SALT TOLERANCE OF PIGEONPEA (*Cajanus cajan*) GENOTYPES ITS RHIZOBIA AND SYMBIOTIC NITROGEN FIXATION

SALT TOLERANCE OF PIGEONPEA (*Cajanus cajan*) GENOTYPES ITS RHIZOBIA AND SYMBIOTIC NITROGEN FIXATION

THESIS SUBMITTED IN PARTIAL FULFILMENT
OF THE REQUIREMENTS FOR THE DEGREE
OF

MASTER OF TECHNOLOGY
IN
APPLIED BOTANY

BY
G. V. SUBBARAO

UNDER THE GUIDANCE OF
Prof. M. K. Jana
Dr. J. V. D K. Kumar Rao

APPROVED :

(Dr. M. K. Jana)
Project-in-charge

(Dr. D. N. De)
Head of the Department
Agricultural Engineering Department
Indian Institute of Technology
Kharagpur-721302

DEPARTMENT OF AGRICULTURAL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY
KHARAGPUR
DECEMBER, 1984

ACKNOWLEDGEMENTS

I wish to record my deep sense of gratitude to my guide Dr. M. K. Jana, Professor in the Department of Agricultural Engineering, I.I.T., Kharagpur, for suggesting the problem, supervision throughout the work and unstinted help in the preparation of the thesis. I am also most grateful to my Co-guide Dr. J. V. D. K. Kumar Rao, Pulse Microbiologist at ICRISAT for providing extensive experimental facilities, help and valuable comments towards the preparation of the manuscript.

I am very grateful to Prof. D. N. De. Head of the Agricultural Engineering Department for providing facilities and encouragement in this research. I am also grateful to all my teachers and colleagues in Applied Botany.

The laboratory and other facilities provided by ICRISAT, Patancheru, where part of the experimental work was done, is gratefully acknowledged. I am particularly indebted to Dr. D.L. Oswalt, Principal Training Officer and Dr. C. Johanson, Principal Pulse Agronomist at ICRISAT for their interest and valuable comments on the work. I am particularly thankful to Dr. Murari Singh, Statistician, ICRISAT for guidance in data analysis.

I wish to express my sincere thanks to Dr. C. P. Rupela and Dr. Y. S. Chauhan for their suggestion during experimentation and to Dr. A. S. Murthy, Senior Training Officer, ICRISAT for his encouragement.

I wish to express my heartfelt thanks to Mr. B. V. S. T. Sai Mrs. M. Usha Kiran, Mr. M. R. Sudarsan, Mr. R. Gururaj and all others in Pulse Agronomy subprogramme at ICRISAT for their cooperation in several ways whenever sought.

V. Subba

(G. V. Subba Rao)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

One of the most likely possibilities for increasing world food production is the expansion of agriculture into marginal lands. A large portion of these lands is in arid and semi-arid regions, and soils and waters in these areas are frequently saline. Legumes are usually found to be not very salt tolerant, but their potential has not been fully exploited. For legumes one must take account of the plant as well as its symbiosis with Rhizobium. Pigeonpea is an extensively cultivated leguminous crop in the semi-arid regions where the salinity problem is becoming severe.

In making these salt affected lands productive, an important approach may be to modify the crops genetically to adapt them to saline environment. It is known that there is no biological incompatibility between plant life and even highly saline conditions, as evidenced by halophytes. There is much genetic diversity in crop species in respect to many traits including salt tolerance and it has been already demonstrated that it is possible to transfer a trait like salt tolerance from a wild salt tolerant species into related crop species (Rush and Epstein, 1976).

Screening for genetic diversity for salt tolerance has been attempted in crops like rice (Akbar and Yabuns, 1975), wheat (Shannon, 1979), barley (Epstein, 1977; Jana et al.,1979), and lentil (Jana and Slinkard, 1979). The lack of variation for salt tolerance in tomato was overcome by making wide

crosses with the exotic tomato (Lycopersicon cheesmanii) collected from the Galapagos Islands (Rush and Epstein, 1976).

Legumes are usually found to be not very salt tolerant but their potential has not been fully exploited. Legumes present additional challenges in finding salt tolerance, as compared with cereals or other non legumes. For legumes, one must take account of the plant as well as its symbiosis with Rhizobium. Although most legumes appear to be salt sensitive, certain legumes such as Prosopis, Acacia can show extreme tolerance to salinity. Prosopis tamarugo can even fix nitrogen symbiotically in sea water which has a salt concentration of 3.5%. Thus previous generalizations on susceptibility of legumes to salt (Bernnstein, 1964) should no longer be made. It is well known that the Rhizobium can tolerate high level of salinity than its host. Among cultivated legumes there is considerable genetic diversity for salt tolerance (Bernstein and Ogata, 1966).

Pigeonpea (Cajanus cajan L. Millsp.) was chosen for the present study mainly because it is one of the pulse crops which is extensively cultivated in semi-arid regions, where salinity problems tend to be more acute. In semi-arid regions, salts move from deeper soil layers to the soil surface due to total evaporation exceeding total rainfall. As a deep rooted crop pigeonpea roots may be able to penetrate to deeper layers of soil where salt stress is relatively less than near the surface.

An extensive world collection of germplasm lines of pigeonpea is maintained in the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). In addition to this ICRISAT has a large collection (400) of pigeonpea-positive rhizobial strains from different parts of the world. These sources may have genetic diversity for salt tolerance as they do for many other characters. This possibility has not been well explored so far.

Lastly, it should be pointed out that eventhough the symbiosis is susceptible to salt stress, in most cases it depends mainly on the host's ability to provide a congenial microenvironment to it's microsymbiotic partner, and the microsymbiont's symbiotic ability under saline stress conditions. If we can find tolerant pigeonpea lines as well as Rhizobium strain which can perform well under salt stress, operation of the symbiosis may not be a limiting factor in saline substrates as may be in the case of Prosopis tamarugo.

Little information is available regarding genotypic variability for salt tolerance in pigeonpea, it's microsymbiont (Rhizobium) and symbiotic behaviour under salt stress conditions. The information is important for starting any pigeonpea breeding programme for salt tolerance. The thesis presented here is an attempt in this direction and the present investigation was undertaken to develop a technique to screen large numbers of pigeonpea germplasm and to determine the amount of genetic diversity for salt tolerance. Pigeonpea-positive rhizobial

strains of diverse origin were also examined for tolerance to salinity in nutrient media. The symbiotic performance of pigeonpea genotypes inoculated with different Rhizobium strains (both legume host and Rhizobium varying in their tolerance) was determined to decided even if both of the symbiotic partners are tolerant the symbiosis itself may be susceptible.

2.LITERATURE REVIEW

2.1. GENERAL RESPONSE OF PLANT TO SALT STRESS

Salts mainly effect plant metabolism in two ways. One is by creating an osmotic pressure and thereby reducing the physiological availability of water though physically available. The second by specific ionic toxicity. The toxicity of salts is directly correlated to their permeability; the more rapidly salts penetrate and accumulate, the more toxic are they to the plants (Neger 1913). According to Arnold (1955) the effect of salts on the plant is determined by the ratio of the adsorbed to the free ions, an increase in the free ions, even of nitrates or sulphates, has an adverse effect on the plant and according to him only the amount of free ions, and not their physical properties determines the condition of the plants in a saline environment.

Salinity induces changes in the anatomy of plants (Batalin, 1875; Lesage 1925; Chermazon, 1910). Most of them take the view that salinity induces features typical of succulence ie. the leaves are thickened, the size of epidermal cells increases, the number of stomata per unit area in the leaf decreases, the palisade and spongy mesophyll layers of the leaf develop extensively, while the conductive layer is poorly developed and differentiated. The tendency to develop succulence is an adaptive response of the plant to salinity and it is accepted. As a result, quite often the degree of succulence is associated with the degree of salt tolerance of the same plant.

However, a number of findings show that salinity induces xeromorphic features or more accurately haloxerism ie.,'together with a thickening of the leaf, the decrease in the size of the epidermal cells, the number of stomata per unit area increases, the conductive system is well developed and differentiated etc (Strogonov and Muradona, 1959).

The type of substrate determines the rate of water exchange of plants; plants from sulphate type of salinity absorb water from the soil and expend it intensively, whereas chloride type of salinity decrease the rate of transpiration and an increase in the volume of the cells, which apparently begin to function as water storage organs due to the penetration of chlorides in to the plant parts (Strogonov, 1953).

2.1.1. SALT TOXICITY AND ADAPTATION OF PLANTS TO SALINITY

Under conditions of strong salinity, salt poisoning is often observed. The first signs of salt poisoning in some plants takes the form of bleaching of chlorophyll while in others browning of isolated parts of the leaves occurs. Bleaching of chlorophyll is accompanied by a decrease in the strength of the bond between the greeen pigment and the protein of the chloroplast. This condition of the necrobiosis is reversible. Under favourable conditions those parts of the leaves which previously became yellow, again became green.(Strogoner and Ivanilskaya 1954, a)

The substances found in the necrotic areas in plants, under conditions of chloride or sulphate salinity, differ in their chemical properties and their distribution. The cells in the state of necrobiosis act as centers for the accumulation of toxic organic substances. These substances, while being translocated through normal cells, poison them and thereby cause a progressive necrobiosis and necrosis in isolated parts of the organ (Strogoner et al., 1961).

Plants under saline conditions, changes in nitrogen metabolism, were accompanied by the accumulation of ammonia, amines, diamines(putrescene, cadaverine), aminoacids (hydroxyproline, proline, l.leucine, isoleucine, d.alanine, phenylalalanine, and tyrosine) will have an adverse affect on the physiological processes in the plant. Accumulation of certain aminoacids as arginine and lysine may occur which serve as precursors for the formation of toxic diamines like putrescine (Strogonov, 1940). The increase in content of the amides glutamine and asparagine, in some organs of plants under saline conditions, can be considered as a protective adaptive response of the plant, binding of ammonia inorder to reduce its concentration and it seems that in some plants the dicarboxylic aminoacids serve as acceptors of ammonia, and in this way neutralizing its toxic effect in the cell (Strogonov, 1958).

2.1.2. MECHANISM OF SALT TOLERANCE

High ion uptake is the principal for halophytic adaptation (Flowers, 1977). These halophytes generate turgor by high internal Na and Cl concentrations. Additional adaptive features which contribute to the avoidence of high ion concentrations in the leaves of some species include salt glands and bladders and increase in leaf volume associated with succulence. The latter is often found in dicotyledons, even in the most salt sensitive non-halophytes.

In case of low ion uptake, the possible adaptation involves the use of organic solutes for example photosynthates for osmotic adaptation. For example the amount of hexose needed to balance an increase of 100mM NaCl would be 20-30% of the total dry weight for highly vacuolated cells compared with about 30% for cells without vacuoles (Greenway, 1973). However, the requirement for solutes would be less if there were structural modifications such as increases in wall extensibility, permeability of the roots to water, or leaf thickness (Greenway and Munns, 1980).

In several species the salt sensitivity of certain varieties is due to the absorption of relatively large amounts of Cl and Na ie. these varieties suffer from "Ion excess" in their expanded leaves. "Ionexcess"can be defined as a condition where high internal ion concentration reduce

growth and the sensitivity in these varieties to ion excess is mainly due to the inadequate cellular compartmentation of ions in the leaves(Greenway and Munns, 1980).

Salt sensitivity of some non-halophytes may be due to insufficient uptake of electrolytes for osmotic pressure or volume maintenance, particularly in the expanding tissues. Yet a mere increase in rate of uptake would not remedy the situation, because several salt sensitive species have a high rate of uptake to the shoots. The key is a synchronization of ion compartmentation by the leaf cells with a high rate of ion transport to the shoot and there is a general assumption that a number of species contain genes for efficient ion compartmentation (accumulation in the vacuole)(Greenway and Munns, 1980).

2.2 SALT TOLERANCE IN RHIZOBIAL STRAINS

Rhizobia are considered to be more tolerant than their host legumes to salinity. Salts of sodium, calcium are known to be toxic to **Rhizobium** at high concentrations (Vincent, 1977). However, there are differences among species and strains of **Rhizobium** with respect to their tolerance to different salts. Berseem strains were inhibited from 0.2% to 0.4% of chlorides and sulphates of sodium and potassium whereas dhaincha strains were tolerant upto 1.8%, and gram, groundnut, cowpea, and guar Rhizobia were found to be stable even at 3% salt level in the growth medium (Yadav and Vyas, 1971).

The resistance of Rhizobia to salts is dependent on the type of salt. Berseem isolates were tolerant to sodium chloride, but susceptible to potassium chloride and potassium sulphate and sodium sulphate; lucerne and dhaincha isolates were tolerant to chlorides and sulphates of sodium (Ethiraj **et al.**, 1972). Magnesium salts stimulated growth at lower concentrations (<1% MgCl2) in **R.trifolii** whereas cowpea, gram, groundnut and guar strains were neither stimulated nor affected (Yadav and vyas, 1971; Ethiraj **et al.**, 1972). The growth rate of Rhizobia isolated from berseem, cowpea, gram, was lower at higher (>1%) sodium chloride concentrations (Gandhi and Vyas,

1969). In **R.trifolii** there was a progressive decrease of growth with increasing salinity of the medium (Pillai and Sen, 1966). In fast growing Rhizobia the polysaccharide gum formation increased with increasing salinity (NaCl 0-1%) and there was a variation in the capacity to form gum among strains in presence of equal amounts of salts; and the production of gum by a strain may be a measure of protection against excess salinity (Pillai and Sen, 1969).

Bharadwaj (1972) reported that the inoculant strains should be isolated from the problem soils because the Rhizobia from normal soils could not do well in problem soils. But in 1975 he reported that he did not find any differences between native (collected from saline soils) and exotic strains (collected from normal soils) in terms of their growth as well as their symbiotic efficiency.

Steinborne and Roughley (1975) have reported a reduction in growth rates of **R.trifolii** and **R.meliloti** in the presence of salt. Carr and Ballard (1979) found that a strain of **R.trifolii** was able to withstand a short exposure to fertilizer solutions with ECs in excess of 60mmhos/cm. Lauter **et al.,** (1981) reported that the Rhizobial growth rates were unaffected by sodium chloride at 120mM and only moderately depressed by 250mM. Singleton **et al.,** (1982) examined the effect of salinity on the growth and survival of **Rhizobium** sp. in culture media and soil and reported that the growth of all strains and species tested decreased when the electrical conductivity of the culture medium was raised from 1.2mmhos/cm to 6.7mmhos/cm or 13.1mmhos/cm. They further pointed that many strains of **Rhizobium** could grow and survive at salt concentrations which are inhibitory to most agricultural legumes.

"nif" genes are thought to be associated with plasmids (Dennican, 1971). It is not known whether Rhizobial salt resistence is plasmid associated or not. But recent reports suggest that in case of lentil isolates salt resistant strains were shown more resistence to antibiotics also (Rai, 1983). It is well known that in general antibiotic resistance is associated with plasmid . So it seems that there is a possibility of salt tolerance assoication with the plasmid and if it is so, it may be a serious barrier to improvement of the efficiency of symbiotic nitrogen fixation in areas of saline soils by

adapting salt resistant mutants of effective Rhizobium.

2.3. SALT TOLERANCE IN LEGUMES

Eventhough legumes are considered to be sensitive to NaCl there is a large variation among genera and species (Bernstein, 1964). **Lupinus luteus** can tolerate upto 100mM NaCl and there is an increase in the fresh and dry weights of the foliage from 50mM to <100mM NaCl. So it can be considered as salt resistant. Peanut (**Arachis hypogaea**), chickpea (**Cicer arietinum**), soybean (**Glycine max**) cv. 'Jackson', beans (**Phaseolus sp.**) and pea (**Pisum sativum**) are most sensitive to salinity. While **Lupinus angustifolius**, the clovers (**Trifolium alexandrinum**), soybean cv.'Lee', alfalfa and **Phaseolus coccineus** are salt tolerant (Lauchli, 1984). But Shelvel **et al.**, (1969), noticed that in peanuts (**Arachis hypogaea**) salt tolerance was more during germination than subsequent growth and he observed 50% reduction in germination at 13mmhos/cm.Ece., and seedling development at 7.2 mmhos/cm.Ece. He reported that the yield was reduced to 50% at Ece., 4.7 mmhos/cm and 20% at 3.7mmhos/cm. Greenbeans tend to die at 8mmhos/cm.Ece (Bernstein and Ayers, 1951 cited from Jana and Slinkard, 1979.). Broad beans (**Vicia faba**) was not seriously affected at 8mmhos/cm E.ce., (Ayers and Edward, 1960 cited from Jana and Slinkard.). In cowpea and mungbean (**Vigna aureus**) NaCl retarded growth .Root growth of mungbeans seems to be more sensitive than that of cowpea (Balasubramanian and Sinha, 1976).

In soybean significant varietal differences to salt stress were noticed and in this case there was no apparent relation between the salt tolerance during germination and later growth phases (Abel and Mackenzie, 1964). Salt tolerant varieties control the chloride accumulation in shoot, whereas the susceptable ones accumulate large quantities in their shoots. Salinity increased the root phosphorus content in **Glycine** (Cates, 1970);there was an opinion that phosphorus may be associated with mechanisms for controlling the salt entering the roots and preventing it, especially the sodium, from passing to the shoot. Abel(1969) found that in **Glycine** the translocation of chlorides to plant tops is genetically controlled. The gene symbols "NCl" and "ncl" were proposed as the dominant for chloride excluders and the recessive for chloride includers respectively. He reported that the chloride includers develop severe leaf necrosis from chloride toxicity, whereas the chloride excluders did not develop necrosis.

In lentil considerable genetic variability for salt tolerance was noticed (Jana, 1979). It was further demonstrated that the critical stages of salt stress in lentil are germination and initial seedling growth, and the seed yield of the salt tolerant lines decline beyond 6-8mmhos. He found that salt stress had relatively less effect after flowering and the response of salinity greatly differed with the type of salt tested (MgsO4, NaCl, Na2SO4, MgCl2) at equal conductivity levels. Lentil responds to

specific ion toxicity. Germination and growth were most severely inhibited by MgSO4, followed by MgCl2. Jana (1979) concluded that it may be possible to select and grow suitable cultivars of lentil in marginal or moderately saline soils.

In certain varieties of Alfalfa (**Medicago sativa**) tested the average yield was reduced to 79% of the control at 3000 ppm salt (NaCl:CaCl2) level, 60% at 6000 ppm level and 42% at 9000ppm level(Brown and Hayward, 1956). Based on callus cultures Smith(1981) reported that certain varieties of **M.sativa** as salt sensitive. **T.fragiferum** was considered a moderately salt resistant one. West **etal.,** (1981)found significant cultivar differences in salt tolerance in **T.subterraneum,** and poor coorrelation between salt tolerance at germination and later stages of growth. Russel(1980) tested the response of a number of tropical and temperate legumes to salinity and found **Medicago sativa** as the most tolerant legume. Among tropical legumes **Macroptilium** lathyroides and Macroptilium atropurpureum were almost equivalent to **M.sativa** in their tolerance. **Desmodium uncinatum** and **Trifolium semipilosum** are considered to be least tolerant.

