, 1939)

1) Major elements	Concentration of stock solution	Volume (ml) per litre of culture solution	Final concentration in culture solution
KNO_3	1M	6	6 mM
$Ca(NO_3)_2$	1M	4	4 mM
$MgSO_4$	1M	2	2 mM
KH_2PO_4	1M	1	1 mM
2) Minor elements A			
H_3BO_3	2.68g l^{-1}	1ml per litre	4.33×10^{-2}mM
$ZnSO_3$ 7 H_2O	0.222g l^{-1}		4.72×10^{-4}mM
$CuSO_4$ 5 H_2O	0.079g l^{-1}		3.16×10^{-4}mM
$MnSO_4$	1.0151g l^{-1}		6.72×10^{-3}mM
3) Minor elements B			
MoO_3	22.96mg in water	each in 100ml then 10ml of each combined in 0.1%$NaHSO_4$ to final volume of 1 litre 1ml final solution per 1 litre culture solution	1.22×10^{-5}mM
NH_4VO_3	96.02mg in 5%H_2SO_4		1.96×10^{-5}mM
$CrK_2^{3/4}SO_4)_4 \cdot {}^{24}H_2O$	44.78mg in 5%H_2SO_4		1.02×10^{-5}mM
$NiSO_4$ $6H_2O$	49.38mg in 5%H_2SO_4		1.7×10^{-5}mM
$Co(NO_3)_2$ $6H_2O$	17.94mg in water		5.44×10^{-6}mM
Na_2WO_4 $2H_2O$			
4) Iron			
FeNa-EDTA	1% aqueous		2.72×10^{-2}mM

The same artificial sea water concentrations were also used for *D. aegyptium* after 5 days of acclimatisation

All the seedlings were brought to these salinity levels by stepwise increments at the rate of 25 $molm^{-3}$ per day. The artificial sea water (Instant Ocean) composed primarily of sodium chloride. Molar ratios of Na^{+} /Mg^{2+} /Ca^{2+} in the artificial sea water is 1/0.022/0.024. The balancing anions, chloride, sulphate were present in a ratio of 1/0.081.

Salinity trials for the two rice cultivars, *P. coarctata* and *D. aegyptium* were conducted in the greenhouse of the University of Sussex with a temperature of 29 ± 4^{o} C during the day and 23 ± 2^{o} C at night. The saturation vapour pressure deficit was 1-2 KPa during the day and 0.6KPa at night. The light flux density ranged from a maximum of 400 umol cm^{-1} S^{-1} P.A.R (high pressure sodium) for 12 hours to a maximum of 1000 umol m^{-2} s^{-1} (natural day light).

Subsequent harvest of 10 plants per treatment were made by cutting at the soil line at the interval of seven days for the two rice cultivars and *D. aegyptium.* The harvested plants were made from an arbitrary first row on the working bench. There were five harvest for the two rice varieties and *D. aegyptium.*

There was only a terminal harvest for *P. coarctata* and this was five weeks after the salinity tolerance experiment started. This was because of the slow growing characteristic of the plant. For each treatment, there were four pots, each containing six

plants, these six plants were used for all the analytical determinations made.

For the determination of growth yield, the shoots were weighed fresh. Subsamples of fresh material were taken for chlorophyll analysis, sugar contents and water potential determination. Later the fresh materials were dried at 80 °C in a forced draught oven (Townsend and Mercer Ltd, Croydon, England) for 24h. The dried material were weighed again and then used for the determination of the ion contents.

Analysis of Sugar: The shoots were weighed fresh and the sugar extracted in 30ml of 80% aqueous ethanol at 80 °C in a water bath for 30 mins. The extract was removed from the boiling tubes and stored, and the shoots were extracted further for sugar in 10ml of 100% ethanol for a further 15 mins. The ethanol extract were then combined and evaporated to dryness. 5 ml of water was added to redissolve the mixture. The amount of sugar in the plant extract was then determined using the anthrone reagent.

Two millilitre of anthrone reagent was slowly added in an ice bath to 0.1 ml of ethanol extract and 1 ml of standard (glucose stock solution, 1.5 mg/ml dilution 10 x , 150 ug/ml), running the reagent carefully down a glass rod. The mixture was stirred carefully and the tubes were covered with glass marble and heated for 15 mins in boiling water. After cooling in ice the absorbance was read at 630nm using a Pye Unicam ultraviolet spectrophotometry model SP1800.

Water potential measurements: Small quantities of undamaged lamina were immediately frozen during the harvest using

solid carbondioxide in an expanding polystyrene box. The frozen material was stored at -10^{o}C for about 3 days before it was thawed and used for measurement of osmotic potential. The Wescor C-52 chamber in conjuction with a HR 33T microvoltmeter was used for the measurement. These values were converted to Megapaskals by extrapolation and by assuming an osmotic coefficient of 1.0 and $osmol\ Kg^{-1}$ =2.479 mPa at 25^{o}C (Yeo and Flowers, 1986).

Chemical analysis of dried material

Dry ashing and Extraction

For each treatment oven dried material were finely ground in a rotary grinder. Weighed samples ranging from 50-100mg were then placed in a crucible and ashed for 4 hours at 550^{o}C in a muffle furnace. When cooled, the ash was dissolved in 0.1 ml, IM HNO_3 and then made up to 10ml volume with 100mM HNO_3.

Analysis of Inorganic ions:

Mineral cations: Sodium, potassium, magnesium and calcium were determined by atomic absorption spectrophotometry (Pye Unicam SP9).

Mineral anions

Chloride: Weighed samples of oven dried material were extracted in 100 mol m^{-3} acetic acid at 90^{o}C for 2 hours. The amount of chloride was estimated using a specific ion electrode EIL used in conjuction with a high impedance voltmeter (Vibron).

Nitrate: Nitrate was determined spectrphotometrically by the phenoldisulphonic acid reaction in aqueous solution extracts of dried material. Phenoldisulphonic acid was prepared

by heating 225ml of sulphuric acid with 25g of phenol in a boiling water bath for 8 hours.

One millilitre samples of the extract (or a suitable volume, containing about 1 ueg of nitrate diluted to 10 ml) were evaporated to dryness in conical flasks. When cool, 0.4 ml of concentrated phenoldisulphonic acid was added and the residue was dissolved by warming gently on a hot plate. The sample was diluted to about 5 ml with water and 1.5 ml of 10 M NaOH was added. The solution was made up to a volume of 10ml with water. The optical density was then determined at 410 nm. Standard curve was prepared by taking known quantities of KNO_3 through the same procedure.

Sulphate: This was estimated spectrophotometrically by the chloranilic acid reaction (Yeo and Flowers, 1985).

One millilitre of the samples was adjusted to pH between 4 and 5 with acetic acid /Na acetate buffer. Excess barium chloranilic acid was added, about enough to cover the end of a spatula. The mixture was boiled for 10 mins in a water bath and cooled afterwards.

Four millilitre of 60% aqueous ethanol was added and the whole mixture centrifuged for 10 mins with MSE bench centrifuge. The optical density was estimated at 530 nm. Standard curve was prepared by taking known quantities of sulphate through the same procedure.

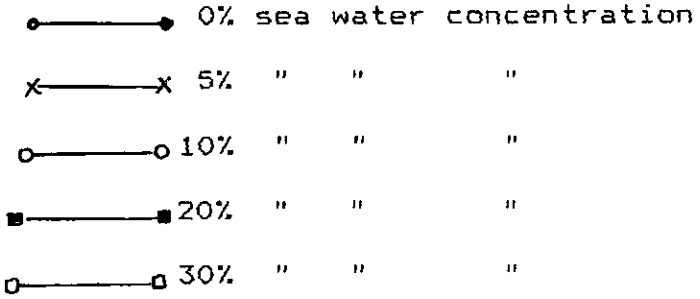
RESULTS

The fresh and dry weights for the two rice varieties, KAU 2 and HG 2153 at the five different sea water concentrations used are shown in figures 1 and 2. The shoot fresh weight at the last harvest was reduced by about 50% at 10% sea water concentration for the two rice varieties relative to the control at 0% sea water and by between 80 and 90% at 30% sea water. The effect of salt treatment on the pattern of dry matter accumulation of these two rice varieties were generally similar to the fresh weight pattern. The varieties HG 2153 grew more slowly than KAU 2 under the same conditions and for the same period, and they differred significantly in shoot dry weight ($P < 0.05$).

Figure 3 shows the relationship between shoot dry and fresh weight for each of the salinity treatment for P. coarctata The yield was generally good between 0 and 20% sea water and there was no significant difference ($P > 0.05$) between their values. Thereafter there was a significant loss of yield weight as salinity increased. At 50% sea water the fresh and dry weights were reduced by 67% and 50% respectively relative to the control.

For D. aegyptium, the shoot fresh weight reduced as the salinity increased (Figure 4). At the last harvest at 50% sea water the fresh and dry weights (Figures 4 and 5) were reduced by 75 and 73% respectively relative to the control. The difference in the dry weight at 0 and 10% sea water was only 5% in favour of 10% sea water but about 10% in fresh weight in favour of 0% sea water. The difference in dry weight between 10 and 20% sea water was 37% and in fresh

Figure 1: Effect of salinity on the shoot fresh and dry weights at 5 harvests of the rice cultivar, KAU 2. Mean values of six replicates (g) ± SD at last harvest.



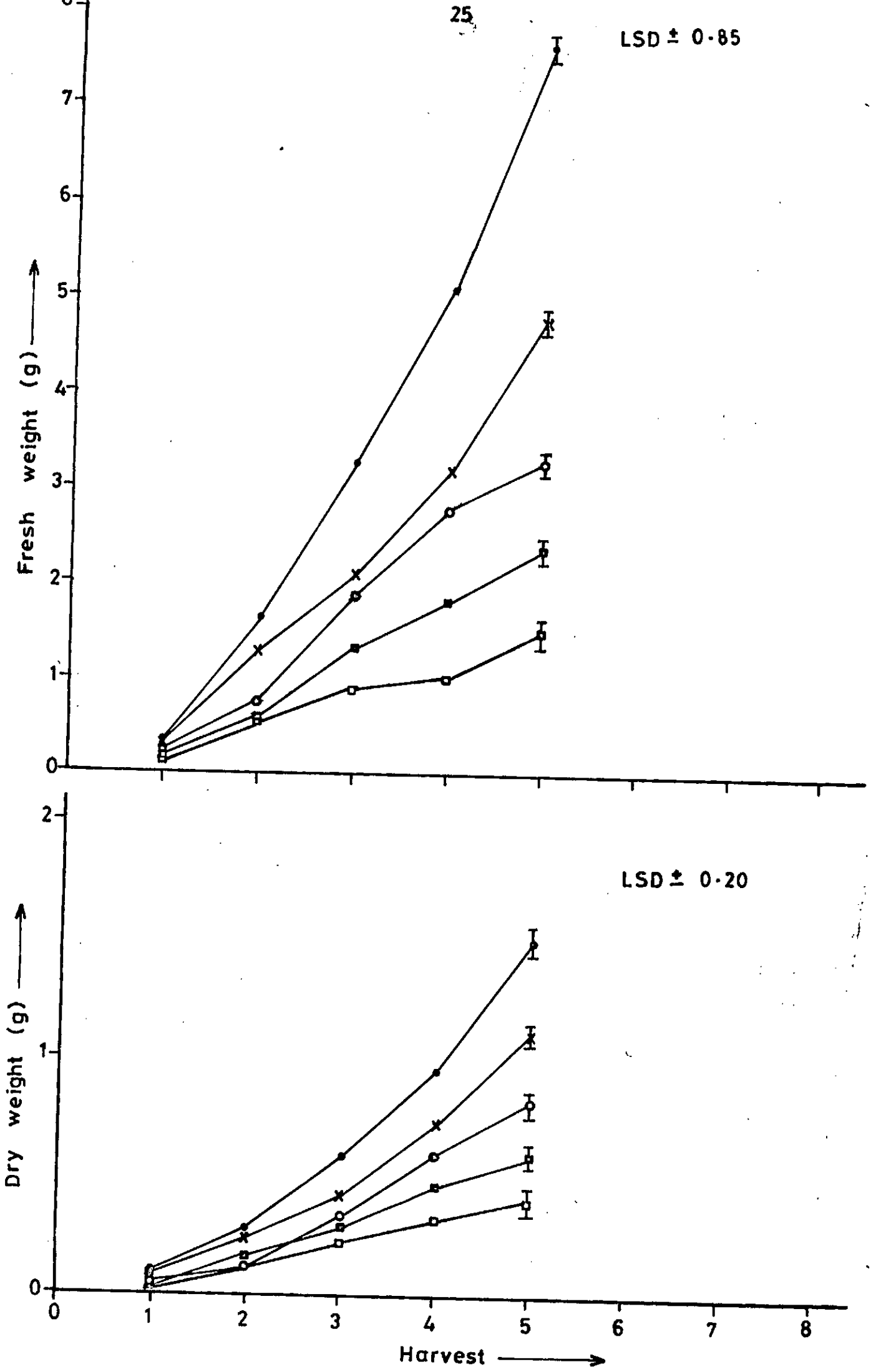
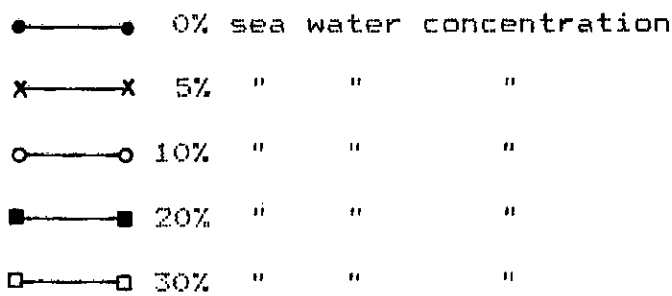
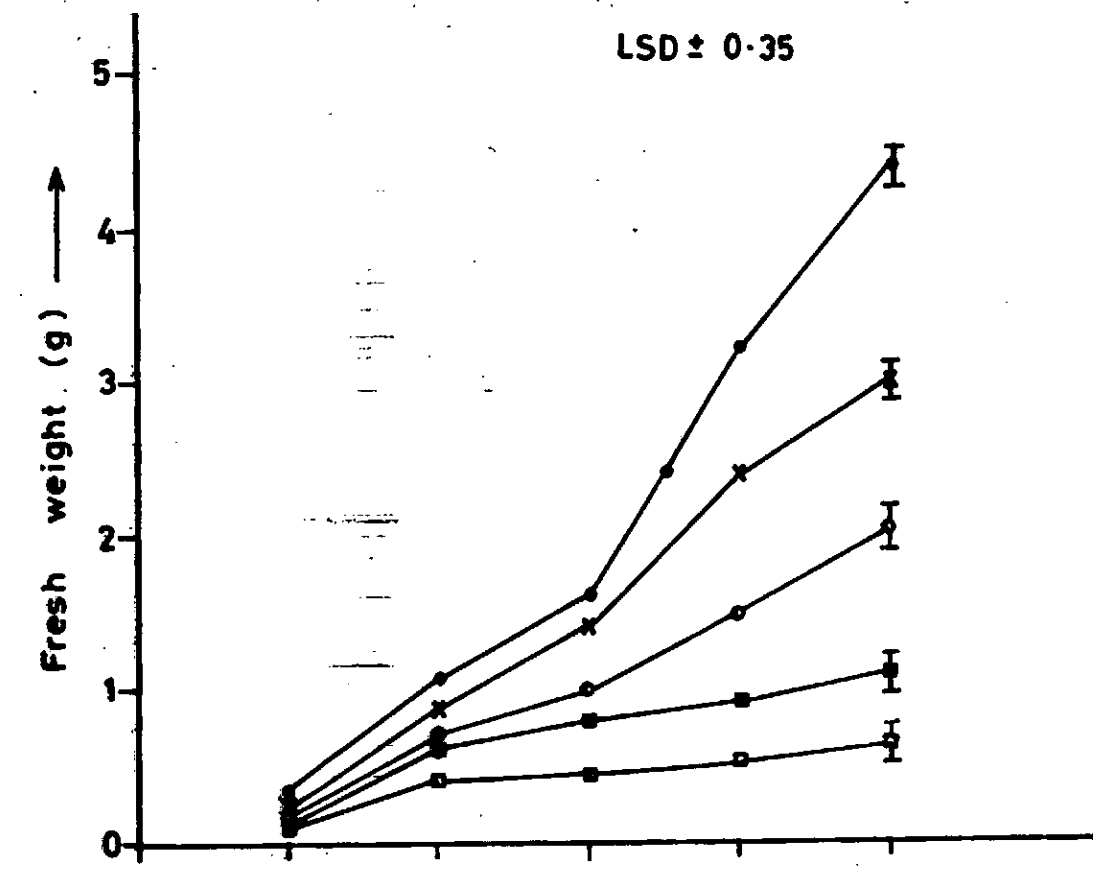
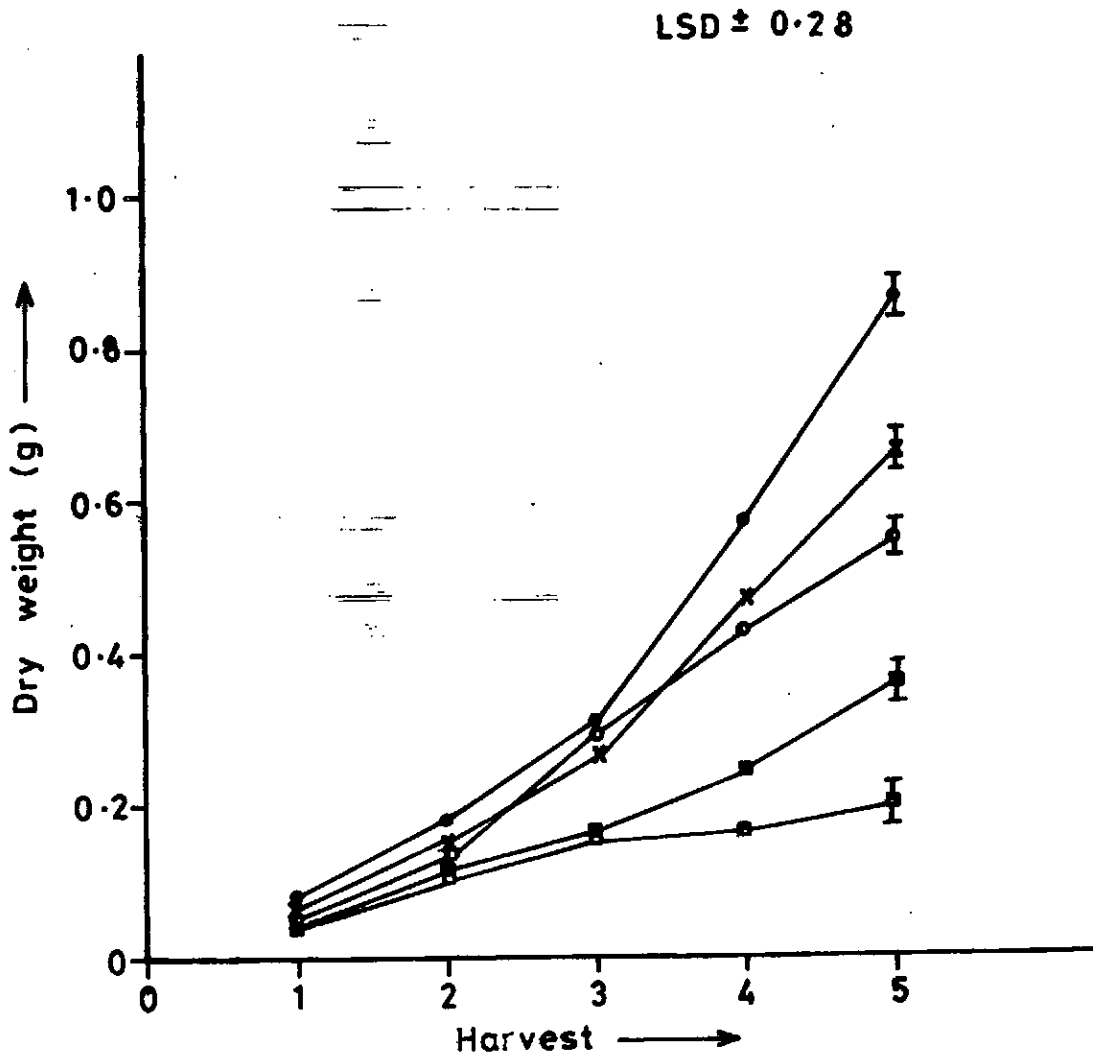

LSD ± 0·85
Fresh weight (g)
0
1
2
3
4
5
6
7
LSD ± 0·20
Dry weight (g)
0
1
2
0
1
2
3
4
5
6
7
8
Harvest

Figure 2: Effect of salinity on the shoot fresh and dry weights at five harvests of the rice cultivars, HG 2153

Mean values of six replicates (g) ± SD at last harvest.




LSD ± 0·35
Fresh weight (g)
0
1
2
3
4
5


LSD ± 0·28
Dry weight (g)
0
0·2
0·4
0·6
0·8
1·0
0
1
2
3
4
5
Harvest

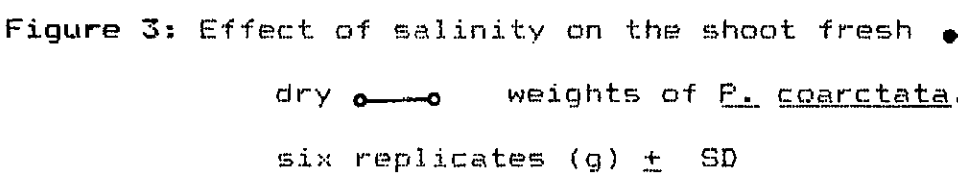


Figure 3: Effect of salinity on the shoot fresh ●——● and dry o——o weights of P. coarctata. Mean values of six replicates (g) ± SD

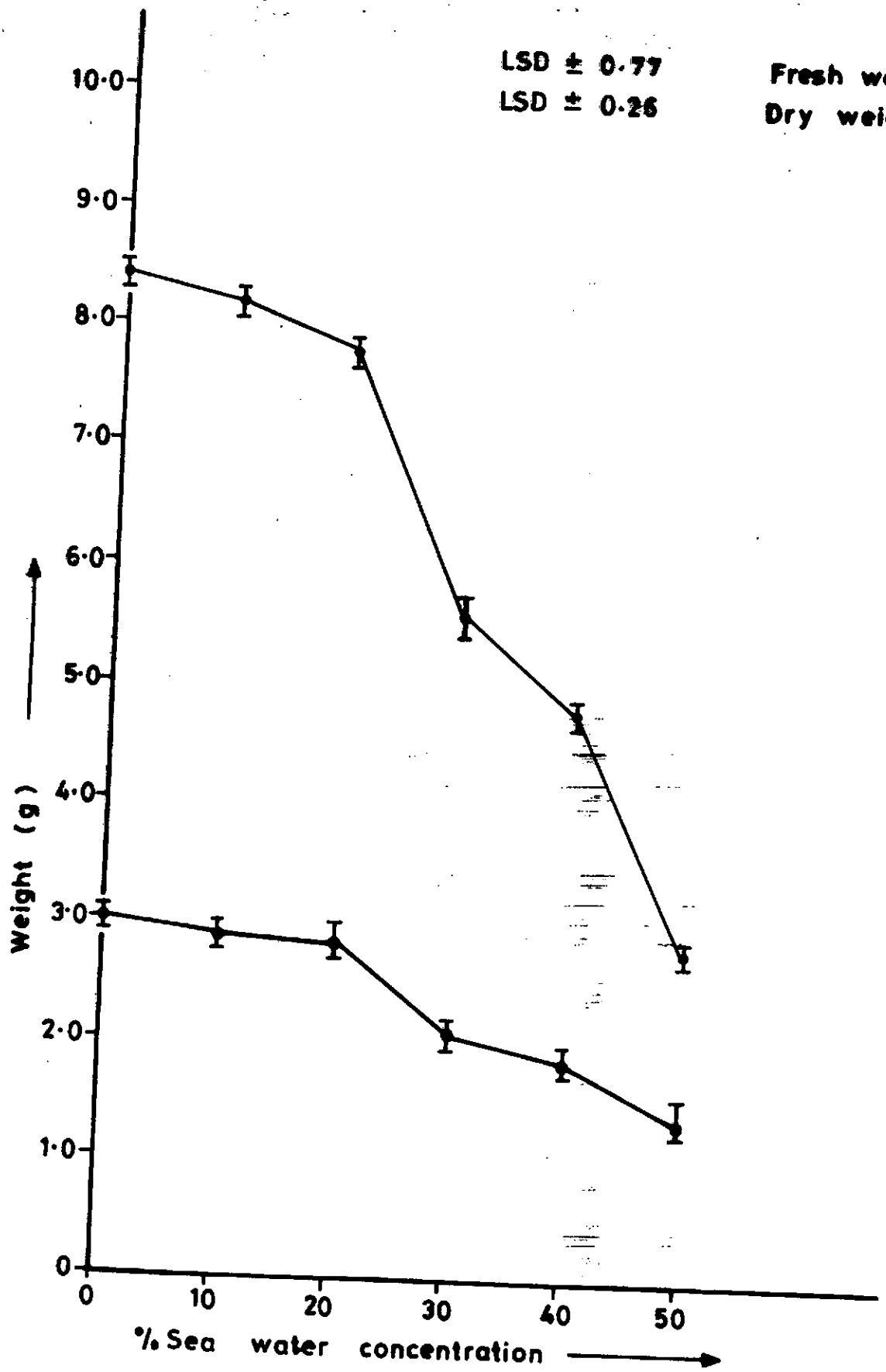
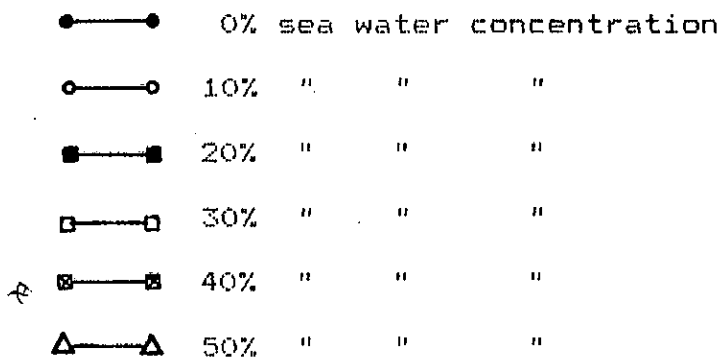

LSD ± 0.77
LSD ± 0.26
Fresh weight
Dry weight
10·0
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
Weight (g)
0
10
20
30
40
50
% Sea water concentration

Figure 4: Effects of salinity on the shoot fresh weight of *D. aegyptium* at 5 harvests.

