# DOCTOR OF PHILOSOPHY

## Salt tolerance in Oryza sativa L.(rice)

Akhtar, Nasim

*Award date:*
2001

*Awarding institution:*
University of Wales, Bangor

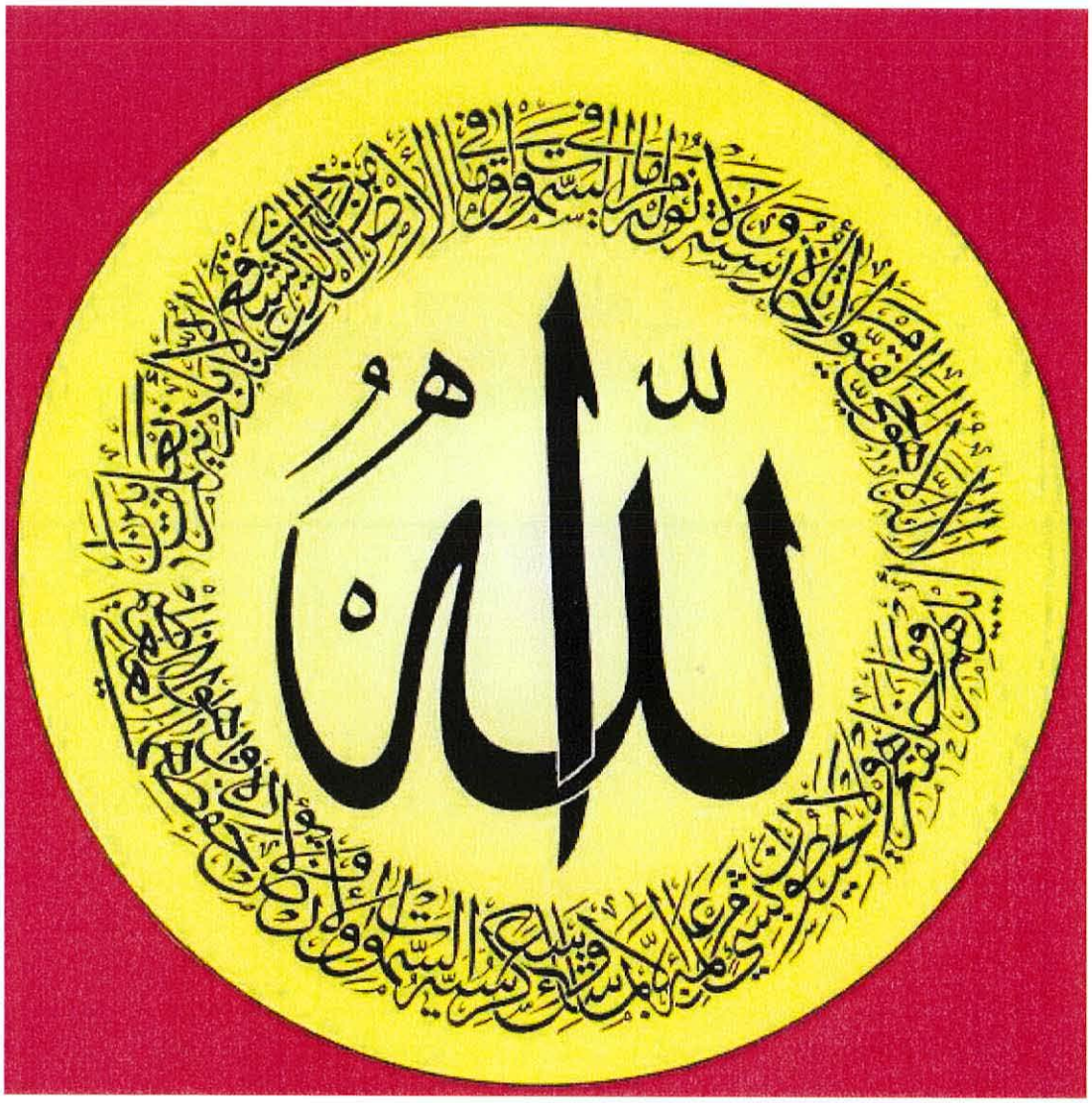
**In the name of Allah,**

**The most Gracious, The most Merciful**

**Thanking**

**Him**

**With whole heart and devoted tongue**



**From The Holy Quraan, Chapter 3, Surah Al-Baqarah, Verse 255 (Ayat-ul-Kursi).**

**Translation**

Allah! There is no god But He (Allah), the Living, The self-subsisting supporter of all No slumber can seize Him Nor sleep. His (Allah's) are all things In the heavens and on earth. Who is that can intercede. In His presence except as He permits? He knows what {appears to His creatures As} Before or After Or Behind them. Nor shall they Compass ought (anything at all) of His knowledge Except As He wants. His Throne does extend Over the heavens And the earth, and He feels No fatigue in guarding And preserving them For He is the Most High, The Supreme in (glory).

# Salt Tolerance in *Oryza sativa* L. (rice).

**Thesis submitted to the University of Wales, Bangor,**
**in candidature for the degree of Philosophiae Doctor**

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**Dedicated to**

All prophets, great women in Islam, Martyrs of Islam, my grandfathers Ch. Fathe-Din, Khushi Muhmmad, Abdullah, Sikandar, grandmothers Lado, Aaishah, Mqabar, Jeanie, my uncles Aabd-ul-Majeed, Ahmad Din, Aabd-ul-Jabbar (died during Ph.D.), Gulam Muhammad, Jalal-ul-Din, Kamal-ul-Din, Commissioner Ali Ahmad, Extra-Engineer Aali Muhammad, Maqbool Ahmad, Shahzadah, Headmaster Noor Muhammad (died during Ph.D.), my Aunt Aazazian (died during Ph.D.) Hajirah, Fadhallan, Raziah (died during Ph.D.) my brothers Yusuf and un-named one day brother, my unseen mother Aazizan, sister Tahira, un-named one day sisters[2], Shamim[2], one of them my beloved sister Dr. Shamim who passed away during my studies for Ph.D. and Dr. Shamim's two children {Rashidah and Imran (Saddam Husain)} who died together in a sudden fire occasion.

My husband's mother Umm-i- Kulthum, my husband`s father Ch. Din Muhammad Numberdar (died during Ph.D.) uncles Aabdul Majeed (died during Ph.D.), Sardar Muhammad, Shafi Muhammad, Noor Muhammad, Aunts Irshad, Beebo and Hussi grandfathers Gulam Ghouth, Muhammad Ismail, Allah Bukhsh Maula Bukhsh, Karim Bukhsh, Khuda Bukhsh, , Qutub-ul-Din, Ramadhan and Muhammad Ali (died during Ph.D.) grand mothers Aaminah, Aalum, Rabo and Ghando, unnamed sisters and Noor Bibi and brother Muhammad Boota (died during Ph.D.).

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## Acknowledgements

All praises to Almighty Allah, the most merciful and the most compassionate, Who enabled me to complete this study. I am also highly indebted to bow my head in front of Him for his countless blessings on me always, for the solution of all the problems and hurdles and for the patience and courage to face the regretful death of my beloved sister during my studies for Ph.D. I am thankful to Muhammad P.B.U.H. whose education is the source of knowledge for all creatures for ever.

I am thankful to Govt. of Pakistan, Ministry of Education Islamabad for COT scholarship and Pakistan High Commission, London for their nice dealing. Reward is going to Extra-Engineer Nazir Ahmad and Dr. Aslam as well for their in time assistance. I am proud of Dr. Altaf-u-Rrehman Rao, Dr. Ferozah Baig, Dr. Humaiun Aarif, Dr. Ijaz Rasool, Dr. Javaid Akhtar, Dr. RH. Qureshi, and Dr. Shafqat Nawaz for their recommendation of this institution for Ph.D.