In cowpea (**Vigna sinensis**) Paliwal and Maliwal (1973) found significant varietal differences to salt tolerance during germination and early growth stages. In case of pea, Cerda (1982) reported that a cultivar 'Durana' was a moderately tolerant to salt stress and Sp-290 was a

moderately sensitive one. He reported that the Ece. values for the Sp-290 and 'Durana' cultivars were respectively 2.5 and 4.,5ds/m.

In pigeonpea (**Cajanus indicus**) Paliwal and Maliwal (1973) noticed significant varietal differences to salt stress during germination and early stages of growth. Based on field screening Rao **et al.,** (1981) reported that pigeonpea genotypes ICP 7623, ICP 7118, ICP 7182, ICP 7035, ST 1, and **Atylosia scaraboides** showed better survival than the tolerant standard variety C 11 under salt stress conditions. Gururajarao **et al.,** (1981) reported that germination and seedling growth of ICP 7035 and ICP 7065 showed a high degree of tolerance to 0.4% (NaCl+CaCl2). In pigeonpea, NaCl induces succulence and other anatomical changes by increasing the palisade and sponge parenchyma tissues. It was also associated with reduced dimensions of the vessel lumen and increased thickening of the vessel wall, presence of thick cuticle and accumulation of leaf epicuticular wax: (Rao and Rao, 1982).

Salt stress lowered the leaf area (Rao and Rao, 1981) reduced the stomatal frequency, reduced stomatal opening, deranged pigment composition and lowered the activity of Ru-Dp carboxylase leading to a reduction in photosynthesis. Deshpande and Nimbalkar (1982) reported that under salt stress conditions the rate of translocation of photosynthates from the leaves to the other plant parts was affected. Lauter **et al.,** (1981) reported that in chickpea,

1550 is tolerant upto 50mM NaCl.

.4. SYMBIOTIC NITROGEN FIXATION UNDER SALT STRESS CONDITIONS

Salt stress may differentially affect each phase of the legume-**Rhizobium** symbiosis: a. Rhizobial survival and growth in the rhizosphere of the host, b.Rhizobial infection of the host root hair, c. nodule initiation and development, d. nodule functioning (nitrogen fixation) and e. growth of the host legume in general. Distinguishing which phase is primarily affected may not be easy due to the close interdependency of these phases.

The symbiotic susceptibility to salt stres is not a generalised phenomenon, it varies from host to host. In **Prosopis tamarugo** the symbiosis was not affected even at 3.6% NaCl level. Singh **et al.,** (1972) reported that failing of the initiation of nodules in lucerne was mainly due to the abortion of the infection threads under salt stress conditions. But Lakshmi kumari **et al.,** (1974) found that salt stress suppressed the root hairs and the mucilaginous layer, leading to the elimination of the rhizosphere and infection thread formation resulting in reduced number of nodules. Tu (1981)attributed the failure of soybean to nodulate at high salinity to decreased Rhizobial colonization, and shrinkage of root hairs. Singleton and Bohlool (1984) reported that in soybean the early processes involved in nodule initiation were extremely sensitive to even low concentrations of NaCl than nodule function and development and probably due to the salt sensitivity of root infection sites.

However, the response to salt stress on nodulation, nitrogen fixation and growth differs with legume species. Nodulation of alfalfa was relatively resistant to salinity (NaCl), whereas nodulation of soybean was severely affected by salinity (Bernstein and Ogata, 1966). Differences were also found between cowpea and mungbean with respect to nodulation and nitrogen fixation, (Balasubramanian and Sinha, 1976) as mungbean was more sensitive than cowpea. In case of berseem (**Trifolium alexandrinum**) salinity (NaCl) did not affect the nodulation and the yield of plants increased with salinity up to 0.5% NaCl. In**Vicia faba** though salinity (NaCl) suppressed the nodule number, the nodule size was increased(Yousef and Sprent, 1983).

Several studies have emphasized that the main effect of salinity on nitrogen fixation resulted from salt injury to the host. Nodules themselves effectively excluded Na and Cl (Wilson, 1970). In soybean the reduced nitrogen fixation under salt stress conditions was mainly due to reduction in photosynthesis (Singleton and Bohlool, 1985).

The symbiotic susceptibility to salt stress also varies between salts. In case of lucerne 0.7% NaCl totally supressed the nodule formation ie. plants were totally devoid of nodules, In case of KCl and MgCl2 (Singh **et al.**, 1973) successful nodulation or symbiosis was possible up to 1% salt. Eventhough lucerne could tolerate upto 3% NaCl, nodulation was affected from 0.4% NaCl onwards with a maximum affect at 0.7%. This resulted in total suppression

of the nodules. Thus it appears that the degree of salinity conducive for good nodulation is definitely different from the limits of tolerance of **Rhizobium** and the host respectively.

In several genotypes of **Vigna radiata** inoculated with **Rhizobium**, nodulation was not affected at salinity levels which are otherwise critical for the plant growth (Rai and Prasad, 1984). The symbiotic behaviour of a native Rhizobial strain (collected from the saline soil) need not be superior than an exotic strain (collected from the normal soil)(Bharadwaj, 1975).

So the symbiotic susceptibility to salt stress is not a generalised phenomenon and it may vary from host to host. In case of lentil under salt stress, significant interactions between Rhizobial strains and genotypes resulted in a differential response of nitrogen fixation (Rai, 1983).

3. MATERIALS AND METHODS

3.1. SCREENING RHIZOBIA FOR SALT TOLERANCE

14 **Rhizobium** isolates able to nodulate pigeonpea were used for this study. The origin and type of growth on yeast extract mannitol (YEM) agar plates are given in Table 1. The cultures were obtained from pigeonpea **Rhizobium** culture collection of Pulse Microbiology, ICRISAT, Patancheru, A.P. 502324, India. They are all effective in fixing nitrogen with pigeonpea and represent diverse locations and soil types (normal and saline). All cultures were maintained on yeast extract mannitol agar slopes (Vincent, 1970). The composition of YEM (g/liter): mannitol 10.0, K2HPO4 0.5; MgSO4 7H2O 0.2; NaCl 0.1; Yeastextract 0.5; agar, 15; distilled water 1000ml, PH 6.8; Congored at the rate of 10ml of 1/400 aquous solution per liter of yeat extract mannitol agar medium was used.

Yeast extract mannitol agar medium with different NaCl levels, namely 0%, 0.25%, 0.5%, 1%, 2%, 3%, 4%, 5% was prepared and autoclaved at 15 lbs/sq.inch pressure for 20 minutes. After sterilization, the YMA medium amended with NaCl was poured into petri plates at the rate of 20 ml/plate and allowed to solidify. After solidification a loopful of young growing culture taken from the growth on YMA slopes was streaked and incubated at 27C. Three replicate plates were used for each treatment per **Rhizobium** culture. Observations on growth and colony size were recorded after 3 days for fast growing cultures and after 7 days for slow growing cultures. For recording colony size, well isolated

Table:1. Origin and growth characteristics of Rhizobium cultures used for salt tolerance study

Sl. No.	Rhizobium	Legume host	Soil type	Growth on YEM agar plates	Source
1	IHP 24	Pigeonpea	Black soil	F b	ICRISAT, Hyderabad
2	IHP 506	Pigeonpea	Saline	F	ICRISAT, Hyderabad
3	IHP 100	Pigeonpea	Saline	F	ICRISAT. Hyderabad
4	IHP 70	Sesbania	Saline	F	ICRISAT, Hyderabad
5	BDN-92	Pigeonpea	a	F	Pulse Research Station, Badnapur
6	IHP 434	Pigeonpea	Black soil	S	ICRISAT, Hyderabad
7	IHP 87	Pigeonpea	Black soil, Saline	S	ICRISAT, Hyderabad
8	IHP 213	Pigeonpea	Red soil	M	ICRISAT, Hyderabad
9	CC 1	Pigeonpea	-	S	TNAU,Coimbatore
10	IHP 69	Indigofera	Saline soil	S	ICRISAT, Hyderabad
11	F4	Pigeonpea	-	S	IARI, New Delhi
12	IHP 35	Pigeonpea	Black soil	S	ICRISAT, Hyderabad
13	KA1	Pigeonpea	-	S	Agricultural College,Kanpur
14	IHP 195	Pigeonpea	Red soil	S	ICRISATA, Hyderabad

a = Not known; b - F, fast grower; M = Medium grower; S = Slow grower

colonies were used.

3.2. Screening pigeonpea genotypes for salt tolerance

29 pigeonpea genotypes were used for the present study. These are breeders promising lines and presently in multilocational field tests under the All India Coordinated Pulse Improvement Project. They represent early, medium and late maturity groups. The details of pedigree, origin and maturity group are given in Table 2.

Pigeonpea seeds were surface sterilised with 0.2% HgCl2 solution for 5 minutes, then washed with sterile and deionised water ten times. The growth pouches manufactured by Scientific Products, 1210 Leon Place, Evanston, Illinois, USA, were used in this experiment. They were sterilised as per the instructions of the manufacturer before use. The growth pouches supplied with 20ml of Arnon's nutrient solution ammended with NaCl at 0mM, 30mM, 60mM, 90mM, 120mM concentrations were arranged in growth pouch racks (Fig.1). The composition of Arnon's nutrient solution is given in Table.3. Seeds were placed in the cleft of growth pouch, ten per pouch.

The experiment was laid as split plot with salt level as main treatment and genotype as subtreatment replicated three times. The nutrient solution containing 20ppm nitrogen as ammonium nitrate was sterilised and added by an automatic syringe as and when required. The racks with growth pouches were incubated at room temperature (27C) for 7 days after which they were transferred and kept in a glass

le:2 Pedigree, naturity and origin of pigeonpea genotypes* used for screening for salt tolerance

Genotype	Pedigree	Maturity	Origin
ICPL-332	ICP-1993-E1-4E8	Medium	Field collection,A.P.
ICPL-310	ICP-7198-W30-W130-W10-W20-888-G9	Late	Kanpur,U.P.
ICPL-311	ICP-74428-W130-1-62-88-88	Late	ICRISAT
ICPL-358	ICP-74367-W90-1-63-8E0-62	Late	ICRISAT
ICPL-366	ICP-7105-12-22-2-2-830-880-8E8	Late	Burhanpur,M.P.
ICPL-42	ICP-155-9	Medium	Field collection,A.P.
ICPL-43	ICP-2223-1	"	" "
ICPL-227	ICP-1-6	"	ICRISAT(Sarada collection
ICPL-230	ICP-7855-360-30-E0	"	IARI,New Delhi
ICPL-236	ICP-102-120-10-10-190-80	"	Mysore
Bahar		Late	Dholi(Bihar)
ICPL-362	ICP-4234-360-70-20-50-40-35	Late	U.P.Field collection
ICPL-211	ICPX-77047-27-1-2-2-1-WI MDT2-1-P	Medium	ICRISAT
ICP-7035		Medium	Pedagat,M.P.
T-15-15		Medium	U.P,Collection for Badnapu
ICPL-295	ICP-7118-W25-W130-W10-W50-50	"	Maharashtra
HY-3C		Medium	Hyderabad,A.P.
LRG-36		Medium	Guntur,A.P.
C-11		Medium	Maharashtra
D 1	ICPX-73031-W1	Medium	ICRISAT
ICP1-1	ICP-6971-83-3-5-3-8-8-80	Early	U.P.
ICPL-304	ICPX-75033-52-WI MDT23-2-8-8	Medium	ICRISAT
ICPL-2	ICP-6971-83-3-5-9-8-8-80	Early	U.P.
T 7		Late	Lucknow,U.P.
ICPL-265	ICP-8518-860-70-88	Medium	A.P.
ICPH-2	MS-4A x BDN-1		ICRISAT
ICPH-6	MS-3A x AS-71-37		ICRISAT
ICPH-7	MS-3A x ICPL-227		ICRISAT
ICPL-87	ICPX-73052-211-1-1-WIDT2-8-80	Early	ICRISAT

ource is ICRISAT Pigeonpea Breeder

1.5: EFFECT OF SALT (NaCl) STRESS ON SHOOT DRY MATTER AMONG PIGEONPEA GENOTYPES (21 DAYS AFTER SOWING)

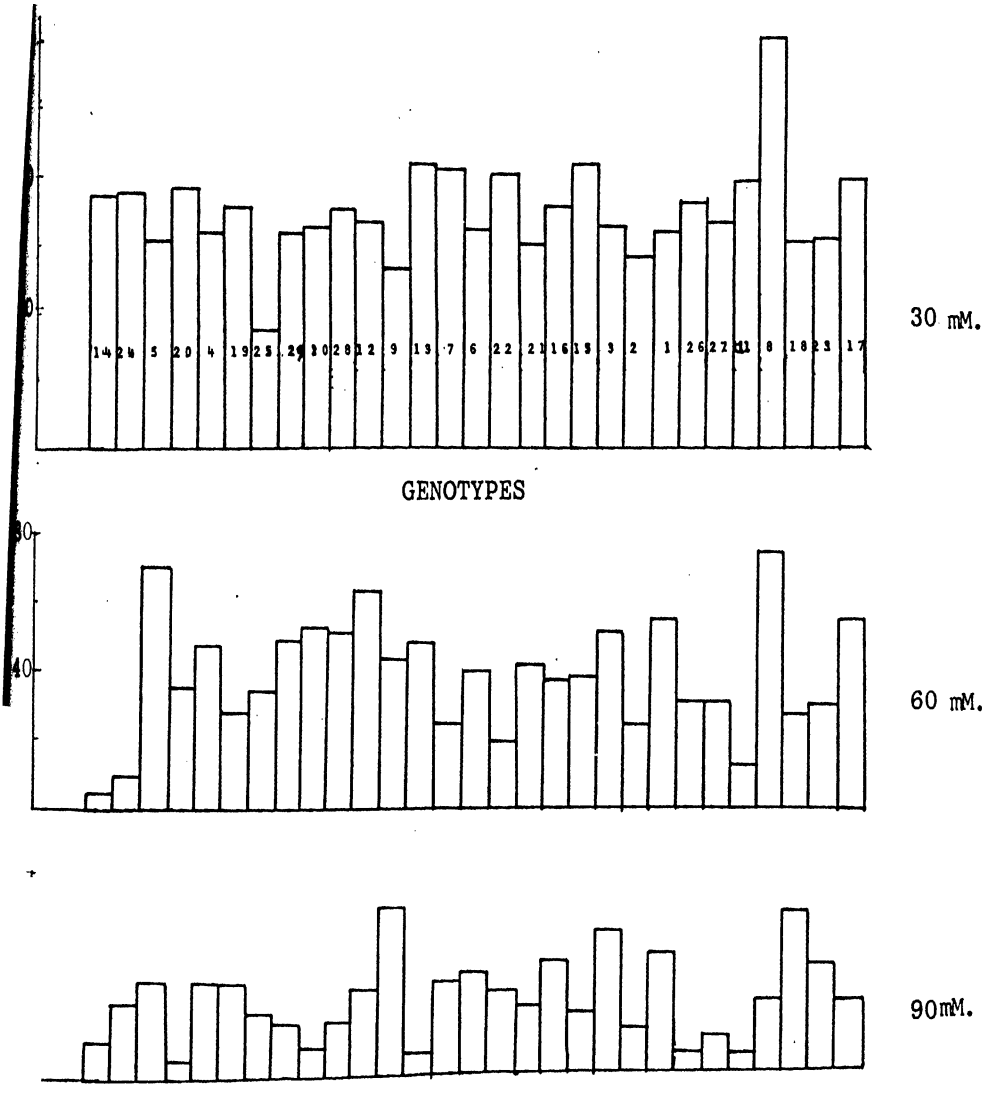


Fig. 1. Pigeonpea plants in growth poach containing nutrient solution.

Composition of Arnon's nutrient solution for pot experiments in Pulse Microbiology.

(This replaces Reading's nutrient solution w.e.f. 15-5-1981)

	Compound	mg/l	for stock solution g/l	
1.	KH_2PO_4	122	12.2	100 times
	KCl	155	15.5	
	$MgSO_4 7H_2O$	250	25.0	
2.	$CaCl_2\ 2H_2O$ or	215	21.5	100 times
	$(CaSO_4 2H_2O)$	(250)	(25.0)	
3.	$MnSO_4 H_2O$	1	1.0	1000 times
	$ZnSO_4 7H_2O$	0.25	0.25	
	$CuSO_4 5H_2O$	0.25	0.25	
	H_3BO_3	0.25	0.25	
	$Na_2MoO_4 2H_2O$	0.05	0.05	
4.	$FeC_6H_5O_7 5H_2O$ (Ferric Citrate) or	30	30	1000 times
	$(FeCl_3)$ Ferric Chloride	(15)	(15)	
	Na Fe L D T A	5 ?	5 ?	1000 t...

To make 1 litre of nutrient solution, take stock solution No.1, 10 ml, No.2, 10 ml, No.3, 1 ml, No.4, 1 ml and add to 1000 ml of deionised water.

Adjust pH of the nutrient solution to 6.5 with either 1 N NaOH or 1 N Hcl.

Source:

Arnon, D.I. 1938. Micro elements in culture solution experiment with higher plants. Amer. J. Bot. 25:322-325.

house for 14 days where the day and night temperatures were around 29C and 23C respectively.

On 3rd and 5th day after sowing, the number of seeds germinated was counted. On 7th day the growth pouches were wraped with a thick paper to prevent light falling on to the roots. On 11th day the total number of seedlings established well in each pouch were counted and the plants were thinned to leave 4 per pouch, which represented the majority of the seedlings in the respective pouch.

Three weeks after sowing the plants were harvested, separated into root and shoot and kept for drying at 70C for two days. The dry weight of shoot and roots were recorded. Since the samples were too many to handle on a single day, they were harvested replication wise; first replication on 21st day, second replication on 22nd day, third replication on 23rd day. All the results were statistically analysed.

For the germination and establishment observations the results were subjected to Angular transformations before analysis to equalise the variance. The salt effect on shoot and root dry matter of pigeonpea genotypes was evaluated by comparison (ratio) with the respective control ie., 0mM NaCl treatment.

3.3. POT TRIAL ON THE EFFECT OF SALT(NaCl) STRESS ON GROWTH, NODULATION, NITROGEN FIXATION AND PHOSPHOROUS UPTAKE BY PIGEONPEA

Four pigeonpea genotypes - ICPL 358, ICPL 332, C 11, ICPL 227 which varied in their response to salinity in growth pouches, were used for this study.

Two pigeonpea **Rhizobium** strains, namely, IHP 100 and IHP 195 both effective with pigeonpea but different in growth characters were used.

Six salinity levels (0mM, 15mM, 30mM, 45mM, 60mM, 75mM of NaCl) were tested in the present experiment.

Seven inches diameter polypropylene pots washed and steamed for 1hr were used. The culture medium consisted of sand:vermiculite:grit mixture (SVG) in the ratio of 1:2:2 (Volume basis). Sand, vermiculite and grit were washed several times in running tap water to remove the dirt and fine particles and air dried before mixing. The SVG medium was sterilized by autoclaving at 15lb/sq in. pressure for 1 hour. After cooling, the SVG medium was filled in the pots at the rate of 2.5 Kg per pot.