Mean values of six replicates (g) ±SD at last harvest.



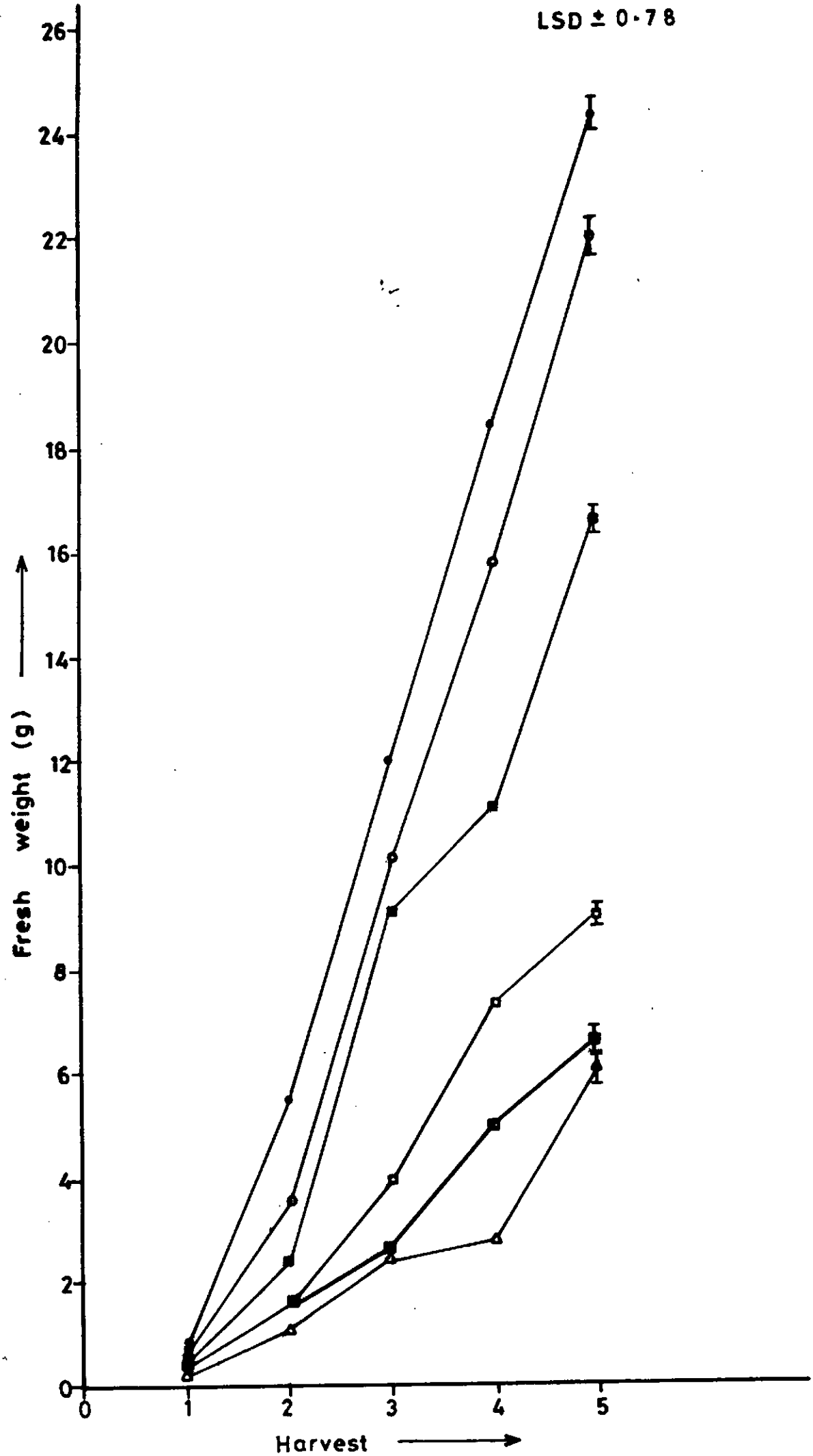
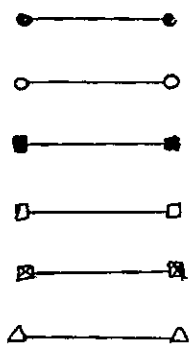
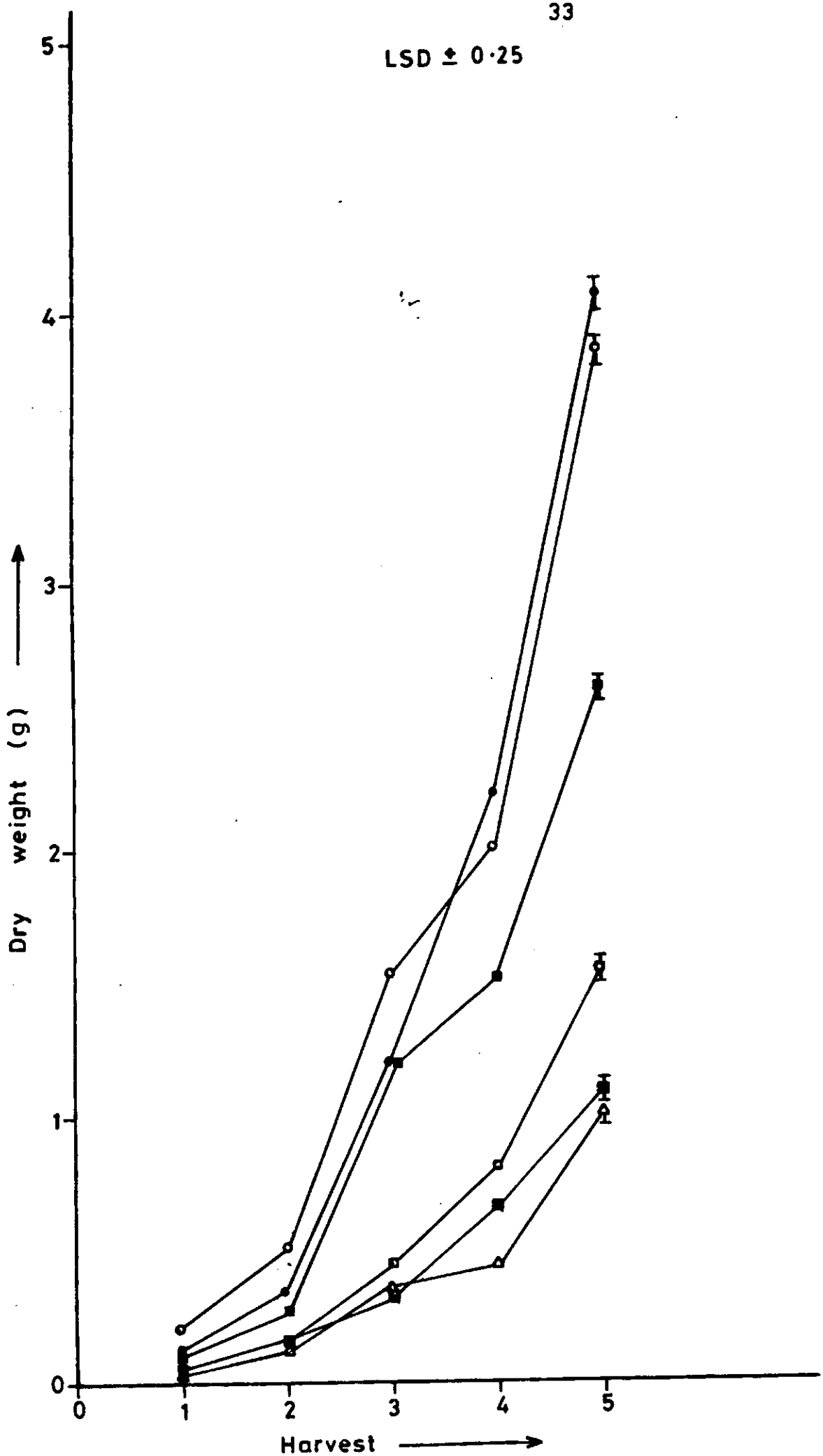

LSD ± 0·78
Fresh weight (g)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
0
1
2
3
4
5
Harvest

Figure 5: Effect of salinity on the shoot dry weight of *D. aegyptium* at 5 harvests. Mean values of six replicates (g) ± SD at last harvest.



0% sea water concentration
10% " " "
20% " " "
30% " " "
40% " " "
50% " " "


LSD ± 0·25
Dry weight (g)
0
1
2
3
4
5
0
1
2
3
4
5
Harvest

weight it was 28%. Between 20 and 30% sea water, a difference of 45% was found in dry weight while 38% difference occurred in fresh weight. Also between 30 and 40% Sea water differences of 31 and 26% were found in dry and fresh weights respectively. And between 40 and 50% sea water differences of 9.0 and 7.5% was observed for the dry and fresh weights respectively. Thus the dry weights of the shoot were affected more dramatically than the fresh weights as larger differences occurred between one salinity level and another.

Tables 3 gives the water content values of the test species. Generally, the content at each harvest decreases as salinity increases in each test plant. In KAU 2, at the first harvest, the ratio of the water content at 0 and 30% sea water was in the order of 1.5 : 1 and the same ratio was maintained at the last harvest, showing that the variety maintained more or less the same water content throughout the experiment. However in the other rice variety, HG 2153, the ratio at 0 and 30% sea water at the first harvest was 1.3 : 1 while at the last harvest it rose to 4.5 : 1. This was as a result of a marked decrease in water content of plants in the 30% sea water at the last harvest relative to the first.

In P. coarctata, the ratio of the water content was 1.7 : 1 at the only harvest in 0 and 50% sea water while between 0 and 30% sea water it was in the region of 1:1.

In D. aegyptium, at the first harvest the ratio of water

TABLE 3: Changes in water contents (gg shoot dry wt^{-1}) of seedlings of KAU 2, HG 2153, P. coarctata and D. aegyptium to varying salinity and at five harvests. There was only one harvest in P. coarctata

Sea water concentration/	Harvest 1	2	3	4	5
			KAU 2		
0	4.00	3.34	4.66	4.58	3.88
5	3.63	394	4.55	398	3.22
10	3.69	4.10	4.11	3.65	3.09
20	3.20	3.21	3.76	2.89	299
30	2.62	2.81	2.74	2.43	2.65
			HG 2153		
0	3.73	4.71	4.23	4.58	4.15
5	3.64	4.63	4.07	4.04	3.50
10	297	4.20	391	3.85	2.70
20	2.78	3.47	3.12	2.16	2.35
30	2.62	3.21	2.40	1.67	091
			D. aegyptium		
0	9.15	9.66	793	7.53	5.25
10	8.75	8.80	7.86	7.48	5.01
20	7.15	8.24	6.89	7.10	4.43
30	6.16	7.05	6.46	7.02	491
40	5.66	6.12	6.44	6.36	4.87
50	5.48	5.35	6.32	5.33	4.19
	P. coarctata				
0	1.83				
10	1.75				
20	1.72				
30	1.67				
40	1.46				
50	1.06				

content at 0 and 50% sea water was in the region of 2:1, while at the last harvest a near 1:1 ratio was observed due to a reduction of water content at 0% sea water. This is the opposite of what occurred in the rice variety, HG2153. However between 0 and 30% sea water in D. aegyptium, the ratio of 1.5 to 1 was observed at the first harvest, while the last harvest was about 1 : 1 ratio.

Ion contents : Potassium was the most abundant cation of the four ions determined at 0 % sea water (Tables 4 and 6). The potassium ion concentration decreased generally in an ubiquitous phenomenon in all the species with increase in salinity.

At the first harvest in KAU 2, the ratio of potassium ion at 0 and 30% sea water was 1.4 : 1, while at the last harvest it was 1.2 : 1, showing a slight reduction in accumulation. In the other rice variety HG 2153, a ratio of 1.2 : 1 was found at the first harvest between 0 and 30% sea water, while at the last harvest it was 1.1 : 1, showing also a slight reduction in potassium ion accumulation.

Between 0 and 30% sea water in the species P. coarctata the potassium ion ratio was 1.2 : 1 at the only harvest and also at the first harvest in D. aegyptium, while at the last harvest it was 1.1 : 1. However, the ratios for 0 and 50% sea water is 1.2 : 1 in P. coarctata at the only harvest and 2.0 : 1 in D. aegyptium at the first harvest; while at the last harvest the ratio fell to 1 : 1.

There was a continous rise in the sodium ion concentration in

TABLE 4: K^+ and Na^+ content (mmolg shoot dry wt^{-1}) in shoot of KAU 2, HG 2153, P. coarctata and D. aegyptium at various sea water concentrations and at five harvests. There was only one harvest in P. coarctata

Sea water concentration	Harvest 1		2		3		4		5	
	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+
%					KAU 2					
0	0.77	0.14	1.08	0.18	1.10	0.18	1.25	0.25	1.38	0.24
5	093	0.39	1.07	0.16	1.41	0.35	1.11	0.30	1.08	0.55
10	098	0.54	1.09	0.67	1.13	0.34	1.11	0.52	1.07	0.75
20	0.73	079	0.84	0.70	1.12	0.68	1.12	098	1.09	096
30	0.54	1.46	098	1.19	1.08	0.78	1.06	1.04	1.04	1.15
					HG 2153					
0	1.08	0.30	1.18	0.13	1.11	0.18	1.07	0.22	0.86	0.29
5	1.04	0.40	1.21	0.27	1.09	0.24	1.05	0.40	091	092
10	0.83	1.34	096	0.38	096	0.38	1.01	0.47	098	1.71
20	097	1.04	099	0.89	096	0.89	0.83	1.31	0.80	1.70
30	093	1.27	094	091	0.82	1.32	0.88	2.08	0.73	1.79
					D. AEGYPTIUM					
0	2.10	0.42	2.22	0.38	2.16	0.42	1.84	0.38	1.34	0.38
10	1.60	1.18	1.60	1.20	1.56	1.54	1.58	192	1.08	2.20
20	1.70	1.62	1.58	1.66	1.80	2.32	1.28	298	1.18	2.64
30	1.54	1.00	1.54	1.84	1.58	2.34	1.44	3.08	1.20	2.70
40	1.24	2.16	1.14	2.28	1.30	2.48	1.20	3.18	1.22	2.82
50	1.02	2.00	092	2.66	1.44	296	1.24	3.30	1.30	3.46
	P. coarctata									
0	0.86	0.20								
10	0.82	0.28								
20	0.89	0.58								
30	0.74	0.72								
40	0.84	090								
50	0.74	1.26								

all the species as the sea water concentration increased (Table 4). At the first harvest, the ratio of sodium at 30 and 0% sea water is 10:1 in KAU 2; 4.2:1 in HG2153; 3.6 : 1 in P. coarctata at the only harvest and 2.3 : 1 in D. aegyptium. At the last harvest the ratios were 4.8 : 1 in KAU 2, 6.2:1 in HG 2153 and 7:1 in D. aegyptium. The ratios for 50 and 0% sea water in P. coarctata at the only harvest is 6.3:1 and in D. aegyptium 4.8 : 1 at the first harvest and 9 : 1 at the last harvest. This increase in sodium ion concentration in all the test species as the salinity increased resulted in a gradual increase in the Na^+ to K^+ ratio; a function of tolerance to salinity for all species (Table 5). While noting that at the final harvest Na : K ratio only exceeded one in KAU 2 at 30% sea water, in HG 2153 it exceeded one at 5-30% sea water, in P. coarctata at 40 and 50% sea water and in D. aegyptium at 10-50% sea water. Also Na : K ratio is greater than 1.0 at 30% sea water concentration for the two rice varieties at all harvests, at 40 and 50% sea water for P. coarctata and 20-50% sea water for D. aegyptium.

The divalent cations Mg^{2+} and Ca^{2+} were present (Table 6) at much lower levels than the monovalent cations and were not as affected by changes in the external salinity as the potassium and sodium ions. In KAU 2, the magnesium ion increased at each harvest, as the salinity increased, but between the first and the last harvests, the concentrations decreased slightly at all the salinity levels. In HG 2153, there was an increase in magnesium

TABLE 5: Na:K molar ratios of the shoots of KAU 2, HG 2153, *P. coarctata* and *D. aegyptium* at varying salinities and five harvests. There was only one harvest in *P. coarctata*

Sea water concentration/	Harvest 1	2	3	4	5
			KAU 2		
0	0.18	0.07	0.16	0.12	0.17
5	0.42	0.14	0.25	0.27	0.51
10	0.55	0.61	0.31	0.46	0.70
20	1.08	0.83	0.61	0.87	0.88
30	2.70	1.21	0.72	098	1.10
			HG 2153		
0	0.28	0.11	0.16	0.21	0.38
5	0.38	0.22	0.22	0.38	1.01
10	1.16	0.39	0.40	0.47	1.74
20	1.07	090	093	1.57	2.13
30	1.36	097	1.16	2.36	2.45
			D. aegyptium		
0	0.20	0.17	0.19	0.21	0.28
10	0.74	0.75	099	1.21	2.04
20	095	1.05	1.29	1.70	2.24
30	1.30	1.19	1.48	2.13	2.25
40	1.74	2.00	2.01	2.25	2.31
50	196	2.09	2.06	2.66	2.66
	P. coarctata				
0	0.23				
10	0.34				
20	0.65				
30	097				
40	1.07				
50	1.70				

ion concentration at all the harvests as sea water concentration increased and also between the first and last harvest even though slightly at the only harvest in P. coarctata the magnesium ion concentration increased dramatically as salinity increased. In D. aegyptium, there was virtually no change in the concentrations between the first and last harvests and also as the salinity increased at each harvest.

The calcium ion concentration increased as salinity increased at all harvests and the concentrations also increased from the first to the last harvest in the two rice varieties and P. coarctata.

In D. aegyptium there was generally a decrease in concentration from the first to the last harvest and at each harvest, the calcium ion concentration decreased (Table 6).

Table 7 gives the chloride content of the test species. Generally the content at each harvest increased as salinity increases with each species and the values also increased in each salinity concentration with harvest. In KAU 2, at the first harvest the ratio of chloride content at the 30 and 0% sea water was in the order of about 3:1 while at the last harvest it went down to 2:1 showing a reduction in the level of accumulation at 30% sea water over time. In the other rice variety, HG2153 at the first harvest, the ratio was 4:1 and also the same ratio was maintained at the last harvest.

P. coarctata has a ratio of 2.5:1 at 50 and 0% sea water and 2:1 at 30 and 0% sea water concentration, while in D. aegyptium at the first harvest the difference at 50 and 0% seawater was in the

TABLE 6: Magnesium and calcium contents (mmol/g) shoot dry weight in the shoots of KAU 2, HG 2153 P. coarctata and D. aegyptium to varying salinities and five harvests. There was only one harvest in P. coarctata

Sea water concentration	Harvest 1		2		3		4		5	
	Mg^{2+}	Ca^{2+}	Mg^{2+}	Ca^{2+}	Mg^{2+}	Ca^{2+}	Mg^{2+}	Ca^{2+}	Mg^{2+}	Ca^{2+}
%					K A U 2					
0	0.11	0.11	0.11	0.13	0.09	0.11	0.09	0.11	0.09	0.14
5	0.11	0.14	0.11	0.14	0.09	0.15	0.11	0.14	0.09	0.14
10	0.12	0.15	0.11	0.15	0.07	0.16	0.13	0.15	0.09	0.16
20	0.14	0.15	0.09	0.16	0.11	0.16	0.15	0.16	0.11	0.17
30	0.16	0.16	0.12	0.17	0.12	0.18	0.16	0.17	0.12	0.18
					H G 2153					
0	0.14	0.15	0.11	0.15	0.15	0.19	0.18	0.16	0.18	0.17
5	0.15	0.15	0.14	0.17	0.16	0.21	0.17	0.16	0.18	0.17
10	0.16	0.19	0.15	0.21	0.17	0.21	0.17	0.16	0.18	0.18
20	0.16	0.19	0.16	0.21	0.18	0.22	0.18	0.20	0.19	0.22
30	0.17	0.20	0.16	0.22	0.18	0.22	0.20	0.20	0.20	0.22
					D. aegyptium					
0	0.21	0.30	0.23	0.32	0.24	0.30	0.22	0.28	0.21	0.28
10	0.21	0.20	0.22	0.26	0.24	0.24	0.22	0.24	0.18	0.22
20	0.21	0.24	0.22	0.26	0.24	0.24	0.22	0.22	0.18	0.22
30	0.21	0.24	0.20	0.26	0.21	0.24	0.23	0.22	0.22	0.20
40	0.22	0.24	0.19	0.22	0.21	0.22	0.22	0.22	0.20	0.20
50	0.22	0.26	0.19	0.22	0.20	0.20	0.22	0.22	0.20	0.20

	P. coarctata	
0	0.35	0.12
10	0.40	0.12
20	0.41	0.12
30	0.44	0.12
40	0.46	0.13
50	0.52	0.14

TABLE 7: Chloride ion content (mmol g^{-1}) in shoots of KAU 2, HG 2153, P. coarctata and D. aegyptium at different salinities and harvests. There was only on harvest in P. coarctata

Sea water concentration/‰	Harvest 1	2	3	4	5
			KAU 2		
0	0.31	0.22	0.38	0.43	0.55
5	0.57	0.38	0.46	0.48	0.59
10	0.65	0.75	1.04	1.04	0.64
20	0.86	1.00	1.00	0.89	0.70
30	1.01	1.04	1.00	1.12	1.08
			HG 2153		
0	0.35	0.26	0.31	0.36	0.41
5	0.53	0.31	0.40	0.55	0.96
10	1.14	0.50	0.55	0.59	1.22
20	1.45	0.70	0.89	1.08	1.18
30	1.23	1.00	1.08	1.22	1.64
			D. aegyptium		
0	0.34	0.22	0.22	0.11	0.82
10	0.68	0.53	1.00	1.18	1.18
20	0.75	0.70	1.04	1.04	1.04
30	0.97	1.00	1.08	1.18	1.18
40	0.82	1.08	1.13	1.22	1.22
50	0.86	1.13	1.22	1.22	1.27
	P. coarctata				
0	0.30				
10	0.38				
20	0.59				
30	0.56				
40	0.72				
50	0.75				

order of 2.5:1 while at the last harvest it was 1.5:1. However between 30 and 0% sea water of the same plant a 3:1 ratio was observed at the first harvest while 1.5:1 ratio was observed at the last harvest showing an increase in accumulation as sea water concentration increased.

The osmolarity of the cell sap calculated from the water contents and the sum of the cations Na^+, K^+, Mg^{2+} and Ca^{2+} according to the method of Storey, Ahmad and Wyn Jones, (1977) is given in Table 8. The values were used to estimate the osmotic potential gradient between the plant tissues and the external solution assuming that the salts in the plants are osmotically active (Storey et al.,1977). The osmolarity increased with salinity in all species at each harvest. At the first harvest, the ratio at 30 and 0% seawater is 3:1 in KAU 2, 2:1 HG2153 1:5:1 in P. coarctata and D. aegyptium. At the last harvest, the ratio went down to 2:1 in KAU 2, it went up to 4:1 in HG 2153 and 2:1 in D. aegyptium. However the ratio between 50 and 0% sea water in P. coarctata is 3:1 and 2:1 in D. aegyptium at the first harvest and 3:1 at the last harvest.