I am very thankful to great scientist Dr. Wyn Jones for his help. I have no appropriate words to express the deep appreciation, whole hearted obligations, gratitude and thankfulness to my venerable supervisor Dr. John Gorham for his keen and valuable guidance and rewardless suggestions, during the course of investigation and for the inspiration to achieve high quality work. I would like to appreciate his patience especially during coffee time's.

It is a matter of great honour for me to offer my humble thanks towards my chairman, Dr. Cris Marshall for his guidance, consistent encouragement, moral support and decent behaviour.

I am sincerely obliged to my internal examiner, Dr. David Wright for his guidance, precious suggestions, motivation and very ethical attitude.

I am really thankful to all ELCOS staff for helping me and especially to Pany Drower for her rewardless help. I offer my regards to Mrs. Mavis Jones for her suggestions and full help at each and every step. I am also very thankful to Miss Paula and Miss Janat I am really indebted to appreciate the special, in time and quick help of Professor Deri Tomos whenever I was in trouble. I am very thankful to all accommodation and library staff, of the University of Bangor.

I offer my thanks to, whole SBS and Pen-y-Ffridd staff, teachers and friends. I am very thankful to Mr. Julian Bridges for his excellent co-opration and tolerance. I pay my regards to Dr. Katherine Steele, and Beverly Moore for their friendly behaviour and assistance. I am very thankful to Dr. Dave Maggs and Dr. Simon Retallick for their assistance. I am blissful to say thanks to, Mr. Abdullah, and family Dr. Aabd-ul-Rrashid, Mr. Anwar, Dr. Anna Sen, Anne-Marie Fangor, Mrs. Aasyah Khan, Andria, Mr. Arthour, Mr. Andrew Dr. Antonet, Mr. Asmerom, Dr. Charlotte Ager, Cengiz, Mr. Charles, Diana, Dr. Farhah Mansoor, Dr. Farrukh, Dr. Georg, Dr. Gill, Gwen, Mrs. Hajira Salim, Mr. Sultan (Maha Bharat) and family Dr. Habib, Dr. Ibrahim and family, Dr. Ilhan, Inga, Dr. Iftikhar, Dr. Iqbal Javaid, Mr.Javaid Khan, Mr. Javaid Chuhan, Dr. Jamil and family, Dr. Javaid Memon, Mr. Jimmy, Katrin, Dr. Mahar, Mr. Maqbool Ahmad & brothers, Mr. Melood, Qari Mirwaz Khan and family, Dr. Muhammad, Dr. Murtdhaa Randhawa, Dr. Mutlaq, Mrs. Fozia Mutlaq and Children, Mrs. Matin, Dr. Saalah and family, Dr. Nafeesa, Mrs. Nusrat Anwar, Ms.Naris and children, Mrs. Parveen Iqbal, Dr. Rafique Randhawa, Mrs. Riffaat Habib, Mrs. Lubna Rafiq, Mr. Radic, Mrs. Rabiah, Mr. Robin and family, Dr. Rosei. Mrs. Rubina Aftab, Mr. Salim, Mr. Shabab, Dr. Shahid, Dr. Shallet, Mrs. Shahnaz Maqbool, Dr. Sheela Gunn, Mrs. Shirley Edwards, Mr. Seyum, Dr. Sona, Dr. Sue

Jones, Dr. Sayyed, Mrs. Tahira Shahid, Mr. Tariq and family, Dr. Wendy, Dr. Waqqas Khan, Yamane, Dr. Yusuf, Mr. Yusuf Mansoor, Dr. Zahrah and family and Muslim women group in Bangor for their help and sufferance. My whole hearted regards are offered from the core of my heart to my sincere friend Dr. Riaz Mansoor (Physician Consultant) for his valuable suggestions, moral support and sincere guidance. I pay my thanks to all my previous and present neighbours in Pen Castle, all my friends in Bangor, Pakistan and any where in the world. I am very thankful to my affectionate teachers (at any stage, any where in the world), parents, sisters, brothers, aunts, uncles, friends and relatives for their contributions and especially to my uncle Aabd-ul-Majeed (late) for helping to set the foundations required to enable me to develop a well-balanced personality. I am thankful to Mian Rafique for his Duaa (Prayer) from Allah for my Ph.D. High contributions of my mother Iqballan, father Saif-u-llah (Bullah Saitth), aunt Ghulam Fatimah, sisters Hajirah, Lecturer Khadijat-ul-Kubra, Dr. Shamim (late), Zarrin and brothers Dr. Aabd-ul-Rrehman, Journalist Khalid Masood, Director Sultan Mahmood, Barrister Muhummad Yusuf, Director Muzzammil Pervaiz (Majeed) and Commissioner Tariq Ayyub Chauhdry cannot be ignored. Passions of my mother Iqbalan, grandmother Salamat, aunts Aalum, Jameelah, Niazan, uncles Ch. Muhammad Boota, Muhammad Yusuf, Muhammad Akbar, Muhammad Iqbal, Major Umar Din, Aabd-ul-Aaziz, Aabd-ul-Rrashid, Secretory Law Session Judge Shafique Ahmad, sisters Abidah[2], Dr. Aaminah, Anwar, Lecturer Khalidah, Teacher Karim, Majeedah, Mumtaz Kousar, Munuwwar, Nazirah, Teacher Nadimah, Saidi, Sakinah, Salmaa, Sairah, Sajidah, Shahnaz, Hameeda, Farhat, Fridah Khanum, Teacher Nabilah, Lecturer Nasim, Nazinah, Saimah, Samiah, Samina, Shafqat, Zarina[2], Nawaz, Tariq, Rukhsana Javaid, Saidah, Sunmbal, , and brothers Ch. Muhammad Shahzadah Salim Sarwar, Ch. Irfan Mahmood, Amir Muhammad, Electrician Arshad, Taj Muhammad, Mechanic Afzal, Ashfaq, Govt. Cotractor Ahmad Hassan, Headmaster Atta-u-llah, Manager Ali Muhammad, Advocate Fazal, Railway officer Bashir Ahmad, Advocate Bashir Ahmad, Ch. Khushi Muhammad, Khalid, Nazir Ahmad, Munir Ahmad, Anees Ahmad, Director of Agriculture Faqir Muhammad, Engineer Gulam Qadar, Police Inspector Nasir Ahmad, Muhammad Tahir, Bashir Ahmad, Mushtaq, Muhammad Mukhtar, Manager Masood Akhtar, Barister Iqbal-ul-Din, Dr.Afzal, Irshad Ahmad, Ashfaq Ahmad, Police Inspector Sardar Muhammad, Sharif, Engineer Shahzad, Engineer Shoukat, Sana-u-llah, Liaqat, Lecturer Zaid-ul-Ddin, my niece Aamnah[2], Aasifah, Aasmah, Anilah, Aqsah, Anam, Hafiz,Arjmund, Bismillah, Fatimah[2], Firdous, Teacher Gulnaz (Hafiz), Iqraa, Jameelah, Marium, Teacher Mehnaz, Nargis, Rahilah[2], Ruqayyah, Saadiah, Shaheen, Shahidah, Shakeelah, Sunilah, Tunzeelah, and nephews, Aabd-ul-Munnan, Aadil, Aabdullah[2], Ali Akbar, Ahsan, Arslan, Hashim, Imran, Luqman, Majeed-ul-Rrehmaan, Habib-ul-Rrehmaan Mohsin, Mubashshar, Nasar-u-llah, Rehan, Rizwan, Salman, Shahid, Shoaib[2], Dr. Tauqeer, Umar[2], Qasim, Hafiz Waheed-ul-Din, Yasir, Zafar Iqbal, Zulfiqar and my husband's mother Ghulam Fatimah, niece Aiman brothers Saaid[2], Javaid, Zafar, Aabd-ul-Rrahmaan, Asghar Ali, Irshad, Afzal[2], Naeem, Aazaim, Arshad[2], Shafiq Ahmad and Naqshband, sisters Waseem, Bilquis, Farzanah Musarrat, Nasim[4], Rubinah, Nusrat[2], Rashidah, Najmah[2] and Yasmin, Aunts, Bukhshi, Ghouth, Karmat, Khurshid, Munirah, Fazlan, Akbari, Rani, Razia, Uazmat, uncles Muhammad Ali, Asghar Ali, Gulam Rasool, Abdul-Majeed Suliman, grandfathers Gulam Gouth, Abdullah, Muhammad Ali, and grand mothers Aaminah, Karmat Haleemah and Kulthum, cannot be ignored.