Pigeonpea seeds were surface sterilized with 0.2% $HgCl_2$ acqueous solution for 5 minutes, washed in several changes (at least 10) of sterile and deionised water. The seeds were then inoculated with a slurry of peat cultures of IHP 100 and IHP 195 separately using methyl ethyl cellulose as

an adhesive. Peat inoculants had a **Rhizobium** population of about 10^9 cells/g. of inoculant. The treated seed carried about 10^5 Rhizobia/seed and was sown in pots at a constant depth of 2cm at the rate of 7 seeds /pot.

The design of the experiment was a split-split plot with salt level as main plot, pigeonpea genotype as subplot and **Rhizobium** strain as a sub-sub-plot and replicated three times. The experiment was conducted in a temperature controlled glass house where the day and night temperatures ranged 27-30C and 20-23C respectively. On 14th day after sowing the seedlings thinned were to leave 4 per pot.

Arnon's nitrogen free nutrient solution prepared with deionised water and amended with different levels of NaCl as indicated above was used for watering the plants upto 24th day after sowing. The pots were maintained at 70 percent waterholding capacity of the growth medium. The pots were flushed through with once a week with the respective treatment nutrient solution to prevent salt accumulation. On 25th day, the plants growing particularly at salt levels 30mM Nacl and above looked sick probably because of salt toxicity. Hence, the pots were flushed through deionised water for a week. After this, half strength Arnon's nitrogen free nutrient solution without NaCl was used till 45th day.

At harvest, 46th day after sowing, dead plants in each pot were counted . Healthy plants height measured. Plant shoot was cut with a secature and leafarea was measured with the help of an automatic leafarea meter Model no. Ll3100 (made by LICOR, USA).

The nodulated roots were carefully removed from the pots and assayed for nitrogenase activity by acetylene reduction technique (Dart **et al.,** 1972). The excised roots and nodules were placed in a glass container of 300ml volume and with a rubber septum fitted in the lid. After a 30 min incubation in a 10% atmosphere of C2H2 at ambient air temperature in the glasshouse, a 5.0ml gas sample was removed and stored in pre-evacuated 10ml Venoject tubes (made by Terumo corporation, Tokyo, Japan). The sample was analysed for ethylene (C2H4) on a Pye Unican 104 gas chromatograph fitted with a flame ionization detector and a glass column 150cm long and 0.6cm O.D., packed with Porapak N. The oven temperature of the gas chromatograph was 100C and the carrier gas (N2) flow rate 45 ml/min.

After the acetylene reduction assay, roots and nodules were cleaned of adhering sand: vermiculite:grit mixture by washing in water and the nodules separated and counted. Plant shoot, roots and nodules were dried at 70C for 48hr, weighed and finely ground by Cyclone mill (made by UDY corporation, Colorado, USA) for chemical analysis. The fallen leaves were collected from time to time and included for observations.

Chemical analysis of plants for nitrogen and phosphorous:

Plant parts - shoot, roots and nodules were analysed separately. All the three replicate samples were pooled and analysed. 100mg of dried sample was digested by adding 4ml. of concentrated sulphuric acid containing 0.5% (W/V) selenium and heating on the hot plate of microKjeldahl digestion apparatus. After digestion, the sample was diluted by making upto 75ml with distilled water. 3ml of this diluted digested sample was fed to the Technicon Autoanalyzer II(manufactured by Technicon Industrial systems, Tarrytown, New York) and analysed for N and P contents.

Principle for Phosphorous:

Determination of phosphorous utilizes the reaction between phosphorous and molybdovanadate (supplied during analysis) to form a phosphovanadate complex, which was measured colometrically at 420nm.(method from: Technicon Autoanalyser Industrial method no. 144.71A)

Principle for Nitrogen:(Kjeldahl)

The quantitation of ammonia is achieved utilising the Berthlot reaction in which the formation of a blue indophenol complex occurs when ammonia is reacted with sodium phenate followed by the addition of sodium hypochlorate. The quantitation of indophenol complex was

measured by calorimeter at 630nm.(method from Technicon Autoanalyzer, Industrial method no. 218-72A)

Statistical analysis:

The data was analysed on the VAX 11/780 computer using GENSTAT programme.

4. RESULTS

4.1. SALT TOLERANCE AMONG PIGEONPEA RHIZOBIA

The response of pigeonpea Rhizobia to different levels of NaCl in the yeast extract mannitol agar medium(YMA), is presented in Table. 4. Significant variation in tolerance to salt was observed among pigeonpea Rhizobia. In fast growing Rhizobia Viz. IHP 24, IHP 506, IHP 100, IHP 70 and BDN-A2 the salt tolerance limit ranged between 1 and 5% NaCl, while in slow growing Rhizobia Viz. IHP 484, IHP 87, IHP 213, CC 1, IHP 69, F4, IHP 35, KA 1,and IHP 195, it ranged between 0.25% and 1% NaCl.

Among fast growing Rhizobia, IHP 24 was able to grow upto 5% NaCl with little change in colony size. Further studies (data not presented) revealed that it could grow up to 7% NaCl in the YMA medium-the growth at 6% was similar to growth at 5%, while at 7% was greatly reduced. Strains IHP 100 and IHP 506 could grow normally upto 2% NaCl, while the growth at 3%, 4% and 5% NaCl consisted of small colonies. Strains IHP 70 and BDN-A2 grew normally upto 1% NaCl but could tolerate up to 3% NaCl as evident by faint growth.

Among slow growers IHP 484 was able to grow upto 1% NaCl while at 2% NaCl only faint growth was seen. Strains IHP 87, IHP 213, CC 1, IHP 69, IHP 35, and KA 1 did not grow at more than 0.5% NaCl while strains IHP 195 and F4 could not grow even at 0.5% NaCl in YMA medium.

Table 4: Effect of salt (NaCl) stress on the growth response of pigeonpea Rhizobium cultures

Sl. No.	Rhizobium strain	NaCl (%)							
		Control (0)	0.25	0.5	1	2			
1	IHP 24	+++ a	++ b	++	++	++	++	++	++
2	IHP 506	+++	++	++	++	++	+ c	+	+
3	IHP 100	+++	++	++	++	++	+	+	+
4	IHP 70	+++	++	++	++	+	+	-	-
5	BCN-42	+++	++	++	++	+	+	-	-
6	IHP 484	+++	+++	++	++	+	-	-	-
7	IHP 97	+++	++	++	-	-	-	-	-
8	IHP 213	+++	++	+	-	-	-	-	-
9	CC 1	+++	++	+	-	-	-	-	-
10	IHP 69	+++	++	+	-	-	-	-	-
11	F 4	+++	++	-	-	-	-	-	-
12	IHP 35	+++	++	+	+	+	-	-	-
13	KA 1	+++	++	+	-	-	-	-	-
14	IHP 195	+++	+++	-	-	-	-	-	-

a +++ = Good growth; b ++ = Moderate growth; c + = Little growth

In general, the strains ability to tolerate NaCl in the growth medium seemed related to their growth character. Fast growers were able to tolerate NaCl more than the slow growers. We did not notice any major difference between native (isolated from saline fields) and exotic (from normal soils) rhizobial strains in their salt tolerance. The most tolerant **Rhizobium** strain IHP 24 was isolated from the normal soil.

4.2. SCREENING PIGEONPEA GENOTYPES FOR SALT TOLERANCE

4.2.1. Effect on seed germination:

The germination of pigeonpea genotypes was retarded and delayed with increasing level of salt (from 0 to 120mM NaCl) in the growth medium (Table.5 and 6; Fig.2 and 3). There were differences among genotypes in ability to germinate at a given level of salt.

At 0mM salt level, the mean germination of pigeonpea genotypes was 81% with a range between 59 and 90% on 3rd day after sowing. Two days later ie. on the 5th day after sowing, the mean germination was 83% ranging between 64 and 90%. At 30mM salt level, the mean germination of pigeonpea genotypes was 90% (range 60-115%) on the 3rd day and 91% (range 70-115%) on the 5th day after sowing compared to the respective controls at 0mM NaCl. In genotypes 11 and 17, the germination was slightly stimulated at 30mM NaCl.

At 60mM NaCl level, the mean germination of pigeonpea genotypes was 59% (range 20-85%) and 75% (range 45%-115%) on the 3rd and 5th day after sowing respectively compared to the control. Germination though delayed was stimulated in genotypes 6 and 8.

At 90mM NaCl level, the mean germination of pigeonpea was 44%(range 10-83%) on 3rd day and 69% (range 45-100%) of the control on 5th day after sowing. Although the germination of genotype 27 was delayed it did not appear to

Fig 2: EFFECT OF DIFFERENT LEVELS OF SALT (NaCl) STRESS ON GERMINATION AMONG PIGEONPEA GENOTYPES (3 DAYS AFTER SOWING)

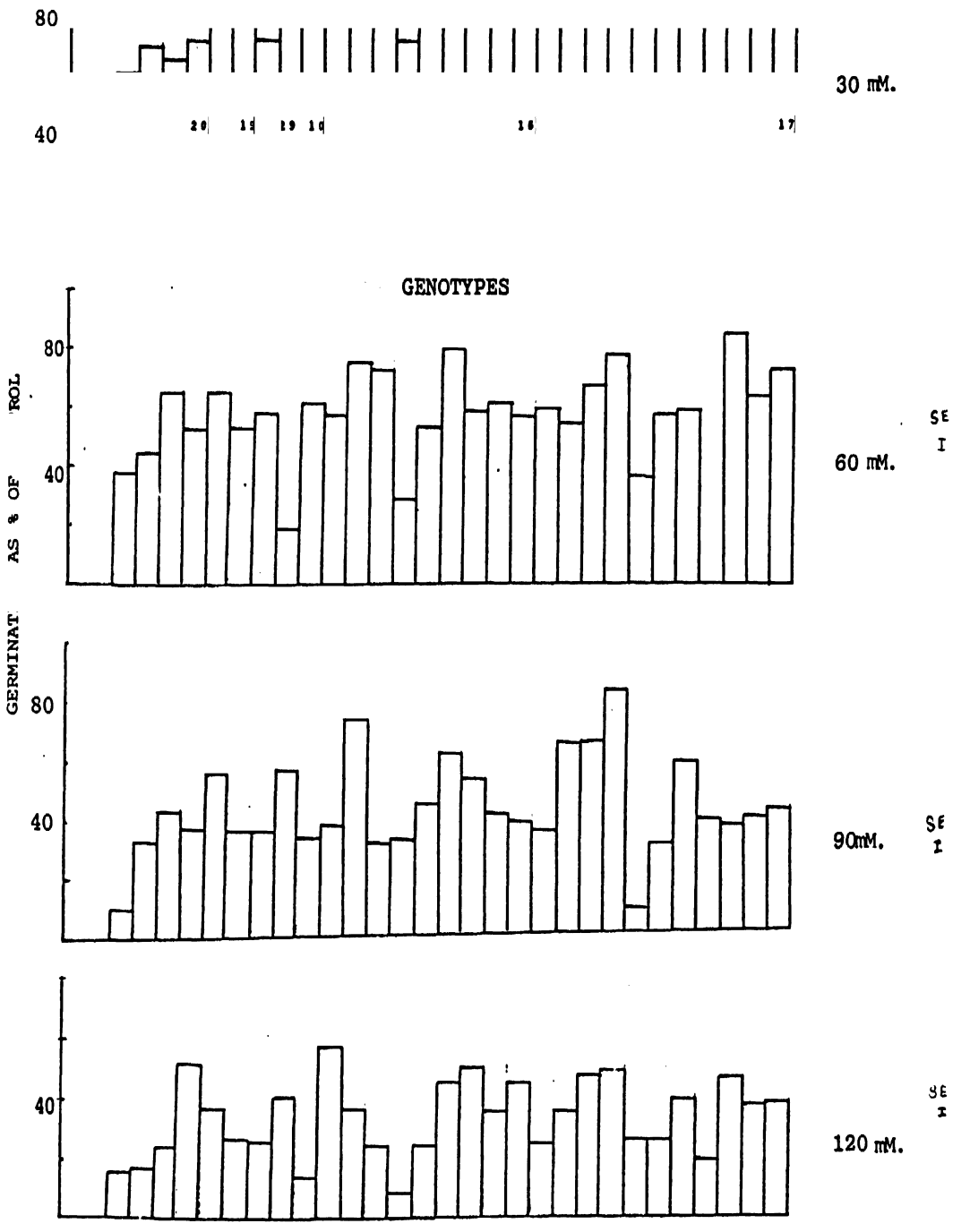


Fig.3: EFFECT OF SALT (NaCl) STRESS ON GERMINATION AMONG PIGEONPEA GENOTYPES (5 DAYS AFTER SOWING)

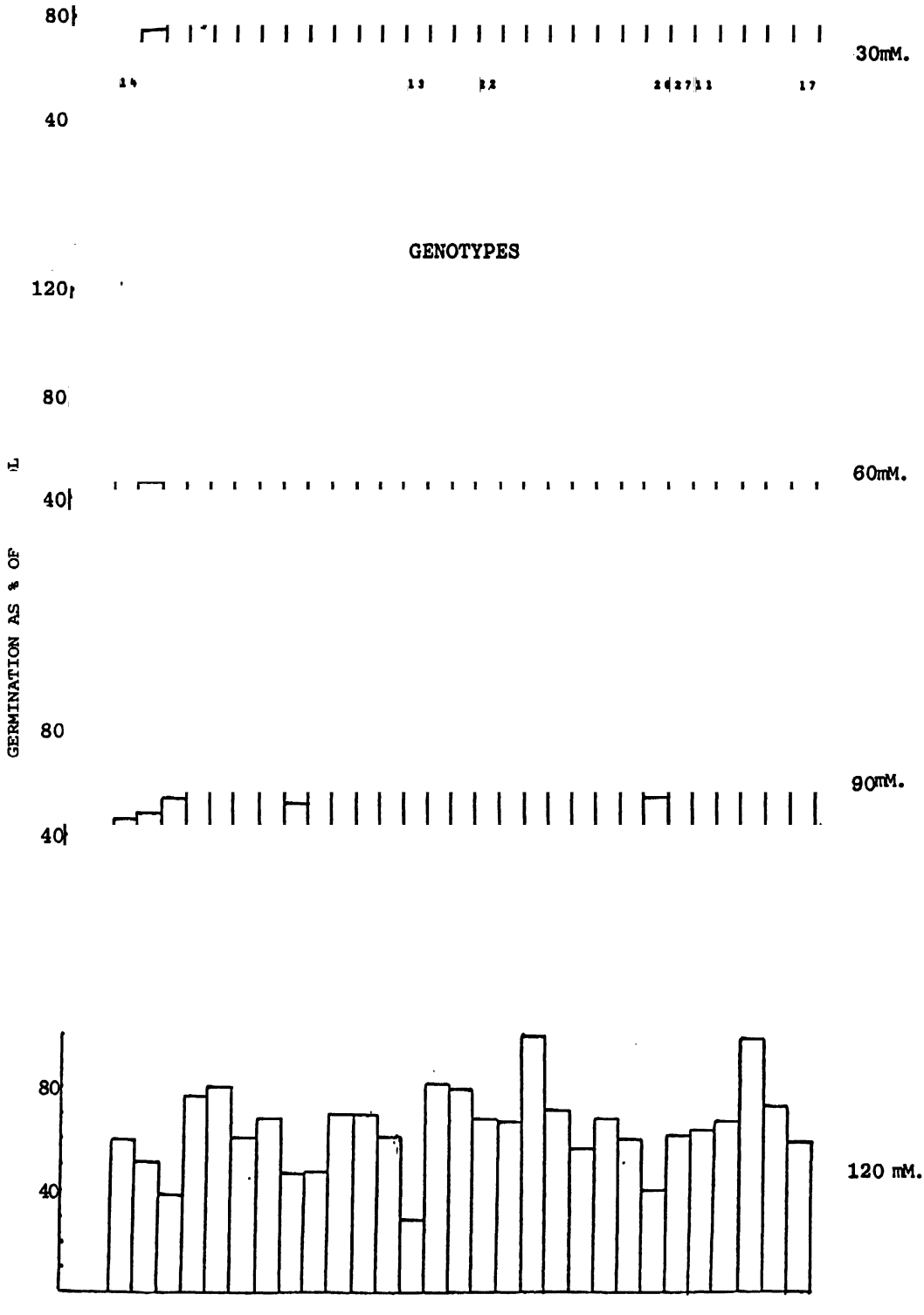


Table.5

Effect of salt(NaCl)stress on germination % among pigeonpea genotypes.

(3days after sowing)

Geno-type.	Cont. (0mM)	germination as % of control at			
		30mM	60mM	90mM	120mM
1	84	107±9.6	79±7.0	84±7.4	49±4.3
2	78	97±9.3	68±6.6	65±6.3	48±4.5
3	90	93±7.8	55±4.6	66±5.4	36±2.9
4	90	83±6.9	66±5.5	57±4.7	37±3.0
5	84	67±5.9	66±5.9	43±3.8	24±2.1
6	84	89±8.0	80±7.1	63±5.6	45±4.0
7	84	93±8.3	54±4.8	44±3.9	24±2.1
8	78	91±8.8	61±5.8	40±3.8	19±1.8
9	84	86±7.7	74±6.6	31±2.7	23±2.0
10	90	88±7.3	61±5.1	34±2.8	14±1.1
11	59	117±4.8	59±7.5	58±7.3	40±5.0
12	78	97±9.3	76±7.3	75±7.2	36±3.5
13	78	72±7.0	28±2.7	33±3.1	7±0.7
14	59	62±7.9	39±4.9	10±1.3	15±1.9
15	90	90±7.5	60±5.0	36±2.9	24±2.0
16	90	86±7.2	57±4.7	38±3.1	46±3.8
17	72	115±1.9	73±7.5	41±4.2	39±3.9
18	83	107±9.6	87±7.8	36±3.2	47±4.1
19	81	79±7.3	53±4.9	35±3.2	26±2.4
20	90	74±6.1	52±4.3	38±3.1	52±4.3
21	90	93±7.8	61±5.1	41±3.4	34±2.8
22	90	90±7.5	59±4.9	52±4.3	50±4.1
23	84	107±9.6	63±5.7	39±3.4	37±3.3
24	78	72±6.9	45±4.3	36±3.2	17±1.6
25	90	72±6.0	59±4.9	36±3.0	26±2.1
26	84	93±8.3	36±3.2	7±0.6	0
27	72	101±0.6	57±6.0	31±3.2	26±2.7
28	64	86±0.1	58±6.8	37±4.3	58±6.7
29	64	89±0.4	19±2.2	57±6.7	41±4.7

SE for control±3.4

Table.6

Effect of salt(NaCl)stress on germination% among pigeonpea genotypes

(5Days after sowing)

geno-type.	cont. (0mM)	germination as % of control at 30mM	60mM	90mM	120mM
1	90	100± 9.1	79± 7.2	83± 7.5	61± 5.5
2	78	97±10.2	72± 7.6	82± 8.6	68± 7.2
3	90	93± 8.5	71± 6.5	80± 7.2	57± 5.1
4	90	83± 7.6	80± 7.2	71± 6.4	81± 7.3
5	84	80± 7.8	87± 8.5	53± 5.2	39± 3.7
6	84	93± 9.1	107±10.5	82± 8.0	80± 7.8
7	84	93± 9.1	74± 7.2	76± 7.3	81± 7.9
8	67	106±13.1	117±14.3	86±10.5	68± 8.3
9	84	89± 8.7	89± 8.7	64± 6.2	62± 6.0
10	90	88± 8.0	73± 6.7	69± 6.2	48± 4.3
11	64	104±13.4	83±10.6	70± 9.0	64± 8.2
12	84	89± 8.7	76± 7.4	77± 7.5	69± 6.7
13	78	91± 9.6	58± 6.1	62± 6.5	28± 3.0
14	75	69± 7.5	57± 6.3	47± 5.1	60± 6.5
15	90	93± 8.5	69± 6.3	66± 5.9	72± 6.5
16	90	93± 8.5	80± 7.2	77± 6.9	100± 9.1
17	73	115±13.0	84± 9.5	82± 9.2	59± 6.6
18	84	107±10.5	100± 9.8	74± 7.2	0
19	81	85± 8.6	76± 7.7	66± 6.7	61± 6.1
20	90	80± 7.2	71± 6.4	73± 6.6	77± 6.9
21	90	93± 8.5	68± 6.2	71± 6.4	67± 6.0
22	90	93± 8.5	71± 6.5	78± 7.0	69± 6.2
23	84	107±10.5	71± 7.0	74± 7.2	73± 7.1
24	84	76± 7.4	46± 4.5	49± 4.7	51± 5.0
25	90	86± 7.9	71± 6.5	60± 5.4	69± 6.2
26	90	100± 9.1	71± 6.5	51± 4.6	41± 3.7
27	72	105±12.0	68± 7.8	102±11.6	63± 7.2
28	75	88± 9.6	57± 6.3	63± 6.8	71± 7.7
29	90	86± 7.9	62± 5.6	50± 4.5	47± 4.2

SE for control±3.6

be adversely affected at 90mM level.