The total ethanol soluble sugar are shown in Table 9. The concentration of sugar increased generally in the four test species at each harvest as the sea water concentration increased. At the first harvest KAU 2 and HG2153 had a ratio of 3:1 between 30 and 0% sea water, while P. coarctata had a ratio of 1:1 and D. aegyptium had 1.5:1. At the last harvest, KAU 2 had 1.2:1, HG2153

TABLE 8: Osmolarity of the cell sap (mmol kg^{-1}) of KAU 2, HG 2153, P. coarctata and D. aegyptium at five harvests. There was only one harvest in P. coarctata.

Sea water concentration/.	Harvest 1	2	3	4	5
			KAU 2		
0	292	425	328	397	487
5	435	381	448	470	609
10	482	490	423	515	676
20	556	570	551	530	786
30	884	851	748	580	932
			HG 2153		
0	346	356	387	369	468
5	475	399	420	438	623
10	865	395	442	483	1140
20	849	617	718	950	1220
30	954	688	1070	1390	1510
			D. aegyptium		
0	337	332	402	409	417
10	382	372	469	547	727
20	497	476	600	682	946
30	527	543	678	711	876
40	616	657	682	752	901
50	624	733	764	925	1205
	P. coarctata				
0	857				
10	893				
20	1167				
30	1187				
40	1553				
50	2530				

TABLE 9: The concentration of total ethanol soluble sugars (mg g fwt $^{-1}$) in fresh leaves of KAU 2, Hg 2153, *P. coarctata* and *D. aegyptium* at 5 harvest, There was only one harvest in *P. coarctata*.

Sea water concentration%	Harvest 1	2	3	4	5
			KAU 2		
0	0.68	1.84	3.62	3.51	5.55
5	0.77	2.44	3.91	4.13	4.62
10	0.83	4.59	4.10	4.09	4.10
20	1.18	4.79	6.35	6.53	6.89
30	1.72	5.23	5.33	5.78	6.24
			HG 2153		
0	0.55	2.55	3.03	3.82	4.00
5	0.64	2.90	3.64	4.10	4.51
10	0.82	2.55	3.06	4.36	4.95
20	1.07	3.09	4.62	5.20	6.10
30	1.51	5.26	5.96	6.44	6.20
			D. aegyptium		
0	0.84	1.67	1.75	1.82	2.07
10	0.94	2.17	3.27	3.82	3.94
20	1.02	2.56	3.58	3.81	4.02
30	1.10	2.92	4.04	4.12	4.21
40	1.20	3.70	5.27	5.29	5.31
50	1.91	5.14	5.60	5.77	5.89
	P. coarctata				
0	2.02				
10	2.23				
20	2.55				
30	2.90				
40	3.09				
50	4.26				

1.5:1 and *D. aegyptium* 2:1. At 50 and 0% sea water the ratio was 2:1 for *P. coarctata* and *D. aegyptium* while the latter had 3:1 at the last harvest.

For water potential, the leaf had a relatively high osmotic potential in the absence of sea water, that is at 0% sea water (Table 10). The osmotic potential decreases with increase in salinity in the three species tested for it. At 30 and 0% sea water, at the first harvest a 1.6:1 ratio was found in KAU2 and *D. aegyptium* and 1.3:1 in HG 2153. At the last harvest there was a ratio of 1.2:1 in KAU 2 and 1.3:1 in both HG2153 and *D. aegyptium* while between 50 and 0% sea water in *D. aegyptium* at the first harvest a 2:1 ratio was found and 1.4:1 at the last harvest.

At 0% sea water, HG 2153 had the highest water potential values at the first harvest while KAU 2 and *D. aegyptium* had similar values. But at the last harvest *D. aegyptium* had the highest followed by HG2153 and then closely followed by KAU 2. At 30% sea water in the first harvest, the same pattern as observed at 0% sea water was found. Also at the last harvest *D. aegyptium* still had lower value than the other two rice varieties, which had the same values. Even at 50% sea water at the last harvest, *D. aegyptium* still maintained low osmotic potential.

Table 11 gives the nitrate and sulphate concentrations of the four test species. The sulphate concentrations decreased slightly as salinity increased in all species except *P. coarctata* which appears not to be affected by increase

TABLE 10: Water potential (-mPa) of leaf sap of KAU 2, HG 2153 and D. aegyptium at five harvests and at varying sea water concentrations. The water potential of P. coarctata was not determined.

Sea water concentrations%	Harvest H - 1	H - 2	H - 3	H - 4	H - 5
			KAU - 2		
0	0.33	0.48	1.44	1.51	1.58
5	0.48	0.69	1.59	1.65	1.73
10	0.62	0.85	1.73	1.73	1.87
20	0.55	1.00	1.81	1.80	1.87
30	0.55	1.29	1.89	1.87	195
			HG 2153		
0	0.24	0.40	1.29	1.43	1.51
5	0.33	0.48	1.44	1.43	1.58
10	0.24	0.55	1.44	1.58	1.73
20	0.24	0.88	1.59	1.73	195
30	0.33	1.00	1.73	1.87	195
			D. aegyptium		
0	0.33	0.40	0.69	0.88	1.14
10	0.40	0.55	0.88	092	1.44
20	0.48	092	0.88	1.00	1.51
30	0.55	1.00	1.07	1.15	1.58
40	0.69	1.29	1.29	1.36	1.80
50	0.69	1.44	1.36	1.43	1.87

TABLE 11: Nitrate (mol/gdwt) and sulphate (mmol gwt^{-1}) of seedlings of KAU 2, HG 2153, P. coarctata and D. aegyptium at five harvests. There was only one harvest for P. coarctata.

Sea water	1		2		3		4		5	
	NO_3^-	SO_4^{2-}	NO_3^-	SO_4^{2-}	NO_3^-	SO_4^{2-}	NO_3^-	SO_4^{2-}	NO_3^-	SO_4^{2-}
%					K A U 2					
0	81.08	0.024	8191	0.023	80.09	0.024	81.09	0.022	81.11	0.023
5	86.07	0.024	87.11	0.022	87.22	0.024	87.12	0.022	87.14	0.022
10	87.09	0.023	87.61	0.022	87.88	0.022	87.81	0.021	87:89	0.021
20	87.88	0.023	87.89	0.021	87.89	0.022	87.89	0.021	8795	0.021
30	8795	0.022	8799	0.020	87.88	0.021	88.02	0.020	88.05	0.020
					H G 2153					
0	80.11	0.023	80.41	0.024	80.55	0.023	80.67	0.024	81.11	0.024
5	80.67	0.023	8478	0.023	84.81	0.023	8496	0.023	85.21	0.023
10	85.11	0.023	85.46	0.022	85.71	0.023	8598	0.022	86.11	0.023
20	86.15	0.020	86.23	0.022	86.51	0.23	86.61	0.022	8695	0.022
30	8694	0.019	8696	0.020	87.08	0.22	87.11	0.021	87.31	0.021
					D. aegyptium					
0	118.06	0.031	120.08	0.030	120.11	0.031	120.12	0.031	120.08	0.030
10	119.01	0.032	122.11	0.031	121.09	0.030	121.11	0.032	122.11	0.030
20	120.11	0.029	122.34	0.028	121 .21	0.030	120.87	0.030	122.67	0.031
30	120.67	0.029	122.67	0.028	121.47	0.030	12097	0.029	123.00	0.031
40	123.08	0.029	123.51	0.027	122.00	0.029	124.11	0.029	123.11	0.029
50	124.06	0.029	123.41	0.027	122.09	0.030	124.21	0.029	12497	0.029
	P. coarctata									
0	98.08	0.020								
10	101.07	0.021								
20	121.34	0.021								
30	126.46	0.020								
40	12892	0.020								
50	12991	0.021								

in sea water concentration. From the first to the last harvest, there was apparently no change in concentration in all the species.

The nitrate concentration increased generally in all species at each harvest as the sea water concentration increased. However between the first and the last harvests, the changes in concentration in the species were marginal at each concentration.

DISCUSSION

It is difficult to make exact quantitative comparison of the present results with other studies of grasses due to the varying time length of the experiment and the possible differences in environmental conditions. The experiment ranged from 7 weeks for the rice varieties to 8 weeks in D. aegyptium and 19 weeks for P. coarctata. However it is clear from the experiments that the rice varieties show a reduction in growth in terms of fresh and dry weights as sea water concentrations increased. D. aegyptium showed insignificant stimulation at 10% sea water and in P. coarctata, there was no significant difference in dry and fresh weights at 0, 10 and 20% sea water. On the basis of the response of the species to salinity, the test species can therefore be divided into two main groups namely, those that have their growth suppressed as sea water concentration increased, that is, the two rice varieties and those that are either not affected in growth or have insignificant stimulation of dry weight at low salinity; that is P. coarctata and D. aegyptium respectively.

In general stimulation of growth at low salinity could be as a result of salt uptake (Greenway, 1968), induction of enzymatic activity by the uptake of certain ions (Baxter and Gibbons, 1957) or the provision of favourable osmotic potentials within the cell (Okusanya, 1979b).

In D. aegyptium, there was an increase in sodium and calcium concentrations (Tables 4 and 6) and magnesium concentrations remained about the same from the first to the last

harvest. This might be responsible for the increase in growth as they may provide favourable internal osmotic potential for the species.

The accumulation of less Na^+ and Cl^- in the shoots of the most tolerant species P. coarctata where no significant reduction in growth occurs at 0 to 20% sea water accords with the result of Luard and El-Lakany (1984), and it means that this tolerant species accumulates relatively less Na^+ and Cl^- in their shoots as a salt tolerant mechanism. Using the result of the last harvest as a basis of comparison, the tolerant species (P. coarctata) maintained the lowest Na:K ratio (0.97) at 30% sea water compared with the other tolerant species, D. aegyptium with 2.25 and the salt sensitive species 1.10 for KAU 2 and 2.45 for HG2153. Ahmad, Wainright and Stewart (1981) working with ecotypes of Agrostis stolonifera and Gorham et al., (1984) working with members of the Triticeae, found that the most salt tolerant forms had the lowest sodium levels in the shoot tissues under salt stress, a condition similar to what obtains in P. coarctata with the least Na^+ accumulation at 30% sea water. However for the two rice varieties and D. aegyptium greater sodium uptake occurred resulting in high Na:K ratio.

In obligate halophytes, osmoregulationin response to a severe salt stress is achieved mainly if not solely by accumulation of inorganic ions from the external medium (Wallace and Kleinkopf, 1974). In the gramineae and cyperaceae, osmoregulation is mainly due to

active potassium ion uptake. This ion accounts for 85% of the regulation, and sodium chloride accounting for the remaining 15% (Albert and Kinzel, 1973). This is not assuming that P. coarctata is an obligate halophyte, but it may be using similar mechanism for salt tolerance. Potassium appears to be an important part of the osmoticum up to 30% in P. coarctata as the tissue level increased far and above that of sodium resulting in Na:K ratio of less than 1.0.

In the response ofD. aegyptium, to high salinity, osmotic adjustment may be achieved partially through the uptake of sodium ions and to a less extent potassium ions for plants in 10% sea water. At 10% sea water insignificant stimulation occurred as the tissue level of potassium decreased with salinity. This is similar with the result obtained from studies with the salt tolerant grasses and the sedge Heleocharis acicularis (Glenn, 1987).

The poor growth in saline conditions in the two rice varieties may be due to the inability of the plants to absorb mineral nutrients from the soil. This is similar to reports by William and Ungar (1972); Chambers, (1973); Okusanya and Ebong, (1984); Okusanya and Ungar, (1984). It may also be due to the uptake of sodium and chloride ions to the detriment of nutrient ions especially potassium ion which is reduced in the shoot. In the two rice varieties the sodium, calcium, chloride and magnesium concentrations increased, the nitrate and sulphate remained about the same while the potassium concentration and water content decreased. Similar results were reported by Levitt, (1980); and Okusanya and Ungar,

(1984). Solov'ev (1969) observed a potassium deficiency in pumpkin (Curcurbita pepo) when subjected to sodium chloride salinization. Further experiments led him to conclude that the main cause of sodium chloride induced growth inhibition is difficulty in uptake of mineral nutrients due to competition with the sodium ions. In favour of this conclusion was the restoration of potassium ion supply and normal growth when an isolated root strand was placed in a nutrient solution as opposed to when placed in pure water when growth was not restored. Similar results have been reported by Okusanya and Ungar (1984) with two salt-tolerant and one salt-sensitive species of Spergularia.

There were increases in the level of calcium, chloride, sodium and magnesium in the shoots of the species tested except D. aegyptium, where the magnesium concentration remained about the same. The decrease in water contents with salinity (Table 3) in all test species probably resulted in the increase in concentration of the salt in the cell sap of the shoot measured as osmolarity (Table 8). This may have lead to osmotic dehydration which may have been an immediate cause of salt injury, such as the depression of growth and dry weight in the species tested especially at higher salinities. Similar results were reported by Levitt (1980).

The sugar concentration data (Table 9) can be considered on the basis that there are either negligible requirements or that there are substantial requirements for organic solutes to help generate an appropriate osmotic pressure. However at high

salinity in these test species, there could be higher respiration and conversion of sugar to amino acids to serve as osmoticum which is used to counter the osmotic potential in the medium. (Gale and Zeroni, 1985). The suggestion that the supply of photosynthate is not limiting growth at high sea water concentration is consistent with the observation with Spartina alterniflora (Longstreth and Strain, 1977), Atriplex amnicola (Aslam, Jeschke, Barrett- Lennard, Setter, Walkin and Greenway, 1986).

The decrease in leaf sap osmotic potentials (Table 10) as salinity increased may be due largely to the result of accumulation of Na^+ and Cl^- in the leaves (Tables 4 and 7). It could also be due to decrease in water content (Table 3) and increase in concentration of sugars (Table 9). Plants must maintain mean water potential lower than those of the external medium in order that water can move into the plant. Since positive turgor pressures must be maintained, low water potential can among other ways be achieved in saline environments by solute and sugar accumulation as is reported in this experiment. Similar decreases in osmotic potential in halophytes have been reported for Suaeda australis (Robinson and Downton, 1985) and Avicennia marina (Downton, 1982).

Despite all the quantitative differences both the two rice varieties and D. aegyptium appeared to be affected in their growth at high salinity by similar mechanism, that is, sodium uptake and water loss which leads to concentration of solutes in their cell sap, a condition inimical to their growth. Thus the species are unable to adjust osmotically to the external saline

medium, a condition reported by Levitt (1980) as responsible for poor growth in glycophytes. Based on the result of the last harvest, of the two rice cultivars whose dry weights were suppressed at all salinity levels, HG 2153 had the highest sodium ion uptake and also the lowest water content at 30% sea water. This shows that KAU 2 is adjusting better osmotically than HG2153. This is because the percentage reduction in dry weight at 30% sea water was 28% in HG 2153 and 23% in KAU2.

The sodium and water contents in D. aegyptium were the highest at 30% sea water compared with those of the two rice varieties and P. coarctata showing that the species is adjusting better than the rice varieties by possible dilution of absorbed solutes.

Whatever the mechanism that the tolerant species P. coarctata uses, lower sodium and chloride ion concentration in the shoot leading to lower Na:K ratio may be the major reason for its relatively high salt tolerance. However, this tolerance is achieved at the expense of its dry weight which increased rather slowly and therefore made it a slow growing plant. That is tolerance is at the expense of good growth as in Paspalum vaginatum (Lakanmi and Okusanya, 1990).

The reduced growth at high sea water concentration in the two rice cultivars and D. aegyptium may presumably be due to high osmolarity caused by high sodium, chloride and calcium as well as a decrease in water content. Also reduction of potassium ion concentration and toxicity of ions may play some parts. There

could also be a diversion of carbohydrates away from the synthetic processes involved in cell growth to the synthesis of organic solutes such as glycerol and organic compounds for osmotic regulation. There could also be other adverse effects on growing cells such as inadequate rates of accumulation of osmotic solutes or accumulation of ions to toxic levels in an attempt to increase the osmotic potentials of the cell sap (Levitt, 1980).

P. coarctata appears to be the most tolerant of the species as there was no significant difference in dry and fresh weight at 0, 10 and 20% sea water. This tolerant species accumulated relatively less Na^+ and Cl^- in their shoots and maintained the lowest Na:K ratio (0.97) at 30% sea water compared with the other species. The next tolerant species is D. aegyptium followed by the rice cultivar KAU 2 and lastly the other rice cultiver HG 2153.

CHAPTER 3

EFFECTS OF VARYING NUTRIENT LEVELS ON GROWTH OF D. AEGYPTIUM AT 10 AND 25% SEA WATER CONCENTRATIONS: GROWTH STUDIES.

INTRODUCTION

The effects of salinity on field plants already studied, show that the glycophytes achieve their best growth in non-saline conditions and their growth is reduced as salinity is increased. On the other hand, halophytes achieve their best growth in non-saline conditions as well as in varying level of salinity depending on their degree of salt tolerance, although their growth is also retarded at high salinities. (Ashby and Beadle, 1957; Greenway, 1968; Waisel, 1972; Ungar 1974; Okusanya, 1979a). The reduction in growth is caused by inhibition of growth and development which is also accompanied by one or more metabolic disturbances.

At high salinity, the plants are salt stressed and this condition may result in toxicity of ions, low uptake of essential mineral ions, reduced enzymatic action, chloroplast damage, necrosis etc. When the plant is under salt stress, virtually all the cells are under this condition, consequently a reversal of salt stress in the plant must of necessity start with the cells. In doing so, the salt-stressed cells may eliminate the osmotic decrease in cell turgor and therefore in cell growth by the process of osmoregulation. However, there may still be a significant decrease in growth, due to the salt stress as it must increase the concentration of ions in its protoplasm to a level suitable for the normal functioning of the cell. But over and above this increase in

concentration of ions, it must decrease the concentration of the sodium salt or ions in its protoplasm below that in the soil, in order to maintain ionic concentrations and balances that support normal functioning of the cells. This maintenance of a lower sodium salt concentration in its protoplasm than in the surrounding soil requires the expenditure of energy that would otherwise be available for growth processes. Growth must therefore be decreased (Levitt, 1980).

The various metabolic processes affected by the salt-induced growth inhibition are photosynthesis, respiration, water deficit, protein metabolism, nucleic acid synthesis and enzymes activity (Levitt, 1980; Termaat and Munns, 1986).

Levitt(1980) reviewed various theories put forward to explain the causes of reduced growth in plants at high salinity. One of these is the inability of the roots to absorb mineral nutrients due to competition with sodium ions in the medium. In nature and in the various laboratory salinity experiments carried out with halophytes, there are usually adequate levels of nutrients in the medium even at high salinities, but many species still show signs of mineral deficiency (Zhukovskaya, 1962; Solov'ev, 1969; Okusanya, 1979b). It thus appears that the above theory is true. Even though much attention has been focused on the mechanism of salt tolerance in halophytes (Stewart and Lee, 1974; Flowers et al., 1977; Yeo, 1983 ; Wyn Jones et al., 1984), not much has been done for many glycophytes (Greenway and Munns, 1980). The question is whether an increase in the levels of some

nutrients would result in a corresponding increase in the uptake of nutrients which could lead to amelioration of the salinity effects.

Additional phosphate enhanced growth in saline conditions (40% sea water) in the halophytes Lavatera arborea (Okusanya and Fawole, 1985) thereby increasing the limit of salt tolerance. In the glycophytes Luffa aegyptiaca the addition of low level ($0.12 g l^{-1}$) of calcium nitrate to the saline medium, significantly reversed the adverse effect of salinity. However the addition of high level ($0.94 g l^{-1}$) of calcium nitrate or low ($0.06 g l^{-1}$) and high ($0.35 g l^{-1}$) levels of sodium dihydrogen phosphate to the saline medium further depressed growth of the species (Okusanya and Ebong, 1984). Amelioration of salt stress with the addition of extra nutrients was also observed in two halophytes species of Spergularia (Okusanya and Ungar, 1984), while the glycophyte S. rubra showed no favourable response, rather the combination of some nutrients further reduced its growth.

Different nutrients have been found to ameliorate the adverse effects of high salinity in different plants: nitrate and phosphate on Spartina alterniflora (Broome, Woodhouse and Seneca, 1975), phosphate on Arachis hypogea (Malakondaiah and Rajeswararao, 1979), calcium on Bromus mollis (Bassett, 1980) and calcium, nitrate and phosphate on Spergularia marina and S _ rupicola (Okusanya and Ungar, 1984). It is apparent that the ability to ameliorate the adverse effect of high salinity is exhibited by several nutrients. the action of these nutrients are

also apparently not plant specific.

The effect of salinity on the growth of D. aegyptium (chapter 2) shows that it was significantly inhibited above 10% sea water concentration. These effects are superimposed on quite large changes in shoot fresh and dry weights (Figures 4 and 5) and ion contents (Tables 4, 5 and 6). There was no significant difference ($P > 0.05$) in dry weight at 0 and 10% salinity levels, while at 20% sea water there was a significant (50%) reduction ($P < 0.05$) of dry weight. Therefore two sea water concentrations of 10 and 25% were used to determine the response of the species to additional nutrients based on the findings from salinity tolerance experiment in chapter 2.

In this chapter, the laboratory experiment was intended to determine the effects of varying nutrient levels on the salinity tolerance and mineral composition of D. aegyptium.

In the Stout and Arnon (1939) solution used in the salinity tolerance studies for D. aegyptium, the main macronutrients (Table 2) present were potassium nitrate (6mM per litre), calcium nitrate (4mM per litre), magnesium sulphate (2mM per litre) and potassium dihydrogen phosphate (1mM per litre). The final concentration of phosphate in the culture solution was small and therefore not worth interferring with. The decision was to vary potassium, nitrate and sulphate levels in this study to determine the effects on growth.

MATERIALS AND METHODS

Seeds of *D. aegyptium* collected as described in the previous chapter were used in this study. They were germinated in sand in seed trays as described in chapter 2 on the salinity tolerance studies.

The sea water concentration (10 and 25%) were used based on the insignificant enhancement of growth at 10% sea water and the 50% reduction of growth at 25% sea water in the previous chapter on salinity tolerance. There were seven treatments and six replicates for each of the sea water concentration.

Treatment 1 was the full strength Stout and Arnon (1939) solution which served as the control. Treatment 2 and 3 had 3mM and 12mM of potassium ion which represented half and double the levels of this ion in the full strength culture solution respectively. Treatments 4 and 5 had 5mM and 20mM of nitrate, equivalent to half and double the levels of this ion, and treatments 6 and 7 had 1mM and 4mM of sulphate in the culture solution, which are also equivalent to half and double the levels of sulphate respectively compared to the control solution. The composition of the culture solutions were based on those listed by Hewitt (1966).

Uniform seedlings were transferred 14 days after germination, into pots of commercial sand, moistened with the appropriate treatment solution. They were brought to the 25% salinity level by incremental adjustment at the rate of 25 mol M^{-3} per day (10% sea water). The pots were arranged in a

complete randomized design and the plants grown under uniform environmental conditions in the University of Sussex greenhouse, the condition of which were stated in chapter 2 of salinity tolerance studies.

The experiment went on for 6 weeks and there were two separate harvests at the second and sixth week of growth. At each harvest, six plants per treatment were cut at the soil line, the shoot were weighed fresh and then dried at 80 ^{o}C for 24 hours in a forced draught oven and the dried materials were weighed and then used for the determination of the ion contents. Sub-samples of fresh materials were taken for sugar and water potential determination. Details of the determination of ion contents, sugars and water potentials are as given in chapter 2 of salinity tolerance studies.

RESULTS

Analysis of variance of the fresh and dry weight data on the effect of the two levels of potassium, nitrate and sulphate on seedlings at 10 and 25% sea water shows that the treatment had significant effect on growth.

At the first harvest, $F\text{-ratio}_{6,70} = 6.64^{**}$ for fresh weight and 20.14^{***} for dry weight. At the second harvest, $F\text{-ratio}_{6,70} = 53.97^{***}$ for fresh weight and 27.24^{***} for dry weight (Appendix 1 - 4).

The pattern observed in relation to fresh and dry weights of the species at the first harvest was slightly different from that at the second harvest (Figures 6 and 7).

At the first harvest, at 10% sea water only the two levels of potassium had significantly higher fresh and dry weight values ($P < 0.05$) relative to the control showing a significant enhancement of growth. At 25% sea water, the two levels of potassium and nitrate had significantly higher ($P < 0.05$) fresh and dry weights data (Figures 6 and 7) relative to the control, thereby showing amelioration of salt stress at this salinity by these elements.