Last but not least I am thankful to my Parents, father Saif-ullah and mother Iqbalan, husband Mr. Muhammad Salim and children, Muhammad Mizfar Salim and Muhammad Minwar Salim who suffered a lot but gained a lot as well due to my studies for Ph.D in Great Britain.

# Abstract

The effect of salinity was influenced by various parameters including systems of salt application, variety, salts, duration of salinity and leaf position. The effect of salinity in *Oryza sativa* L., as in barley, was influenced by methods of salt application. CM-67 barley, Maratelli rice were salt susceptible and salt resistant under foliar and root treatments respectively on a shoot fresh weight basis but Maratelli on dry weight basis as well. However the opposite was true for Moroberekan.

High accumulation of ions cause dehydration of tissues leading to ion toxicity and low turgor. Transpirational bypass flow was not a major pathway for $Na^+$ transport in these varieties because there was a negative relationship between fluorescence and $Na^+$ concentration in the varieties. Ion concentrations were variable and low $Na^+$ and high $Cl^-$ concentrations were observed in all varieties and in RILs (Co39 x Moroberekan). Although $Cl^-$ concentrations were higher than $Na^+$ in rice, it is not clear which is more toxic.

Salt was toxic to parental varieties and 170 recombinant inbred lines from $F_8$ generation (Co39 x Moroberekan) on shoot fresh weight and resistance score basis. In general, Moroberekan had higher accumulation of ions particularly $Na^+$ and $Cl^-$ than Maratelli and Co39 under salinity. Maratelli had earlier anthesis than Moroberekan. There were significant negative correlations for $Cl^-$, $Na^+$ and the sum ($Na^+$+$K^+$) with resistance score, SFW and water content under salinity. $Na^+$ was significantly but negatively correlated with SDW under salinity. A significant and negative correlation was found between the sum and water content at later stages of salinity.

Various QTL for physiological and growth traits were detected on different chromosomes of rice. Marker RZ276, and the region around it on chromosome 1 is important for salt tolerance in rice because of the presence of consistent QTL for $Na^+$ accumulation.

# List of abbreviations

| Abbreviation | Description |
|---|---|
| A | Assimilation rate |
| AFLP | Amplified Fragment Length Polymorphism |
| ANOVA | Analysis of Variance |
| $c_0$ | Mole fraction of $CO_2$ in outlet air from leaf chamber |
| ci | Internal $CO_2$ concentration, |
| Conc. | Concentration |
| DNA | Deoxyribo Nucleic Acid |
| dS $m^{-1}$ | deci Siemen per meter - unit of conductance |
| DW | Dry Weight |
| E | Transpiration rate (mol $m^{-2}$ $s^{-1}$) |
| EC | Electrical Conductivity |
| ECo | Electrical conductivity for zero yield level |
| ECt | Electrical conductivity for threshold value |
| ESP | Exchangeable Sodium Percentage. |
| Expt. | Experiment |
| f | Mole flow of air (mol $s^{-1}$) |
| F2 | $2^{nd}$ Filial generation |
| F8 | $8^{th}$ Filial generation |
| F9 | $9^{th}$ Filial generation |
| Fig | Figure |
| FMS | Fluorescence Monitoring System |
| Fm | Maximum fluorescence |
| Fo | Minimal or initial fluorescence |
| Fv | Variable fluorescence |
| $f_v$ | Volumetric flow of air ($cm^3$ $min^{-1}$) |
| Fv/Fm | Photochemical efficiency |
| FW | Fresh Weight |
| FW/DW | Fresh Weight Dry Weight ratio |
| g $l^{-1}$ | gram per litre |
| g | Grams |
| GLM | General Linear Model |
| gs | Stomatal conductance (mol $m^{-2}$ $s^{-1}$) |
| h | Hour |
| IRGA | Infra-Red Gas Analyser |
| kg $kg^{-1}$ dw | Kilogram per kilogram dry weight |

| | |
|---|---|
| L | Root hydraulic conductance |
| l | litre |
| mOsmol $kg^{-1}$ | milli Osmomole per kilogram - unit of osmolality |
| m | meter |
| min | minute |
| mg $l^{-1}$ | milligram per litre |
| ml $l^{-1}$ | Millilitre per litre |
| mM | milliMolar |
| mmol $kg^{-1}$ dw | Milli mole per kilogram dry weight |
| mmol $kg^{-1}$ | millimole per kilogram |
| MO | Moroberekan - rice variety |
| mol $m^{-3}$ | mole per meter cube |
| MPa | Mega pascal |
| MR | Maratelli - rice variety |
| n | No. of replicates |
| No. | Number |
| p | Atmospheric pressure during measurement (kPa) |
| Pn | Net photosynthesis rate |
| ppm | parts per million |
| PTS | Trisodium-8-hydroxy-1,3,6-pyrenetrisulphonic acid |
| QTL | Quantitative Trait Locus/Loci |
| RAPD | Randomly Amplified Polymorphic DNA |
| RFLP | Restriction Fragment Length Polymorphism/ Polymorphisms |
| RH | Relative Humidity |
| RIL/RILs | Recombinant Inbred Line/Lines |
| rpm | Revolution per minute |
| RWC | Relative Water Content |
| SAR | Sodium Absorption Ratio |
| SDW | Shoot Dry Weight |
| SFW | Shoot Fresh Weight |
| SPAD | Soil Plant Analysis Development - For chlorophyll measurements |
| T | Absolute temperature |
| T1 | Actual leaf temperature |
| Treat | Treatment |
| VPD | Vapour Pressure Deficit |
| Vs | Shoot volume |
| W | Watt |

| | |
|---|---|
| $x_0$ | Mole fraction of water vapour at leaf chamber outlet (mol $mol^{-1}$) |
| $x_e$ | Mole fraction of water vapour at leaf inlet (mol $mol^{-1}$) |
| $x_s$ | Mole fraction of water vapour at saturation. |
| xs | Saturated vapour pressure |
| Ym | Maximum yield under non-saline conditions |
| $\psi$ | Water poential |
| $\Delta\psi$ | Total water potential difference between the leaves and the root |
| $\Delta c$ | $CO_2$ differential between reference and analysis streams (mol $mol^{-1}$) |
| $\mu g\ l^{-1}$ | microgram per litre |
| $\mu g\ ml^{-1}$ | microgram per millilitre |
| $\psi L$ | Decline in leaf water potential |
| $\psi p$ | Turgor potential |
| $\psi s$ | Solute potential |

# Chapter 1

# General Introduction

## 1.1 Introduction

Salinity is the accumulation of salts such as sodium chloride, calcium chloride, sodium sulphate and calcium carbonate in the soil in any proportions, possibly accompanied by high levels of boron and selenium. Magnesium salts may also be present. At the same time some nutrients may become deficient, especially N, P and K. Because of this, salinity itself is a complex phenomenon and plants show different symptoms and responses under varying salinity conditions. Additionally there are some interactions with other environmental factors that can modify the effects of salinity on plants. These factors could include pollution, temperature, humidity, topography, net radiation, water content in the root zone and the availability of oxygen or carbon dioxide. It was observed by Cerda *et al.* (1977) that factors like plant species, salinity concentration and nutrient levels influence the effects of salinity. It is clear that not only salinity itself but plant responses and overall effects are very complicated. Therefore salinity cannot be studied in isolation (Flowers, 1985; Gorham, 1992).