At 120mM salt level the mean germination of pigeonpea was reduced to 33% (range 5-60%) on the 3rd day but rose to 65% (range 30-100%) on the 5th day compared to the controls. Only two genotypes, namely 16 and 18 had 100% germination on the 5th day while it was only about 45% on the 3rd day.

4.2.2. Effect on establishment of seedlings:

The results of establishment of seedlings as influenced by salinity 11 days after sowing are presented in Table 7; Fig4. The establishment of pigeonpea seedlings was adversely affected with increasing salt level. Though there were differences among genotypes tolerance at a given salt level, the performance was not consistent across the salt levels.

At 30mM NaCl, the mean establishment of the pigeonpea seedlings over all genotypes was 89% (range 65-135%) compared to the control treatment, a figure very close to the percent germination observed 3 days after sowing. In genotype 17, the establishment of the seedlings was 30% greater than in control, an indication of the stimulatory effect of salt at low concentrations on germination as well as establishment.

At 60mM NaCl level, the mean establishment of the seedlings was 60% (range 22-89%) compared to control. Genotypes 8 and 18 showed greater establishment than the

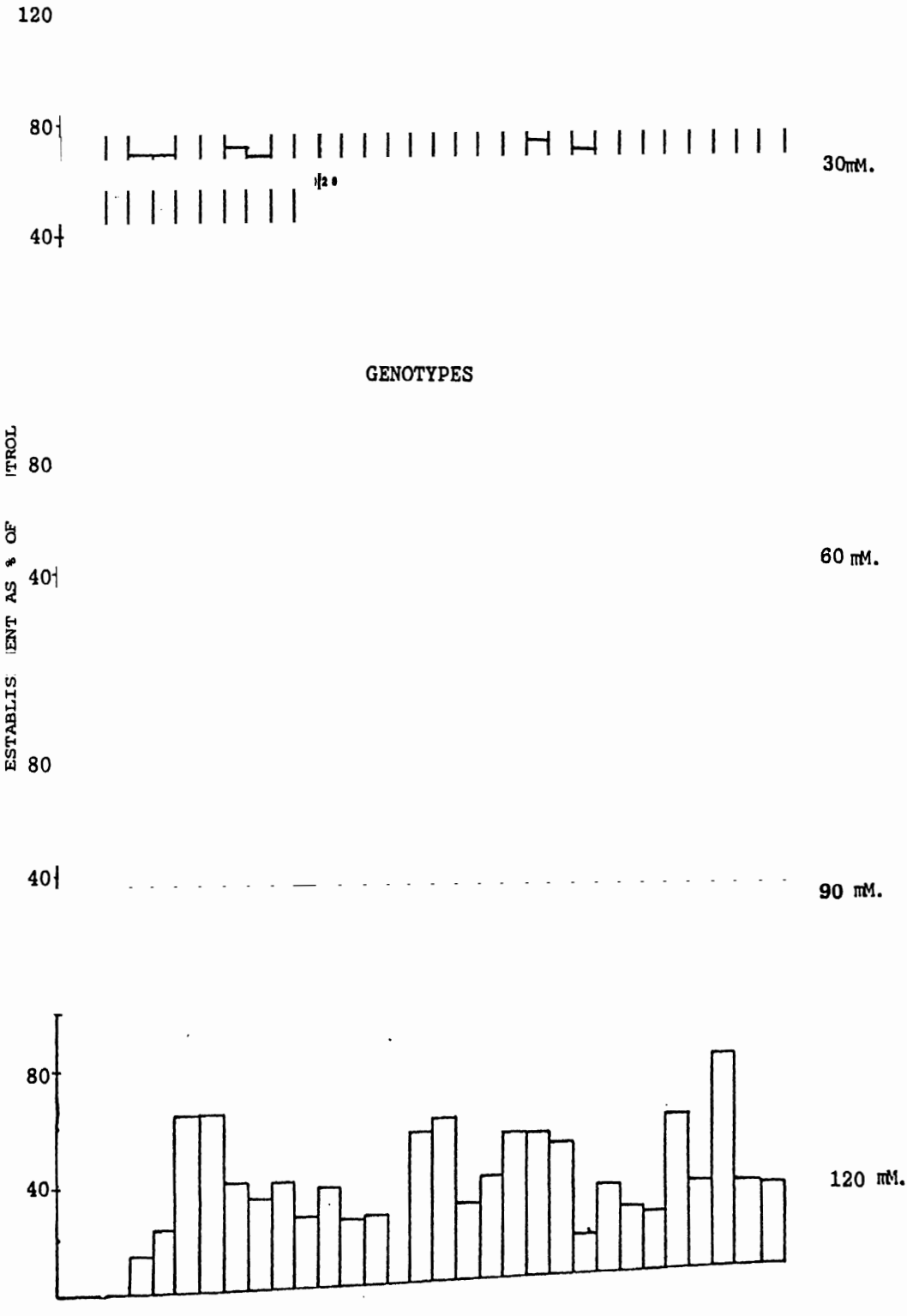
Table.7

ect of Salt(NaCl) stress on establishment of seedlings among pigeonpea genotypes (11 days after sowing)

genotype.	cont. (0mM)	establishment as % of control at			
		30mM	60mM	90mM	120mM
1	75	112±12.2	71± 7.7	77±8.4	32±3.4
2	81	71± 7.1	39± 4.0	35±3.5	14±1.3
3	68	102±12.1	63± 7.4	80±9.5	47±5.5
4	71	102±11.6	81± 9.2	74±8.5	63±7.1
5	81	68± 6.8	71± 7.2	45±4.5	23±2.2
6	77	102±10.7	71± 7.4	60±6.3	58±6.1
7	90	93± 8.5	41± 3.7	55±4.9	52±4.7
8	66	95±11.7	86±10.7	33±4.1	30±3.7
9	75	79± 8.6	85± 9.3	30±3.3	24±2.5
10	90	79± 7.2	62± 5.6	33±3.0	24±2.2
11	49	96±16.1	53± 8.9	49±8.1	56±9.3
12	75	83± 9.0	73± 8.0	43±4.6	23±2.5
13	75	83± 9.0	40± 4.4	46±4.9	0
14	56	78±11.5	38± 5.5	0	0
15	90	74± 6.7	51± 4.6	43±3.9	50±4.5
16	84	86± 8.4	65± 6.4	38±3.7	51±5.0
17	51	135±21.8	81±13.0	60±9.5	30±4.7
18	78	108±11.4	89± 9.3	50±5.2	77±8.1
19	81	72± 7.2	52± 5.3	45±4.5	38±3.8
20	67	89±10.9	61± 7.5	61±7.5	62±7.5
21	84	89± 8.7	51± 5.0	56±5.4	36±3.4
22	72	98±11.2	46± 5.3	49±5.5	27±3.0
23	72	98±11.2	53± 6.1	37±4.2	31±3.5
24	69	69± 8.2	22± 2.6	38±4.5	13±1.5
25	90	69± 6.3	41± 3.7	29±2.6	31±2.8
26	81	100±10.1	39± 4.0	11±1.1	23±2.2
27	72	101±11.6	43± 5.0	46±5.2	21±2.4
28	62	98±13.0	39± 5.2	67±8.9	35±4.6
29	68	97±11.7	51± 6.2	38±4.6	38±4.5

SE for Control±3.6

Fig.4: EFFECT OF SALT (NaCl) STRESS ON ESTABLISHMENT OF PIGEONPEA GENOTYPES (11 DAYS AFTER SOWING)



others.

At 90mM NaCl level, the mean establishment of pigeonpea seedlings over genotypes was 40% (range 0-80%) compared to control. Genotype 14 was very sensitive hence failed to establish, while genotypes 3 and 1 ranked top in establishment at 90mM salt in the nutrient solution.

At 120mM salt level, the establishment of pigeonpea seedlings was poor, and the mean over genotypes was 3% (range 0 - 77%) compared to control. Genotypes 13 and 14 failed to establish, while genotype 18 was the best among others.

4.2.3. Effect on shoot dry matter

The results of shoot dry matter of 29 pigeonpea genotypes as affected by different salt levels are presented in Table 8 and Fig.5. There was a significant decline in shoot dry matter with increasing salt concentration. There were differences among genotypes in tolerance to salinity, however, they were not consistent at all the salinity levels tested.

At 30mM NaCl, the mean shoot dry matter produced by pigeonpea genotypes was 71% (range 54 to 120%) compared to control. The shoot dry matter was stimulated in only genotype 8 while in others it was reduced by 30mM NaCl.

Table.8

:t of salt(NaCl)stress on the shoot dry weight(mg/plant)among pigeonpea genotypes(21days after sowing)

geno-type.	cont. (0mM)	shoot dry weight as % of control at			
		30mM	60mM	90mM	120mM
1	80	64± 6.7	55± 5.9	35±3.6	23±2.4
2	77	56± 6.1	24± 2.7	12±1.3	6±0.6
3	60	64± 9.1	52± 7.3	41±5.8	8±1.1
4	77	62± 6.8	47± 5.2	28±3.0	12±1.2
5	66	60± 7.7	71± 9.0	28±3.6	7±0.9
6	54	64±10.1	41± 6.4	31±4.8	8±1.3
7	57	82±12.2	24± 3.5	27±4.0	13±1.9
8	62	120±16.3	75±10.2	21±2.8	21±2.8
9	57	57± 8.4	42± 6.3	50±7.3	0
10	78	64± 6.9	54± 5.8	9±0.9	8±0.8
11	87	79± 7.6	13± 1.2	4±0.4	4±0.4
12	55	67±10.2	64± 9.7	26±3.9	13±1.9
13	96	83± 7.4	48± 4.3	7±0.6	1±0.1
14	107	74± 5.9	4± 0.3	11±0.8	4±0.3
15	93	83± 7.5	39± 3.5	18±1.6	9±0.8
16	69	72± 8.7	38± 4.6	33±4.0	16±1.9
17	85	79± 7.8	56± 5.5	20±1.9	0
18	68	70± 8.7	27± 3.4	47±5.8	10±1.2
19	85	70± 6.9	26± 2.6	28±2.7	15±1.4
20	72	76± 8.9	35± 4.1	6±0.6	17±2.0
21	68	60± 7.4	42± 5.2	19±2.4	4±0.5
22	66	80±10.2	20± 2.5	25±3.1	10±1.2
23	69	71± 8.7	31± 3.7	31±3.7	5±0.6
24	74	75± 8.5	9± 1.1	22±2.5	5±0.6
25	65	54± 7.1	34± 4.4	20±2.5	0
26	74	73± 8.3	30± 3.5	6±0.6	0
27	75	67± 7.5	31± 3.5	11±1.2	0
28	77	70± 7.7	51± 5.6	17±1.8	12±1.3
29	83	62± 6.3	49± 4.9	16±1.6	10±1.0

SE for Control±4.6

At 60mM NaCl, the shoot dry matter was further reduced with a mean of 38% over genotypes (range 4 to 75%) compared to control., Genotypes 8 and 5 stood top producing shoot dry matter about 75% of the control while 72% of the genotypes had produced shoot dry matter less than 50% of the control.

At 90mM and 120mM NaCl, the growth of pigeonpea genotypes was very poor and the shoot dry matter produced was only 20% of the control. Though about 35% of the seedlings established 11days after sowing, they became sick showing leaf necrosis initially and drying finally because of salt toxicity. It appears that pigeonpea cannot tolerate NaCl beyond 90mM level.

4.2.4. Effect on root dry matter

The data on the effect of NaCl stress on root dry matter production of 29 pigeonpea genotypes 21days after sowing is presented in Table 9 and Fig.6. The data of the treatment 120mM NaCl was not included as the plants did not survive upto 21days after sowing. The root dry matter decreased with increasing salt concentration. The pigeonpea genotypes varied in their tolerance to NaCl at a given level.

At 30mM NaCl, the mean root dry matter produced over all genotypes was 74% (range 47 to 108%) compared to that obtained at 0mM NaCl. The pigeonpeas that suffered most, with root dry matter less than 50% to control, were genotypes 2 and 6. In genotypes 15 and 26 the roots were

Fig.6: EFFECT OF SALT (NaCl) STRESS ON ROOT DRY MATTER AMONG PIGEONPEA GENOTYPES (21 DAYS AFTER SOWING)

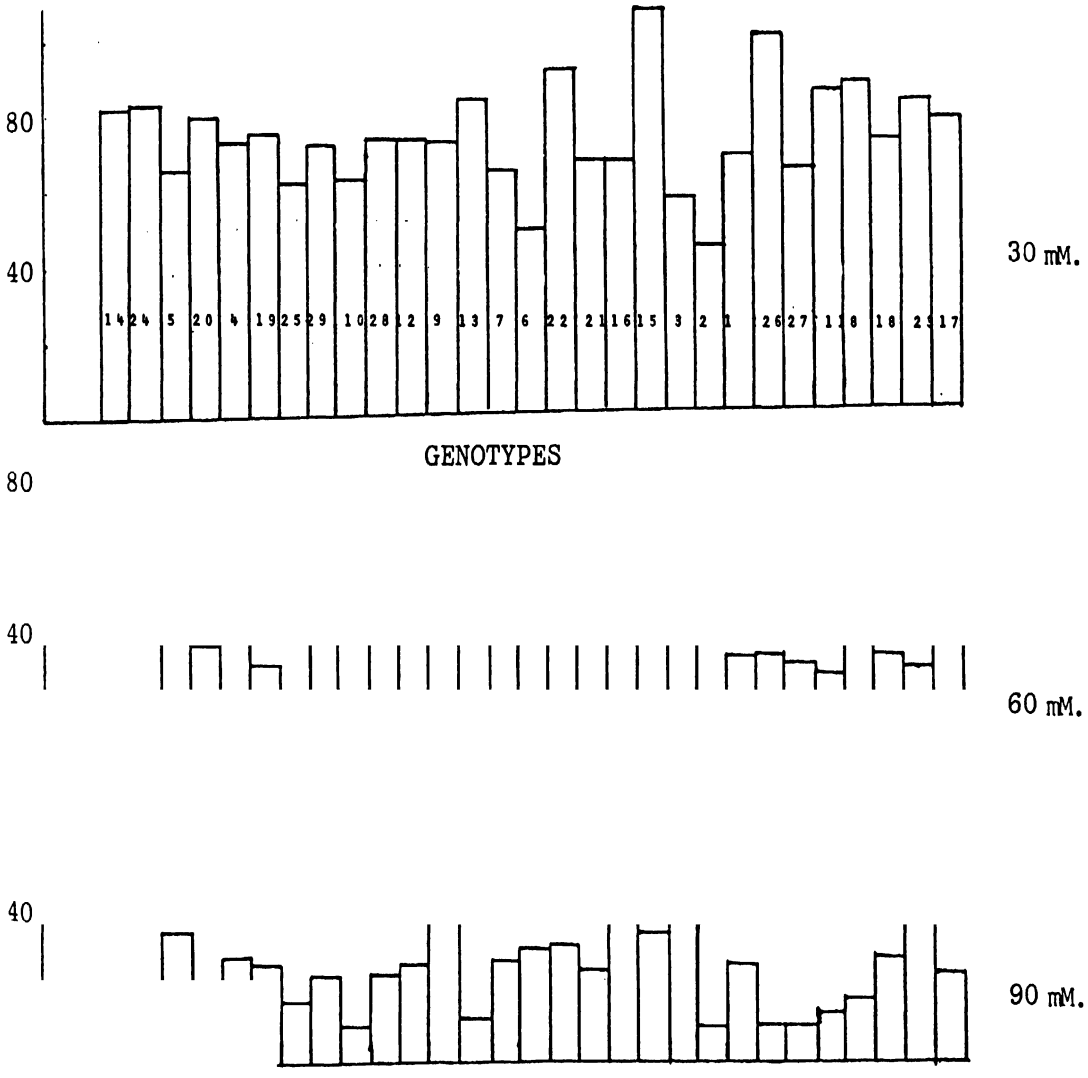


Table.9

ffect of salt(NaCl)stress on the root dry weight(mg/plant) among pigeonpea genotypes(21days after sowing)

geno-type.	cont. (0mM)	root dry weight as % of control at			
		30mM	60mM	90mM	120mM
1	39	68± 7.5	30±3.3	26±2.9	20±2.1
2	40	47± 5.1	19±2.0	10±1.0	2±0.2
3	31	67± 9.2	56±7.7	46±6.2	11±1.5
4	36	74± 8.8	53±6.2	29±3.3	13±1.5
5	37	65± 7.6	62±7.3	36±4.1	9±1.0
6	33	48± 6.3	42±5.4	30±3.9	11±1.4
7	31	65± 8.9	21±2.9	27±3.7	23±3.2
8	42	88± 8.9	59±6.0	18±1.8	19±1.9
9	35	72± 8.9	44±5.4	49±6.0	0±0.1
10	48	63± 5.6	50±4.4	9±0.8	6±0.5
11	49	85± 7.4	25±2.2	14±1.1	9±0.8
12	30	73±10.4	46±6.4	27±3.8	20±2.8
13	50	84± 7.3	52±4.5	11±0.9	3±0.2
14	52	82± 6.8	14±1.1	21±1.7	13±1.0
15	42	108±11.1	71±7.3	34±3.5	20±2.0
16	35	68± 8.2	43±5.2	39±4.7	32±3.9
17	52	77± 6.4	48±4.0	24±2.0	0±0.1
18	39	71± 7.9	30±3.4	29±3.2	16±1.7
19	47	76± 7.0	31±2.8	31±2.4	23±2.1
20	38	81± 9.2	35±4.0	11±1.2	19±2.2
21	33	68± 8.8	37±4.8	24±3.1	10±1.3
22	37	93±10.8	17±2.0	31±3.6	17±1.9
23	35	92±11.4	27±3.3	51±6.2	11±1.4
24	38	84± 9.7	13±1.4	21±2.4	7±0.7
25	38	61± 6.9	20±2.3	17±1.8	0
26	34	101± 2.8	32±4.0	10±1.2	0
27	39	64± 7.1	28±3.1	9±1.0	0
28	37	74± 8.7	48±5.7	24±2.7	18±2.1
29	40	73± 7.8	56±6.0	23±2.4	0

SE for control±2.4

not as well affected as the shoot.