At the second harvest, however, only half the concentration of potassium and the two levels of nitrate had significant enhancement ($P < 0.05$) of fresh and dry weight values (Figures 6 and 7)

Figure 6 : Mean fresh weight ± SD of *D. aegyptium* seedlings at different concentrations of Stout and Arnon (1939) solution at 10 and 25% sea water concentrations after 2 weeks (I) and 6 weeks (II) of transplanting.

A - Full strenght Stout and Arnon (1939)

B - Double Potassium

C - Half "

D - Double nitrate

E - Half "

F - Double sulphate

G - Half "

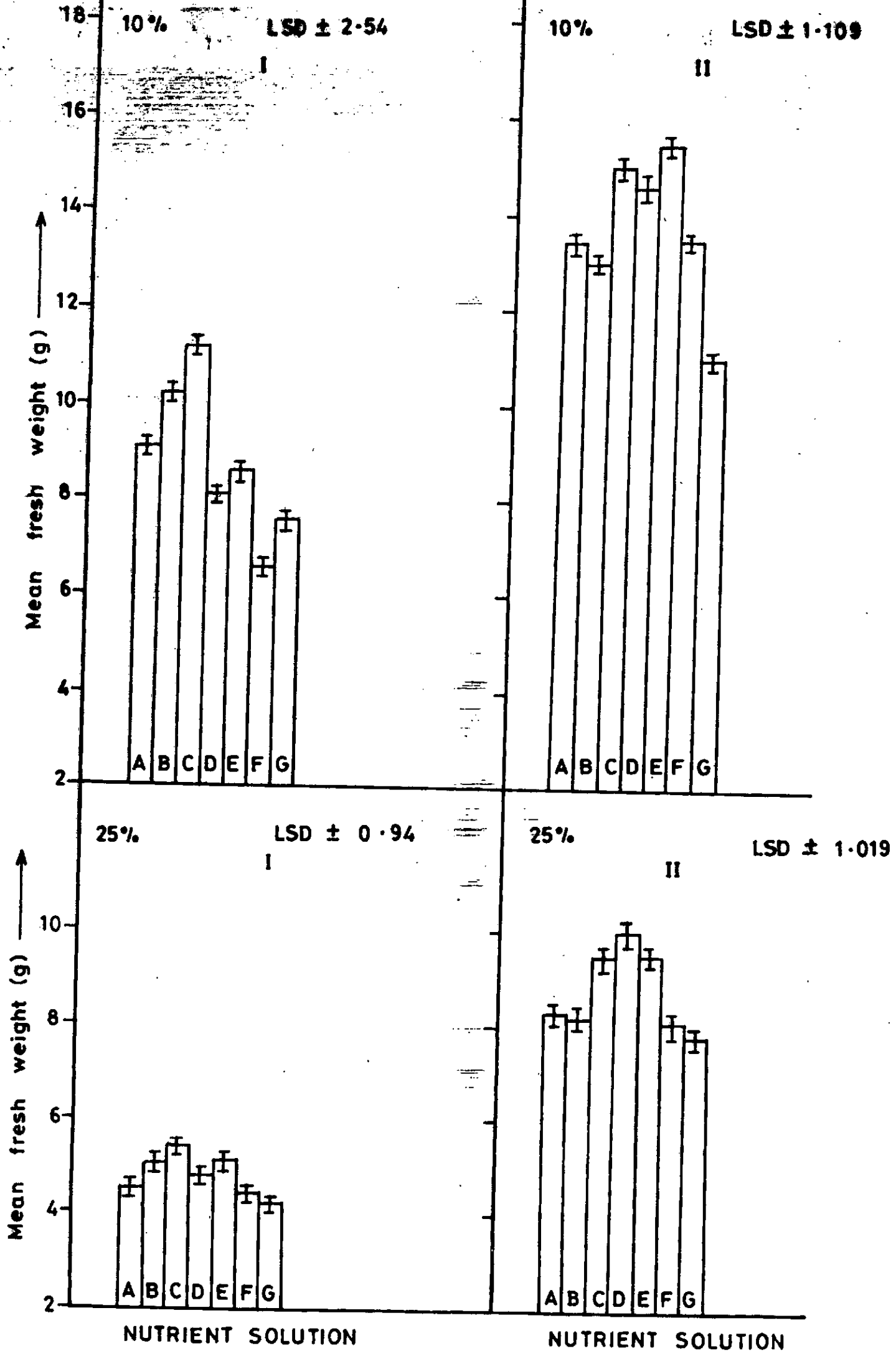

10%
LSD ± 2·54
I
10%
LSD ± 1·109
II
25%
LSD ± 0·94
I
25%
LSD ± 1·019
II
Mean fresh weight (g)
18
16
14
12
10
8
6
4
2
A B C D E F G
NUTRIENT SOLUTION

Figure 7: Mean dry weight of D. aegyptium seedlings at different concentrations of Stout and Arnon (1939) solution at 10 and 25% sea water concentrations after 2 weeks (I) and 6 weeks (II) of transplanting. Bars represent SD

A - Full strenght Stout and Arnon (1939)

B - Double Potassium

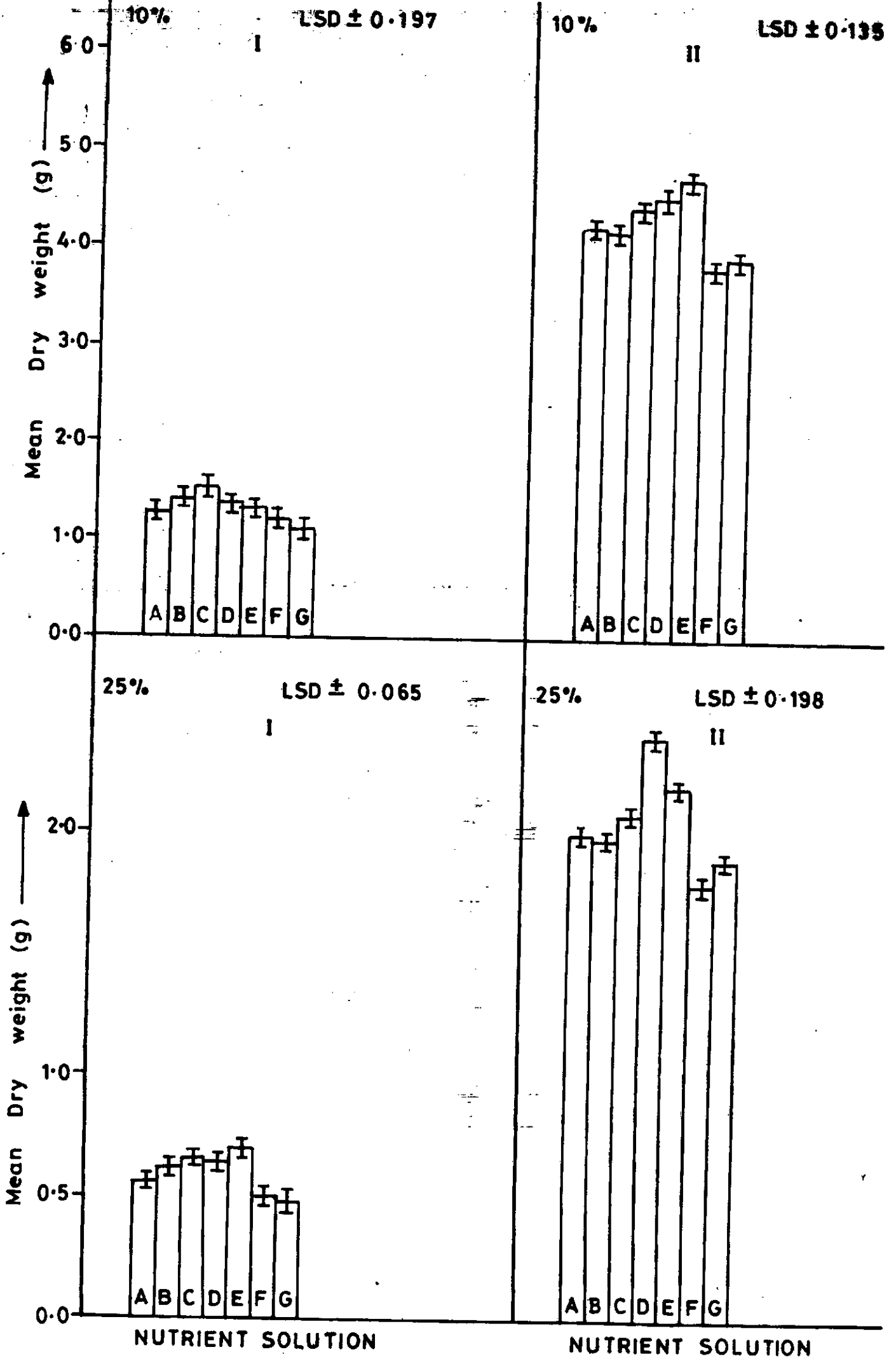
C - Half "

D - Double Nitrate

E - Half "

F - Double Sulphate

G - Half "


10%
LSD ± 0·197
I
10%
LSD ± 0·135
II
Mean Dry weight (g)
6·0
5·0
4·0
3·0
2·0
1·0
0·0
A B C D E F G
25%
LSD ± 0·065
I
25%
LSD ± 0·198
II
Mean Dry weight (g)
2·0
1·0
0·5
0·0
A B C D E F G
NUTRIENT SOLUTION
NUTRIENT SOLUTION

The fresh and dry weight data at the two levels of sulphate (Figures 6 and 7) and at the two sea water concentrations were significantly lower ($P < 0.05$) than those of the control, showing that the two levels of sulphate did not ameliorate the salt stress in D. aegyptium.

The result of the chemical composition of the shoot, shows that the amount of sodium and chloride at the first and second harvests increased only slightlly at double strength of sulphate and fairly well at double strength of potassium and half strength of sulphate when compared with the control (Tables 12a and 12b). The values of sodium and chloride ions were significantly lower ($P < 0.05$) at the two levels of nitrate and half strength of potassium ($P < 0.05$) compared with the control. Generally the amount of sodium and chloride ions from the first to the second harvest increased in all the treatments at the two levels of sea water concentrations.

The potassium ion concentration (Tables 12a and 12b) increased in all the treatments at the two levels of sea water concentration from the first to the second harvest. At the first harvest, the potassium ion concentrations were generally lower or equal to that of the control, while at the second harvest these values were higher than those of the control at half strength of potassium at both sea water concentrations and also at both levels of nitrate and at the two sea water concentrations ($P < 0.01$).

TABLE 12a : The elemental composition of the shoot of D. aegyptium in saline medium with or without additional nutrients at the first harvest.

Treatments	Sea Water Conc.	K^+	Na^+	Cl^-	Mg^{2+}	SO_4^{2+}	Ca^{2+}	NO_3^-
		mmol^{-1} gdwt						moles/gdwt
Complete stout and Arnon	10%	0.70	1.56	0.59	0.53	0.24	0.024	251.05
	25%	0.59	1.84	0.60	0.54	0.24	0.022	215.41
Double Potassium	10%	0.63	1.72	0.66	0.54	0.24	0.014	274.93
	25%	0.54	1.88	0.68	0.53	0.23	0.015	218.47
Half Potassium	10%	0.67	1.44	0.58	0.52	0.22	0.023	714.51
	25%	0.59	1.62	0.51	0.51	0.20	0.019	749.52
Double Nitrate	10%	0.69	1.42	0.51	0.52	0.23	0.017	670.13
	25%	0.54	1.67	0.54	0.54	0.20	0.021	693.20
Half Nitrate	10%	0.64	1.08	0.52	0.52	0.23	0.015	283.02
	25%	0.51	1.48	0.53	0.50	0.20	0.018	279.92
Double Sulphate	10%	0.55	1.58	0.61	0.55	0.22	0.022	265.24
	25%	0.49	1.86	0.64	0.50	0.22	0.020	282.87
Half Sulphate	10%	0.68	1.68	0.61	0.52	0.22	0.016	433.95
	25%	0.49	2.14	0.64	0.48	0.20	0.020	494.06

TABLE 12b The elemental composition of the shoot of D. aegyptium in saline medium with or without additional nutrients at the second harvest.

Treatments	Sea Water Conc.	K^+	Na^+	Cl^-	Mg^{2+}	Ca^{2+}	SO_4^{2+}	NO_3^-
		mmol^{-1} gdwt						moles/gdwt
Complete stout and Arnon	10%	0.90	1.70	0.59	0.56	0.26	0.028	123.09
	25%	0.60	1.94	0.81	0.54	0.24	0.021	120.67
Double Potassium	10%	0.88	2.12	0.92	0.56	0.25	0.014	226.95
	25%	0.57	2.24	1.03	0.55	0.22	0.020	242.62
Half Potassium	10%	0.93	1.50	0.58	0.54	0.24	0.021	130.31
	25%	0.65	1.68	0.74	0.49	0.20	0.016	137.09
Double Nitrate	10%	0.97	1.58	0.53	0.54	0.20	0.013	155.48
	25%	0.69	1.74	0.78	0.55	0.20	0.016	179.91
Half Nitrate	10%	0.99	1.22	0.51	0.50	0.20	0.019	162.55
	25%	0.65	1.49	0.73	0.48	0.18	0.018	183.78
Double Sulphate	10%	0.85	1.98	0.88	0.56	0.24	0.022	133.66
	25%	0.57	2.16	1.28	0.52	0.23	0.021	201.52
Half Sulphate	10%	0.86	2.04	1.04	0.49	0.24	0.019	216.94
	25%	0.58	2.36	0.94	0.50	0.20	0.021	261.48

Table 13

Water content (gg dry wt^{-1}) of *D. aegyptium* at 10 and 25% sea water concentrations with or without additional nutrients.

Treatment		Water contents	
Solution	Sea water concentration	1st Harvest	2nd Harvest
Full strength Stout & Arnon, 1939	10%	5.30	2.44
	25%	5.18	2.20
Double Potassium	10%	6.42	3.19
	25%	7.36	3.42
Half Potassium	10%	5.48	2.28
	25%	6.49	2,90
Double Nitrate	10%	5.93	2.69
	25%	7.14	3.00
Half Nitrate	10%	5.42	2.54
	25%	6.97	3.01
Double Sulphate	10%	6.47	3.21
	25%	7.48	3.26
Half Sulphate	10%	5.98	3.11
	25%	7.27	3.32

The magnesium, calcium and sulphate concentrations decreased from the first to the second harvest (Tables 12a and 12b) in all the treatments, however these differences were not significantly different ($P > 0.05$). The values of the control and the other treatments were also not significantly different ($P > 0.05$). However, the nitrate concentrations (Tables 12a and 12b) decreased significantly ($P < 0.05$) from the first to the second harvest and all the treatments had a significantly higher nitrate concentration than those of the control at both harvests.

The water contents of each harvest are given in Table 13. For each treatment, the values increased at higher salinity except those of the control where there was a decrease at higher salinity. Also from the first to the second harvest there was a significant decrease ($P < 0.05$) in water content in all the treatments. At the first and second harvests, the water contents at each salinity levels were higher than those of the control.

The water potential values measured (Table 14) increased at higher salinity in all the treatments and these values also increased from the first to the second harvest in all the treatments.

The ethanol insoluble dry weight of the undamaged parts of the shoot sampled for sugars (Table 15) shows that the sugar contents increased at higher salinity in all the treatments. The total ethanol soluble sugar content increased from the first to the second harvest in all the treatments. At the first harvest, the values

Table 14

Water potential of the cell sap (-mPa) of *D. aegyptium* at 10 and 25% sea water with or without additional nutrients.

Treatments		Water Potentials	
Solution	Sea water concentration	First Harvest	Second Harvest
Full strength	10%	0.69	1.29
Stout and Arnon (1939)	25%	0.92	1.36
Double Potassium	10%	1.14	1.29
	25%	1.36	1.95
Half Potassium	10%	1.07	1.27
	25%	1.21	1.81
Double Nitrate	10%	0.69	1.24
	25%	1.00	1.81
Half Nitrate	10%	0.63	1.25
	25%	1.00	1.36
Double Sulphate	10%	1.07	1.89
	25%	1.36	1.95
Half Sulphate	10%	0.92	1.80
	25%	1.07	1.89

Table 15

The concentration of total ethanol soluble sugars (mgg fwt^{-1}) in fresh leaves of *D. aegyptium* in saline medium at the first and second harvest.

Treatments

Solution	Sea water concentration	1St Harvest	2nd Harvest
Full Strength Stout and Arnon, (1939)	10%	59.42	43.58
	25%	77.62	63.24
Double Potassium	10%	81.36	58.75
	25%	80.20	70.45
Half Potassium	10%	82.86	47.11
	25%	84.27	68.27
Double Nitrate	10%	59.06	49.38
	25%	62.14	58.00
Half Nitrate	10%	53.75	48.05
	25%	69.20	51.78
Double Sulphate	10%	57.45	48.56
	25%	59.21	49.78
Half Sulphate	10%	56.45	49.69
	25%	59.80	50.78

at double and half strength of potassium were significantly higher than those of the control ($P < 0.05$), while those of the other treatments were lower. At the second harvest, all the treatments had significantly higher ($P < 0.05$) sugar content at 10% sea water, while at 25% sea water this was only significantly higher than the control at both levels of potassium ($P < 0.05$).

DISCUSSION

It is quite clear from the results presented in Figures 6 and 7 of the above experiment on *D. aegyptium* that twice the concentration of nitrate and half of the strength of nitrate and potassium in the Stout and Arnon (1939) solution at sea water concentration of 25% have an ameliorating effect on salt stress. At 10% sea water significant ($P < 0.05$) enhancement of seedling growth was also observed compared to the control. This suggests that nitrate and potassium concentration may play an important role in the salt tolerance and growth of the species in the salt refinery, from where the collection of the seeds were made.

It appears that several natural processes at the site of collection can have this ameliorating effect: the first is that the unpurified salt stored in the warehouse at the salt mine contains a lot of other nutrients including nitrate and potassium. Secondly, the soil contains high levels of nutrients especially nitrate and potassium ions as shown in the analysis of the soil from the salt refinery (Table 22; chapter 6).

Nitrate appears to be more important for growth than half the concentration of potassium because of the significant difference in the means of their dry weights ($P < 0.05$) at the second harvest. Ingestad and Lund (1979) described nitrogen as a cardinal element whose concentration within the tissue is usually kept within fairly narrow limits. It primarily influences the development of leaf area which subsequently controls other growth

and metabolic activities. Nitrates may increase the uptake of potassium as observed in Tables 12a and 12b and potassium is necessary for optimal growth in plants. Nitrates may result in reduced sodium and chloride uptake (Tables 12a and 12b) which are toxic to the plant. Also, it may supply increased nitrate in the plant which has been shown by many workers to enhance plant growth under saline conditions (Hoffman and Sachert, 1967; Broome, Woodhouse and Seneca, 1975; Linthurst and Seneca, 1981; Loveland and Ungar, 1982). In the case of D. aegyptium the nitrate concentration in the shoot (Tables 12a and 12b) increased significantly with addition of extra nitrate to the Stout and Arnon (1939) solution.

The significantly good growth at low level of potasium (half strength) relative to the control can be explained by the fact that plants can do without the element for long periods (Ingestad and Lund, 1979) and also by the fact that other ions such as NH_4^+ and Na^+ may substitute for potassium (Evans and Sorger, 1966). Also at this low level of potassium in the root external surrounding, there was a decrease in the sodium and chloride contents of the shoot (Table 12a and 12b) and an increase in the concentration of sugars in the shoot (Table 15) which is important for osmotic adjustment.

The accumulation of less sodium and chloride ions in shoots of seedlings treated with the two levels of nitrate and half the level of potassium, means that these seedlings may have been able to reduce the uptake of sodium and chloride ions below toxic

levels and this may aid the survival under the stress conditions. Similar results were obtained by Ball and Farquhar (1984).

The reduced growth associated with the addition of the two levels of sulphate and twice the strength of potassium may be due to either the lower external water potential of the medium which impairs nutrients absorption (Okusanya and Ebong, 1984) and the fact that the increase in sodium and chloride contents in the plants could have reached toxic levels in this plant (Tables 12a and 12b). High sulphate levels of the surrounding root surface could also lead to an increase in the acidic nature of the medium which may be detrimental to growth. The response of the species to different hydrogen ion concentration is contemplated. It has also been reported that high potassium level of the surrounding root surface may be damaging to metabolic activity just as equivalent concentration of sodium (Marschner, 1986).

A survey of the literature of osmotic adjustment patterns of glycophytes and halophytes gives ambiguous indications that the ability of a plant to accumulate any one or group of solutes could be a measure of its salt tolerance. However Weimberg (1986) found that the only biochemical parameter that appears to be reliably correlated with tolerance is the relative ability of glycophytes to exclude Na^+ (or avoid ion excess). D. aegyptium appears to have been able to achieve this at the two levels of nitrate and at low level of potassium which possibly led to the amelioration of salt stress at 25% sea water and a

significant enhancement of growth at 10% sea water concentration relative to the control.

The relative high concentration of sugars present in the treatments at 10% sea water at the two levels of potassium relative to the control (Table 15) may be important for osmotic adjustment. Shannon (1978) found enough sucrose in 32 lines of tall wheat and he suggested that this might be used as markers of salt tolerance in this taxonomic group. Flowers et al., (1986) also found a higher proportion of potassium in the monocotyledonous halophytes than in the dicotyledonous halophytes, and they also had relatively high concentration of sugars in their leaf cells. However, since high potassium concentration may be just as damaging to metabolic activity as equivalent concentration of sodium and in as much as sucrose does not appear to be a compatible solute (Munns, Greenway and Kirst, 1983), it may be that both potassium and sucrose are largely restricted to the vacuole and so contribute to its solute potential in the seedlings of D. aegyptium.

The increase in water content (Table 13) at 25% sea water concentration in all treatments is similar to the results of Halket (1915) and Greenway (1968). This is probably a physiological adaptation in which much water is retained in the plant to dilute the absorbed solutes in the cell sap in order to prevent injury to the cell at 25% sea water.

Despite the similar water content at the two levels of sulphate and at twice the concentration of potassium, growth was

still poorer than in the control and at the two levels of nitrate and half the concentration of potassium. The higher uptake of sodium and chloride ions at these levels of sulphate and potassium (Tables 12a and 12b) could be important. They could be toxic to the plant as they produced higher water potential values (Table 14) which might be unfavourable to the plant.

The results of the above experiments show that the addition of certain nutrients to the seedlings of D. aegyptium growing under high salinity can help overcome some problems associated with the high salt concentration. This could be as a result of a combination of factors which may include better salt or ion uptake like K^+ and NO_3^-, the reduction in uptake of Na^+ and Cl^-, salt dilution through increased water uptake leading to plant succulence, increase in sugar concentration which is used as osmoticum and the provision of favourable osmotic pressure in the cell sap.

CHAPTER 4

EFFECT OF VARYING NUTRIENT LEVELS ON GROWTH OF D. AEGYPTIUM AT 10 and 25% SEA WATER CONCENTRATIONS: ION RELATIONS.

INTRODUCTION

The understanding of the mechanisms by which plants respond to certain form of environmental stress, including salinity, as well as understanding the general uptake and transport of inorganic ions and their roles in cell water relations and metabolism, requires knowledge of the distribution of ions within plant cells (Harvey, 1985).

The rate of transport of ions to the shoot gives a measure of the plants ability to osmotically adjust in the shoot following a change in the external salinity. It also can be seen as a means of preventing high levels of sodium and chloride from accumulating in the roots (Flowers et al., 1977).

When grown in saline conditions, plants respond with a variety of integrated changes in the distribution of ions, cell water relations, biochemical functions and morphology (Harvey and Thorpe, 1986). The importance of inorganic ion accumulation in halophytes and semi-tolerant glcophytes as a mechanism for lowering tissue osmotic potential has been recognised in plants grown over a range of salinity treatments (Eaton, 1927, 1947; Greenway and Munns, 1980; Munnset al., 1983)

Whatever process or processes mediate between the external and internal environment of a halophyte or a semi-tolerant

glycophyte, these plant species must be able to accomplish three major things in saline conditions: 1) They must have the capacity to provide ions for the osmotic adjustment of expanding cells and to adapt to salinity fluctuations and so maintain the necessary negative water potentials gradient from the environment to the plants.
ii) They must acquire ions of nutritional importance, such as potassium.
ii) They must be able to distribute these ions within the plant cells, so as to permit the vascular accumulation of sodium chloride for osmotic adjustment at concentrations which would not be toxic in the adjacent cytoplasm (Yeo and Flowers, 1986).