Salinity is often associated with waterlogging and poor soil structure, or with drought and high temperatures. Stressful environments are often characterised by the occurrence of more than one stress simultaneously, and hence efforts to overcome stresses by breeding or management are also complicated. Any change in environmental conditions that might reduce or adversely change a plant's growth or development is called biological stress. Biological strain is the reduced or changed function (Levitt, 1972). Soil salinity restricts growth in many temperate regions as well as sub-tropical arid regions (Greenway and Munns, 1980).

Soils are classified into three categories (Richards, 1954). Saturated saline soil solution has an electrical conductivity ($EC_s$) greater than 4 dS $m^{-1}$, a value of exchangeable sodium percentage (ESP) less than 15, a sodium absorption ratio (SAR) less than 13 and a pH less than 8.5. Sodic soils have an ($EC_s$) < 4 dS $m^{-1}$, an ESP > 15,

a SAR > 13 and pH > 8.5. There may be deficiency of iron, phosphorus, zinc and sometimes even calcium or potassium. These soils are waterlogged with poor aeration and poor structure. Saline sodic soils have an $EC_s$ > 4 dS $m^{-1}$, but an SAR > 15 and pH > 8.5 (Marschner, 1995).

In a saline environment plants face two kinds of problems. The first is of survival and the other is of productivity. In the natural environment survival is the major problem but productivity has to be considered if competition exists. For agriculture, however, productivity is the major consideration because of its economic value (Barrett-Lennard, 1986).

Plants are categorised on the basis of their responses to salinity. The plants that like sweet (non-saline) water are called glycophytes. Halophytes are those species that normally grow in saline or brackish water or soil. The strict halophytes are those plants that can grow better in the presence of salts than without salts. The euhalophytes are true halophytes that can tolerate or endure high levels of salt. *Halobacterium* cannot grow unless the soil is salty and this group of plants is called obligate halophytes (Gorham *et al.*, 1985).

### 1.1.1 Saline areas

The saline areas of the world consist of salt marshes of the temperate zones, mangrove swamps of the subtropics and tropics and interior salt marshes adjacent to salt lakes. Saline soils are abundant in semiarid and arid regions where the amount of rainfall is insufficient for substantial leaching. Salinity problems occur in non-irrigated croplands and range lands either as a result of evaporation and transpiration of saline underground water or due to salt input from rainfall. Salinity is particularly critical in irrigated areas. Salinity has been an important historical factor and has influenced the life spans of agricultural systems, *e.g.* in Mesopotamia. More recently large areas of the Indian subcontinent have been made non-productive by salt accumulation and poor water management. The problem in Pakistan is that saline-sodic lands already have high water tables.

Arid and semiarid areas in the world particularly face the problem of salinity. Salinity is classified in two groups depending upon its being natural or man-made (artificial). The natural one is called primary and man-made salinity is called secondary

salinisation. Natural (primary) salinity occurs in Australia and some other areas where seas have been eliminated by evaporation and salts are left behind. On the other hand secondary salinity is man-made because it is due to mismanagement of irrigation systems. Secondary salinisation is particularly important in Asia, for example in India and Pakistan. Salinity can cause economic losses of hundreds of millions of dollars per year to agriculture (Ghassemi *et al.*, 1995). About 7 million ha. are under severe salinity but 20 million ha. are potentially saline (Mohan *et al.*, 2000). Different organisations (WAPDA, FAO, UNESCO, UNEP, GLASOD) have given estimates for saline lands in various countries and in the world, but there is variation among the figures due to different techniques and criteria used for measurements. Some figures for the saline area in Pakistan are given in Table 1.1.

**Table 1.1 Estimated salt-affected land area ($10^6$ ha) in Pakistan from Economic Survey of Pakistan, 1999-2000**

| Province | Area of the state | Total of salt-affected land | % Salt-affected land |
|---|---|---|---|
| **Baluchistan** | 34.72 | 0.11 | 0.32 |
| **NWFP** | 10.2 | 0.52 | 5.10 |
| **Punjab** | 20.62 | 2.77 | 13.41 |
| **Sindh** | 14.1 | 2.62 | 18.55 |
| **Total (1998-99)** | 79.61 | 6.67 | 8.38 |
| **From data collected by Singh (1992).** | | | |
| **Total** | 79.64 | 6.01 | 7.55 |

### 1.1.2 Rice

Rice is an economically important and valuable cereal crop. Although rice is a salt-sensitive crop it is very tolerant to flooding. It is not easy to replace rice with a more salt-resistant crop that would not suffer from waterlogging and submergence in the rainy season (Yeo and Flowers, 1989). The Asian cultivated rice, *O. sativa,* is an economically important staple food for more than one half of the world population. Wild relative species in the genus *Oryza*, together with weedy rice and different rice varieties, serve as an extremely valuable gene pool that can be used to broaden the genetic background of cultivated rice in breeding programmes (Brar and Khush, 1997;

Bellon *et al.*, 1998). Rice is economically important for Pakistan. The economic status of rice is shown in the following table (Dr. Javaid Akhtar, personal communication).

**Table 1.2 Information about rice in Pakistan**

| Year | Av.Yield(kg/ha) | Area ($10^6$ ha) |
|---|---|---|
| 1998-1999 | 1928 | 2.4 |
| 1999-2000 | 2050 | 2.6 |

The genus *Oryza* was firstly described by Linnaeus in 1753 to recognise only one species (*O. sativa)* based on samples of cultivated rice from Ethiopia (Hubshah). *Oryza sativa* L. (rice) is a cereal crop with many spikelets and the spikelets are made up of glumes and florets. The genus *Oryza* belongs to the section Euoryza of the tribe Oryzeae, subfamily Oryzoideae of the grass family Poaceae (Gramineae) (Vaughan, 1989; Woodland, 2000). This genus has two cultivated species, *O. sativa* L. and *O. glaberrima* Steud. However, more than 20 wild species are distributed throughout the tropical and subtropical parts of the world (Brar and Khush, 1997; Bellon *et al.*, 1998).

Rice shows high sensitivity to NaCl salinity. Even 50 mol $m^{-3}$ NaCl was lethal for rice at the seedling stage (Yeo *et al.*, 1990). Details of responses of plants to salinity are given in subsequent sections.

## 1.2 Salinity tolerance models

The responses of plants to salinity can usually be described in terms of threshold values, below which yield is not affected and above which yield decreases linearly with salinity. Plant growth responses to salinity can be measured in different ways and at many developmental and growth stages.

### 1.2.1 Linear response model to salinity by Maas and Hoffman

This model (Figure 1.1) was proposed by Maas and Hoffman in 1977. According to the model, crops tolerate salinity up to a certain threshold level. Above the threshold level the yield for each crop decreases linearly. The threshold value was specific for each crop.

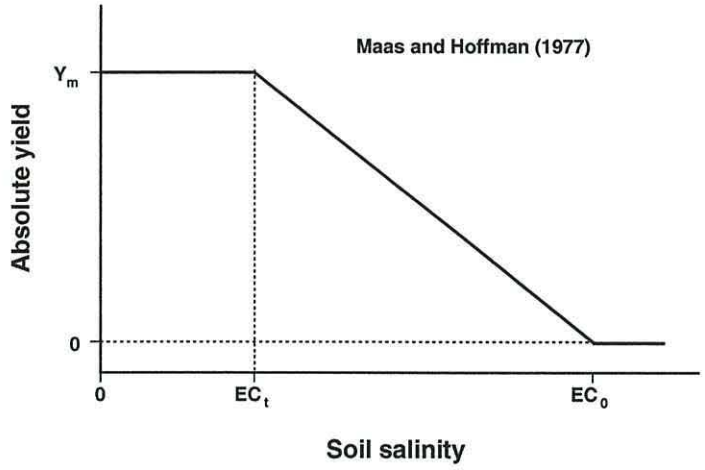


**Figure 1.1 Salt tolerance model of Maas and Hoffmann**

For the calculation of relative yield the following equation was given.

$$Y = 100 - B ( EC_e - A ).$$

In the above equation relative yield is denoted by Y, above the threshold value at any soil salinity level, $EC_e > A$ and can be calculated by the above equation.