At 60mM NaCl, the root dry matter was significantly affected, with a mean of 36% (range 13-71%) over genotypes compared to plants grown at 0mM NaCl. In genotype 15 the reduction in root dry matter was far less than all others. Genotypes 2, 7, 11, 14, 22, 24, 25 were more susceptible to salt than others.

At 90mM NaCl, the root development was severely affected. The mean root dry matter produced over all genotypes was 23% (range 9 to 51%) compared to plants grown at 0mM NaCl. Only 2 genotypes-9, 23 were found less susceptible than others.

4.2.5. Correlations between germination, establishment and plant (shoot and root) dry matter

The results are presented in Table 10. Observations on germination of pigeonpea 3 days after sowing were correlated well with germination counts after 5 days and plant establishment suggesting that early observations on germinating ability might reflect the plant's establishment 11 days after sowing. Germination counts 5 days after sowing did not give any more information on plants establishment than that obtained by germination counts 3 days after sowing. The early observations on germination were also correlated well with shoot and root dry matter. The correlations between establishment 11days after sowing and shoot and root dry matter after 21days were significant.

Table 10: Correlations between germination, establishment and plant (shoot and root) dry matter of 29 pigeonpea genotypes grown at 0, 30, 60, 90 and 120 mM NaCl.

	Germination (5 days after sowing)	Establishment (11 days after sowing)	Shoot dry weight	Root dry weight
1.Germination (3 days after sowing)	**0.79	**0.83	**0.62	**0.67
2.Germination (5 days after sowing)		**0.76	0.53	0.53
3.Establishment (11 days after sowing)			**0.67	0.65
4.Shoot dry weight				**0.94

** Significant at 0.01

4.3. EFFECT OF SALT (NACL) STRESS ON GROWTH, NODULATION, NITROGENASE (ACETYLENE REDUCTION) ACTIVITY, NITROGEN AND PHOSPHOROUS UPTAKE OF 4 PIGEONPEA GENOTYPES INOCULATED WITH 2 RHIZOBIUM STRAINS AND GROWN IN POTS

All the 4 pigeonpea genotypes Viz. ICPL 358, ICPL 332, C 11, ICPL 227 germinated uniformly at all the salt concentrations (0, 15mM, 30mM, 45mM, 60mM, 75mM NaCl) imposed from time of sowing. Till 15th day after sowing, no treatment effects either genotypic, or of **Rhizobium** strain or salt could be seen. On 16th day after sowing, initial symptoms of leaf necrosis appeared in all the genotypes particularly at 60 and 75mM NaCl. With time, the leaf chlorophyll bleached. At 60 and 75mM NaCl the severity of symptoms appeared relatively early in ICPL 358, and C11 particularly those plants inoculated with **Rhizobium** strain IHP 100 but not IHP 195.

By 24th day after sowing, none of the 4 genotypes survived at 60 and 75mM NaCl. Genotypes ICPL 358 and C 11 did not survive at 45mM NaCl while the other genotypes ICPL 332 and ICPL 227 partially survived at 45mM. The survival of different genotypes grown with salt upto 45mM NaCl are given in Table 11. The survival of genotypes ICPL 358 and C11 was not uniform even at 30mM NaCl.

4.3.1. Effect of salt stress on pigeonpea growth

Table 11: Survival of pigeonpea genotypes in pots watered with nutrient solution containing different levels of NaCl, 45 days after sowing.

Salt treatment NaCl (mM)	No. of replicate with living plants/3 in ICPL 355	ICPL 332	C 11	ICPL 227
0 mM				
15 mM				
30 mM				
45 mM				

A = Inoculated with IHP 100; B = Inoculated with Rhizobium IHP 195

The leaf area of pigeonpea an indication of photosynthetic ability, as influenced by salinity is shown in Table 12. There was significant decrease in leaf area with increasing salt concentration up to 30mM. The genotype effects were also significant - ICPL 227 had largest leaf area, while C11 had lowest leaf area, **Rhizobium** effects were significant with IHP 195 inoculation leaf area was greater than with IHP 100. The salt level, genotypic interaction effects were highly significant with ICPL 227 showing greater tolerance at 30mM NaCl, while the others were more susceptible.

fig. 7 & 8

fig. 9

The shoot and root dry matter of different genotypes as influenced by salinity are presented in Tables 13 and 14 respectively. Both shoot and root drymatter were significantly reduced with increasing salt concentration upto 30mM, with severe reduction at 30mM NaCl. ICPL 227 was most tolerant upto 30mM while the others were susceptible. At 45mM NaCl, ICPL 227 and ICPL 332 was the only genotype that survived in some replications while the others did not. Inoculation with IHP 195 produced more dry matter than with IHP 100 suggesting that the former probably fixed more nitrogen at all salinity levels. This is rather surprising as the salt tolerance of IHP 195 was far less than that of IHP 100. Genotypic **Rhizobium** interactions were significant with all genotypes except C 11 producing more dry matter with IHP 195 than with IHP 100.

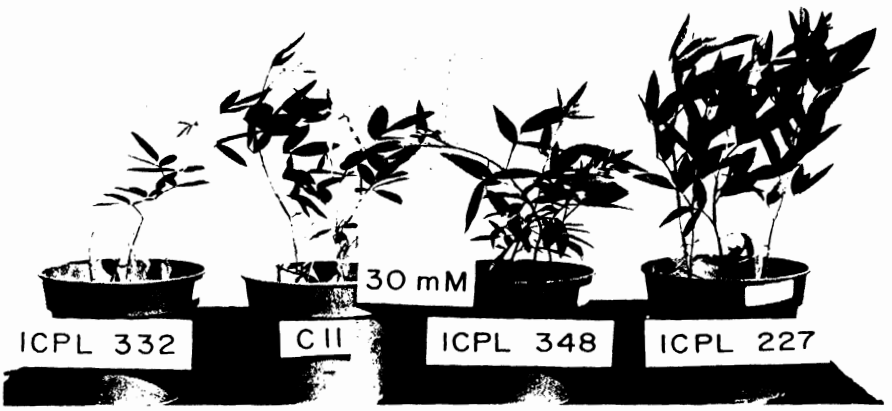


Fig.7 Effect of salt (30 mM NaCl) stress on the growth of four pigeonpea genotypes, 45 days after sowing.

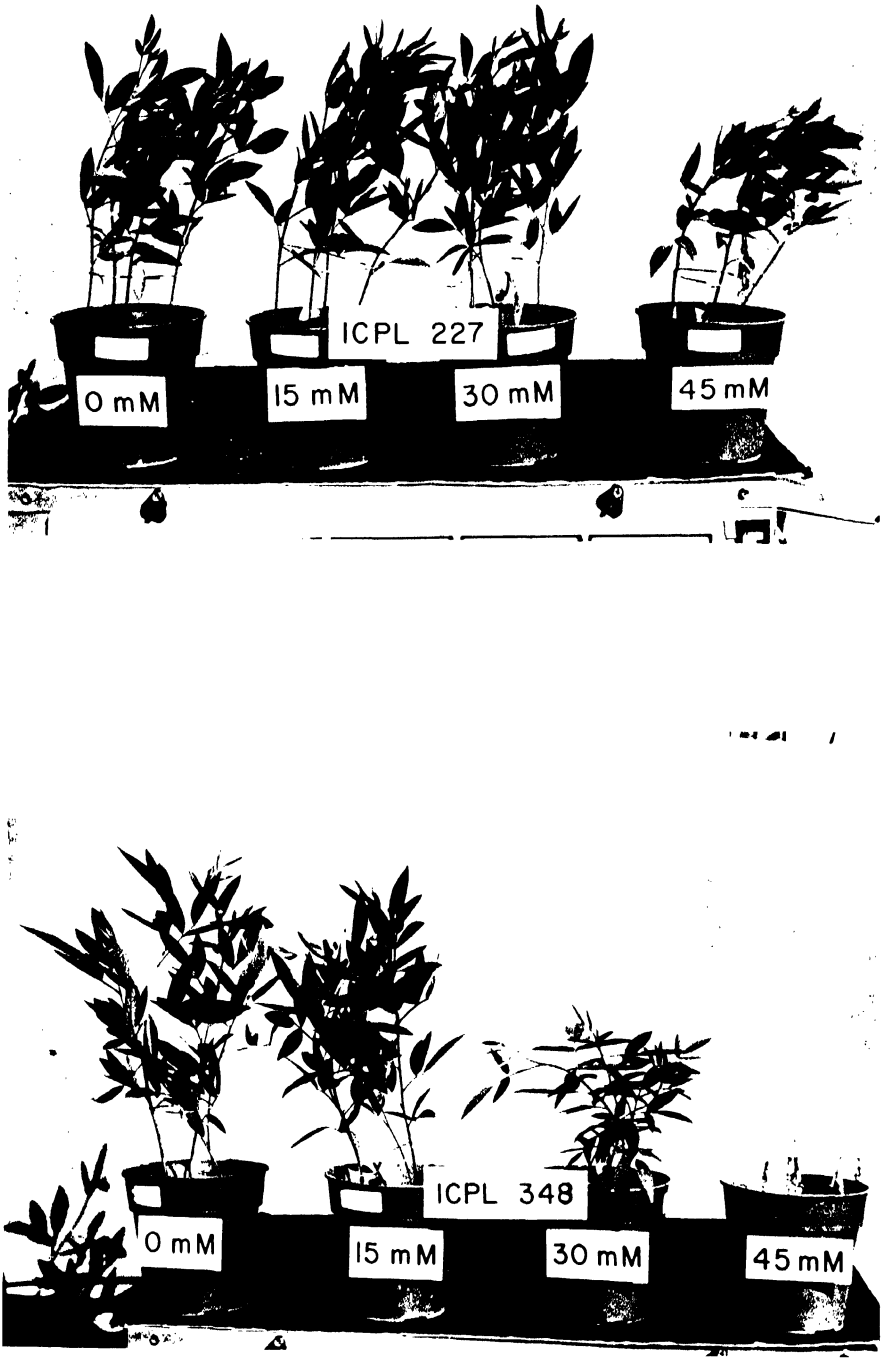


Fig.8. Performance of ICPL 227 (tolerant at 45 mM) and ICPL 358 (susceptible at 45 mM) at various salt levels (0, 15, 30, 45 mM), 45 days after sowing.

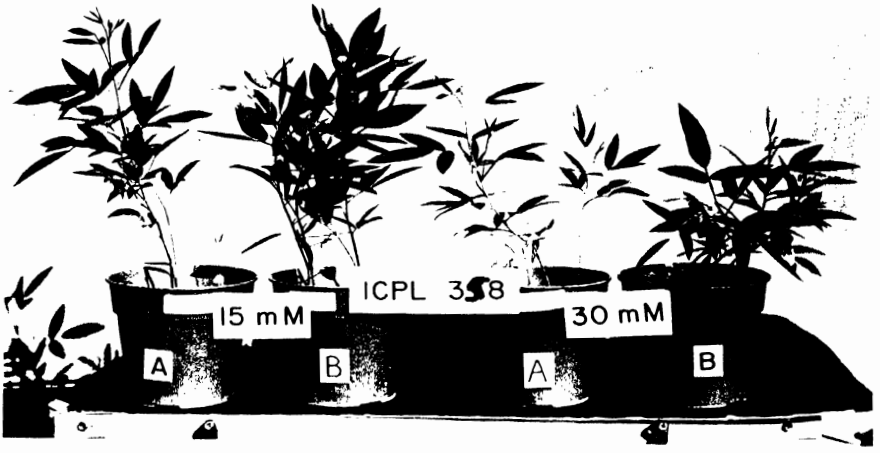


Fig. 9. Effect of Rhizobium strains (A, IHP 100; B, IHP 195) on the growth of pigeonpea genotype ICPL 358, grown at 15 mM, 30 mM salt (NaCl) levels in the medium, 45 days after sowing.

Table:13. Effect of salt (NaCl) stress on shoot dry weight (g/pot) of four pigeonpea genotypes inoculated with rhizobium strains IHP 100, IHP 195 and grown in pots (45 days after sowing).

Salt treatment (NaCl)	Pigeonpea genotypes												Rhizobium strain		
	ICPL 359			ICPL 332			C 11			ICPL 227					
	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean
0 mM	4.58	6.54	5.56	5.25	6.03	5.64	5.70	5.83	5.76	5.57	6.06	5.81	5.28	6.12	5.7
15 mM	3.19	5.82	4.50	4.00	5.88	4.94	3.76	5.00	4.38	4.56	5.80	5.18	3.88	5.63	4.8
30 mM	0.64	1.57	1.10	0.63	2.19	1.41	1.82	1.22	1.52	3.72	4.50	4.11	1.71	2.37	2.0
45 mM a	ND	ND	ND	0.22	ND	ND	ND	ND	ND	1.12	3.43	2.27	ND	ND	ND
Mean	2.8	4.64	3.72	3.31	4.7	4.0	3.76	4.02	3.89	4.61	5.46	5.05	3.62	4.70	4.16

1. Salt treatment = ± 0.243 **
2. Genotype = ± 0.239 **
3. Strain = ± 0.127 **
4. Salt treatment vs. Genotype = ± 0.473 *
5. Salt treatment vs Strain = ± 0.283 *
6. Genotype vs Strain = ± 0.299 *
7. Salt treatment vs Genotype vs Strain = ± 0.533 NS

a = Not included for statistical analysis
* = Significant at P< 0.05
** = Significant at P< 0.01
NS = Not significant

Table 14. Effect of salt (NaCl) stress on root dry weight (mg/pot) of four pigeonpea genotypes inoculated with rhizobium strains IHP 100, IHP 195 and grown in pots (45 days after sowing).

Salt treatment (NaCl)	Pigeonpea genotypes												Rhizobium strain		
	ICPL 358			ICPL 332			C 11			ICPL 227					
	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean
0 mM	1317	1674	1495	1585	1700	1643	1824	1661	1743	1662	1406	1534	1597	1610	1604
15 mM	841	1726	1284	1180	1705	1443	1162	1677	1419	1350	1391	1371	1133	1625	1379
30 mM	121	426	273	171	574	373	591	299	445	1017	1405	1211	475	676	576
45 mM a	ND	ND	ND	63	ND	ND	ND	ND	ND	238	929	584	ND	ND	ND
Mean	760	1215	1017	979	1326	1153	1192	1212	1202	1342	1401	1372	1068	1304	1186

	SE	
1. Salt treatment	= ± 34.58	**
2. Genotype	= ± 75.3	*
3. Strain	= ± 54.95	**
4. Salt treatment vs. Genotype	= ± 118.17	**
5. Salt treatment vs Strain	= ± 75.67	NS
6. Genotype vs Strain	= ± 109.27	NS
7. Salt treatment vs Genotype vs Strain	= ± 179.14	NS

a = Not included for statistical analysis
* = Significant at P< 0.05
** = Significant at P< 0.01
NS = Not significant

The results of shoot height in cm taken at harvest are presented in Table 15. The effects of salinity level, genotypes, *Rhizobium* strains and salinity genotypic interactions were all significant and the trends were similar to those of shoot dry matter.

4.3.2. Effects of salt stress on pigeonpea nodulation and nitrogenase activity

The results of nodule number and weight of pigeonpea genotypes as affected by *Rhizobium* strain and salt stress are presented in Table 16 and 17 respectively. Increasing salt from 0 to 30mM had significantly reduced both nodule number and weight. There were significant differences between genotypes - ICPL 227 produced highest number and dry weight of nodules while ICPL 358 had least. *Rhizobium* strain effects were significant only in nodule number. IHP 195 was significantly better than IHP 100 in nodule number but not in total dry weight of nodules. The salinity genotypic interaction, salinity strain interaction effects were significant in both nodule number and nodule weight. ICPL 227 was least affected by 30mM NaCl in both nodule number and weight whereas the other genotypes showed significant reduction at 30mM NaCl. IHP 195's ability to nodulate at 15mM salt was significantly better than IHP 100.

Salt stress had no effect on nitrogenase activity measured as acetylene reduction (AR) activity per pot per hour (Table 18). However, genotype effects were significant

Table:15. Effect of salt (NaCl) stress on shoot height (cm/pot) of four pigeonpea genotypes inoculated with rhizobium strains IHP 100, IHP 195 and grown in pots (45 days after sowing).

Salt treatment (NaCl)	Pigeonpea genotypes												Rhizobium strain		
	ICPL 359			ICPL 332			C 11			ICPL 227					
	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean
0 mM	148	167	158	150	164	157	162	186	174	142	148	146	151	166	158
15 mM	109	119	114	132	144	138	121	145	133	139	138	138	125	136	131
30 mM	19	59	39	24	52	38	60	39	49	96	117	107	50	67	58
45 mM a	ND	ND	ND	15	ND	ND	ND	ND	ND	36	91	64	ND	ND	ND
Mean	92	115	103	102	120	110	114	123	119	126	135	130	109	123	116

	SE	
1. Salt treamtnet	= ± 11.25	**
2. Genotype	= ± 6.29	*
3. Strain	= ± 11.44	*
4. Salt treatment vs. Genotype	= ± 10.33	*
5. Salt treatment vs Strain	= ± 6.9	NS
6. Genotype vs Strain	= ± 8.86	NS
7. Salt treatment vs Genotype vs Strain	= ± 15.0	NS

a = Not included for statistical analysis
* = Significant at P< 0.05
** = Significant at P< 0.01
NS = Not significant

Table:16. Effect of salt (NaCl) stress on nodule number per pot of four pigeonpea genotypes inoculated with rhizobium strains IHP 100, IHP 195 and grown in pots (45 days after sowing).