Ions are of fundamental importance to the water relations of leaf cells under saline conditions, their continued cellular function depends upon osmotic adjustment with more ions. To be successful, these ions must be accumulated in the protoplast at the rate at which they are supplied. A failure to do this results in dehydration either through underadjustment or water loss to the cell walls (Flowers et al.,1986)

Whilst it is clear that intracellular compartmentation of ions is a necessary condition of salt tolerance in higher plants which adjust osmotically with inorganic ions, it is not by any means the whole answer to their salt tolerance (Flowers et al., 1977). Very small imbalances between the rate of ion supply to the shoot and the rate of ion accumulation into cells would have the grave consequences of excessive cellular concentrations, inadequate osmotic adjustment or of accumulation in the apoplast

(Oertli, 1968).

Regulation of salt transport in relation to shoot growth is clearly not effective in salt sensitive plants where sodium uptake exceeds the demands of growth and osmotic adjustment, and which results in ion toxicity (Yeo and Flowers, 1986).

Osmotic adjustment can be defined as the lowering of tissue osmotic potential by accumulation of solutes such that turgor is maintained at low external water potentials (Shennan, Hunt and Macrobbie, 1987). Presumably such a build up of solutes in cells prevents wilting of the plants by changing the solute to solvent ratios (i.e, the water activity of the solvent) inside cells to match a similar change in the external environment in order to prevent net water movement outward across the cell plasma membrane (Borowitzka, 1981).

There have been numerous studies to identify and quantitatively measure the compounds whose concentrations increased in stressed plants tissues to try to obtain experimental evidence for a relationship between osmotic adjustment and salt or drought tolerance (Briens and Larher, 1982; Voetberg and Stewart, 1984; Wyn Jones and Gorham, 1983).

The responses of halophtyes to salinity are somewhat different from those of glycophytes (Jefferies and Rudmik, 1984). Leaf cells of non-stressed halophytes usually contain much higher levels of inorganic ions than non-stressed glycophtes. Salinity, especially NaCl is needed by a number of halophytic plants at low to moderate concentrations (Ca 50 - 125mM) for optimal growth.

Consequently salt must be present in the tissue of the halophytes (Greenway, 1968; Ungar, 1974; Okusanya, 1979b; Levitt, 1980).

An active movement of sodium out of the cell has been found in the roots of Triglochin maritima under saline conditions (Jefferies, 1973). The general relationship between metabolic activity and sodium movement are unclear and vary with species and/or concentration (Ansari, 1982). Sodium has been found to be active in roots of Helianthus annus (Bowling and Ansari, 1971) and in five aquatic species. The extrusion of sodium ions from barley roots has also been discussed (Nassery and Baker, 1972, 1974).

It is clear from the result of the experiment on the salinity tolerance of D. aegyptium (chapter 2) that inorganic ion accumulation (Tables 4 and 5) plays a major role in osmotic adjustment. Sodium and calcium in the shoots increases, potassium decreases, magnesium, calcium, nitrate and sulphate remain about the same from 0 to 50% sea water concentrations. The work described in this chapter therefore looks at the role of ion uptake and accumulation in relation to plant growth as this is critical to the plants ability to grow in saline conditions.

MATERIALS AND METHODS

The collection and germination of the seeds of D. aegyptium used in this study were as described in chapter 2 of this thesis.

Fourteen days after germination, the seedligs were transferred to solution cultures in plastic boxes of 3-litre capacity painted black to prevent algal growth. Details of culture techniques, growth conditions and the seven cultures used were outlined in chapter 3 on the effect of varying nutrient levels on growth at 10 and 25% sea water concentrations.

Plants were selected for uniformity of size at the time of transfer to solution culture. Therefore variation between plants used in this study were those arising from the treatments used.

Five days after transfer to solution culture, a period long enough to allow for stability and acclimatization of root growth, the seedlings were salinized to 25 mol m^{-3} d^{-1} sea water (10% sea water concentration).

The first harvest was a day after salinization to 10% sea water. Immediately after the first harvest, salinization in the other boxes was increased to 62.5 molm $^{-3}$ d^{-1} sea water (25% sea water concentration). This concentration was maintained until the end of the experiment. After the increase in salinization as there were daily harvest for 4 days.

A complete randomized design was used for the experiment. One seedling was supported in each of the 25 holes in the lid with a non-absorbent cotton wool. This was used so as to reduce to the barest

minimum loss of the culture solution in the boxes through evaporation.

On the whole, five plants were harvested daily for 5 days for analysis and weighing. At harvest the roots of salt-treated plants were rinsed for 5 minutes in iso-osmotic mannitol solutions, and those of control plants in deionized water. Shoots of salt plant were also rinsed in deionized water to remove any surface salt. They were all blotted on paper towels before being weighed. Shoots and roots were dried seperately for 24 hours at 80 ^{o}C, on forced draught oven.

Analysis of chloride content was carried out using Specific ion electrode after extracting weighed dried material in acetic acid (100 mol m^{-3}) for 2 hours at 90 ^{o}C. Na, K, Ca and Mg were determined following dry ashing (Muffle furnace for 2 hours at 550 ^{o}C) by atomic absorption spectrophotometry (Pye Unicam SP 9). Sulphate and nitrate were determined on aqueous extracts by a turbidometric method and the phenoldisulphonic acid reaction respectively.

Na:K ratios were estimated from ion concentrations in roots and shoots on the basis of dry weight. K:Na selectivity ratio (Sk,Na) was calculated according to Pitman (1976).

$$Sk,Na = \frac{\text{K content}}{[K]\ \text{medium}} \cdot \frac{\text{Na Content}}{[Na]\ \text{medium}}$$

Culture fluxes (J_k , J_{Na} , J_{Cl}) were calculated following Pitman (1975).

Ion fluxes, $$J = \frac{M_2 - M_1}{w(T_2 - T_1)}$$

where $w = \dfrac{W_2 - W_1}{\text{Log}_e \frac{W_1}{W_2}}$

M_2 and M_1 are total ion content, that is content per unit dry weight multiplied by dry weight at times T_2 and T_1 and W_2 and W_1 are the root weights at the two harvests being considered.

RESULTS

The effect of the two levels of nitrate, potassium and sulphate on dry weight of D. aegyptium after 24 days of treatment shows that the two concentrations of nitrate and half strength of potassium had significantly ($P < 0.05$) higher total dry weight relative to the control (Table 16). Twice the strength of potassium and the two levels of sulphates resulted in significant decrease ($P<0.05$) of total dry weight relative to the control. This shows that these treatments retarded the growth of the plants. The increase in total dry weight at half strength of potassium was due to the increase in shoot weight as there was no difference in root weight relative to that of the control. At the two levels of nitrate, the significant increase in total dry weight was due to increase in both root and shoot weights but possibly more to root in half strength of nitrate. The significant decrease at both levels of sulphate and at twice the concentration of potassium relative to the control was due only to shoot reduction.

Generally, there was a continuous uptake of K^+, at each harvest which is significantly greater ($P < 0.05$) than that of the control at the two levels of nitrate and half strength of potassium in both shoot and root throughout the course of the experiment, with a corresponding decrease in Na^+ concentration in both shoot and root relative to the control (Table 17). From the first to the last harvest at the two levels of sulphate and at twice the potassium ion concentration, the potassium ion increased daily but this was significantly lower

TABLE 16: Effect of different nutrient levels on the shoot and root growth of D. aegyptium at the last of five harvest. (±SD)

Treatments	Shoot dry weight(g)	Root dry weight(g)	Total dry weight(g)
Culture Solution	0.47 ± 0.02	0.093 ± 0.02	0.563
Double potasium	0.40 ± 0.07	0.123 ± 0.06	0.523
Half potassium	0.55 ± 0.04	0.098 ± 0.09	0.648
Double nitrate	0.64 ± 0.05	0.146 ± 0.12	0.786
Half nitrate	0.50 ± 0.04	0.130 ± 0.07	0.630
Double sulphate	0.42 ± 0.07	0.098 ± 0.09	0.518
Half sulphate	0.44 ± 0.03	0.110 ± 0.03	0.550

TABLE 17: Potassium and Sodium concentration (mmolgdwt^{-1}) in the shoot and root of D. aegyptium in different nutrient levels at five harvest.

Treatments	1st Harvest		2nd Harvest		3rd Harvest		4th Harvest		5th Harvest	
	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+
					Shoot					
Culture Solution	0.76	0.75	0.77	1.01	0.79	1.13	091	1.43	092	1.48
Double potassium	0.68	0.69	0.69	0.74	0.74	099	0.87	1.38	091	1.56
Half potassium	0.78	0.63	0.80	0.75	090	0.84	099	0.89	1.14	1.01
Double nitrate	0.81	0.63	0.86	0.69	095	0.89	1.09	0.83	1.19	1.07
Half nitrate	0.80	0.64	0.87	0.68	1.10	0.85	1.17	093	1.17	1.10
Double sulphate	0.64	0.73	0.70	0.70	0.75	0.79	0.81	1.12	0.89	1.53
Half sulphate	0.59	0.71	0.60	0.74	0.79	0.89	0.83	1.26	0.85	1.52
					Root					
Culture Solution	0.63	0.72	0.63	0.78	0.71	0.74	0.81	0.84	0.82	0.85
Double potassium	0.56	0.61	0.58	0.74	0.70	0.77	0.77	0.86	0.80	093
Half pottassium	0.70	0.69	0.72	0.69	0.80	0.73	0.84	0.75	090	0.80
Double nitrate	0.72	0.70	0.74	0.72	0.76	0.73	0.79	0.74	092	0.83
Half nitrate	0.69	0.67	0.75	0.70	0.80	0.72	0.82	0.75	093	0.81
Double sulphate	0.54	0.66	0.56	0.68	0.65	0.74	0.70	0.79	0.73	096
Half nitrate	0.54	0.67	0.56	0.69	0.61	0.76	0.63	0.81	0.70	099

than that of the control ($P < 0.05$). However the increase in sodium ion concentration exceeded that of the control significantly ($P < 0.05$). In all the treatments, the daily concentration of the ions in the shoot was significantly greater ($P < 0.01$) than the levels in the root at all the harvests.

The Na:K ratio increased in both shoot and root in response to salinity at the two levels of sulphate and at twice the strength of potassium. This ratio showed an increase with increase in age of plants under saline conditions as sodium progressively replaced potassium in all the treatments. However, at the two levels of nitrate and half strength of potassium, as the age of the plant increased, the Na:K ratio decreased in both shoot and root (Table 18) showing a decrease in the daily accumulation rate of sodium in these plant.

The chloride contents in the shoot and root increase with each harvest in all the treatments relative to the control, but the increase was only significantly different ($P < 0.05$) at the two levels of nitrate and half strength of potassium (Table 19)

Table 20 shows the rate of net ion transport (J_{Na}, J_{K}, J_{Cl} in plants, that is a minimal estimate of the unidirectional influx which it would equal were efflux equal to zero. For plants treated with double and half strength of nitrate and also half strength of potassium, the net transport of potassium (J_{K}) and chloride (J_{Cl}) was significantly higher than the control ($P < 0.05$) while the net transport of sodium (J_{Na}) was significantly lower than that of the control ($P < 0.05$) at the

TABLE 18: Effect of salinity on the Na:K ratio of shoot and root of D. aegyptium

Treatments	Harvest 1	2	3	4	5
		SHOOT			
Culture solution	0.98	1.31	1.43	1.57	1.61
Double potassium	1.01	1.07	1.33	1.58	1.71
Half Potassium	0.81	0.93	0.93	0.89	0.88
Double Nitrate	0.78	0.80	0.94	0.76	0.89
Half Nitrate	0.80	0.78	0.79	0.79	0.94
Double sulphate	1.14	1.00	1.05	1.38	1.72
Half Sulphate	1.18	1.23	1.12	1.52	1.78
		ROOT			
Culture solution	1.14	1.23	1.05	1.05	1.03
Double Potassium	1.07	1.27	1.10	1.11	1.16
Half potassium	0.98	0.95	0.91	0.89	0.88
Double nitrate	0.97	0.97	0.96	0.93	0.90
Half nitrate	0.97	0.93	0.90	0.91	0.86
Double sulphate	1.22	1.21	1.13	1.12	1.31
Half sulphate	1.24	1.23	1.26	1.27	1.41

TABLE 19: The effect of different nutrient levels on chloride concentration ($molm^{-3}$) in the shoot and root of D. aegyptium.

Treatments	Shoot Harvest					Root Harvest				
	1	2	3	4	5	1	2	3	4	5
Culture Solution	0.25	0.30	0.31	0.35	0.40	0.25	0.26	0.29	0.32	0.35
Double potasium	0.27	0.30	0.31	0.36	0.45	0.27	0.29	0.37	0.38	0.41
Half potassium	0.34	0.41	0.49	0.55	0.57	0.29	0.31	0.37	0.39	0.45
Double nitrate	0.28	0.34	0.39	0.46	0.56	0.29	0.31	0.37	0.39	0.45
Half nitrate	0.43	0.48	0.52	0.58	0.59	0.36	0.38	0.39	0.43	0.45
Double sulphate	0.27	0.30	0.35	0.38	0.47	0.30	0.31	0.34	0.36	0.37
Half sulphate	0.29	0.35	0.38	0.39	0.46	0.34	0.38	0.40	0.44	0.46

TABLE 20: Net transport of ions (J) in relation to growth in *D. aegyptium* ($mmolg^{-1}$ dry wtd^{-1})

Treatments		Harvest flux			
		1	2	3	4
Culture Solution	JNa	1.32	1.09	1.09	1.73
	Jk	0.90	0.88	0.90	0.99
	JCl	0.64	0.67	0.62	0.66
Double potassium	JNa	1.20	1.44	1.71	1.93
	Jk	1.06	0.66	0.49	0.31
	JCl	0.79	0.76	0.80	0.81
Half Potassium	JNa	1.22	1.03	0.92	0.85
	JK	1.53	1.73	1.90	1.94
	JCl	0.85	0.82	0.84	0.99
Double nitrate	JNa	1.23	1.20	1.01	0.96
	JK	0.71	0.87	1.29	1.56
	JCl	0.47	0.49	0.51	0.63
Half nitrate	JNa	1.30	1.23	1.11	0.97
	JK	0.63	0.68	0.98	1.22
	JCl	0.48	0.51	0.59	0.65
Double sulphate	JNa	1.98	1.78	1.82	1.86
	JK	1.01	1.00	0.98	0.81
	JCl	0.59	0.50	0.63	0.86
Half sulphate	JNa	1.46	1.39	1.52	1.82
	JK	0.89	0.86	0.60	0.58
	JCl	0.47	0.46	0.44	0.49

daily harvest. In the other three treatments, that is, the two levels of sulphate and at twice the concentration of potassium, the net transport of sodium, (J_{Na}) was significantly higher ($P < 0.05$) than the control, while J_K and J_{Cl} were significantly lower ($P < 0.05$).

Selectivity values of both shoots and roots (Table 21) showed a significant increase ($P < 0.05$) in response to salinity and a progressive increase with increase in age of plants in all the treatments relative to the control ($P < 0.05$).

TABLE 21: Effect of salinity on the selective ratio of shoot and root of D. aegyptium.

Treatment		Harvest 1	2	3	4	5
Culture Solution	Shoot	4.40	5.10	5.27	5.45	5.93
	root	3.35	3.79	3.60	4.16	4.22
Double potassium	Shoot	4.01	4.89	5.28	5.57	5.99
	root	2.49	4.92	4.97	5.42	5.56
Half potassium	Shoot	4.11	5.62	5.66	6.04	6.40
	root	2.30	4.79	5.81	5.73	6.16
Double nitrate	Shoot	4.97	5.25	6.24	6.40	7.15
	root	3.89	4.33	5.67	6.74	6.77
Half nitrate	Shoot	4.29	4.31	5.41	6.63	6.81
	root	2.51	3.10	4.67	5.67	6.61
Double sulphate	Shoot	2.46	3.52	5.50	5.90	6.01
	root	1.68	2.64	3.80	5.00	5.10
Half sulphate	Shoot	4.03	4.80	5.20	5.40	6.40
	root	2.90	3.25	5.09	5.74	5.94

DISCUSSION

The nutrients and their levels differred in their amelioration of the stress caused by sea water concentration of 25%. In glycophytes, salinity tolerance may depend at least in part, on the efficiency with which the root system can limit transport of sodium chloride to the shoots. The two levels of sulphate and at twice the level of potassium all had higher rate of accumulation of sodium and chloride than the control. In glycophytes usually, if the rate of transport of sodium chloride to the shoots exceeds that at which those ions can be accumulated in leaf cell vacuoles, then the plant will die either of ion toxicity or cellular dehydration (Greenway and Munns 1980; Flowers and Yeo, 1986). This appears to be the cause of poor yield at the two levels of sulphate and at twice the level of potassium. In the two levels of nitrate and half of the level of potassium, the rate of transport of sodium to the shoot (Table 20) was significantly ($P < 0.05$) lower than the control which therefore excludes ion toxicity or cellular dehydration in the shoot resulting in a corresponding higher yield under these treatments.

A difference between the nutrients in selectivity of their roots might reflect differences in selectivity at the primary site of uptake and/or the tonoplast of the cortical cells, (Jeschke, 1983). Since selectivity in the shoot was greater than that in the root (Table 21), this suggests that there is also selective release of ions to xylem in root

(Jeschke, 1983). Although reabsorption from the xylem to the xylem parenchyma may also contribute to the apparent selectivity of the shoots (Flowers et al.,1977) such a system is likely to be rapidly saturated at high NaCl ($62.5 molm^{-3}$) external salinities because of its relatively small volume. The salinized culture solution with two levels of nitrate and half strength of potassium had the lowest Na:K ratio (Table 17) which were significantly different ($P<0.05$) from those values obtained with other nutrients levels. This shows an inclination again towards the halophytic properties of the plant, which were however improved by the two levels of nitrate and half the level of potassium compared with the control at the daily harvest. Ingestad and Lund (1979) describe nitrogen as a cardinal element whose concentration within the tissue is usually kept within fairly narrow limits. It primarily influences the development of leaf area which subsequently controls other growth and metabolic activities. It may also increase nitrate concentration in the plant which usually enhances growth (Loveland and Ungar, 1982).

As dicussed with the growth studies in chapter 3 the significantly good growth at low level of potassium can be explained by the fact that plants can do without the element for fairly long periods (Ingestad and Lund, 1979) and also by the fact that other ions such as NH_4^+ and Na^+ may substitute for potassium (Evans and Sorger, 1966).

In most glycophytes, the cytoplasm is thought to contain 100 - 200 $molm^{-3}$ K (Leigh and Wyn Jones, 1984) and has a high K:Na

ratio (Wyn Jones, Brady and Speirs, 1979). The data on the two levels of nitrate and half the concentration of potassium on ion contents are fully consistent with the general concept of halophytes, having a high cytoplasmic requirements (Flowers and Lauchli, 1983; Wyn Jones, Gorham and McDonnell, 1984).

In general, salinity raised the sodium and chloride ion concentration in all compartments of the cell at the two levels of sulphate and at twice the concentration of potassium. However, the increase of chloride concentration was much less than that of Na^+ since prior to salinization, plant chloride content was much greater than that of sodium. This was due to the concentration of chloride in the culture solution being greater than that of sodium.

The major difference between the treatments that had twice the concentration of potassium and the two levels of sulphate which resulted in significantly poor yield ($P < 0.05$) and the two levels of nitrate and half the concentration of potassium which had significantly ($P < 0.05$) higher yield than the control, were the higher concentrations of sodium and chloride accumulated in the shoot. The high shoot concentration of Na^+ and relative low K^+ might be expected to have an effect on protein synthesis (Wyn Jones et al.,1979; Flowers and Lauchli, 1983). A high chloride concentration would also be detrimental in this respect (Brady, Gibson, Barlow, Speirs and Wyn Jones 1984; Gibson Speirs and Brady, 1984). In Vitro protein synthesis requires 100 to 150 $molm^{-3}$ K and a high K:Na ratio, and any large changes from these

conditions may affect both the quantity and type of protein produced (Gibson et al., 1984; Wyn Jones et al., 1984). However, what is most important for the long-term survival of the plant is the net transport of sodium and chloride to the shoot. If regulated, this must be effected through root cytoplasmic ion concentrations.

In the absence of any major component of ion transport from root to shoot by-passing the symplasm, transport is the product of ion concentration into the cell, and the rate of radial movement through, the cytoplasm. Ion transport to the vacuoles can limit net transport by reducing the symplastic ion concentration. The capacity of this vacuoles depends upon the ion concentration in the root vacuoles, the root volume and growth rate.

It is now well accepted as seen in this experiment that the root acts as a primary barrier to ion movement into the plant, resulting in concentrations in the shoot being radically different from those occurring in the external medium.

That there is a more detrimental effect of salinity on root than shoot growth is borne out by the general increase in shoot; root ratios and the marked decrease in the root dry weight in contrast to the small increase in the shoot dry weight. Results similar to the above had also been obtained with other halophytes (William and Ungar, 1972; Chambers, 1973).

D. aegyptium was found to be a cumulative halophyte as there was daily accumulation of ions in the shoot and root. The rate of accumulation of the K^+, Na^+ and Cl^- varied in the seedlings that had enhanced growth and those in which there was

poor yeild. In all the treatments the ion content of the shoots were significantly higher than those of the roots and there was a more detrimental effect of salinity on root than shoot growth.

CHAPTER 5

EFFECT OF SALINITY ON STOMATAL CONDUCTANCE, TRANSPIRATION AND PHOTOSYNTHETIC CAPACITY OF D. AEGYPTIUM.

INTRODUCTION.

During photosynthesis, high energy-containing compounds like carbohydrates are synthesized in the chlorophyll from low energy-containing compounds like carbon dioxide and water using light energy from sunlight. The final products of photosynthesis, that is carbohydrates are distributed to all parts of the plant and oxygen is given off as a gas through the stomata, back to the atmosphere in exchange for the carbon dioxide that was taken in (Bannister, 1976).

Many factors affect the photosynthetic ability of plants. One of them is transpiration. Firstly, if the carbon in carbon dioxide is to be trapped and used to build organic molecules, it must enter the plant through stomata situated mostly in the leaves. Secondly if the gas is to enter the cells inside the plant, and hence reach the chloroplast where photosynthesis takes place, the cells must be moist. As the plant takes in carbondioxide gas, the plant tends to lose water by evaporation; and the more water it loses, the more it must draw in from the roots (Moore, 1981). For plants in saline areas the uptake of water may be further decreased by the soil osmotic potential. Consequently soil salinities will affect photosynthetic ability of the plants.

Another problem in photosynthesis is photorespiration, the

term which refers to oxygen competing with carbon dioxide and preventing its fixation. The net effect of photorespiration is that oxygen in the atmosphere depresses the rate of photosynthesis. Oxygen interferes with the initial combination of carbon dioxide with ribulose biphosphate. Oxygen apperantly competes with carbon dioxide for sites on the enzyme so that instead of combining with the carbon in CO_2 to form one molecule of phosphoglyceric acid and glycollate. The glycollate breaks down to produce carbon dioxide again. (Bannister, 1976; Moore, 1981).

Generally increased salinity results in decreased growth in non-halophytic plants (Greenway and Munns, 1980; Seemann and Critchley, 1985), but halophtyes can grow at much higher salt concentrations than glycophytes (Strogonov, 1964). Usually growth is not affected at low salinity and may even be enhanced but it decreases at very high salt concentrations. Of course, the limit survived varies among the halophytes themselves (McMillan and Moseley, 1967). Different physiological processes have been put forward to account for this reduction in growth. This reduction in growth may result from salt effects on dry matter allocation, ion relation, water status, physiological processes, biochemical reactions or a combination of such factors (Seemann and Critchley, 1985).

The rate of photosynthetic carbon dioxide fixation declined with salt stress in barley (Munns et al., 1983), sugar beet (Papp, Ball and Terry, 1983) and sunflower (Rawson and Munns, 1984), due to poor leaf elongation and hence the poor development

of photosynthetic surface area. On the other hand, Downton (1977), attributed the growth reduction in grapevines under salt stress to decrease in photosynthetic capacity. This decline in photosynthetic carbon dioxide fixation is also found to be at least partially a consequence of stomatal closure, although the extent to which such closure limits photosynthesis during salt stress (by reducing the intercellular carbon dioxide concentration) has been less often quantitatively determined (Seemann and Critchley, 1985).

The net rate of carbondioxide uptake (Pn) of many plant species declines with increasing rhizosphere salinity (Downton, 1977; Helal and Mengel, 1981; Walker, Torokfalvy, Grieve and Prior, 1983). The decline in Pn has been attributed to salinity effects on both non-stomatal (Longstreth and Nobel, 1979) and stomatal controls (Kemp and Cunningham, 1981). Declining stomatal conductance could reduce Pn by lowering the carbon dioxide concentration in the leaf, as hypothesized for spinach (Robinson, Downton and Millhouse, 1983). In contrast, reductions in stomata conductance coupled with lowered potential of Pn, could act to maintain a relatively constant intercellular carbondioxide concentration (Osmond, 1980; Farquhar and Sharkey, 1982). These fits the response of Avicennia marina grown at 50 to 500mM NaCl and vapour pressure deficit of 0.6 kPa (Ball and Farquhar, 1984).