A = Threshold salinity

B = The reduction in yield (percentage) per unit salinity increment (slope)

In research and management the threshold-slope model of Maas and Hoffman (1977) proved to be very useful. Maas and Hoffman collected and normalised all available salt tolerance data from the previous 30 years to present this model for the salt tolerance of agricultural crops. This model includes only the data correlating plant response to the total soluble salts in the root medium. Absolute yield reductions for crops grown under saline conditions are subject to numerous cultural and environmental effects including the interactions between salinity and various soil water and climatic factors that could change the salt-tolerance of the plant (Maas, 1985). This model was updated by Maas in 1993.

### 1.2.2 Sigmoidal salinity response model by Van Genuchten

The salt tolerance database of Maas and Hoffman (1977) was re-analysed by Van Genuchten in 1983. A curve (Figure 1.2) was obtained with a non-linear least-squares

parameter, which fits the three unknown coefficients ($Y_m$,$c_t$ and S) of the threshold slope model to the given data (Van Genuchten, 1983).

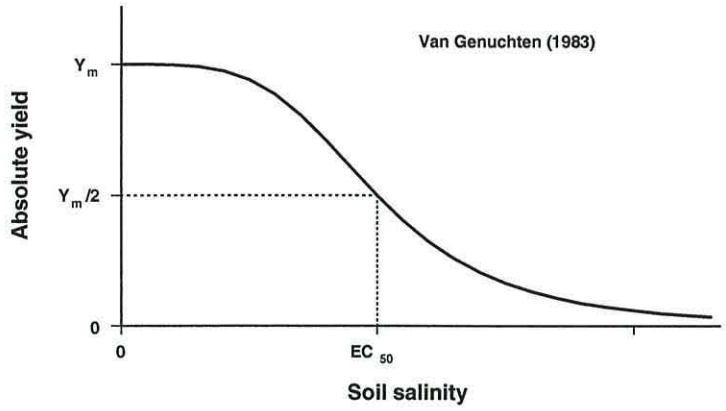


**Figure 1.2 Salt tolerance model of Van Genuchten**

An improved S-shaped salinity response model was described by Van Genuchten and Hoffman in 1984 in an alternative form. To re-analyse the salt tolerance database of Maas and Hoffman (1977) one of the smooth S-shaped response functions of Van Genuchten and Hoffman (1984) was used. A single dimension-less curve could be used to represent the salt tolerance of most crops (Van Genuchten and Gupta, 1993).

### 1.2.3 Dalton's salt loading concept model

The xylem ion flux, Js, controlling salt loading to the shoot was described in terms of ion and water flux across the root surface by Dalton *et al.* in 1975. Molar flux in terms of nutrient solution, osmotic potential and the root coefficient could be calculated.

Molar ion flux was heavily dependent on transpiration and the biophysical properties of the root (Dalton *et al.*, 1975). Instead of osmotic permeability and reflection coefficient, a metabolically active term, the metabolic component of the ion flux represented by k was important to both the salt loading processes and water use. A link to yield was provided by this salt loading concept of plant responses to salinity in terms of root zone salinity and bio-physical properties affecting transpiration as well as plant geometry as it pertained to root and shoot surface areas across which ion and water transport occurred (Dalton and Gardner, 1978).

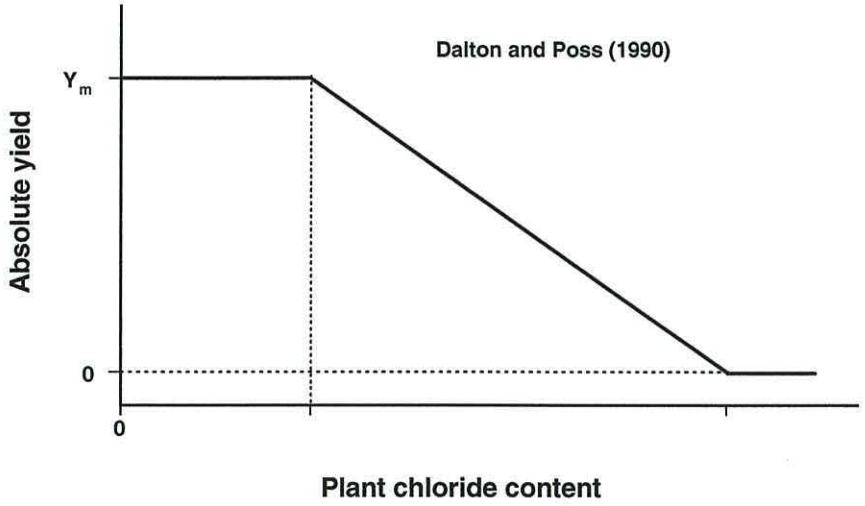


**Fig 1.3 Salt tolerance model of Dalton**

The relationship shown in Figure 1.3 was an improved form of Dalton's model, reformed by Dalton and Poss (1990). Salinity stress index was introduced by this model and it was given in terms of Js and Vs, that is xylem solute flux and the shoot volume production rate respectively. The shoot volume production term was supposed to be an intrinsic property of the plant and it could be taken into account experimentally with fresh weight measurements. The ion flux could be calculated in terms of osmotic potential of the soil solution water and ion transport coefficients of the root, the volume flux of water into the root and the active root surface area (Dalton and Poss, 1990).

## 1.3 Mechanism of Growth Reduction

In *Hordeum vulgare* water deficit occurred in the elongating leaf tissue due to insufficient rates of uptake of $Cl^-$ and ($Na^+ + K^+$), and growth was limited by slow rates of increase in vacuolar volume rather than by slow cell division (Munns *et al.*, 1983). Reduction in photosynthesis was the primary cause for growth reduction at high NaCl (Munns *et al.*, 1983). The reduction in the hydraulic conductance of the pathway from the external medium to the expanding leaf cells might also be the cause of reduction in leaf size under salinity (Yeo *et al.*, 1991). Turgor and growth both decline with increasing NaCl because active ion influx probably becomes saturated if the uptake of $Cl^-$ and $Na^+$ is mainly by active processes (Oertli, 1975). The contribution of $Cl^-$ and $Na^+$ uptake by shoots of halophytes (*Suaeda maritima*) to osmotic adjustment was

dependent on the ion fluxes across the plasma membrane and as a result ion concentrations in the cell walls would be determined by the rates of supply and uptake into the symplast (Oertli, 1966,1968; Greenway and Munns, 1980,1982). High ion accumulation in the apoplast of the mature cells might be responsible for low turgor, a probable cause of low growth rates (Flowers and Yeo, 1988). Similar ideas were proposed for both halophytes and glycophytes by various scientists (Greenway and Munns, 1980; Leigh and Tomos, 1983; Munns and Passioura, 1984; Clipson *et al.*, 1985; Flowers *et al.*, 1985*a*; Flowers *et al.*, 1985*b*; Yeo *et al.*, 1985*b*). Both the cytoplasm and apoplast are small compartments. The effects of excess salt may be enlarged by imbalance between compartments, resulting in toxicity in spite of apparently moderate overall tissue concentrations (Yeo and Flowers, 1989).

Water relations are responsible for the sudden changes in leaf elongation in wheat resulting from sudden changes in water stress, but changes in leaf elongation are transient if induced by sudden changes in humidity, heat or minor changes in soil salinity (Cramer and Bowman, 1991; Neumann, 1993). However, permanent changes in leaf elongation rate were found with sufficiently large changes in salinity (Munns *et al.*, 2000).

Changes in root growth rate were associated with changes in cell wall properties such as plasticity and elasticity, and not with changes in turgor pressure (Pritchard *et al.*, 1987,1991). Reduced root elongation growth was correlated with hardening and rigidity of the cell walls in the growing region (Pritchard *et al.*, 1988). A small number of physical parameters such as cell wall rheology, membrane and tissue hydraulic conductivity, membrane and tissue solute transport contribute towards the control of uniform and differentiated growth of cells (Tomos *et al.*, 1989).