Salt treatment (NaCl)	Pigeonpea genotypes												Rhizobium strain		
	ICPL 353			ICPL 332			C 11			ICPL 227					
	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean
0 mM	199	454	327	256	471	364	273	450	362	335	461	398	266	459	362
15 mM	129	524	327	163	402	283	186	489	338	282	356	319	190	443	316
30 mM	47	175	111	42	195	119	114	103	109	302	380	341	126	213	170
45 mM a	ND	ND	ND	9	ND	ND	ND	ND	ND	85	215	150	ND	ND	ND
Mean	125	385	255	154	356	255	191	348	269	306	399	353	194	372	283

1. Salt treatment = ± 6.73 **
2. Genotype = ± 20.5 *
3. Strain = ± 15.05 **
4. Salt treatment vs. Genotype = ± 31.68 *
5. Salt treatment vs Strain = ± 19.73 *
6. Genotype vs Strain = ± 29.7 NS
7. Salt treatment vs Genotype vs Strain = ± 48.55 NS

a = Not included for statistical analysis
* = Significant at P< 0.05
** = Significant at P< 0.01
NS = Not significant

Table:17. Effect of salt (NaCl) stress on nodule dry weight (mg/pot) of four pigeonpea genotypes inoculated with rhizobium strains IHP 100, IHP 195 and grown in pots (45 days after sowing).

Salt treatment (NaCl)	Pigeonpea genotypes												Rhizobium strain		
	ICPL 352			ICPL 332			C 11			ICPL 227					
	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean
0 mM	502	515	509	554	522	538	664	651	658	670	563	617	597	563	580
15 mM	321	541	431	439	529	484	490	552	521	504	607	556	438	557	497
30 mM	58	177	118	79	191	135	229	104	166	451	480	466	204	238	221
45 mM a	ND	ND	ND	8	ND	ND	ND	ND	ND	98	383	241	ND	ND	ND
Mean	294	411	352	357	414	385	461	436	448	541	550	546	413	453	433

1. Salt treatment = ± 22.55 **
2. Genotype = ± 26.25 **
3. Strain = ± 13.99 NS
4. Salt treatment vs. Genotype = ± 46.39 *
5. Salt treatment vs Strain = ± 28.33 *
6. Genotype vs Strain = ± 32.87 NS
7. Salt treatment vs Genotype vs Strain = ± 58.86 NS

a = Not included for statistical analysis
* = Significant at P< 0.05
** = Significant at P< 0.01
NS = Not significant

Table no. Effect of salt (NaCl) stress on nitrogenase activity (u M C2 H4/pot/hr) of four pigeonpea genotypes inoculated with rhizobium strains IHP 100, IHP 195 and grown in pots (45 days after sowing).

Salt treatment (NaCl)	Pigeonpea genotypes												Rhizobium strain		
	ICPL 359			ICPL 332			C 11			ICPL 227					
	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean
0 mM	10.8	16.4	13.6	15.1	9.9	12.0	34.9	38.3	36.7	19.3	18.1	18.8	20.0	20.4	20.2
15 mM	25.0	13.0	19.0	19.8	21.7	20.8	34.6	21.6	28.1	16.5	18.9	17.7	24.0	18.8	21.4
30 mM	6.2	7.3	6.8	9.4	25.3	17.3	29.5	14.5	22.0	22.5	17.5	20.0	17.0	16.1	16.5
45 mM a	ND	ND	ND	1.9	ND	ND	ND	ND	ND	10.1	18.1	14.4	ND	ND	ND
Mean	14.0	12.2	13.1	14.3	18.6	16.7	33.0	24.9	29.0	19.4	18.1	19.0	20.3	18.5	19.4

	SE	
1. Salt treatment	= ± 3.16	NS
2. Genotype	= ± 2.8	**
3. Strain	= ± 1.35	NS
4. Salt treatment vs. Genotype	= ± 5.25	NS
5. Salt treatment vs Strain	= ± 3.56	NS
6. Genotype vs Strain	= ± 3.38	NS
7. Salt treatment vs Genotype vs Strain	= + 6.2	NS

a = Not included for statistical analysis
* = Significant at P< 0.05
** = Significant at P< 0.01
NS = Not significant

with C 11 showing highest AR activity. The AR activity of ICPL 358, ICPL 332 and ICPL 227 were low and did not differ significantly from one another. In ICPL 358 and ICPL 332 the AR activity would have been greater if only the replications where the plants survived were alone considered. **Rhizobium** strains did not differ significantly indicating that IHP 100 and IHP 195 were equally effective in fixing nitrogen. The interaction effects of salt, genotypes and strains were not significant. The specific nitrogenase activity (SNA = AR activity/g.dry nodule weight/hr) data is presented in Table 19. The specific activity increased significantly with increase in salt concentration. There were significant differences in SNA among genotypes. ICPL 332 and C 11 had higher SNA compared to ICPL 358 and ICPL 227. Salt genotype interaction effects were also significant. At 30mM NaCl, ICPL 332 and C 11 showed largest SNA, while ICPL 358 and ICPL 227 did not.

4.3.3. Effect of salt stress on N and P uptake by pigeonpea

The nitrogen content was highest in nodules (mean 6.24%) followed by shoot (mean 3.10%) and roots (mean 1.88%).

Shoot nitrogen content (%) increased up to 30mM NaCl (Table 20). The N content increased significantly with increase in salt concentration even at 45mM NaCl in the tolerant genotype ICPL 227. Genotypes varied significantly

Table:9 Effect of salt (NaCl) stress on specific nitrogenase activity (u M C2 H4/g dry wt. of nodule/hr) of four pigeonpea genotypes inoculated with rhizobium strains IHP 100, IHP 195 and grown in pots (45 days after sowing).

Salt treatment (NaCl)	Pigeonpea genotypes												Rhizobium strain		
	ICPL 359			ICPL 332			C 11			ICPL 227					
	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean
0 mM	23.3	33.3	28.3	30.0	16.7	23.3	50.0	60.0	55.0	30.0	33.3	31.7	33.3	35.8	34.6
15 mM	90.0	26.7	53.3	46.7	40.0	43.3	80.0	40.0	60.0	33.3	33.3	33.3	60.0	35.0	47.5
30 mM	36.7	26.7	31.7	90.0	263.3	176.7	113.3	86.7	100.0	50.0	40.0	45.0	72.5	104.2	88.3
45 mM a	ND	ND	ND	230.0	ND	ND	ND	ND	ND	110.0	50.0	80.0	ND	ND	ND
Mean	46.7	28.9	37.8	55.6	106.7	81.1	81.1	62.2	71.7	37.8	35.6	36.7	55.3	58.3	56.8

	SE	
1. Salt treaatnet	= ± 6	**
2. Genotype	= ± 9	**
3. Strain	= ± 8	NS
4. Salt treatment vs. Genotype	= ± 16	**
5. Salt treatment vs Strain	= ± 10	NS
6. Genotype vs Strain	= ± 16	NS
7. Salt treatment vs Genotype vs Strain	= ± 27	NS

a = Not included for statistical analysis
* = Significant at $P<0.05$
** = Significant at $P<0.01$
NS = Not significant

Table:20.Effect of salt (NaCl) stress on shoot nitrogen percent of four pigeonpea genotypes inoculated with rhizobium strains IHP 100, IHP 195 and grown in pots (45 days after sowing).

Salt treatment (NaCl)	Pigeonpea genotypes												Rhizobium strain		
	ICPL 358			ICPL 332			C 11			ICPL 227					
	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mea
0 mM	3.0	3.1	3.1	2.8	2.9	2.9	2.9	2.9	2.9	2.8	3.0	2.9	2.9	3.0	2.9
15 mM	3.1	3.6	3.4	3.1	3.1	3.1	2.9	3.1	3.0	3.0	3.2	3.1	3.0	3.3	3.1
30 mM	3.2	3.5	3.4	3.1	3.3	3.2	3.3	3.2	3.3	3.2	3.2	3.2	3.2	3.3	3.3
45 mM a	ND	ND	ND	2.4	ND	ND	ND	ND	ND	3.3	3.5	3.4	ND	ND	ND
Mean	3.1	3.4	3.3	3.0	3.1	3.0	3.0	3.1	3.1	3.0	3.1	3.1	3.05	3.18	3.1

	SE	
1. Salt treamtnet	=	± 0.04 **
2. Genotype	=	± 0.05 *
3. Strain	=	± 0.03 **
4. Salt treatment vs. Genotype	=	± 0.08 NS
5. Salt treatment vs Strain	=	± 0.05 NS
6. Genotype vs Strain	=	± 0.06 NS
7. Salt treatment vs Genotype vs Strain	=	+ NS

a = Not included for statistical analysis
* = Significant at P< 0.05
** = Significant at P< 0.01
NS = Not significant

in N content with highest in ICPL 358, while the remaining 3 genotypes had similar N content. **Rhizobium** strains varied significantly in their effect on N content of shoot. IHP 195 was more effective than IHP 100 in N fixation resulting in high N content in plant shoot. The interactions between salt level, genotype and **Rhizobium** were not significant.

In roots also the N content (%) was increased significantly with increase in salt concentration in all genotypes and even at 45mM NaCl in the tolerant genotype ICPL 227 (Table 21). In nodules the N content increased with increasing level of salt up to 30mM (Table 22). At 45mM NaCl, the nodules of surviving plants accumulated even greater N content. Pigeonpea genotypes had significant effect on N content of nodules- ICPL 227 nodules contained the highest N of 6.7% while C 11 nodules contained the least of 5.83%. Strain effects were also significant as nodules formed by IHP 100 had greater N content than those formed by IHP 195. There was no interaction of salt level, genotype and **Rhizobium** strain in nodule N content.

Phosphorous uptake

The phosphorous content was highest in nodules (mean 0.45%) followed by shoot (mean 0.39%) and roots (mean 0.34%).

Table 21: Effect of salt (NaCl) stress on root nitrogen percent of four pigeonpea genotypes inoculated with rhizobium strains IHP 100, IHP 195 and grown in pots (45 days after sowing).

Salt treatment (NaCl)	Pigeonpea genotypes												Rhizobium strain		
	ICPL 359			ICPL 332			C 11			ICPL 227					
	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean
0 mM	1.32	1.39	1.36	1.83	1.88	1.86	1.86	1.83	1.85	1.81	1.85	1.83	1.83	1.86	1.85
15 mM	1.74	1.95	1.85	1.87	1.92	1.92	1.92	1.75	1.84	1.76	1.88	1.82	1.82	1.94	1.88
30 mM	2.01	1.92	1.97	2.14	2.10	2.12	1.88	1.95	1.92	1.96	1.99	1.97	2.00	1.99	1.99
45 mM a	ND	ND	ND	1.72	ND	ND	ND	ND	ND	1.77	1.76	ND	ND	ND	ND
Mean	1.86	1.92	1.89	1.95	1.99	1.97	1.89	1.91	1.90	1.84	1.91	1.88	1.88	1.93	1.91

1. Salt treatment = ± 0.019 **
2. Genotype = ± 0.02 NS
3. Strain = ± 0.01 NS
4. Salt treatment vs. Genotype = ± 0.03 NS
5. Salt treatment vs Strain = ± 0.02 NS
6. Genotype vs Strain = ± 0.02 NS
7. Salt treatment vs Genotype vs Strain = ± NS

a = Not included for statistical analysis
* = Significant at $P < 0.05$
** = Significant at $P < 0.01$
NS = Not significant

Table:22 Effect of salt (NaCl) stress on nodule nitrogen percent of four pigeonpea genotypes inoculated with rhizobium strains IHP 100, IHP 195 and grown in pots (45 days after sowing).

Salt treatment (NaCl)	Pigeonpea genotypes												Rhizobium strain		
	ICPL 358			ICPL 332			C 11			ICPL 227					
	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean
0 mM	6.28	5.89	6.09	6.5	5.81	6.15	5.46	4.2	4.83	6.79	7.35	7.07	6.26	5.81	6.03
15 mM	6.49	5.74	6.11	6.32	6.09	6.20	6.62	5.66	6.14	6.54	5.77	6.16	6.49	5.82	6.15
30 mM	6.36	6.15	6.26	6.51	6.28	6.40	6.66	6.40	6.53	6.98	6.81	6.90	6.63	6.41	6.52
45 mM a	ND	ND	ND	8.15	ND	ND	ND	ND	ND	6.64	9.09	7.87	ND	ND	ND
Mean	6.38	5.93	6.152	6.44	6.06	6.252	6.25	5.42	5.83	6.77	6.64	6.70	6.46	6.01	6.24

	SE	
1. Salt treamtnet	= ± 0.11	NS
2. Genotype	= ± 0.13	*
3. Strain	= ± 0.09	*
4. Salt treatment vs. Genotype	= ± 0.23	*
5. Salt treatment vs Strain	= ± 0.16	NS
6. Genotype vs Strain	= ± 0.18	NS
7. Salt treatment vs Genotype vs Strain	= ±	NS

a = Not included for statistical analysis
* = Significant at P<0.05
** = Significant at P<0.01
NS = Not significant

In shoots, the P content (%) increased significantly with increase in salt level above 15mM (Table 23). Neither genotypes nor **Rhizobium** strains and interactions had any significant effects on P content of shoot.

In roots also the P content increased with salt level and there were no significant changes due to genotypes (Table 24). Inoculation with IHP 100 resulted in increased P content than with IHP 195. It will be interesting to elucidate the role of **Rhizobium** in the P uptake by pigeonpea.

In case of nodules the P content was considerably high at 30mM salt concentration compared to 0 and 15mM salt levels (Table 25). The P uptake was particularly enhanced in plants surviving at 45mM NaCl level. Among the genotypes ICPL 227, ICPL 358 and ICPL 332 took up significantly more P than C 11. It is interesting to note that P uptake was relatively greater in nodules formed by IHP 100 than by IHP 195. The interaction effect between salt levels, genotypes and strains were not significant.

Table:23.Effect of salt (NaCl) stress on shoot phosphorus percent of four pigeonpea genotypes inoculated with rhizobium strains IHP 100, IHP 195 and grown in pots (45 days after sowing).

Salt treatment (NaCl)	Pigeonpea genotypes												Rhizobium strain		
	ICPL 358			ICPL 332			C 11			ICPL 227					
	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean
0 mM	0.33	0.34	0.34	0.39	0.37	0.39	0.38	0.37	0.38	0.37	0.37	0.37	0.37	0.37	0.37
15 mM	0.32	0.34	0.33	0.41	0.36	0.39	0.39	0.36	0.39	0.39	0.36	0.38	0.38	0.36	0.37
30 mM	0.45	0.46	0.46	0.51	0.41	0.46	0.36	0.46	0.41	0.43	0.40	0.42	0.44	0.43	0.44
45 mM a	ND	ND	ND	0.42	ND	ND	ND	ND	ND	0.47	0.42	0.445	ND	ND	ND
Mean	0.37	0.39	0.37	0.44	0.39	0.41	0.38	0.40	0.39	0.40	0.39	0.39	0.39	0.39	0.39

1. Salt treatment = ± 0.01 *
2. Genotype = ± 0.01 NS
3. Strain = ± 0.01 NS
4. Salt treatment vs. Genotype = ± 0.02 NS
5. Salt treatment vs Strain = ± 0.01 NS
6. Genotype vs Strain = ± 0.02 NS
7. Salt treatment vs Genotype vs Strain = ± NS

a = Not included for statistical analysis
* = Significant at P< 0.05
** = Significant at P< 0.01
NS = Not significant

Table:24 Effect of salt (NaCl) stress on root phosphorous percent of four pigeonpea genotypes inoculated with rhizobium strains IHP 100, IHP 195 and grown in pots (45 days after sowing).

Salt treatment (NaCl)	Pigeonpea genotypes												Rhizobium strain		
	ICPL 359			ICPL 332			C 11			ICPL 227					
	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean
0 mM	0.43	0.27	0.35	0.21	0.29	0.25	0.40	0.31	0.36	0.35	0.30	0.33	0.35	0.29	0.32
15 mM	0.33	0.25	0.29	0.40	0.29	0.35	0.40	0.31	0.36	0.37	0.26	0.32	0.38	0.28	0.33
30 mM	0.32	0.39	0.36	0.47	0.38	0.43	0.39	0.38	0.39	0.39	0.35	0.37	0.39	0.38	0.38
45 mM a	ND	ND	ND	0.51	ND	ND	ND	ND	ND	0.39	0.49	0.44	ND	ND	ND
Mean	0.36	0.30	0.33	0.36	0.32	0.34	0.40	0.33	0.37	0.37	0.30	0.34	0.37	0.32	0.34

1. Salt treatment = ± 0.021 NS
2. Genotype = ± 0.024 NS
3. Strain = ± 0.017 NS
4. Salt treatment vs. Genotype = ± 0.425 NS
5. Salt treatment vs Strain = ± 0.03 NS
6. Genotype vs Strain = ± 0.034 NS
7. Salt treatment vs Genotype vs Strain = ± NS

a = Not included for statistical analysis
* = Significant at $P<0.05$
** = Significant at $P<0.01$
NS = Not significant

Table:25.Effect of salt (NaCl) stress on nodule phosphorous percent of four pigeonpea genotypes inoculated with rhizobium strains IHP 100, IHP 195 and grown in pots (45 days after sowing).

Salt treatment (NaCl)	Pigeonpea genotypes												Rhizobium strain		
	ICPL 358			ICPL 332			C 11			ICPL 227					
	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean	IHP 100	IHP 195	Mean
0 mM	0.45	0.43	0.44	0.45	0.42	0.43	0.37	0.29	0.33	0.46	0.53	0.50	0.43	0.42	0.43
15 mM	0.50	0.42	0.46	0.45	0.43	0.44	0.41	0.39	0.40	0.38	0.41	0.40	0.43	0.41	0.42
30 mM	0.50	0.49	0.50	0.50	0.48	0.49	0.50	0.45	0.48	0.52	0.50	0.51	0.51	0.48	0.49
45 mM a	ND	ND	ND	0.96	ND	ND	ND	ND	ND	0.49	0.68	0.56	ND	ND	ND
Mean	0.48	0.45	0.47	0.47	0.44	0.46	0.43	0.38	0.40	0.45	0.48	0.47	0.46	0.44	0.45

1. Salt treamtnet = ± 0.009 **
2. Genotype = ± 0.01 *
3. Strain = ± 0.008 NS
4. Salt treatment vs. Genotype = ± 0.01 NS
5. Salt treatment vs Strain = ± 0.01 NS
6. Genotype vs Strain = ± 0.01 NS
7. Salt treatment vs Genotype vs Strain = ± NS

a = Not included for statistical analysis
* = Significant at P<0.05
** = Significant at P<0.01
NS = Not significant

5. DISCUSSION

5.1. SALT TOLERANCE AMONG PIGEONPEA GENOTYPES

Successful agriculture on saline soils requires the use of crop varieties tolerant to salinity. Screening large pools of genetic diversity and selecting genotypes are the first logical step towards genetic approach to salinity . Salt tolerance has been reported in crops like barley, wheat, rice (Epstein, 1977, Shannon, 1977, Akbar and Yabuns, 1975), and it has been possible to transfer a trait like salt tolerance from the wild species to its related crop species (Rush and Epstein, 1976).