The effect of salinity on transpiration of halophytes was summarized by Waisel (1972). Apparently, halophytes do not comprise a homogenous group and cannot be separated from

glycophytes on the basis of their transpiration characteristics (Eshel and Waisel, 1984). Numerous studies of the transport/transport interractions have been done over the years and have been reviewed by Van den Honert, Hooymans and Volkers (1955) and Pitman (1982). These workers have shown that the results depend on the plant species, age, the ion in question and the growth conditions.

Results in Chapters 2 and 3 showed that at sea water concentration above 10% there was a gradual decrease in dry weight of D. aegyptium and an increase in the total ion contents of the plant. There was therefore a need to find out the physiological basis for the growth reduction in this plant. The present study describes the influence of changes in stomatal conductance, transpiration and photosynthetic metabolism in D. aegyptium grown under different salinity regimes.

MATERIALS AND METHODS

Seeds of *D. aegyptium* were germinated in seed trays containing sand in the greenhouse of the University of Sussex under controlled environmental conditions as described in salinity tolerance studies of this thesis (Chapter 2).

After 2 weeks of germination, equal size seedlings were transplanted into boxes of solution culture of Stout and Arnon, (1939). Salinization started 5 days afterwards. The salinity levels used were 0, 10, 25 and 50% sea water and they were brought to these salinity levels by stepwise increments at the rate of 25 $mol\ m^{-3}$ sodium chloride per day (10% sea water concentration).

Measurements of carbondioxide exchange rates and transpiration of attached leaflets were made using a conventional 'open' gas exchange system with an ADC 225 Infra red gas analyzer. Each of the experiments were repeated. Net photosynthesis of the leaf lamina was determined at the quantum flux of 500 $umol\ m^{-2}\ s^{-1}$ (PAR). Leaf temperature was $25 \pm 1\ ^{o}C$. Leaf and air temperatures within the cuvette were measured with copper constantan thermocouples. The zero setting of the humidity sensor was regularly checked with dry air and the span calibrated against air of known humidity. Air was sampled from outside the laboratory and mixed in a small mixing chamber before being pumped into the leaf chamber.

The principle of operation is that a reference gas (usually air) is passed through one tube while the sample gas passed through the other analysis tube. Both tubes pass infra red radiation into the detector, consisting of two receiving chambers

separated by a thin diaphragm. The detector is filled with carbon dioxide gas, which makes it selective to the energy of the wavelength that excites the molecules of that gas. The resultant heating causes a pressure rise, which acts upon the diaphragm. The sample gas absorbs radiation proportional to the concentration present, reducing the input on the analysis compared with the reference side of the detector.

This imbalance in radiation produces a differential pressure on the diaphragm which moves and changes the electrical capacitance. It is this movement which is amplified and displayed to show the gas concentration.

Following measurements of their photosynthetic characteristics, the plants were harvested for the determination of their fresh and dry weight. The measurements of photosynthetic characteristics were made in the dark for 12hours and also 12 hours in light.

Photosynthesis, stomatal conductances and transpiration were calculated according to equations in von Caemmerer and Farquhar (1981).

RESULTS

The photosynthetic response of *D. aegyptium* to increasing salinity levels in culture solution is shown in Fig. 8. There was a linear steady reduction in photosynthetic rate as sea water concentration increased. The ambient photosynthetic rate declined by approximately 75% over the range of 0 - 50% sea water. By extrapolation, the photosynthetic rate will be zero at about 75% sea water concentration.

There was a similarly high reduction in stomatal conductance to carbondioxide and water (Figure 9).

The decline in both attributes was approximately 73% over the range of 0 - 50% sea water. The two attributes show a similarity in the shape of their graphs although conductance to carbondioxide lagged behind stomatal conductance to water.

The rate of transpiration expressed per gram dry weight was reduced by 64% at 50% sea water over that of the control (Fig 10).

Figure 8: Net photosynthesis of *D. aegyptium* at various sea water concentrations ± SD

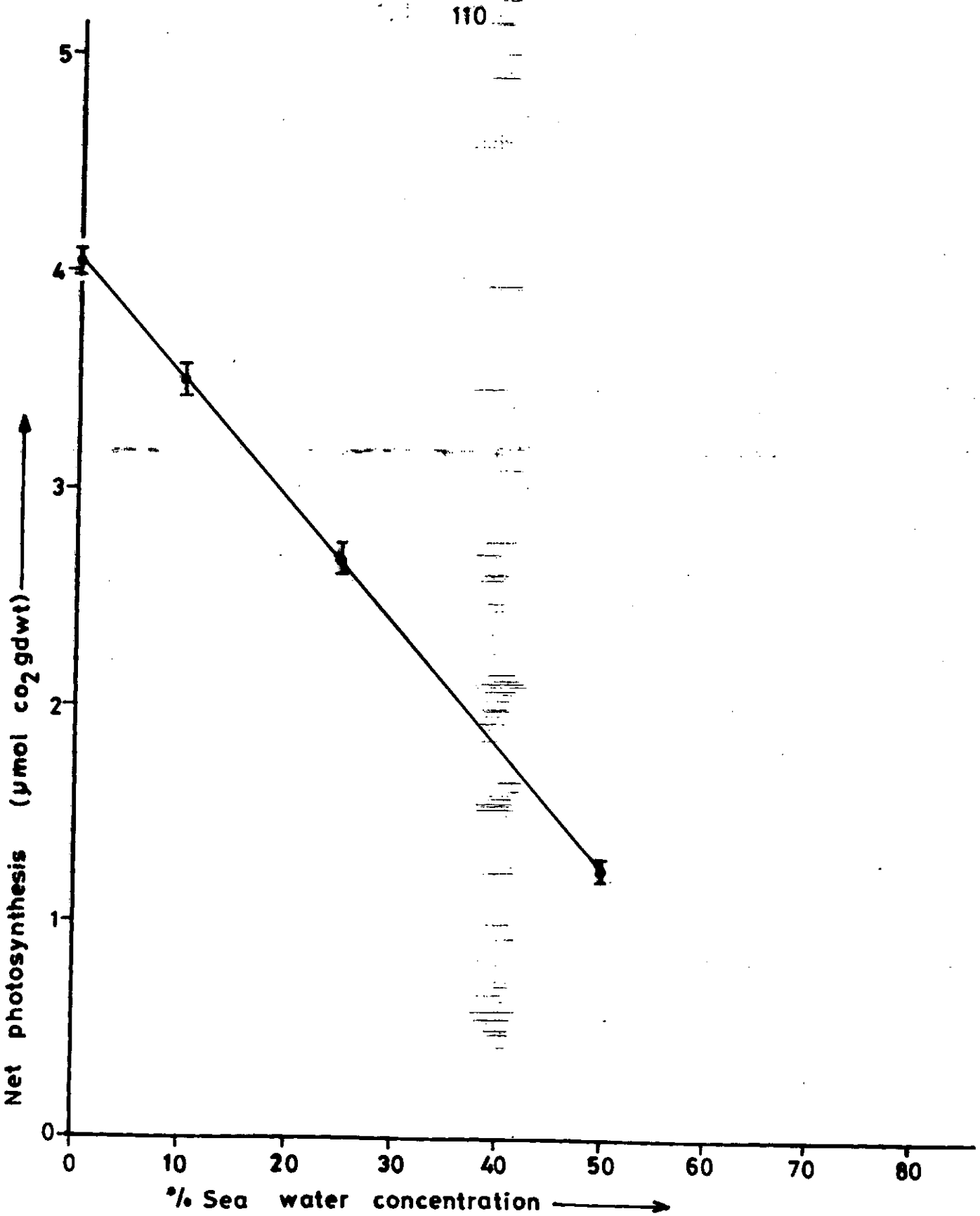

Net photosynthesis (µmol co_2 gdwt)
0
1
2
3
4
5
0
10
20
30
40
50
60
70
80
% Sea water concentration

Figure 9: Changes in stomatal conductance in *D. aegyptium* to water ●——● and carbondioxide ○——○ with salinity changes. Bars represent SD.

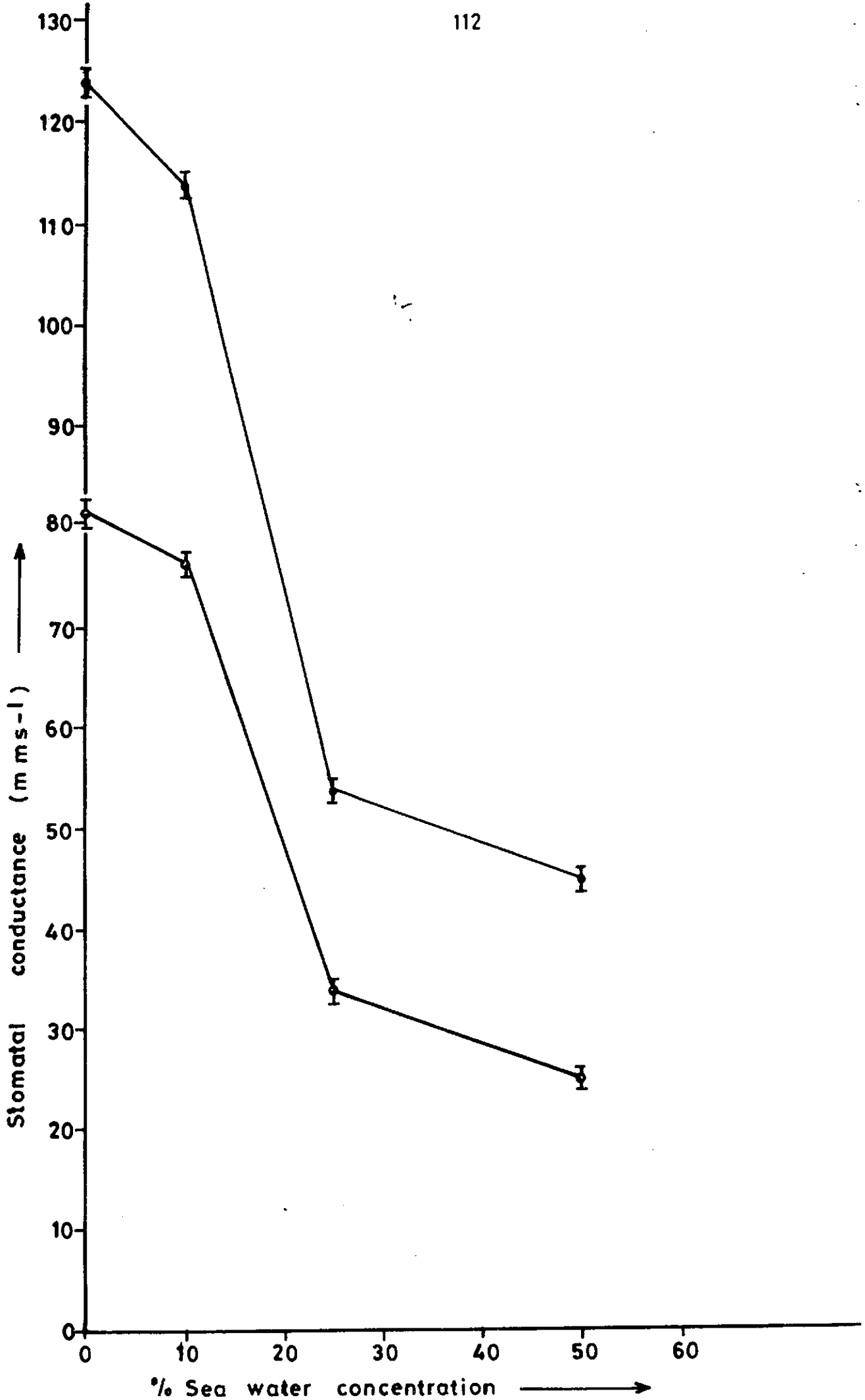
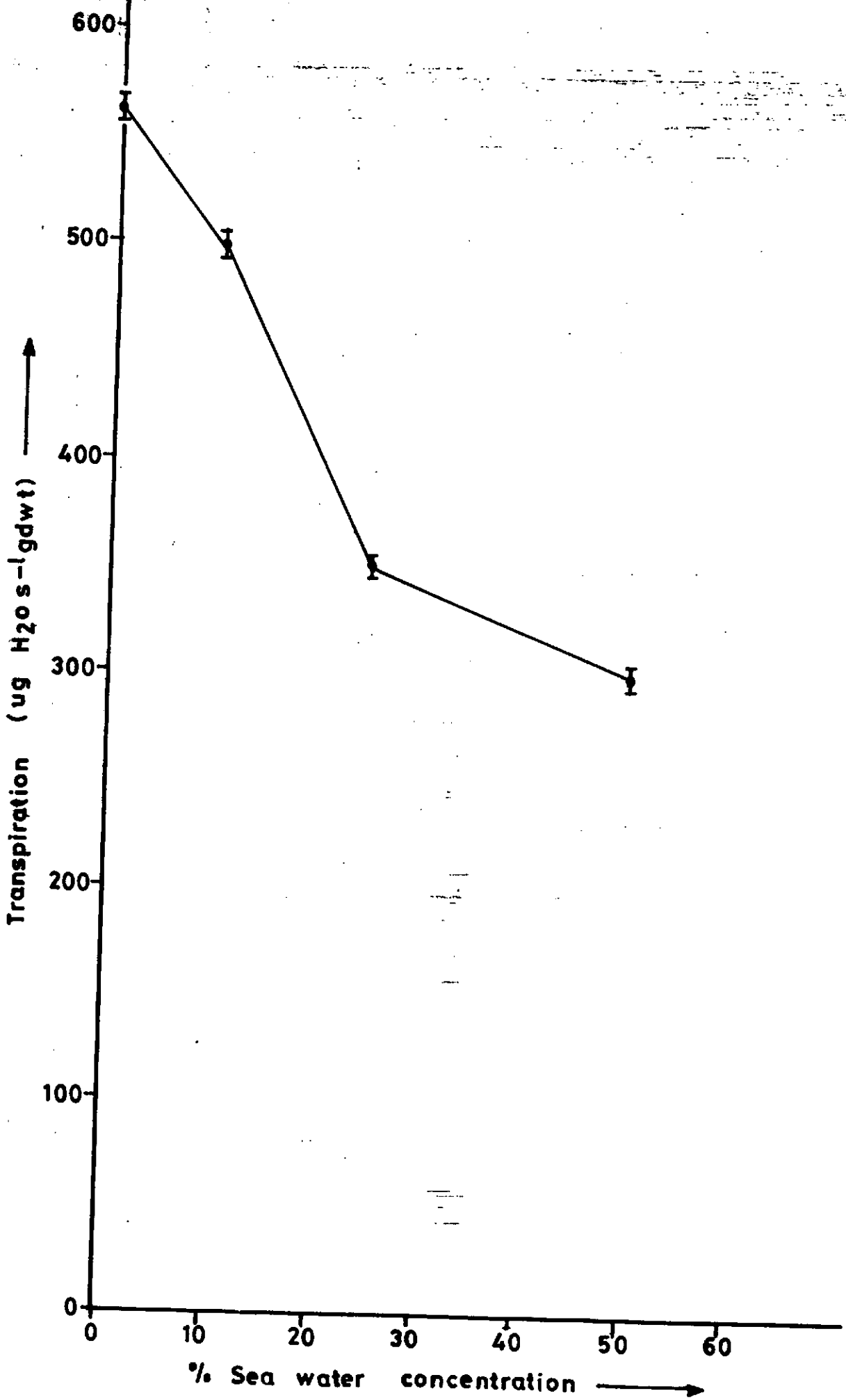

130
120
110
100
90
80
70
60
50
40
30
20
10
0
Stomatal conductance (m ms-1)
0
10
20
30
40
50
60
% Sea water concentration

Figure 10: Transpiration (ug H_2O S^{-1} g dwt $^{-1}$) in *D. aegyptium* under various sea water concentrations. Bars represent SD


600
500
400
300
200
100
0
Transpiration (ug H_2O s^{-1} $gdwt^{-1}$)
0
10
20
30
40
50
60
% Sea water concentration

DISCUSSION

Salinity effects on photosynthetic processes fall into two major categories; a) the response of stomata to salinization of the plant and b) the effects of salt on the capacity of the plant for carbondioxide fixation, independent of altered diffusion associated with salinization of salt sensitive species (Gale, Kohl and Hagan, 1967; Downton, 1977; Longstreth and Nobel, 1979; Walker *et al* ., 1983), whereas the reverse is generally the case for salt tolerant species (Levitt, 1980; Osmond, Bjorkman and Anderson, 1980).

The data presented here for *D. aegyptium* showed that stomatal conductance is reduced by salinity. However the extent to which stomatal closure affects photosynthesis is indicated by the 73% reduction in stomatal conductance to both water and carbondioxide at 50% sea water concentration over the control. This result is similar to that obtained with *Phaseolus vulgaris* (Downton, 1977), a species in which growth is particularly sensitive to the presence of sodium chloride in the root zone (Greenway and Munns, 1980).

The salt induced decline in stomatal conductance (73%) to water and carbondioxide (Figure 9) a phenomenom also seen in various species after abscisic acid treatment (Seemann and Critchley, 1985) and in some instance with water stress (Farquhar and Sharkey, 1982) could be responsible for a nearly equal decline (75%) in the photosynthetic rate at 50% sea water concentration over the control.

The high reduction in stomatal conductance by salt stress in this species (73%) is in contrast to that of halophytes, where salinity has a lesser effect (24%) upon the extent to which stomatal conductance limit photosynthesis (Osmond et al., 1980). Therefore it appears that D. aegyptium does not behave like a true halophyte with respect to stomatal closure under salt stress.

Since there was a 74% reduction in shoot dry weight at 50% sea water concentration relative to the control in the salinity experiment (Chapter 2) with D. aegyptium, this could therefore be directly related to the similar (75%) decline in photosynthetic activity. This implies a low production of dry matter at 50% sea water. In contrast, there was a slight increase in dry weight at 10% sea water concentration over that at 0% (Fig 2) while there was a decline in the photosynthetic activity (Fig 8). This could be linked to ion accumulation at 10% sea water concentration which increases the shoot dry weight. Yeo (1974) has reported that 45% of the total dry weight of some plants could be due to ion accumulation.

A reduction in photosynthetic capacity resulting from salinity stress has also been suggested to be a consequence of end product inhibition of certain processes of carbon metabolism because of other salt inhibited reactions (Greenway and Munns, 1980; Munns et al., 1983; Rawson and Munns, 1984). These workers suggested that reduction in photosynthesis does not limit growth directly, since for example carbohydrates were found to accumulate in leaves of certain species suffering from salt

stress. Instead, they hypothesized that inhibition of photosynthesis could be a consequence of an altered source - sink relationship inhibiting certain reactions of photosynthesis, rather than excess ion directly affecting photosynthesis. However inference could not be drawn from the results obtained here that end product inhibition of certain processes could be limiting photosynthesis.

Other workers suggest that the decline in photosynthetic capacity with increasing salinity may be related to internal ion concentration particularly the $Na^+ : K^+$ ratio. In this species the Na:K ratio increased generally with increase in salinity (Table 4, chapter 2). The Na^+ to K^+ ratio at the roots would remain constant in dilutions of sea water, whereas the ratio varied as the Na^+ level increased from 10 to 50% sea water concentrations. The decline in the K^+ concentration in the leaves (Table 4, chapter 2) was accompanied by decline in photosynthetic capacity (Fig 8) which can be a symptom of K^+ deficiency (Terry and Ulrich, 1973 a,b; Peoples and Koch,1979).

In a review, Lauchli and Pfluger (1978) stressed the role of K^+ as the dominant counter ion to light-induced H^+ flux across the thylakoid membranes and the establishment of the transmembrane pH gradient necessary for the synthesis of ATP (photophosphorylation), in analogy to ATP synthesis in mitochondria. Potassium ion also plays a role in stomatal regulation; it has the major responsibility for turgor changes in the guard cells during stomatal movement. An increase in the K^+

concentration in the guard cells results in the uptake of water from the adjacent cells and a corresponding increase in turgor in the guard cells and thus stomatal opening (Hsiao,1970; Marschner, 1986). It is possible that D. aegyptium may have experienced some metabolic dysfunction relating to their requirements for K^+ at high salinity since there was reduced k^+ as salinity increased (Table 4, Chapter 2).

A strong influence of sea water concentration on the transpiration characteristics of D. aegyptium was demonstrated in this study. The sea water concentration of 50% brought about a 64% reduction in transpiration. The ecological importance of this to the plant may be that it affords the plant protection from excessive water loss. This could mean a higher water content in the plant for the dilution of solutes at higher salinity and therefore lowering of the plant osmotic potential below that in the surrounding soil for effective water intake by the roots. However, in this species the water content decreases as salinity increases at each harvest (Table 3, chapter 2). This therefore implies that water is not conserved in the plant inspite of stomatal closure at high salinity. The low water content could be due to the inability of the roots to absorb enough water because of the lower osmotic potential in the growth medium, caused by the soil salinity despite the fact that water was available in the surrounding soil. It may also be suggested that the plant probably fixes carbondioxide with the C_4 metabolism. This allows plants to reduce internal carbondioxide concentrations to zero

when stomata are closed and then maintain a steep gradient across the leaf epidermis when they are almost closed. The initial fixation to oxaloacetate takes place in the cells in the middle layers of the mesophyll and the oxaloacetate so formed is then converted to four-carbon malate or aspartate. But the four-carbon products are then transported to specialised cells which surround the vascular bundles in the leaves, the tubes and columns of cells that convey water and nutrients through the plant. In these specialised bundle cells, carbondioxide is released from the four-carbon molecules, and then fixed with ribulose diphosphate.

The C_4 mechanism enables plants to utilize both carbondioxide and water more efficiently (Bannister, 1976; Moore, 1981). However, this C_4 mechanism cannot be the cause of low water content in the shoot of D. aegyptium since the photosynthetic capacity was measured in light with adequate water supply to the plant.

It could be concluded that high salinity depressed growth through reduced photosynthetic capacity, stomatal conductance to water and carbondioxide. This was made worse by reduced tranpiration and inability of the plant to absorb water which in turn led to dehydration of cells and increased turgor.

CHAPTER 6

GERMINATION STUDIES

INTRODUCTION

The germination stage is probably the most critical period for species establishment since without it, seedling growth cannot occur (Okusanya, 1976). Germination process consists of two phases: radicle emergence and seedling growth (Bewley and Black, 1978). During the first phase, water absorption plays a key role (Corchete and Guerra, 1986).

Many stringent demands are placed on the seeds that arrive in a particular habitat, if they are to be able to complete satisfactorily, the rest of their life cycle (Harper, Lovell and Moore, 1970). Conversely, the habitat should be capable of satisfying the requirements of the new arrivals (Gleason, 1939). Thus the continued existence of a species in a given habitat is possible only if the enviromental factor complex of the habitat lies within the limits of the physiological tolerance of the species.

Stages of germination and seedling establishment, which are sometimes accompanied by extremely high mortality, in the life cycle of higher plants are the important phases determining the population size of a species and its spatial and temporal distribution (Grime, 1979; Solbrig, 1980).

A variety of physiological mechanisms are known to exist to ensure safety in these critical phases: the germination requirements for each species or population should keep the seeds

in safer pre-germination phases when the probability of successful seedling establishment is low. Therefore seed germination has been assumed to depend upon sites offering the required conditions for germination, in which case they are termed 'safe sites' (Harper, 1977).

Germination responses to factors of the environment such as moisture, light, temperature and salinity vary among and within populations of plant species (Dale, 1974; Edward, 1975). The effects of environment on seed germination are important agricuturally. The topic is relevant to efficient crop establishment, the stability, longevity and production of forage crops, and also the dynamics of weed infestation (Thornley, 1986).

When seeds are sown in an environment with an imposed water deficit, both the rate and percentage of germination decrease (Mayer and Poljakoff-Mayber, 1982; Prisco and O'Leary, 1970; Gonzalez-Murua, 1985). It is known that in the absence of various avoidance mechanisms, plants subjected to water or salt stress must regain turgor through osmotic adjustment to resume growth (Corchete and Guerra, 1986). Seeds of many species have evolved ways to prevent dessication of the embryo in response to the moisture regime of the habitat. Harper and Sagar (1963) and Harper and Benton (1966) have shown that the failure of seeds of some plants species to germinate was the result of delayed or insufficient hydration of the seeds, a condition brought about by level of water table in the soil.

Water uptake and available water have been shown to be two important aspects of moisture requirements of many plant species (Lazenby, 1955; Toole, Hendricks, Borthzick and Toole, 1965). An experiment was therefore set up to determin the effects of different soil moisture regime on the seed germination of D. aegyptium.