Physiological and biochemical responses to salt stress and dehydration stress were very similar (Cushman *et al.*, 1990). These were reduced numbers of mitochondrial christae, hypertrophy of golgi apparatus and endoplasmic reticulum in *Zea mays* (Yeo *et al.*, 1977) and reduced oxidative phosphorylation in *Pisum sativum* (Flowers, 1974). Such changes presumably reduced levels of ATP in leaves of salt-stressed plants of pepper (*Capsicum annum*) and safflower (*Carthamus tinctorious* L.) (Nieman *et al.*, 1988). The reductions were most extensive in the more salt-sensitive pepper. Cytoplasmic ATP is generated entirely or predominantly by respiration, so dependence

of growth on respiration could explain the carbohydrate accumulation in non-halophytes when subjected to salt stress. Chloroplast ATP pools were not affected initially by salt stress so that photosynthetic carbon reduction continued while growth was reduced by a depletion in cytoplasmic ATP pools and a reduction of cytoplasmic phosphate potential.

Decreased efficiency of ribulose bis-phosphate carboxylase-oxygenase was observed in bean (Seeman and Sharkey, 1986) and loss of assimilates was thought to be responsible for the reduction of growth. In salt excluders (mostly monocots and especially legumes) the predominant reason for injury is water deficit resulting in loss of turgor followed by a decrease in cell expansion (decrease in cell size) and reduced photosynthesis (Wyn Jones, 1981; Gorham *et al.*, 1985). Growth might be reduced due to limited delivery of solutes by the phloem transport system under salt stressed conditions to enlarging tissues (Gorham *et al.,* 1985).

### 1.3.1 Plant age and stage of growth

Changes in salt resistance during rice development were reported by Heenan *et al.* (1988). They observed that young seedlings and flowering stages were the most sensitive. Changes in salinity resistance with growth stage might not exhibit the same pattern in different genotypes, therefore the relative resistance of various varieties might not be the same at each stage. It was doubtful that resistance at one stage implied resistance at other stages (Lutts *et al.*, 1995).

Lutts *et al.*, (1995) observed the effects of stress at 20, 30, 40, and 50 mol $m^{-3}$ NaCl on the indica rice cultivars Nona Bokra, Buhra Rata, Panwell, and Pokkalli, and the japonica cultivars varieties I Kong Pao (IKP) and Tainung 67 and the elite breeding lines IR-4630, IR -2153 and IR-31785. All indica cultivars were found to be relatively salt resistant. Under the same saline conditions, japonica lines showed fluctuating resistance, especially the lines IR-4630, IR 31785 and IKP which exhibited the greatest variability in response to salt stress during the vegetative phase as well as during the reproductive developmental stage.

According to Francois *et al.* (1986) semi-dwarf bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* L., Durum group) were more tolerant to salinity of NaCl and $CaCl_2$ (1:1 by weight) at ECs from 1.5 to 20.5 dS $m^{-1}$ after the three-leaf stage of growth than they were at germination. Up to the booting stage, the

morphological development of the plants was not affected by salinity. In the high salt treatments the inflorescence emerged from the boot approximately 10-12 days earlier than in the control treatment. The duration of plant development was also affected by salinity and the time from planting to maturity for many cereal crops decreased with increasing salinity stress (Maas and Poss, 1989*a*). In bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* L.) reduction in grain yield was less than in vegetative growth when plants were grown under saline water having equal weights of NaCl and $CaCl_2$ (Gorham *et al.*, 1986). Salinity enhanced the rate of maturation of plants of cv. Probed and Aldura (Maas and Grieve, 1990). It was found by Rashid *et al.* in 1999 that there was variation in accumulation of $Na^+$ in cell sap of apex, leaf 1, 2, and 3 (with the increase of age) in different varieties (LU26S, Blue Silver, Lyallpur 73, Pak 81, Indus 79) of wheat at 100 and 200 mol $m^{-3}$ NaCl in hydroponics (Rashid *et al.*, 1999).

Reduction in vegetative growth associated with increasing levels of salinity of two salts (NaCl and $CaCl_2$) was very similar for two cultivars, Kinman and Essar, of guar (*Cyamopsis tetragonoloba* ) (Francois *et al.*, 1990). The average threshold was 4.9 dS $m^{-1}$ and a reduction in vegetative growth of 9.6 % for each unit increase in salinity above that 4.9 dS $m^{-1}$ was observed. Vegetative growth was more sensitive to salt stress than seed production, which suggested that photosynthate distribution to the seed might be less affected by salinity than vegetative growth. Soil water salinity up to 8.5 dS $m^{-1}$ had no significant effect on emergence. Salt levels greater than 8.5 dS $m^{-1}$ delayed, but did not significantly reduce, final emergence percentage. The result indicated that guar appeared to be as tolerant during plant emergence as during reproductive growth, while vegetative growth was significantly more salt-sensitive (Francois *et al.*, 1990).

It was observed by Nassery *et al.* (1978) that there was less effect of salinity on the later stages of expansion growth than on leaf initiation of sesame (*Sesamum indicum* L.). Phosphate deficiency in cv. Long Pod was intensified by salinity, and the effect was obvious because the older leaves of salt-stressed plants became senescent and abscised 7 to 16 days after the transfer to Pi-free media.

Cowpea (*Vigna unguiculata* L.) Walp. was found to be most sensitive during the vegetative and reproductive stages, less sensitive during flowering, and least sensitive during the grain filling stage (Maas and Poss, 1989*b*). Therefore it might be possible to irrigate it with saline water during the more tolerant stages of growth and use low-

salinity water only during the sensitive stages of growth. Salt tolerance of many crops including pinto beans (*Phaseolus vulgaris L.*) varies with growth stage. Development in wheat might also be reflected in the timing of the subsequent life-cycle phases that lead to the differences in the time to maturity (Grieve *et al.,* 1993). Early ear emergence, anthesis and grain maturity in *Triticale* (Francois *et al.*, 1988), *Triticum aestivum* (Rawson, 1986) and *Sorghum bicolor* (Francois and Clark, 1980) were observed under salinity stress. Khatun and Flowers (1995*b*) concluded that salinity levels of 0, 10, 25, and 50 mol $m^{-3}$ NaCl reduced fertility in rice cultivar IR-36 (*Oryza sativa* L.). The number of fertile florets and viability of pollen decreased with the increase of salinity in the medium.

### 1.3.2 Seed germination

High seed viability and early or late emergence are genetically-controlled characters (Thornberry and Smith, 1955). The rate and percentage of emergence were decreased by salinity in 6 varieties (N53-509, B54-842, Improved Pellican, Jackson, N53-505, Lee) of soybean (Ogasa, 1939; Rudolfs, 1921). Possible reasons for delayed emergence could be the combined effects of osmotic pressure and ion toxicity (Uhvits, 1946; Bernstein and Hayward, 1958). The first effect of soil salinity on physiological reactions in plants is the reduction of water absorption *via* roots (Bernstein, 1961). Rudolfs observed in 1921 that absorption of water was reduced to 9% in 15 hours with increased (0-7 atmospheres) external osmotic pressure due to NaCl addition. According to Ogasa (1939) seed germination of soybean variety KO 561 was inhibited by a 0.2 % NaCl solution at 30°C but it was necessary to increase the concentration to 0.3% NaCl to cause inhibition at 15°C. Uhvits (1946) observed considerable reduction in seed germination of alfalfa in solution cultures supplemented with NaCl, possibly due to the toxic salt effect at osmotic pressures higher than 7 atmospheres. According to Ayers and Hayward (1948) salt tolerance during germination is not positively correlated with later stages of growth.