Pigeonpea is an important grain legume of the semi-arid regions where the salinity problem is increasing every year, however, there is little information available on genetic diverity of pigeonpea for salt tolerance.

To find out genotypic variability for salt tolerance in pigeonpea, a laboratory technique for rapid screening has been developed involving growth pouches containing nutrient solution with different salt levels. Growth pouches provided uniform salt stress throughout the growth period and occupied minimum space for testing a large number of genotypes at a time. In this experiment single salt (NaCl) was used because of the report by Ayers and Hayward (1948) that mixed salts were less toxic than single salt. Hence it was assumed that a genotype tolerant to monosalt (NaCl) will be having fair chances of more tolerance to mixed salts, likely to occur under field conditions. Among the salt concentrations used to find out the threshold level of salt

stress in pigeonpea, 60mM salt level was found to be acceptable salinity level, in almost all the 29 genotypes of pigeonpea; beyond which (90 and 120mM) seeds could germinate but the seedlings failed to survive beyond two weeks.

Considerable genotypic diversity for salt tolerance was noticed in pigeonpea. ICPL 227 stood best among 29 genotypes by showing relatively more tolerance in their growth at 60mM salt level. Since there was no survival of genotypes beyond two weeks after germination, 90 and 120 mM salt levels were not considered. At 30mM salt level the shoot growth was stimulated in ICPL 227 but not root growth. Similar observations of stimulation of shoot growth by salt (NaCl) were made in Lupinus luteus (Vansteveninck et al., 1982). In case of T 15-15, the root growth was stimulated but not the shoot growth. The reasons for this differential response of shoot and root growth of a genotype at a particular level of salt stress are not known although such differences have been reported in other crops (Maliwal and Paliwal, 1969).

In the present investigation with pigeonpea, the level of resistance for salt stress varied with genotype. Some genotypes, like ICP 7035, ICPL 304, T7, Bahar were not affected at 30mM salt level, but were severely affected at 60mM salt level. Other genotypes like ICPL 366, ICPL 331, ICPL 227, ICPL 362, ICPL 332 were least affected at 60mM NaCl. In most of the genotypes both shoot and root growth were equally affected. A number of genotypes were intermediate in their tolerance.

Paliwal and Maliwal (1973) reported that there were significant varietal differences in pigeonpea in salt tolerance during germination and early stages of growth. Rao et al., (1981) based on field screening reported that some pigeonpea genotypes ICP 7623, ICP 7118, ICP 7182, ICP 7035 and Atylosia scaraboides (wild species closely related to pigeon pea) were more tolerant in growth at 0.4% salt stress than their tolerant check C 11. Gururaja Rao et al., (1981), on the basis of early screening in laboratory reported that ICP 7035 was salt tolerant at 0.48 salt (NaCl + $CaCl_2$), while in our study both ICP 7035 and C 11 turned out to be susceptible. This difference is probably because of the difference in salts used. Further, under field conditions the salt stress involves a mixture of salts and the composition and concentration vary from place to place and time to time. So it may not be advisable to evaluate the tolerance level of a genotype based on the field screening alone.

In pigeonpea the salt tolerance during germination and early stages of growth was relatively greater than at later stages of growth. The seed germination was delayed with increasing salt concentration. However at lower levels of salt stress the final germination percentage was stimulated in some of the genotypes ICPH 6, Bahar, LRG 36, ICPL 227, ICPL 296, HY 3C, ICPL 42.

The critical stage of salt stress varies with crop species. In case of lentil the critical stage of salt stress was germination and early seedling growth (Jana, 1979), whereas in peanuts,

salt tolerance was more during germination than subsequent growth (Shelwel *et al.*, 1969). From the present results in pigeonpea, it is evident that the evaluation of the genotypes for salt tolerance should not be based on germination and/or early seedling growth alone but based on growth at later stages i.e. about 2 weeks after sowing as well. Eventhough, the tolerant genotype ICPL 227 showed good performance at all stages of its 21 day growth period, many genotypes did not show good performance consistently after germination. In view of this it would be advisable to work out thoroughly the criteria for salt tolerance based on performance at various growth stages of crop.

The present study indicated considerable genotypic variability for salt (NaCl) tolerance. This was based on a study of only breeders promising lines which are usually considered to be having a narrow genetic base due to the continuous inbreeding followed by a selection in a given set of agronomic condition for a particular character usually connected with its yield ability. This being so, greater genotypic diversity can be expected for salt tolerance in pigeonpea germplasm collection which has about 10,000 accessions. With few minor modifications the screening method adopted can be used for large scale screening of pigeonpea germplasm for salt tolerance.

5.2 SALT TOLERANCE AMONG PIGEONPEA RHIZOBIA

Legume-*Rhizobium* symboisis in saline soils may be limited by many factors. One of the important factors is the ability of

Rhizobium to survive and multiply in the rhizosphere of host legume under saline conditions. Rhizobia are considered to be more tolerant to salt than their hosts and, considerable variation among species and strains of Rhizobium with respect to their tolerance to salts has been reported by several authors (Yadav and Vyas, 1971 ; Ethiraj et al., 1972 , Singleton et al., 1982, Rai and Prasad, 1984). In the present experiment with pigeonpea, Rhizobium isolates also showed significant variation in tolerance to salt stress (NaCl) and the tolerance ranged from 0.25% to 5% NaCl in the yeast extract mannitol agar medium.

Interestingly, considerale variation was observed between the fast and slow growing Rhizobia. In case of fast growers, the tolerance ranged from 1% to 5% salt in the medium, while in case of slow growing Rhizobia the tolerance ranged from 0.25% to 2% salt level. Out of the 5 fast growers tested at 8 different salt levels in the medium (0 to 5%) only one strain IHP 24 could grow up to 5% salt level in the medium with roughly 50% reduction in colony size. Further tests revealed that this strain could grow even up to 7% salt level but with colony size drastically affected. This is the first report of a Rhizobium being able to grow up to a salt concentration of 7% NaCl in the medium. The relative tolerance of the 5 fast growing Rhizobia can be shown as : IHP 24 > IHP 506 > IHP 100 > BDNA 2 > IHP 70. Of these, IHP 24 and BDNA 2 were isolated from the normal soils and the remaining from saline soils (native). Bharadwaj (1972) reported that Rhizobia from normal soils could not be well under saline

stress conditions. But we did not find any difference between the native and exotic (from normal soil) strains, in tolerance to salt; in fact the most salt tolerant Rhizobial strain IHP 24 was isolated from the normal soil. Bharadwaj (1975) later reported that he did not find any difference in survival as well as symbiotic ability between native and exotic rhizobial strains. Recently, Singleton et al.,(1982) reported that the isolates from saline soils are not consistently more tolerant to salt than isolates from non-saline soils.

In case of slow growing Rhizobia growth was slightly affected between 1% and 2% salt level. The relative tolerance of the 9 slow growing strains is shown here : IHP 484 > IHP 87 > IHP 213 > IHP 35 > KA 1 > CC 1 > IHP 69 > IHP 195 = F4. All these strains except IHP 69 and IHP 87 originated from normal soils and here also no major difference between native and exotic strains in salt tolerance was observed.

Fast growing Rhizobia were relatively more salt tolerant than slow growers. Pillai and Sen (1969) reported that polysaccharide gum formation in fast growing Rhizobium strains increased with increasing NaCl in the medium, and also there was variation in the capacity of strains to form gum in presence of equal amounts of salts. The production of gum by a strain may be a measure of protection against excess salinity. This explains the reason why fast growers are more salt tolerant than slow growing Rhizobia. Singleton et al., (1982) reported that

the slow growers were not more tolerant than fast growers in case of soybean Rhizobium isolates.

Pillai and Sen (1966) reported that in case of R.trifolii there was a progressive decrease of growth with increase in salinity of the medium. In pigeopea-Rhizobium isolates also there was a progressive decrease in colony size with increasing salinity of the medium ; but with IHP 24 grown up to 3% NaCl there was not much difference in the colony size.

The results of this experiment indicate that many pigeonpea Rhizobia could grow normally at NaCl concentrations that are inhibitory to the host plant i.e. 0.5% NaCl in the medium. The minimum tolerance limit in most of the strains was more than 0.5% salt. So it seems that the survival and multiplication of Rhizobium may not be a limiting factor for establishing pigeonpea-Rhizobium symbiosis under saline conditions.

It may be always better to screen and select Rhizobia for salt tolerance based on growth and symbiotic ability under saline stress conditions rather than taking growth alone as a criterion. Eventhough the host tolerance plays an important role in deciding the symbiotic performance, presence of a tolerant as well as symbiotically efficient Rhizobium is very essential for the successful symbiosis under saline stress conditions.

5.3. EFFECT OF SALT STRESS ON GROWTH, NODULATION, NITROGENASE (ACETYLENE REDUCTION) ACTIVITY, NITROGEN AND PHOSPHOROUS UPTAKE OF 4 PIGEONPEA GENOTYPES INOCULATED WITH 2 RHIZOBIUM STRAINS

Nodule initiation in the legume-Rhizobium symbiosis involves a complex interaction between host root, Rhizobial strain, and the environment. Any assessment of the feasibility of growing legumes under saline conditions needs to consider the effects of salinity on legume-Rhizobium symbiosis. In the present investigation, the effects of salt stress on the symbiotic ability of 4 genotypes of pigeonpea inoculated with two different Rhizobia were studied to find out the involvement of host tolerance to salt in the symbiotic nitrogen fixation. Of the 2 Rhizobium strains, IHP 100 collected from the saline soil was a fast grower and highly salt tolerant, while IHP 195 an exotic strain was a slow grower and sensitive to salinity. Both these strains are effective in N2 fixation in symbiosis with pigeonpea.

In pigeonpea, increasing salt stress decreased the number of nodules and the total nodule dry weight. At 30mM, the number of nodules was severely reduced in case of ICPL 358, ICPL 332 and C 11, but not in the tolerant genotype ICPL 227. However, even at 60 and 75 mM NaCl there was evidence of nodule formation even though the host plant did not survive beyond 25 days. In legumes, the degree of salinity conducive for good nodulation is different from the limits of tolerance of either of the symbiotic partners (Singh et al., 1973). However, the response to salt stress in

nodulation and nitrogen fixation varies with legume species. Nodulation of alfalfa was relatively resistant to salinity (NaCl), whereas nodulation of soybean was severely affected (Bernstein and Ogatta, 1966). In Trifolium alexandrinum, salinity did not affect the nodulation (Pillai and Sen, 1966), whereas in Vicia faba salinity suppressed the number of nodules (Yousef and Sprent, 1983).

It was reported that the early processes involved in nodule formation of soybean were extremely sensitive to salinity(NaCl) than nodule function and development (Singleton and Bohlool, 1984). Several studies indicated that the main effect of salinity (NaCl) on nitrogen fixation resulted from salt injury to the host (Wilson, 1970). Tu (1981) described that the decreased Rhizobial colonization, and shrinkage of root hairs were the main reasons for symbiotic failure in soybean at high salinity while Singleton and Bohlool (1984) reported that in soybean the colonization on the root surfaces was not affected even at high salt stress conditions. Lakshmi Kumari et al., (1974) reported that in lucerne the reduction in nodule number under salt stress was due to suppression of root hairs as well as mucilaginous layer leading to the elimination of rhizosphere and infection thread formation. However, in Prospis tamarugo the symbiosis was not affected even at 3.6% NaCl level (Felker et al., 1981). It is evident that the failing of symbiosis in saline conditions was mainly due to either the host's inability to provide congenial microenvironment to its symbiotic partner Rhizonium or the

Rhizobium's inability to infect the host and cause nodule initiation. If both are efficient, symbiosis may not be a limiting factor under saline stress conditions.

In pigeonpea, IHP 195 was symbiotically more effective than IHP 100 under salt stress conditions, eventhough the latter was a strain collected from the saline soil and highly salt tolerant. The role of Rhizobium in enhancing the tolerance ability of the host is further established with superior performance of a genotype with one strain of Rhizobium and not with other. It appears that in presence of a tolerant host and a symbiotically effective Rhizobium strain under saline stress conditions, failing of symbiosis may not be a limiting factor inc case of pigeonpea.

In pigeonpea, genotypes grown at various salt levels showed no adverse effect on the total nodule activity even though the nodule number and leaf area were considerably affected. It is quite surprising that the total nitrogenase activity was not affected even up to 30mM NaCl, although there was a significant increase in the specific activity under similar salt stress. This is contrary to the reports of Yousef and Sprent (1983), Wilson (1970), Rai (1983), who noticed low nodule nitrogenase activity under salt stress conditions in fababean, soybean and lentil respectively and according to them the reduction of nodule activity was mainly due to the reduction of leaf area and reduction in the photosynthesis rather than the primary salt toxicity to the nodule activity.

The percentage nitrogen in the shoot, root and nodules have shown gradual increase with increasing salt stress. It is not known whether the increase in the specific activity with salinity was due to the quick recovery response of the genotypes (as the nodule activity was measured 20 days after the salt stress was removed) or due to accumulation of intermediate nitrogenous compunds (Strogonov, 1940) or due to protective-adaptive response of the plants binding ammonia etc. (Strogonov, 1958) susceptible genotypes, showed high specific activity than the tolerant genotypes. The high nitrogen percentages in the shoot as well as in the root dry matter may be due to the quick growth after removing salt stress. But whatever may be the nitrogen demand of the plant the nodule functioning is dependent upon the energy supplying capacity of the plant which is directly related to leaf area. Eventhough, the leaf area in pigeonpea was reduced considerably due to the salt stress, the higher demand for nitrogenous compounds during the quick recovery period of growth might have triggered the nodule activity and the plant may try to pump most of the photosynthates to the nodules so that it can assimilate more nitrogen, which is an essential requirement for the growth of the plant. Another possible reason for high specific nodule activity and higher nitrogen per cent in shoot, root and nodules could be the requirement of nitrogenous compounds in its mechanism of tolerance like accumulation of dicartoxylic amino acids in some crops under saline stress conditions to neutralise the toxic effect by accepting of ammonia (Strogonov, 1958).

Under saline condition, phosphorous uptake is likely to be affected (Jana,1979) due to the competition of salt ions with phosphate ions. Since phosphorus has a key role in the nodule development, it is essential to know whether the effect of salt stress on nodule development and nodule activity is due to the primary salt toxicity to the nodules or due to the secondary effect like the nutrient (phosphorous) deficiency. For that, phosphorous content in the dry matter of various genotypes at various treatment salt levels was analysed.

Salinity increased the root phosphorous content in case of soybean (Gates, 1970) and considered to be associated with mechanisms for controlling the salt entering the roots and preventing it, especially the sodium, from passing to the shoot tips. In pigeonpea a higher phosphorous percentage in the shoots, roots, and nodules at 30 and 45 mM salt levels. In case of roots, phosphorous content has been distinctly influenced by Rhizobium strain. All pigeonpea genotypes inoculated with IHP 100 took more phosphorous than with IHP 195. Lie (1971) observed that rhizobia helps in increasing the uptake of phosphorous and other ions by legumes. If the present study is any indication of the involvement of Rhizobium in increased uptake of phosphorous by the host legume, screening of a wide range of isolates might give strains with variable effects on P uptake by pigeonpea. Further studies are needed to elucidate the role of Rhizobia in P uptake and its relation to salinity stress and N fixation in pigeonpea.

The higher percentages of phosphorous in the shoot of pigeonpea and nodules might be due to the necessity of rapid recovery under salt stress and develop tolerance. Since the tolerance mechanism is connected with huge energy requirements, the high phosphorous percentage in the shoot and nodules at higher salt levels may be required for generating more energy (ATP) than what is required for the respiration and carbohydrate metabolism of plants under normal conditions.

In pigeonpea during early stages of growth at 45 and 60mM salt levels leaf necrosis appeared followed by bleaching of chlorophyll and finally to necrosis and death of the plant. Bleaching of chlorophyll is considered to be accompanied by a decrease in the strength of the bond between the green pigment and protein of the chloroplast leading to necrobiosis (Strogonov and Ivanilskaya, 1954). Salinity also induces other changes as Rao and Rao (1982) observed succulence in pigeonpea with NaCl salt stress. The degree of succulence may be associated with the degree of salt tolerance of the plant (Strogonov and Muradova, 1960). In the present experiment, the tolerant genotype ICPL-227 even at 45mM salt level ~~showed~~ no succulence was noticed. A general reduction in the shoot height and plant dry weight at higher salt levels, in addition to the reduction in the leaf area, may be due to inhibition of cell division (Strogonov, 1964) or reduction in the rate of translocation of photosynthates from the leaves to other plant parts as was reported by Deshpande and Nimbalkar (1982) in pigeonpea. There

was considerable genotypic differences in the response of salt stress in pigeonpea.

The present study indicates that considerable genotypic variation exists in pigeonpea in relation to salt tolerance. In the presence of a salt tolerant pigeonpea genotype and symbiotically efficient Rhizobium under saline stress conditions, the failing of symbiosis may not be a limiting factor for normal growth of the pigeonpea. The nodule activity did not seem to be affected by the salt stress whereas, high gram nodule activity and high percentages of nitrogen accumulation was noted in the root, shoot and nodules at various salt levels. The role of rhizobial strains in the phosphorous uptake of pigeonpea is also interesting and worth probing further.

6. SUMMARY

The importance of salt tolerance in pigeonpea is emphasized by the fact that pigeonpea is a major pulse crop of semi-arid land and the area of salt affected soils in arid and semi-arid land is increasing dramatically. One of the approaches of making these lands productive is to modify the crops genetically towards better adaption to saline environment. Since legumes are one of the most important source of protein, a solution in this direction is likely to have a direct impact on the development of agricultural resources contributing towards the economy of nutritional needs. Only a few preliminary studies have been made on the effects of salt stress on edible seed legumes and little is known about the genetics of salt tolerance. Legumes involve an additional challenge, as one must take into account the crop as well as its symbiosis with Rhizobium. Since, there is need for addition and application of genetic dimension to research and development dealing with stress, the present investigation was undertaken with the objective of determining genetic variations in pigeonpea and its rhizobia for salt tolerance as well as the nature of nitrogen fixation. The accomplishments so far have been :

Considerable genotypic variation to salt tolerance in pigeonpea was noticed. Out of 29 genotypes tested at 5 salt levels by solution culture in growth pouches, ICPL 227 grew better at high salt levels than all others and can be considered as a tolerant one. The salt level of 60mM was found to be the limit for survival of pigeonpea genotypes tested.

The salt tolerance during seed germination was relatively ~~greater~~ lower than at latter stages of growth. So it is not advisable to evaluate salt tolerance of pigeonpea based on germination alone but also growth at latter stages (in about 2 weeks after sowing).

A screening technique has been developed which with minor modifications, can be used for large scale screening of germplasm for salt tolerance.

The pigeonpea-positive Rhizobium strains showed significant variation in NaCl tolerance in the yeast mannitol agar medium which ranged from 0.25% to 5% salt level. Strain IHP 24 proved to be most tolerant.

Among the rhizobial strains, fast growers were found to be relatively more tolerant than slow growers, and there was no major difference between native (from saline soils) and exotic (from normal soils) rhizobial strains in their salt tolerance. The most tolerant strain IHP 24 was isolated from normal soil.