The importance of light and its effect on germination of seeds have received much attention (Toole et al., 1965; Cavers and Harper, 1966; Cole 1967; Shontz and Oosting, 1970). When seeds are dispersed they are either partly or completely buried in the soil, or, they lie on the surface and are covered with litter, or occupy cracks or crevices in the soil or are completely exposed on the soil. In these different positions, they obtain different amounts of light.

Germination of seeds in response to light revealed that variations occur in the inter and intra-specific as well as inter and intra-population levels (Cavers and Harper, 1966). It is therefore of ecological interest to determine the effect of light and dark regimes on germination of the seeds of D. aegyptium.

Although choice of an appropriate temprature is often crucial in devicing techniques for germination (Ellis, Hong and Roberts, 1985), optimal temperature regimes particularly for tropical grasses are not well established (Goedert and Roberts, 1986). The International Seed Testing Association Rules (ISTA, 1985), prescribe the same regime for most tropical grasses, namely 35 ^{o}C or sometimes 30 ^{o}C for 8hours in light and 20 ^{o}C for 16

hours in the dark, but this recommendation has been found not to be very satisfactory for seeds of Brachiaria humidicola (Rendle) Schweickerdt (Goedert, 1984). Also D. aegyptium is widespread and abundant in farmland and wasteland throughout most of Nigeria. The Climatic data collected from the Federal Meteorological Station, Oshodi, Lagos, showed clearly that there is a gradient of temperature from the north to the south of the country. All these factors necessitated the setting up of an experiment to determine the temperature requirements of the seeds of D. aegyptium collected from Ijoko via Otta.

For species growing in saline environments, germination is an important stage in the life cycle because it determines the salinity concentration to which later stages of the species will be exposed (Ungar, 1982). The population of D. aegyptium under study was collected from a salt refinery industry at Ijoko via Otta, and the analysis of soil from the site shows that the salinity level is 30% that of sea water (Table 22). Collection of soil was made in April, 1987 when soil salinity could have been reduced by the rain.

The germination of seeds may be affected by salinity through osmotic or specific ion toxicity or both (Uhvits, 1946). These effects may slow down or completely inhibit germination depending on the level of salt in the growth medium (Ayers and Hayward, 1948). The soil microenvironment in which a seed is expected to germinate and become established as a seedling is likely to have a higher salt concentration than the bulk of the soil because of evaporation and capillary rise of saline water to the soil

TABLE 22: Analysis of soil from the National Salt Company of Nigeria, Ijoko via Otta and soils used in the various germination experiments.

Soil Type	pH	Organic matter g/100g dry soil	Soil moisture	K	Na	Ca	Mg	Salinity %	chloride ppm
				m equiv/100g dry soil					
Salt Company	7.9	28.10	13.10	6.00	5.70	11.80	14.70	30	9×10^{-5}
Humus	7.8	19.60	22.10	9.24	2.10	29.61	8.61	5	3×10^{-5}
Sand	6.9	13.70	15.90	2.04	2.40	0.77	2.94	2	2×10^{-5}
Red Earth	5.6	16.20	8.70	0.00	2.30	0.00	2.80	8	4×10^{-5}

surface (Pasternak, Twersky and De Malach, 1979). In general, both halophytes (Okusanya, 1977; Ungar 1978) and glycophytes (Albregts and Howard, 1973) respond in a similar manner to increased salinity stress; with both showing a reduction in the total number of seed germinating and time of germination. The precise salinity concentration causing a delay and reduction in the number of seeds germinating depends upon the salt tolerance of each individual species. An experiment was therefore set up to determine the effect of salinity on the germination of the seeds of D. aegyptium

The soil is the material in which plants root, and from which they draw their water supplies and essential nutrients (Russell, 1968). The detailed microtopography of the surface of the soil determines the density of safe sites within a gap and so is an important feature regulating regeneration. The effect which the microrelief has on germination is largely controlled by the amount of water content which exist between the seed and the soil surface, and on the tension with which water is held in the soil (Fenner, 1985).

The actual places in the soil in which the individuals of a particular species can germinate and establish themselves, must vary almost infinitely in their suitability for that species. Some plants have a narrow, some a wide range of tolerance of various soil conditions. Each plant has a definite range of pH value within which it can germinate and grow, and a narrow (optimum) range within which it germinates and grows luxuriantly. It is

obvious that within the wider range, but outside the narrower, the plant is more or less handicapped in its germination, growth and other life functions (Fenner, 1985).

The pH value obtained depends largely on the proportion of soil to water and on the salts present in the soil. Some of the common soil types in Nigeria are gravels, sand, red earth, loam and limestone soils with varying pH. It was therefore necessary to determine the effect of three soil types namely humic soil, sand and red earth which are the more common soil in wasteland and farmland where populations of D. aegyptium are usually found. It was therefore necessary to determine the effect of pH on the germination of the seeds.

MATERIALS AND METHODS

Seeds of *D. aegyptium* were collected from a population growing at the premises of the National Salt Company of Nigeria Ijoko, via Otta in Ogun State. Seeds were kept dry in the cupboard in the laboratory until used.

For each germination experiment, there were four replicates with twenty-five seeds per replicate. The number of seeds germinating were noted every two or three days. Seeds were considered to have germinated when the radicles were about 5mm long or the plumule has emerged from the soil. The rate of germination (reciprocal of the time taken for germination) and the percentage germination (the percentages of the non-dormant seeds within a seed population under a certain combination of conditions) at the end of the experiment were recorded. The comparisons of the germination percentages were done using the X^2 test as outlined by Clarke (1980).

For the determination of the effect of light and dark regimes, salinity, temperature and pH on germination, sterile disposable Petridishes were used. The seeds were placed on two layers of Whatman qualitative filter papers. The petridishes for the light experiment were placed in a lighted closed germinating cabinet in the Botany Research Laboratory of the University of Lagos. Temperature averaged $30 \pm 2^{o}C$ day and $23 \pm 2^{o}C$ night. Room lighting was supplemented with light supplied by six 60watt fluorescent tubes for 12 hours each day. The total light intensity in the cabinet was about 3000lux.

All other experiments, except the effect of temperature and dark regime took place on the working bench of the Botany Research Laboratory. Temperature averaged 29±2°C (day) and 22±2°C (night). Room lightining was about 2,000 lux which was sunlight supplemented by fluorescent tubes. Relative humidity was between 80 - 60%.

The effect of light and dark on germination were first investigated so as to be sure that most, if not all of the other factors necessary for maximum germination of seeds are provided when the effects of the other factors are determined. Each experiment lasted for 25 days during which time germination usually levelled off.

Effect of light and dark regimes;

Petridishes containing seeds for the light treatment were placed in the lighted germinating chamber in the Botany Research Laboratory, while petridishes containing seeds for the dark treatment were wrapped with black polythene papers and placed in cupboards in the laboratory.

Since there were no light filters to be used to count the germinating seeds at intervals in the dark, three sets of petridishes were provided for the dark experiments. One set was observed for the number of seed germinating at 3 days, another at 5 days and another at 7 days. Seeds which did not germinate in the dark at day 3 and day 5 were brought to light.

All the seeds for the two sets of treatments were moistened with 10ml of distilled water.

Effect of pH

One-fifth strength Hoagland and Arnon (1938) solution adjusted to pH 3.5, 5.0 and 7.0 by using either dilute sulphuric acid or

dilute potassium hydroxide. In addition, distilled water (pH 7.0) was used. Thus there were four treatments. In each case ten millilitre of the solutions were used to moisten the filter papers.

Effect of Salinity

Sea water concentrations of 0, 10, 20, 30, 40 50, 75 and 100% were used. The different concentrations of sea water were prepared by mixing appropriate volume of filtered sea water collected from the Lagos Bar Beach and one-fifth strength Hoagland and Arnon (1938) solution.

Every 5 days, the seeds were removed and then returned to petridishes containing new filter papers moistened with appropriate solution. This procedure was used to reduce any large scale increase and fluctuation in the various salinities due to evaporation (Okusanya, 1979a).

Effect of Soil types

Three soil types designated as humic soil, red earth and sand were used. The humic soil was collected from the botanical garden of the University of Lagos. The soils were sieved to remove stones and debris and thoroughly mixed before being used. The red earth was collected at Ilaro road, Papa near Ewekoro (Ogun State). The sand used was from River Majidun near Ikorodu in Lagos State. The characteristics of the soils are given in Table 22.

The seeds were placed at 0.5 cm depth in each of the three soil types in disposable petridishes in which holes were made at the bottom to facilitate good drainage of water. One layer of whatman qualitative filter paper was placed in the petridishes

before the addition of each soil type to prevent the soil from passing through the holes. Watering was with distilled water once a day to field capacity.

Effect of soil moisture content:

Seeds in humic soil in disposable petridishes were subjected to three watering regimes namely, watering once a day (wet); watering every four days (dry) and continuous watering (waterlogged). the waterlogged treatment was achieved by putting the disposable petridishes in containers of water such that they are completely covered by water.

Petridishes for the wet and dry treatments had holes at the bottom to facilitate good drainage of water and the dishes were lined with a layer of Whatman qualitative filter paper to prevent the soil from sieving through.

Humic soil was used because of the high percentage germination recorded in the soil, in the soil type experiment (Fig 14).

Effect of temperature

This was investigated by subjecting the seeds to the effects of seven constant temperature regimes of 5, 10, 15, 21, 31, 37 and 44 ^{o}C. Distilled water was used to moisten the seeds.

The low temperature incubators manufactured by Astell Hearson London were used for temperatures of 15, 21, 31, 37 and 44 $^{+o}$C. Illumination was provided by two, 15 watt fluorescent tubes. Lighting in the incubator was regulated to give a 12 hour photo period. For temperatures of 5 and 10 ^{o}C, a thermocool

fridge (Thermocool Engineering Co. Ltd., Ilupeju, Lagos) was adapted to these two temperatures and then used for the treatment. However illumination was not provided inside the fridge as this was not considered important in this experiment; the seed of D. aegyptium. achieved 100% germination in the dark after 7 days (Fig 11).

This may be a drawback in the experiment but it cannot be helped in the absence of a lighted incubator that could work at these low temperatures.

RESULTS

The type of germination in D. aegyptium is epigeal. The radicle emerges first and reaches about 20-25 mm in length before the emergence of the cotyledons.

Effect of light and dark regimes: The results of the effect of light and dark regimes on the seed germination are given in Fig 11. There was good germination both in dark and in light but the observed rate of germination in light was significantly higher ($P < 0.001$) than in the dark at 3 and 5 days of germination.

Seeds in light reached 100% germination in 3 days of germination. The percentage germination in the dark increased as the number of days of germination increases reaching 100% germination in 7 days. When seeds which did not germinate in the dark after 3 and 5 days were brought to light for a further period of seven days, 100% germination was reached. The seedlings produced in the dark were weak and etiolated.

Effect of pH: Fig 12 shows that the seeds responded differently to the effect of pH. There was an increase in percentage germination and observed rate of germination as pH increases, but finally the same percentage germination of 100% was achieved after 8 days at pH 5.0 and 7.0. The rate of germination in distilled water reached 100% after only 3 days. In Hoagland solution of pH 7.0 it achieved 60% germination after 3 days, showing a difference in their rate of germination. The differences in percentage germination in Hoagland solution of pH 3.5 and those of 5.0, 7.0 and distilled water were significant ($P<0.05$)

Figure 11: Percentage germination ± SD of *D. aegyptium* in light o——o and in dark ●——●

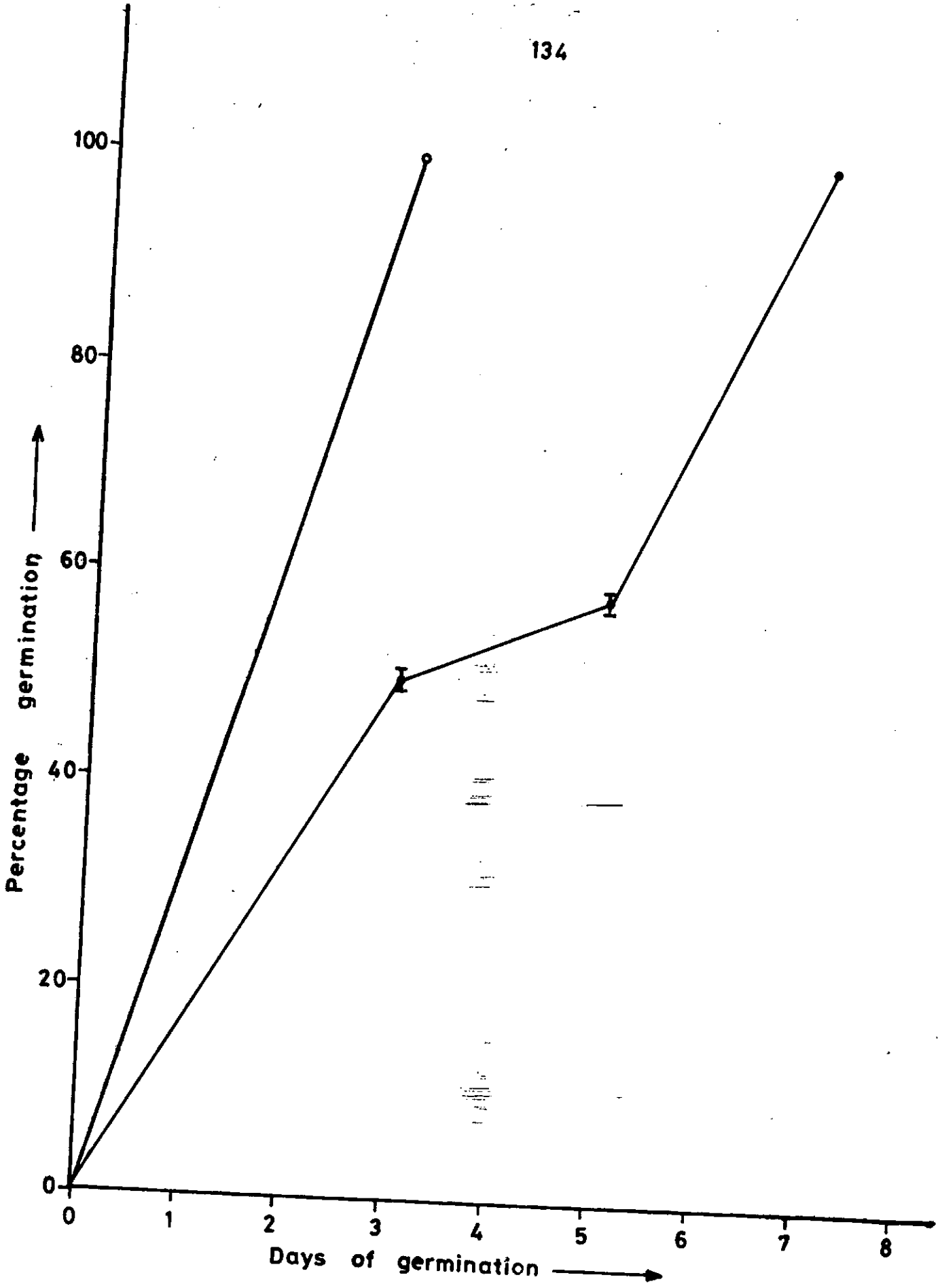
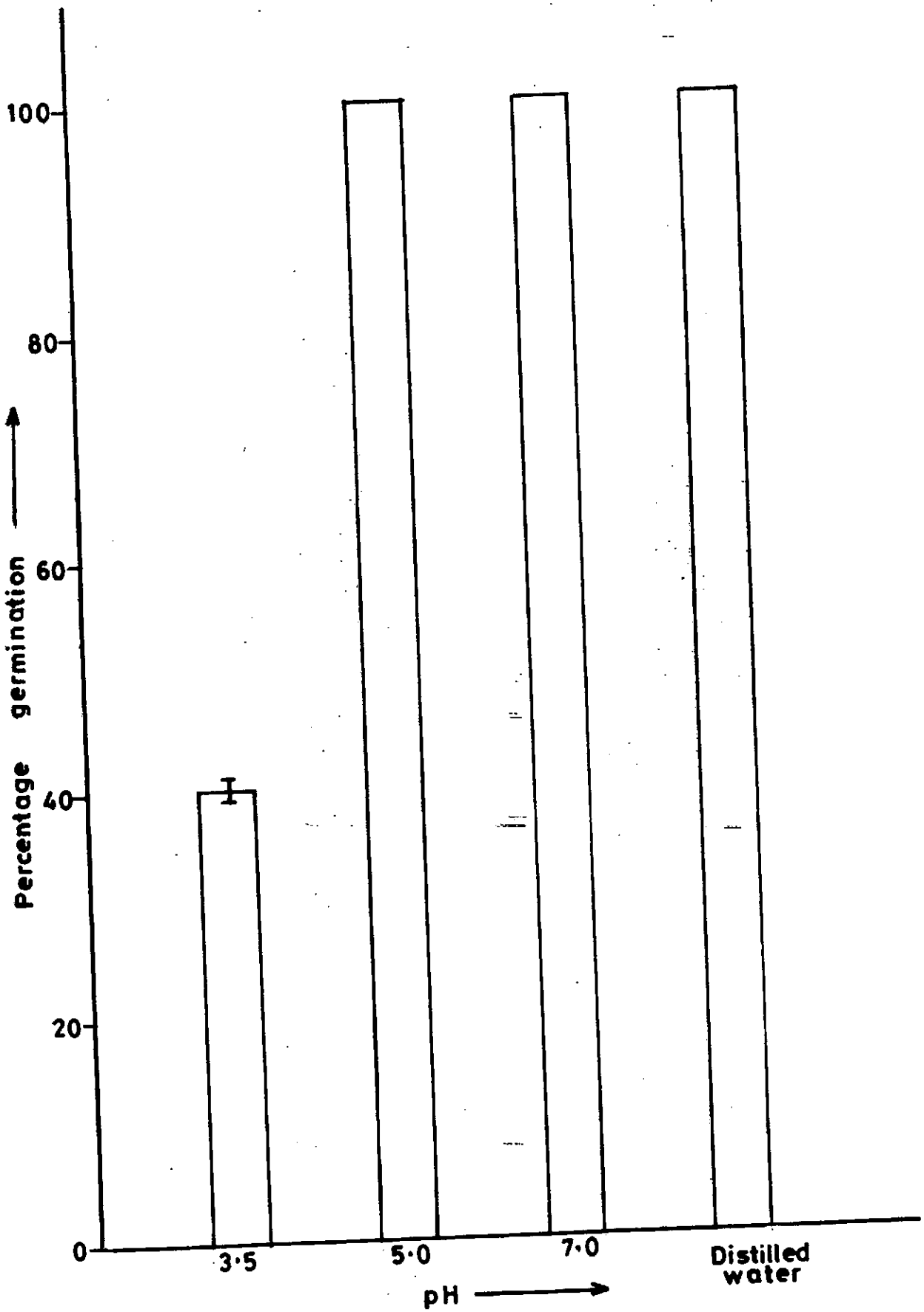

100
80
60
40
20
0
Percentage germination
0
1
2
3
4
5
6
7
8
Days of germination

Figure 12: Percentage germination ± SD of *D. aegyptium* at various pH ranges of Hoagland and Arnon (1938) solution and distilled water of pH 7.0.


100
80
60
40
20
0
Percentage germination
3·5
5·0
7·0
Distilled water
pH

Effect of salinity: Germination values of 100% occurred at 0-50% sea water, showing that an increase of salinity up to 50% sea water does not affect final percentage germination (Fig 13).
The observed rate of germination however decreased as salinity increased. There were sharp decline in germination percentages in salt solutions of 75 and 100% sea water compared to those of 50% sea water ($P < 0.001$).

Recovery experiments in distilled water indicate that increasing osmotic stress did not permanently inhibit germination of the species. Final percentage germination of 45 and 29% were obtained in seeds previously soaked in 75 and 100% sea water respectively after transfer to distilled water for 7 days.
Effect of soil type: Fig 14 shows that the seeds germinated well in all the soil types . There was no significant difference ($P>0.05$) in percentage germination between humic soil and red earth - the two soils types which recorded the highest percentage germination. Germination percentage in sand was significantly lower ($P<0.05$) than in the other two soil types. However the observed rate of germination in red earth was lower than in the humic soil types.
Effect of soil moisture: The results of the effect of soil moisture on germination are given in Fig 15. There was increase in germination percentages as soil moisture decreases. But germination percentage in dry treatmet was not significantly different from the wet treatment ($P>0.05$). Germination in the waterlogged treatment was significantly lower ($P<0.001$) than the other two soil moisture treatments.

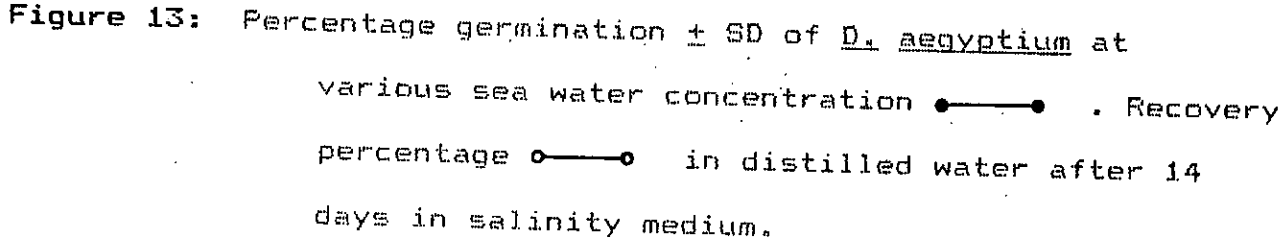


Figure 13: Percentage germination ± SD of *D. aegyptium* at various sea water concentration ●——● . Recovery percentage o——o in distilled water after 14 days in salinity medium.