### 1.3.3 Shoot growth

Reduction in the shoot weights of pepper and safflower (Nieman *et al.*, 1988), kenaf (*Hibiscus cannabinus* L.) (Francois *et al.*,1992), bread wheat (*Triticum aestivum* L. cv. Probred) and *Triticum turgidum* L. cv. Aldura. (Maas and Grieve, 1990) were observed under NaCl and $CaCl_2$ salinity. Yields of shoots and pods in sesame (*Sesamum indicum* L.) were reduced under multiple salts ($NaNO_3$, NaCl, $Na_2SO_4$, $CaCl_2$) (Nassery *et al.*, 1979). At 50 and 250 mol $m^{-3}$ NaCl plant and shoot growth were related to $K^+$ uptake into the leaves of spinach (Chow *et al.*, 1990). Significant reductions (35% and 75% under 50 and 150 mol $m^{-3}$ NaCl respectively) in the leaf elongation rate were observed in rice only in the leaves developed after less than 20 minutes of salt application (Yeo *et al.*, 1991). Changes were transient if plants were exposed to salinity for a short time, but catastrophic in prolonged exposure. Reduced leaf growth in wheat was due to development of small and dark green leaves (Gauch and Wadleigh, 1944; Haward and Wadleigh, 1949; Brown and Haward, 1956). Salinity reduced the final number of leaves in wheat on the main stem (Grieve *et al.*, 1992; Maas and Grieve, 1990; Francois *et al.*, 1994). Reduction in total shoot and stem dry matter yields of both durum and bread wheats were observed under salinity (Grieve *et al.*, 1992; Grieve *et al.*, 1993; Maas *et al.*, 1994). The decrease in yield with increased salinity was attributed to the reduction in both stem diameter and plant height in wheat (Grieve *et al.*, 1992) and in kenaf (Francois *et al.*, 1992). Salinity affected the leaf initiation rate by negatively influencing the phase of primordium initiation differentially in wheat (*Triticum aestivum* L.) cvs. Yecora Rojo and Anza. The leaf initiation rate decreased while leaf duration was unaffected by salinity (Grieve *et al.*, 1993).

### 1.3.4 Root growth

Reduced growth of roots was observed under salinity in *Oryza sativa* (Bhushan and Grover, 1993) and in *Sesamum indicum* L. (Nassery *et al.*, 1979). Root growth of sesame (*Sesamum indicum* L.) was little affected by 0.20 MPa of salt when the Ca:Na ratios were 1 or less (Nassery *et al.*, 1979). Roots of pepper (*Capsicum annuum)* cv. Yolo Wonder were less affected compared with shoots, while no reduction was found in safflower (*Carthamus tinctorius)* L. cv. Gila. The maintenance of sink activity was an important property in determining salt tolerance (Nieman *et al.*, 1988).

### 1.3.5 Tillers and spikelets

Salinity greatly reduced tillering (Maas and Grieve, 1990) and resulted in fewer spikes per plant, fewer kernels per spike, shortened reproductive phase, reduced number of spikelet primordia on the main spike and significantly reduced kernel number and weight in wheat (Grieve *et al.*, 1992; Grieve *et al.*, 1993; Maas *et al.*, 1994). Salinity reduced all growth and yield components significantly with salt imposition throughout the growing season. With imposition of salt prior to terminal spikelet differentiation, the number of spikelets per spike and the number of tillers per plant were significantly reduced. Only kernel number and weight were reduced with imposition of salt after terminal spikelet differentiation. The most pronounced effects of salinity were on the yield components that were growing or developing at the time of salinity imposition (Hoffman *et al.*, 1973; Maas *et al.*, 1973; Maas and Poss, 1989*a*).

## 1.4 Mechanisms of injury

The effects of salinity include components of osmotic stress, ion effects and nutrient interactions (Nieman and Shannon, 1976). Absence of complete osmotic adjustment regardless of the salt exclusion and ionic toxicity due to non-exclusion of salts or due to dehydration could be reasons for plant injury (Gorham *et al.*, 1985). In salt includers (dicots and chenopods) ionic imbalance occurs because some toxic ions like Na and Cl become high enough in concentration to become toxic for the plants, while K and Ca become deficient (Wyn Jones, 1981; Gorham *et al.*, 1985).

Adverse effects of salinity like leaf injury, leaf burning and leaf necrosis are modified by nutrients in various crops like soybean, *Glycine max L.* (Grattan and Maas, 1984, 1985) and *Beta vulgaris* L. (Ogata and Maas, 1973). At 0.20 MPa $Ca(NO_3)_2$ and $CaCl_2$ were more injurious than NaCl in sesame (*Sesamum indicum* L.) but toxic effects of the $CaCl_2$ increased with time. Very shallowly serrated blades or short withered tips were observed in *Sorghum bicolor* and this is a characteristic of mild Ca-deficiency. Severe injury occurred on NK 265 blades at $Na^+/Ca^{2+} = 34.6$. At -0.4 MPa however no blade injury was observed on NB 9040, while at all $Na^+/Ca^{2+}$ ratios a few individual NK 265 plants were at least mildly affected (Grieve and Maas, 1988).

### 1.4.1 Ionic toxicity

The generalised effects of ion toxicity include metabolic inhibition, interference with protein synthesis, cellular dehydration, stomatal closure and early senescence of leaves. Accumulation of any ion in excess, and its interference with nutrition, can cause toxicity. The most abundant ions in nature are $Na^+$ and $Cl^-$ and distinction between their toxicities sometimes becomes difficult because the concentration of both ions increases simultaneously (Shannon, 1997). Ion toxicity is not directly linked with leaf damage in the case of rice (Yeo and Flowers, 1986). The minimum level of leaf chloride in numerous fruit trees associated with leaf burn was 5000 ppm to 18000 ppm chloride (Bernstein and Hayward, 1958). Usually no specific chloride injury was shown by the leaves of field vegetable and forage crops in salinised soil ranging from 17750 ppm to 53000 ppm chlorides. Ionic imbalance leads to more or less equal ionic toxicity in apoplast and symplast (Flowers and Yeo, 1986). In chloride-includer varieties of soybean the chloride concentration was high in the plant tops and caused severe leaf necrosis. However the chloride concentration was low in chloride excluders and they did not show symptoms of leaf necrosis due to chloride toxicity (Abel, 1969).

Except for graminaceous species such as wheat (Gorham, 1993), sorghum or rice (Flowers *et al.*, 1991; Yeo, 1993), sodium toxicity is not as widespread as chloride toxicity and is mainly related to low $Ca^{2+}$ concentrations in the substrate or poor soil aeration. Many crop species with relatively low salt tolerance are $Na^+$ excluders and able to restrict the transport of $Na^+$ at low and moderate salinity levels into the leaves where it is highly toxic in salt sensitive species (Drew and Dikumwin, 1985). Under poor soil aeration conditions, however, the restriction in uptake and shoot transport of $Na^+$ and $Cl^-$ is nullified leading to massive accumulation in the leaves and corresponding salt toxicity (Barrett-Lennard, 1986). There is increasing support, however, for the hypothesis of Oertli (1968) that salt accumulation in the leaf apoplasm is an important component of salt toxicity, leading to dehydration, low turgor and death of leaf cells and tissues (Munns, 1988). In rice, for example, an early symptom of root exposure to only 50 mM NaCl is wilting, although accumulation of ions in the leaves is more than sufficient for osmotic adjustment. In the leaf apoplasm however the $Na^+$ concentrations may reach 500 mM which causes dehydration of the leaf cells (Flowers *et al.*, 1991).

Photosynthesis of the whole shoot is often not informative for causal interpretation of the mechanisms of salt injury as salts primarily accumulate in mature leaves. In rice at low substrate salinity net photosynthesis in the whole shoot was not affected, but it was in the older leaves. Here the decrease in net photosynthesis was inversely related to the $Na^+$ concentration in the leaves, presumably mainly in the apoplasm (Yeo *et al.*, 1985*b*). In the long term, growth responses to salinity can be determined by the maximum salt concentration tolerated by fully expanded leaves because the influx of NaCl with the transpiration stream is high and leads to extremely high internal salt concentrations up to the toxic level (Flowers and Yeo, 1986). If the rate of leaf death approaches the rate of new leaf expansion the photosynthetic area will become too low to support continuous growth (Munns and Termaat, 1986).