In pigeonpea, the survival and multiplication of Rhizobium in the rhizosphere may not be a limiting factor for establishing the symbiosis under saline conditions, as the minimum tolerance limit in most of the strains is more than 0.5% salt.

Screening and selection of Rhizobium for salt tolerance based on growth and symbiotic ability should be a better criterion than growth alone. For example, under salt stress IHP 195 was symbiotically more efficient strain than IHP 100 ,eventhough the latter was found to be more tolerant in terms of growth.

The host tolerance plays an important role in deciding the symbiotic performance but the presence of a tolerant and symbiotically efficient Rhizobium is very essential for a successful symbiosis under saline conditions.

In the tolerant genotypes like ICPL 227 the nodulation and other growth parameters such as leaf-area and dry matter were least affected under salt stress. In case of susceptible or moderately tolerant genotypes nodulation and growth were more affected.

The nitrogen fixing potential of the nodules was not affected by salinity in any of the genotypes though the leaf-area was considerably affected.

The high specific nitrogenase activity and an increase in nitrogen content of shoot, root and nodules under salt stress was surprisingly contradictory to previous reports and raises questions as to the involvement of nitrogenous compounds in the mechanism of salt tolerance.

Under salt stress, phosphorus uptake was not adversely affected and there was an increase in phosphorus concentrations in all the genotypes tested. There was also an effect of rhizobial strain on phosphorus uptake, e.g., pigeonpea inoculated with IHP 100 accumulated more phosphorus than with IHP 195.

7. REFERENCES

Abel, G. H., and Mackenzie, A. J. 1964. Salt tolerance of soybean varieties (Glycine max L.) during germination and later growth. Crop Sci. 4 : 157-161.

Abel, G. H. 1969. Inheritance of the capacity for chloride inclusion and chloride exclusion by soybeans. Crop Sci. 9 : 697-98.

Akbar, M. and Yabuno, T. 1975. Breeding for saline-resistant varieties of rice. III. Response of F_1 hybrids to salinity in reciprocal crosses between Jhona 349 and Magnolia. Japan J. Breed. 24 : 215-220.

Arnon, D. I. 1938. Microelements in culture solution experiment with higher plants. Amer. J. Bot. 25 : 322-325.

Arnold, A. 1955. Die Bedeutung der chlorionen fur die Pflanze. Bot. Stud. 2 : 114-121. *

Ayers, A. D. and Hayward, H. E. 1948. A method for measuring the effects of soil salinity on seed germination with observations on several crop plants. Soil Sci. Soc. Am. Proc. 13 : 224-226.

Balasubramanian, V., and Sinha, S. K. 1976. Effect of salt stress on growth, nodulation and nitrogen fixation in cowpea and mungbeans. Physiol. Planta. 36 : 197-200.

Bates, L. S., Waldren, R. P. and Teare, I. D. 1973. Rapid determination of free proline for water stress studies. Plant and Soil 39 : 205-207.

Bernstein, L. 1964. Salt tolerance of plants. Agric. Inf. Bull. 283. U.S. Department of Agriculture, Washington, D.C.

Bernstein, L., and Ogata, G. 1966. Effects of salinity on nodulation, nitrogen fixation and growth of soybeans and alfalfa. Agron. J. 58 : 201-3.

Batalin, A. 1884. Wirkung des chloronatriums auf die Entwicklung von Salicornia herbacea L. - Bull. Congr. Int. Bot. Hort. St. Petersbourg.*

Bharadwaj, K. K. R. 1972. Note on the growth of Rhizobium strains of dhaincha (Sesbania cannabina) in a saline-alkali soil. Indian J. Agric. Sci. 42 : 432-433.

Bharadwaj, K. K. R. 1975. Survival and symbiotic characteristics of Rhizobium in saline-alkali soils. Plant and Soil 43 : 377-385.

Bhumbla, D. R., Singh, B. and Singh, T. N. 1968. The effect of salt on germination. Indian J. Agron. 13 : 181-185.

Brown, J. W. and Hayward, H. E. 1956. Salt tolerance of alfalfa varieties. Agron. J. 48 : 18-20.

Carr, W. W. and Pollard, T. M. 1979. Effects of fertilizer salt concentration on viability of seed and Rhizobium used for hydroseeding. Can. J. Bot. 57 : 701-704.

Cerda, A., Bingham, F. T., and Hoffman, G. J. 1977. Interactive effect of salinity and phosphorous on sesame. Soil Sci. Soc. Amer. Proc. 41 : 915-918.

Cerda, A., Caro, M. and Fernandez, F. G. 1982. Salt tolerance of two pea cultivars. Agron. J. 74: 796-798.

Chapman, S. R. and Hart, L. 1977. Developing salt tolerant crop varieties. Bull. Montana State Univ. 13 : 10-11.

Chermezon, H. 1910. Recherches anatomiques sur ler plantes littorales. Ann. Sci. Natur. Bot. 9 : 1-12.

Dart, P. J., Day, J. M. and Harris, D. 1972. Assay of nitrogenase activity by acetylene reduction. in IAEA/FAO, Vienna, Technical Booklet "Use of isotopes for study of fertilizer utilization by legume crops". pp. 85-100.

Deshpande, R. and Nimbalkar, J. D. 1982. Effect of salt stress on translocation of photosynthates in pigeonpea. Plant and Soil 65 : 129-132.

Dunnican, I. K. and Cannon, F. C. 1971. The genetic control of symbiotic properties in Rhizobium : evidence for plasmid control. Plant and Soil (Special vol.): 73-79.

Epstein, E. 1977. Genetic potential for solving problems of soil mineral stress : adaptation of crops to salinity. In. M.J. Wright (ed.) Plant adaptation to mineral stress in problem soils, Maryland.

Ethiraj, S., Sharma, H. K. and Vyas, S. R. 1972. Studies on salt tolerance of Rhizobia. Indian J. Microbiol. 12 : 87-92.

Felker, P., Clark, P. R., Leeg, A. E. and Pratt, P. F. 1981. Salinity tolerance of the tree legumes. Plant and Soil 61 : 311-317.

Flowers, T. J., Troke, P. F. and Yeo, A. R. 1977. The mechanism of salt tolerance in halophytes. Ann. Rev. Plant Physiol. 28 : 89-121.

Gandhi, R. C. and Vyas, S. R. 1969. Studies on Rhizobia from different legumes grown in the Punjab, Haryana and Himachal Pradesh. Punjab Agric. Univ. J. Res. 6 : 678-686.

Gates, G. T., Haydock, K. D. and Robins, M. F. 1970. Response to salinity in Glycine : Salt concentration and the content of phosphorous, potassium, sodium and chloride in cultivars of G. wightii. Aust. J. Expt. Agric. Animal Hus. 10: 98-110.

Gleiter, M. E. and Parker, H. E. 1957. The effect of phosphorous deficiency on the free amino acids of alfalfa. Arch. Biochem. Biophys. 71 : 1-2.

Greenway, H. 1973. Salinity, plant growth and metabolism. J. Aust. Inst. Agric. Sci. 39 : 24-34.

Greenway, H. and Munns, R. 1980. Mechanisms of salt tolerance in non-halophytes. Ann. Rev. Plant Physiol. 31 : 149-190.

Gururajarao, G., Mallikarjuna, K., Gopalarao, P. and Rajeswararao, G. 1980. Physiological studies in early seedling growth in green gram (Phaseolus radiatus) under sodium chloride salinity. Indian J. Expt. Biol. 18 : 320-322.

Gururajarao, G., Ramarao, K. V. and Rajeswararao, G. 1981. Studies on salt tolerance of pigeonpea cultivars. Proc.Indian Acad. Sci. 90 : 555-559.

Huber, W. 1974. Uber den Eurifless von NaCl oder abscisinsaure behandlung auf den proteimetabolismus und einige weitere enzyme des aminosaurestoff-weschels in keimlingen von Pennisetum typhoides. Planta 121 : 225-235.*

Imbamba, S. K. 1973. Response of cowpeas to salinity and (2-chloroethyl) trimethyl ammonium chloride (ccc). Physiol. Planta 28 : 346-349.

Jana, M. K. and Slinkard, A. E. 1979. Salt stress in legumes. Proc. Int. Legume Conf., ICARDA, Syria.

Jana, M. K. and Slinkard, A. E. 1979. Screening for salt tolerance in lentil. Lens 6 : 25-27.

Jana, M. K. 1979. Crop improvement in relation to environmental stresses : I. Salt stress in legumes. Harvester 21 : 32-38.

Jana, M. K., Jana, S. and Acharya, N. 1979. Salt stress tolerance in heterogeneous populations of barley. Euphytica 29 : 409-417.

Lakshmi Kumari, M., Singh, C. S.,Sugga Rao, N. S. 1974. Root hair infection and nodulation of lucerne (Medicago sativa L.) as influenced by salinity and alkalinity. Plant and Soil 40 : 261-268.

Lauchli, A. 1984. An adaptation of legumes for crops and pastures under saline conditions. In R. C. Staples and G. H. Toenniessen (Ed.) Strategies for crop improvement. John Wiley & Sons, New York, pp 171-187.

Lauter, D. J., Munns, D. N. and Clarkin, K. L. 1981. Salt response of chickpea as influenced by nitrogen supply. Agron. J. 73 : 961-966.

Lesage, P. 1925. Comparison du chlorcure de sodium, du chlorcure de potassium et de la sylvinite riche dans leur action sur les plantes cultivees. Ann. Soc. Agron.(Paris) 42 : 172-190.

Lie, T. A. 1971. Symbiotic nitrogen fixation under stress conditions. Plant and Soil (Special Vol.) : 117-127.

Lie, T. A. 1974. Environmental effects on nodulation and symbiotic nitrogen fixation in The biology of nitrogen fixation. North Holland Publ. Co. Amstderm.

Maas, E. V. and Hoffman, G. J. 1977. Crop salt tolerance Current assessment. J. Irrig. Drain. Div. ASCE 103 : 115-134.

Maliwal, G. I. and Paliwal, K. V. 1969. Salt tolerance of crops at germination stage. Ann. Arid Zone 8 : 109-125.

Mehta, B. V. and Desai, R. S. 1957. Effect of soil salinity on germination of some seeds. J. Soil Water 6 : 169-176.

Munns, D. N. 1968. Nodulation of Medicago sativa in solution culture : Acid sensitive steps. Plant and Soil 38: 10-15.

Neger, F. W. 1913. Biologie derpflanzen auf experimentiller grundlage (Bionomie). Stuttgart.*

Nieman, H. R. and Clark, R. A. 1976. Interactive effects of salinity and phosphorous nutrition on the concentrations of phosphate esters in mature photosynthesizing corn leaves. Plant Physiol. 57 : 157-161.

Paliwal, K. V. and Maliwal, G. L. 1973. Salt tolerance of some arhar (Cajanus indicus) and cowpea (Vigna sinensis) varieties at germination and seedling stages. Ann. Arid Zone 12 : 135-144.

Philip, J. R. 1958a. Propagation of turgor and other properties through cell aggregations. Plant Physiol. 33 : 1-7.

Philip, J. R. 1958b. The osmotic cell, solute diffusebilitie, and the water economy. Plant Physiol. 33 : 1-4.

Pillai, R. N. and Sen, A. 1966. Salt tolerance of Rhizobium trifolii. Indian J. Agric. Sci. 36 : 80-94.

Pillai, R. N. and Sen, A. 1969. Effects of salinity on the production of gum by Rhizobium species. Indian J. Agric. Sci. 39 : 32-95.

Pillai, R. N. and Sen, A. 1973. Salt tolerance of Rhizobium from Dolichos lablab. Zentralbl. Bacteriol.(Abs) 128 : 538.

Rai, R. 1983. The salt tolerance of Rhizobium strains and lentil genotypes and the effect of salinity on aspects of symbiotic nitrogen fixation. J. Agric. Sci. 100 : 81-86.

Rai, R. and Prasad, V. 1984. Studies on growth and symbiotic nitrogen fixation of Rhizobium of Vigna radiata under stress condtions. J. Agric. Sci. 102 : 399-404.

Ramamoorthy, B. 1964. Some aspects on the management of subwater plant climate complex in the arid zone. Proc. Symp. Problems of Indian Arid Zone. 276-285.

Rao, G. G. and Rao, G. R. 1982. Anatomical changes in the leaves and their role in adaptation of salinity in pigeonpea (Cajanus indicus) and gingelley (Sesamum indicum). Indian Nat. Sci. Acad. Proc. 48 : 774-778.

Rao, G. G. and Rao, G. R. 1981. CO_2 incorporation into leaves of pigeonpea and sesame under salt stress. J. Nuclear Agric. Biol. 10 : 123-126.

Rao, I. M., Venkatratnam, N., Sheldrake, A. R. 1981. Field screening of pigeonpeas for tolerance to soil salinity. Int. Pigeonpea News letter 1 : 23.

Riley, D. and Bamber, S. A. 1970. Salt accumulation at the soybean (Glycine max L.) root soil interface. Soil Sci. Soc. Am. Proc. 34 : 154-155.

Russel, F. S. 1976. Comparative salt tolerance of some tropical and temperate legumes and tropical grasses. Aust. J. Expt. Agric. Animal Hus. 16 : 103-109.

Rush, D. W. and Epstein, E. 1976. Genotypic responses to salinity : differences between salt sensitive and salt tolerant genotypes of the tomato. Plant Physiol. 57 : 162-166.

Shalhevel, J., Reineger, P. and Shimshi, D. 1969. Peanut response to uniform and non-uniform soil salinity. Agron. J. 61 : 384-387.

Shannon, M. C. and Akbar, M. 1979. Breeding plants for salt tolerance In Proc. workshop on membrane biophysics and salt tolerance in plants. Faesalabad, Pakistan, 125-130.

Singh, C. S., Lakshmi Kumari, M., Biswas, A. and Subbarao, N. S. 1972. Nodulation of lucerne under the influence of chlorides of magnesium and potasium. Proc. Indian Acad. Sci. B 76 : 90-96.

Singh, C. S., Lakshmi Kumari, M., Biswas, A. and Sibbarao, N. S. 1973. Effect of carbonate and bicarbonate of sodium on growth of Rhizobia and nodulation in Lucerne (Medicago sativa L.). Indian J. Microbiol. 13 : 125-128.

Singleton, P. W., S. A. El-swaify. and Bohlool, B. B. 1982. Effect of salinity on Rhizobium growth and survival. Appl. Environ. Microbiol. 44 : 884-890.

Singleton, P. W. 1983. A split root growth system for evaluating the effect of salinity on the components of the soybean Rhizobium japonicum symbiosis. Crop Sci. 23 : 259-262.

Singleton, P. W. and Bohlool, B. B. 1983. The effect of salinity on the functional components of the soybean- Rhizobium japonicum symbiosis. Crop Sci. 23 : 815-818.

Singleton, P. W. and Bohlool, B. B. 1984. Effect of salinity on nodule formation by soybean. Plant Physiol. 74 : 72-76.

Smith, M. K. and Mecomb, J. A. 1981. Use of callus cultures to detect sodium chloride tolerance in cultivars of three species of pasture legumes. Aust. J. Plant Physiol. 8 : 437-442.

Sprent, J. 1972. The effects of water stress on nitrogen fixing root nodules. III. Effects of osmotically applied stress. New Phytol. 71 : 451-460.

Steinborne, J. and Roughley, R. J. 1975. Toxicity of sodium chloride ions to Rhizobium spp. in broth and peat culture. J. Appl. Bacteriol. 39 : 133-138.

Strogonov, B. P. 1962. Physiological basis of salt tolerance of plants. Translated from Russian original by Poljakoff-Mayber, A and Mayer, A. M. Jerusalem Israel Program for Scientific translations. (1964).

Storey, R. and Jones, G. W. 1979. Responses of Atreplex spongiosa and Suaeda monoica to salinity. Plant Physiol. 63 : 156-162.

Subbarao, N. S., Kumari M. L., Singh, C. S. and Magu, S. P. 1972. Nodulation of lucerne (Medicago sativa L.) under the influence of sodium chloride. Indian J. Agric. Sci. 42 : 384-386.

Tu, J. C. 1981. Effect of salinity on Rhizobium-root hair interaction, nodulation and growth of soybeans. Can. J. Plant Sci. 61 : 231-239.

Uhvits, R. 1946. Effect of osmotic pressure on water absorption and germination of alfalfa seeds. Amer. J. Bot. 33:

Vincent, J. M. 1962. Influence of calcium and magnesium on the growth of Rhizobium. J. General Microbiol. 28 : 653.

Vincent, J. M. 1977. Rhizobium general microbiology. Pages In R.W.F. Hardy and W. S. Silver, (ed.) A treatise on dinitrogen fixation. John Willey & Sons, N.Y.

Vansteveninck, R. F. M., Vansteveninck, M. E., Stelzer, R. and Lauchli, A. 1982. Studies on the distribution of Na and Cl in the species of lupin (Lupinus luteus and Lupinus angustifolius) differing in salt tolerance. Physiol. Planta. 56 : 465-473.

Weinberg, R. 1970. Enzyme levels in pea seedlings grown on highly salinized media. Plant Physiol. 46 : 466-470.

West, D. W. and Taylor, J. A. 1981. Germination and growth of cultivars of Trifolium subterraneum L. in the presence of sodium chloride salinity. Plant and Soil 62 : 221-230.

Wilson, J. R. and Norris, D. D. 1970. Some effects of salinity on Glycine max and its Rhizolium symbiosis. Proc. Int. Grass Congr. II. 455-458.

Wilson, J. R. 1970. Response to salinity in Glycine. VI. Some effects of a range of short term salt stress on growth nodulation and nitrogen fixation of Glycine weightei (formerly javonica). Aust. J. Agric. Res. 21 : 571-582.

Wilson, J. R., Haydock, K. P. and Robins, M. F. 1970. Response to salinity in glycine. 5. Changes in the chemical composition of three Australian species of G. wightii (G. javanica) over a range of salinity stresses. Aust. J. Expt. Agric. Animal Hus. 10 : 156-165.

Wilson, J. R., Haydock, K. P. and Robins, M. F. 1970b. The development in time of stress effects in two species of Glycine difference in sensitivity to salt. Aust. J. Biol. Sci. 23 : 537-551.

Winter, E. and Lauchli, A. 1982. Salt tolerance of Trifolium alexandricum L. Aust. J. Plant Physiol. 9 : 221-226.

Yadav, N. K. and Vyas, S. R. 1971. Response of root nodule Rhizobia to saline-alkaline and acid conditions. Indian J. Agric. Sci. 41 : 875-881.

Yadav, N. K. and Vyas, S. R. 1971. Effects of salts and pH on the growth of Rhizobium strains. Indian J. Microbiol. 11 : 97-102.

Yousef, A. N. and Sprent, J. I. 1983. Effects of sodium chloride on growth, nitrogen incorporation and chemical composition of inoculated and NH_4NO_3 fertilized Vicia faba L. plants J. Expt. Bot. 34 : 941-950.

* Original not cited.