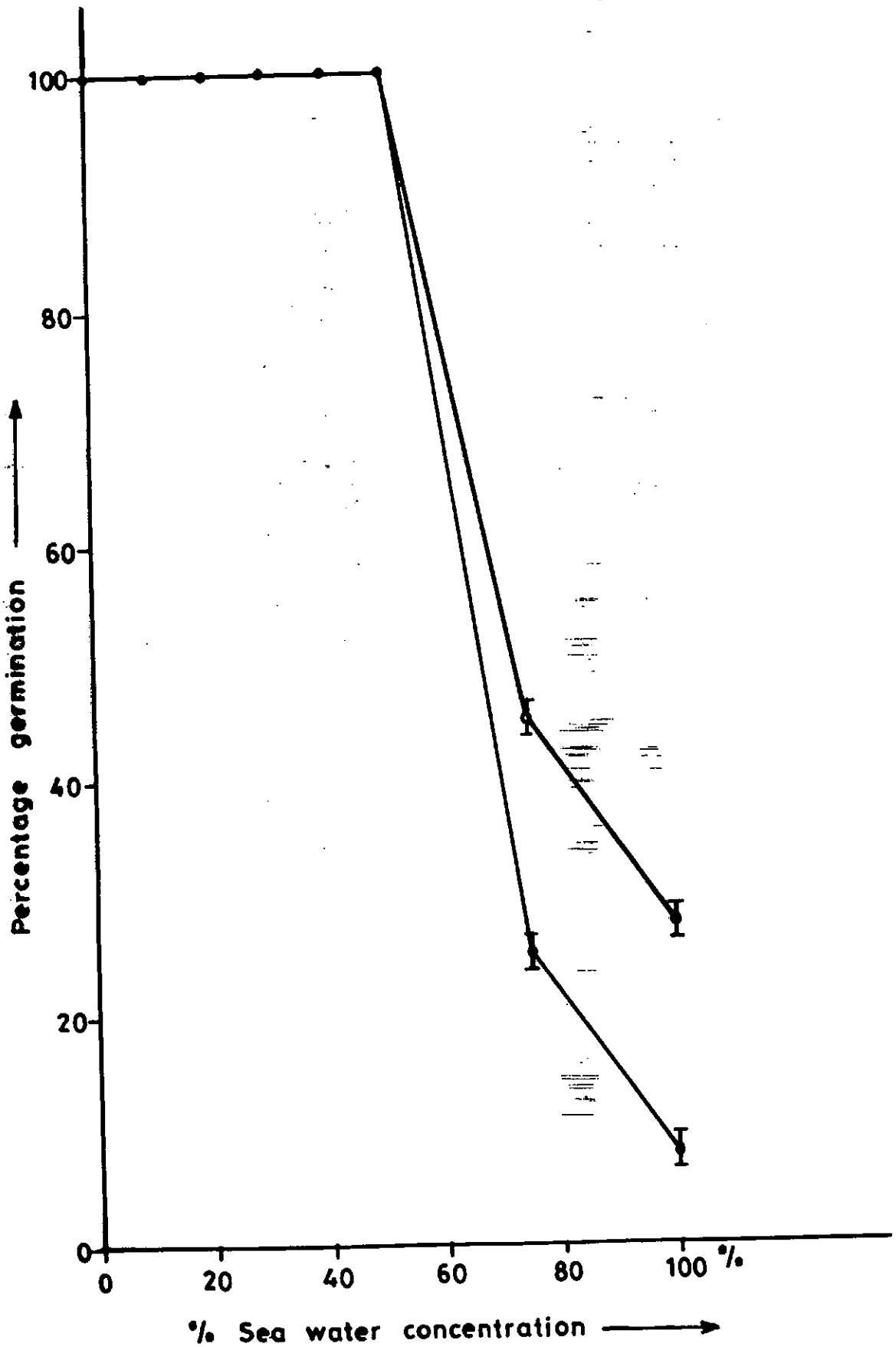
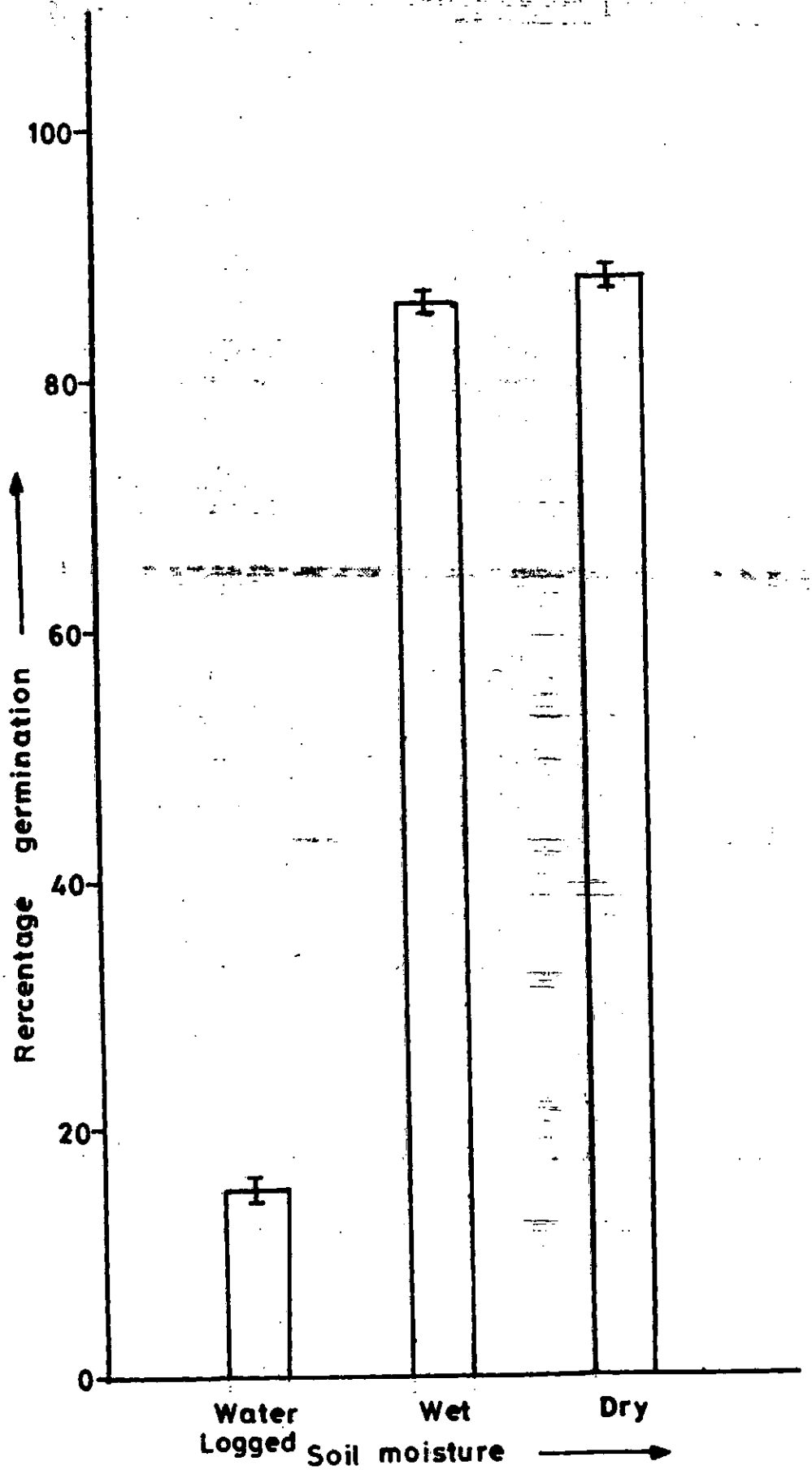

100
80
60
40
20
0
Percentage germination
0
20
40
60
80
100 %
% Sea water concentration

Figure 14: The percentage germinatiom ± SD of D. aegyptium in various soil types.


100
80
60
40
20
0
Percentage germination
Sand
Humus Soil
Red Earth
Soil type

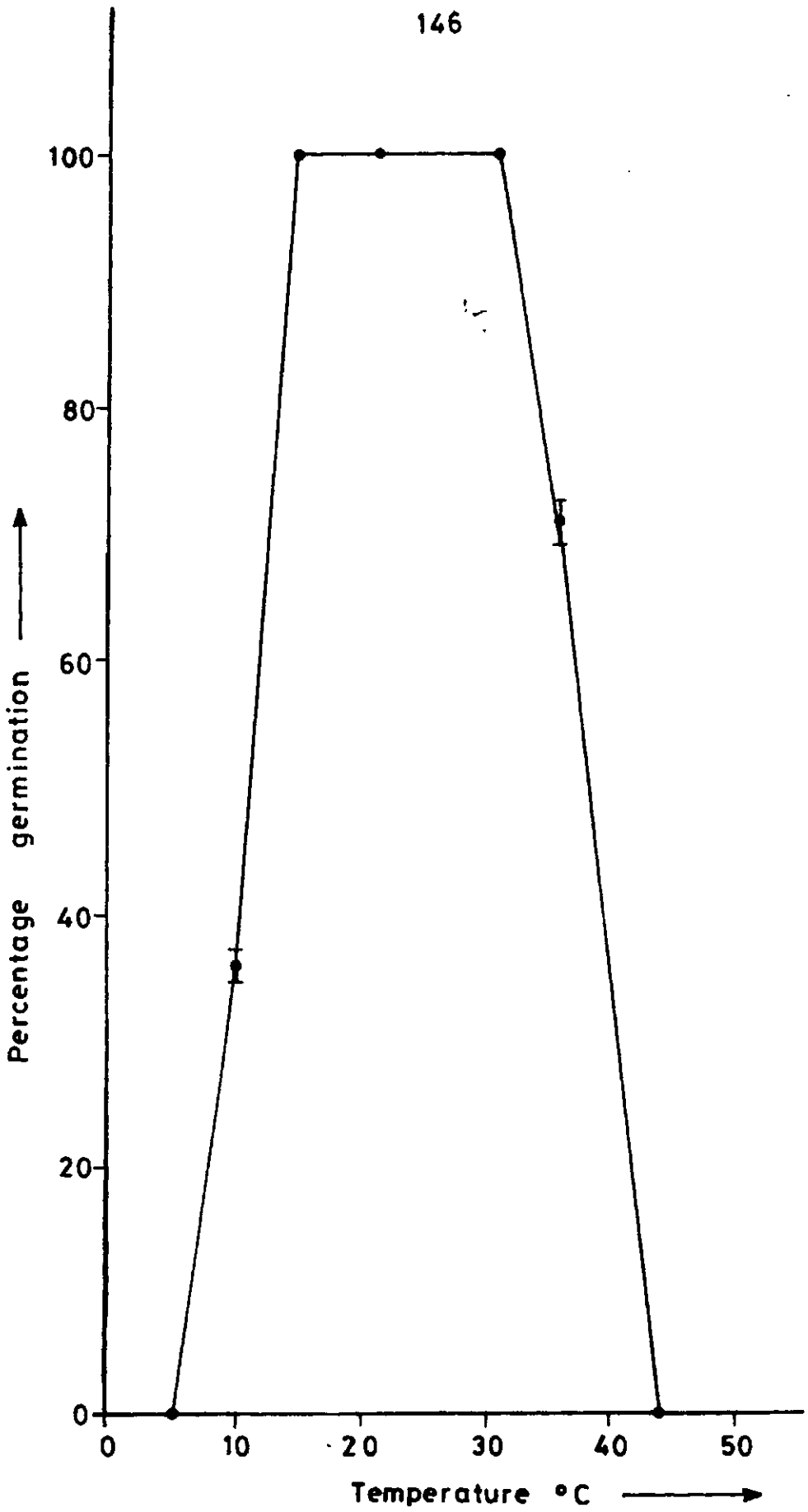
Figure 15: Percentage germination ± SD of *D. aegyptium* under water-logged, wet and dry soil conditions.


100
80
60
40
20
0
Rercentage germination
Water Logged
Wet
Dry
Soil moisture

Effect of temperature : Fig 16 shows the result of the effect of various constant temperature regimes on the germination of the seeds. No germination occurred at 5 °C but as temperature increased up to 31 °C, percentgae germination increased, thereafter the percentage germination decreased significantly ($P<0.05$). No germination was observed at 44 °C. Also the percentage germination at 10 °C was significantly lower ($P<0.05$) compared to that at 15 °C.

The optimum temperature for seed germination is between 15 and 31 °C the minimum is 5 °C and the maximum at 44 °C.

Figure 16: Percentage germination ± SD of *D. aegyptium* at various constant temperatures.


100
80
60
40
20
0
Percentage germination
0
10
20
30
40
50
Temperature °C

DISCUSSION

The satisfactory germination in both light and dark (Fig 11) indicates that the seeds can germinate in cleared and uncleared farmland and wasteland, as well as when buried in soil. The ability to germinate well in both light and dark, aids the spread of the species, consequently it is found in varied light conditions. However, the fact that there is significantly higher rate of germination in light and that light hastens maximum germination in seeds previously placed in the dark can be attributed to the difference in the rate at which the seeds may be producing growth promoting substances (gibberellic acid and kinetin) which will initiate germination processes in the dark (Kahn, 1971). These substances are usually produced faster in light. Fenner (1985), linked the higher rate of germination in light than dark to the conversion of phytochrome to the active germination inducing form in the light, followed by its spontaneous reversion to the germination inhibiting form in the dark.

The results of the effect of pH on germination (Fig 12) showed that there was germination at the three pH ranges tested. The good germination at the range of pH 5.0 to 7.0 and the fairly good germination at pH 3.5 may be a reflection of the species ability to germinate in the acidic and neutral pH range. Species which do this are able to colonize a wide range of habitats (Ingestad 1976; Okusanya, Ola-Adams and Bamidele, 1981).

The reduced germination in the acid solution may be a result of low nutrient concentration, high aluminium concentrations leading to toxicity and high hydrogen ion concentration. It could also result from post-germination failure, because in the acid solution, the testa of many seeds got ruptured but the radicles did not emerge (Okusanya, 1978; Marschner, 1986).
Low nutrient supply cannot be the cause of poor germination at pH 3.5 since there is sufficient mineral elements in the Hoagland solution. Since the experiment did not involve acid soil but solution, the issue of excessive levels of free and exchangeable aluminium did not arise. Therefore the poor germination in D. aegyptium at pH 3.5 may probably be due to high hydrogen ion concentration and or post germination failure. Manganese toxicity was not considered important in this investigation as it is usually associated with soils that have been steamed (Black, 1968; Boyd, 1971).

The differences in the rates of germination in distilled water and Hoagland solution of same pH could be attributed to the greater ease with which distilled water could be absorbed than the Hoagland solution, which has a lower osmotic concentration (Sonaike and Okusanya, 1987). Distilled water may also be able to wash off any substance in the seed coat which may be inhibitory to germination, consequently the rate of germination will be enhanced in distilled water (Sonaike, 1985).

The traditional method of determining the range of tolerance of seed germination in sea water is by the use of filter papers in petridishes moistened with appropriate sea water concentrations

(Rivers and Weber, 1971; Onyekwelu, 1972). However this leads to evaporation of water which would inevitably leave more concentrated solutions in the petridishes. The method of allowing the seeds to germinate while floating on the various concentrations of sea water seems to surmount this problem. Okusanya (1979a) minimized this problems by removing the seeds every five days and rinsing in distilled water before returning to petridishes containing new filter papers moistened with appropriate solution and this was the method used in this investigation.

The result of the effect of salinity on germination of the seed is unusual for a glycophyte. However the result can be ascribed to the influence of the salt being refined in the area where it was collected. The unpurified salt is stored in the open warehouse and is being blown about by the wind.

Good germination up to 50% sea water portrays the plant as a halophyte as most glycophyte only germinate in up to 30% sea water (Ungar, 1974; Okusanya, 1979a). Above 50% sea water there was a rapid decrease in the final germination ($P < 0.05$) and a noticeable delay in the rate of germination (Ungar 1967, 1974; Greenway 1968; Okusanya 1979a, 1983).

Recovery experiments in distilled water for seeds at 75 and 100% sea water was significantly low (20% germination) compared to other halophytes like Crithmum maritimum (100% germination) Okusanya (1979a) and Hordeum jubatum (100% germination) Ungar

(1974). Also Ungar (1982) reported the laboratory results of Zid and Boukhis and indicated that seeds of Atriplex halimus, which did not germinate when exposed to 5% sodium chloride subsequently germinated at levels equal to those of the original distilled water when returned to distilled water. These therefore show that D. aegyptium is not a true halophyte considering the habitat of seed collection and the habitat of other populations from farmland and wasteland which are non-saline. Consequently it could be considered as an ecotype which may be gradually adjusting to the saline environment of the salt refinery. Good recovery response is usually common among halophytes, since it might be of some ecological advantage within highly saline environments, reflecting a physiological response that is strongly selected for during the evolution of halophytes (Ungar, 1978, 1987).

The germination response of this species to salinity could be of ecological significance since germination of seed is in early raining season when salinity could still be as high as 50% sea water concentration. Even towards the dry season when salinity in the habitat is high due to evaporation, a small amount of germination can still occur.

The germination of the seeds in the three soil types (Fig 14) may explain the wide ecological spread of the plant in Nigeria (Townrow, 1959). D.aegyptium must be able to grow in or adapt to a wide range of soils. The significantly low ($P<0.05$) percentage germination in sand compared to the two other soil types could be attributed to low nutrient level of sand and/or

the poor water retention ability (Russell, 1968). However, since there was 100% germination in distilled water (Fig 12) with no nutrient level, it therefore implies that low percentage germination recorded in sand may be due to the poor water retention ability despite the fact that the seeds were watered daily.

The low rate of germination in red earth compared to that in humic soil may be due to the physical properties it possess like slow percolation of water, poor aeration and high water holding capacity (Tansley, 1954).

Also the pH of red earth used was 5.8 and humus 7.8 (Table 22). Acidic pH ranges were found only to slow down the rate of seed germination, but the final percentage germination at pH 5.0 and 7.0 were the same reaching 100% germination (Fig 12). The pH value of the humus soil and the fact that it is well drained might be responsible for the higher rate of seed germination in the soil compared to other soil types used.

Attempt to correlate the percentage germination in red earth with what was obtained in the germination response in the waterlogged condition (Fig 15) shows that defective aeration might be the responsible factor in the waterlogged experiments. The first possible secondary effect of waterlogging is a leaching of mineral nutrients or essential intermediates from the seeds where they are immersed. But this does not apply in this experiment as the working apparatus is compact, as such there is no leaching away of the mineral nutrients and more so the seeds

of D. aegyptium were found to germinate very well n distilled water (Fig 12) with no nutrient content. The second secondary effect linked with waterlogging is the gas stress namely oxygen deficit, carbondioxide excess and ethylene excess (Levitt 1980; Mayer and Poljakoff-Mayber 1982; McIntyre, Mitchell and Ladiges, 1989). This might be the possible reason for the poor germination in the waterlogged condition. Wesson and Wareing (1969) also reported that burial significantly inhibit seed germination in seeds when soil moisture level is in excess of 80% soil capacity. This is similar to the result of the experiment with waterlogged soil condition (Fig 15).

The fact that there was no significant difference ($P>0.05$) at both wet and dry treatments, shows a wide tolerance of soil moisture, a characteristic necessary for a species which inhabit wide ecological zones (Sonaike, 1985). In both the southern and northern parts of Nigeria, rains start after hot dry weather. Seed germination in this species starts immediately. At this time, the temperature is high about 33/25 ^{o}C and since rains are still not regular, the soil is still fairly dry or moist. Prior to this time, the land had been cultivated, bringing buried seeds near to the top and exposing them to light.

All these conditions viz high temperature, dry/moist soil and light are those which enhanced germination of this species.

The limitations encountered in the temperature studies viz the adaptations of the thermocool refrigerator to 5 and 10 °C respectively, the non-provision of extra source of light in the fridge. Also the unavoidable power interruption in the fridge and the low temperature incubators used caused by the change in power supply to generator set and vice versa on campus could not be avoided, but power supply was fairly steady during the period.

The response of the seeds of D. aegyptium to constant temperature (Fig 16) shows that germination is best between 15 and 31 °C which are the mean minimum and maximum temperatures in most parts of Nigeria. This implies that it can germinate all the year round.

At low temperatures of 5 and 10 °C, the non-availability of extra light source could not be the factor responsible for the low rate of germination at 10 °C, but at both temperatures the activity of enzymes involved with respiration, metabolism and consequently germination would be relatively low (Okusanya, 1980). High temperature of 37 °C may cause an increase in respiration leading to high energy production , more rapid ion uptake and in this way cause an apparent increase in specific ion toxicity or osmotic inhibition of enzymatic processes (Ungar, 1982). At 44 °C, the enzymes involved with respiration, metabolism and consequently germination might have been denatured.

Thus in general, the response of the species to temperature follows the usual enzymatic minimum, optimum and maximum curve indicating that the response may be controlled by enzymes.

In conclusion, the germination response of the seeds of D. aegyptium fits very well its ecological requirements and ties well with the nature of the habitat where it was collected.

CONCLUSION

It is clear from the results obtained in these investigation that *P. coarctata* is the most tolerant of salinity followed by *D. aegyptium*, the rice cultivar, KAU 2, and the least tolerant being the other rice cultivar HG 2153. The tolerance in *P. coarctata* is as a result of low accumulation of Na^+ and Cl^- in the shoot as well as the maintenance of relatively low Na : K ratio (0.97) at 30% sea water concentration compared with the other species. The reduction in growth in the two rice cultivar may be due to high osmolarity caused by high sodium, chloride and calcium contents as well as a decrease in water content in the shoot which therefore decreased the osmotic potential of the cell sap which they could not tolerate.

In *D. aegyptium*, the mechanism of salt tolerance at high salinities appears to be by internal osmotic adjustment achieved by means of sodium, calcium and chloride uptake and accumulation and water loss (Tables 3, 4, 6, and 7) which leads to the high concentration of solute in the cell sap. Sugars (Table 9) may also play a major role because the concentration increased at each harvest and at each sea water concentration. This implies that if *D. aegyptium* is grown on saline soil the accumulated sodium chloride in the shoot which is absorbed from the soil will periodically be removed when the shoot is harvested. With time, this helps to reduce considerably sodium chloride levels of soils which are unsuitable for agricultutral purposes by the high salinity. Therefore *D. aegyptium* can be used as a biological soil desalinizer.

The 75% reduction in photosynthetic carbon dioxide fixation at 50% sea water over the control must also influence the rate of growth, as there was also a simultaneous 74% reduction in dry matter production at 50% sea water over that of the control. In genera the trends observed mirror those of the effects of salinity on stomata conductance to water and carbondioxide and also transpiration where there were 73% and 64% reduction respectively as opposed to 24% reduction in photosynthesic carbon dioxide fixation in halophytes (Osmond et al.; 1980). It therefore appears that D. aegyptium does not behave like a true halophyte with respect to stomatal closure, but like an ecological halophyte, a salt tolerant glycophyte, which it is by nature. This is not suprising judging from the habitat where it was collected which had a salinity level of 30 - 50% sea water concentration.

The effect of varying nutrient level on growth of D. aegyptium at 10 and 25% sea water concentrations shows that half the levels of potassium and nitrate and twice the level of nitrate further helped to significantly stimulate growth at 10% sea water and ameliorate the effect of poor growth at 25% sea water concentration. This appears to be as a result of a combination of factors which may include better salt or ion uptake like K^+ and NO_3^-, the reduction in uptake of Na^+ and Cl^-, salt dilution through increase water uptake leading to plant succulence, increase in sugar concentration which is used as osmoticum and the provision of favourable osmotic pressure in the cell sap.

The germination experiment appears to portray D. aegyptium

as a halophyte as it has a tolerance range of 100% sea water, with 100% germination in 0-50% sea water. Recovery experiments in distilled water after pretreatment in 75 and 100% sea water also showed a 20% enhancement of germination, showing that seeds occurring at these soil salinities have a chance of survival when the soil salinities reduce as a result of rain.
However, most halophytes have 100% germination in recovery experiments which shows that D. aegyptium may not be a true halophyte.

The grass, D. aegyptium can also germinate in three soil types namely humic soil, red earth and sand which are the most common soil types in Nigeria. This might be responsible for its wide distribution in the country. In addition it shows that if humus, red earth or sand is saline the seeds can germinate well in them. The species can germinate in both light and dark indicating that the seeds can germinate in cleared and uncleared farmland and wasteland, as well as when buried in soil which must occur during tilling or cultivation or when on the soil top. The faster rate of germination in light is a reflection of its characteristic as earlier coloniser of abandoned farmland.

The good germination at the PH range of 5.0 to 7.0 and the fairly good germination at pH 3.5 may be a reflection of the species ability to colonize a wide range of habitat attesting to its wide distribution in Nigeria where the soils are mostly basic and slightly acidic. The high germination at dry and wet soil moisture is also a reflection of its wide distribution in the country from the wet south to the dry north, while the

poor germination in the waterlogged soil condition reflects its low abundance in swamps. Its optimum temperature for germination (15 - 31 °C) coincides with the mean minimum and maximum temperature in most part of the country and this also reflects its country wide distribution.

Thus the results of the germination experiments show clearly that D. aegyptium can germinate well in all parts of Nigeria with its wide edaphic and climatic variations. The above coupled with its good growth in saline environment and its salt tolerance and also the ability to absorb and accumulate salt make the species a good candidate for selection as a biological desalinizer. This suggestion can only be confirmed after field trials which are now being contemplated. If on the other hand D. aegyptium is being proposed as a fodder, it can be grown without any problem in areas with low salinity where it grows very well. If grown in highly saline areas, it could be fertilized with low or high levels of nitrate or low level of potassium. This is because under these conditions, the species accumulate less Na^+ and Cl^-, thus it reduces the uptake of these ions, high concentration of which detrimental to the growth of most species.

Although D. aegyptium is classified as a glycophyte, the Otta population used in these experiments behaved like an ecological halophyte with respect to its germination in sea water concentration and the consequent survival of its seedlings in 50% sea water. However the germination recovery experiment did not achieve 100% germination. Its ability to accumulate Na^+ and Cl^- under saline condition with the attendant halophytic mechanism

of salt tolerance probably makes this population an ecotype. This is confirmed by the results of a comparative germination studies of this population and another one from the University of Lagos (Okusanya and Sonaike unpublished) which showed that the University of Lagos population had decreased germination with increase in salinity and a tolerance range of 20% sea water as compared to 100% sea water in the Otta population.

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Appendix 1

Analysis of variance (ANOVA) of fresh weight data at the first harvest.

Source of variation	Degree of freedom	Sum of Squares	Mean squares	F - ratio
Salinity	1	8.7	8.7	1.8126
Treatment	6	346.76	57.79	6.64 ***
Interaction	6	60.07	10.01	2.067
Error	70	339.03	4.84	
Total	83	754.58	9.091	

LSD = t (1.276)

APPENDIX 2

Anova of dry weight data at the 1st harvest

Source of variation	Degree of freedom	Sum of squares	Mean squares	F - ratio
Salinity	1	0.076	0.076	0.644
Treatment	6	9.184	1.530	20.140 ***
Interaction	6	1.120	0.1866	1.573
Error	70	8.306	.118	
Total	83	18.687	0.225	

LSD = t (0.198)

Appendix 3

Anova of fresh weight data at the 2nd harvest

Source of variation	Degree of freedom	Sum of squares	Mean squares	F- ratio
Salinity	1	9.41	9.41	1.02
Treatment	6	298.75	49.792	53.97***
Interaction	6	2.45	4.08	4.43
Error	70	64.57	0.92	
Total	83	375.18	4.52	

LSD = t(0.554).

Appendix 4

Anova of dry weight data at the second harvest

Source of variation	Degree of freedom	Sum of squares	Mean squares	F- ratio
Salinity	1	5.14	5.14	1.06
Treatment	6	13.795	2.299	27.24 ***
Interaction	6	1.175	0.195	2.32
Error	70	5.90	8.44	
Total	83	26.01	0.313	

LSD = t (0.1677).