### 1.4.2 Ionic imbalance

Ionic imbalance implies a deviation from the 'normal' ratios of ions found in non-stressed plants. This may be an excess of cations or anions generally, or abnormal $K^+/Na^+$, $Na^+/ Ca^{2+}$ or $Cl^-/NO_3^-$ ratios. Nassery *et al.* (1978) reported that the greater sensitivity of sesame to single salts than to salt mixtures demonstrated the importance of avoiding ionic imbalance in salt tolerance studies. Extreme ratios of $Na^+/Ca^{2+}$ caused ionic imbalance and additional growth reduction (Nassery *et al.*, 1979). Due to high concentrations of $Na^+$, $K^+$ deficiency was induced in barley (Gorham and Wyn Jones, 1989). Several workers report reduced $Ca^{2+}$ concentrations and $Ca^{2+}$ deficiency in crop plants under the influence of NaCl salinity (Grieve and Maas, 1988; Maas and Grieve, 1987; Cramer and Läuchli, 1986; Cramer *et al.*, 1986). Protection of root growth from salt stress by supplemental $Ca^{2+}$ could avoid $Ca^{2+}$ deficiency and imbalance of $K^+/Na^+$ selectivity in cotton (*Gossypium hirsutum* L.) (Cramer *et al.*, 1987). Concentrations of $Cl^-$ and $Ca^{2+}$ in necrotic leaves of sesame remained below those of uninjured NaCl-treated and control leaves, thus necrosis might be due to low Mg concentration or the consequence of ionic imbalance rather than toxicity of $Ca^{2+}$ and $Cl^-$ in the leaves (Nassery *et al.*, 1979).

## 1.5 Nutrition

Essential nutrients are directly involved in plant metabolism, cannot be replaced by other nutrients and are indispensable for the completion of the life cycle of the plant (Arnon and Stout, 1939). Nutrient supply above the adequate or luxury range is toxic to plants and responsible for growth reduction.

Mineral nutrition of plants is disturbed by high concentrations of $Cl^-$ and $Na^+$ (Rains, 1972). $Na^+$ was able to spare the barley plant's requirement for $K^+$ but not to replace it as an essential macro-nutrient (Wyn Jones and Gorham, 1989). $Mg^{2+}$ concentration in cotton was influenced by nutritional status, being inversely dependent on the external $CaCl_2$ concentration (Gorham and Bridges, 1995). At 50 and 250 mol $m^{-3}$ NaCl, changes in $K^+$ status were observed by Chow *et al.* (1990) in spinach plants grown in nutrient solutions with KCl at 0.01, 0.1, 1 and 10 mol $m^{-3}$. Measurements of the absorption of $K^+$, $Ca^{2+}$, $Mg^{2+}$, phosphorus, nitrate and micro-nutrients on plant exposure to salinity indicated that there is no simple effect of NaCl on plant nutrition. The uptake of nutrients by roots did not limit growth of *Lupinus albus* under sub-lethal salinity (Jeschke *et al.*, 1992). An increase in concentration of nutrients in the root medium is able to enhance growth and to improve salinity tolerance of plants (Kafkafi *et al.*, 1982; Glass, 1983; Leidi *et al.*, 1992). Amzallag *et al.* (1992), however, concluded that the improvement was not due to a partial recovery from a nutrition disorder induced by salinity, but rather due to an indirect effect of nutrients on the hormonal balance of the plant.

Transport of $K^+$ from root to shoot was inhibited by 85% in the plants of *Hordeum vulgare*, cv. California Mariout that were treated with 100 mol $m^{-3}$ salts as compared to the control plants, while the decrease in growth depending on the root fresh weight was reduced to 50 % which was less than transport (Jeschke and Wolf, 1985). Net uptake rates of $K^+$ and to a higher extent of its translocation from root to shoot was reduced by 100 mol $m^{-3}$ NaCl and as a result shoot content of $K^+$ was lowered while $K^+$ concentration in the root was higher in maize (*Zea mays* L.) plants (Botella *et al.*, 1997).

## 1.6 Foliar uptake of salts

According to Benes *et al.* (1996) vascular plants, especially halophytes, have the ability to select the ions during their uptake at root level. But if selectivity is present at the root level, it was not necessarily present at the leaf level. For example, in maize (*Zea mays* cv. Juanita), $Na^+$ concentrations in leaf cell sap were higher in plants given saline sprinkler irrigation than in plants continuously exposed to soil salinity. However, the increase was higher in barley compared with maize. $Cl^-$ concentrations in leaf sap of maize cv. Juanita, and barley, cv. Kym were also higher in sprinkler-irrigated plants. Barley leaves as well as maize leaves lacked the ability to selectively exclude $Na^+$ during saline sprinkling. However maize leaves showed selectivity for $Na^+$ over $Cl^-$ via leaves, while barley showed no discrimination during uptake of $Na^+$ and $Cl^-$ during saline sprinkling (Benes, *et al.*, 1996).

## 1.7 Mechanisms of salt tolerance

No simple relationship between salinity tolerance at whole plant and at the cellular levels was found in rice (Flowers *et al.*, 1985*a*). The ability of plants to regulate the influx of salt is obviously one of the major factors determining salt tolerance. Growth and survival of plants at high salinity depend on adaptation to both low water potentials and high $Cl^-$ and $Na^+$ concentrations, but the situation is very complex. Adverse effects of low external water potential can be mediated by uptake of electrolytes, but this uptake also raises the danger of ion excess. Probable adaptations therefore range between exclusion of $Cl^-$ and $Na^+$ and rapid uptake of these ions for use as the principal osmotic solutes in the tissues (Greenway and Munns, 1980).

### 1.7.1 Compartmentation

Solute compartmentation between cytosol and vacuole is presumably an important aspect of salt tolerance (Wyn Jones, 1981). There is compartmentation of ions or solutes between vacuole, cytoplasm, nucleus, mitochondria, plastids and other organelles within the cytoplasm at sub-cellular levels, and between cells and tissues at the cellular level. Sugars, salts and organic acids are considered to be major solutes contributing to the osmotic pressure of the sap. Among the sugars glucose, fructose and

sucrose are the predominant sugars (Yeo, 1981; Storey *et al.*, 1983). NaCl is compartmentalised between leaves, that is protecting the younger ones, and within the leaf, that is between protoplast and apoplast and between cytoplasm, organelles and vacuoles (Yeo and Flowers, 1986).

The contribution of $Na^+$, $K^+$, $Cl^-$ and $SO_4^{2-}$ to the osmotic pressure of leaf sap ranged between 65% and 90% for 14 species of dicotyledonous halophytes and between 45% and 70% for 9 species of monocotyledonous halophytes (Albert and Popp, 1978). In the more successful halophytes, uptake of substantial amounts of $Cl^-$ and $Na^+$ is almost certainly accompanied by maintenance of higher ion concentrations in the vacuole than in the cytoplasm (Munns *et al.*, 1983).

Among the anions $NO_3^-$ is generally the most important inorganic component while $K^+$, $Ca^{2+}$ and $Mg^{2+}$ are the major cations in non-stressed plants. However, the proportion of the solutes varies with the specific plant part, plant age and the habitat in which the plant is growing. Analysis of whole plant tissues provides only limited information and there is a need to know concentrations and changes in concentrations within smaller compartments.

#### 1.7.1.1 Intercellular compartmentation

Exclusion of Cl, but not of Na, from the vacuoles of mesophyll cells of salt-grown or K deficient barley was observed by Leigh and Storey (1993) using X-ray microanalysis. Elements were equally distributed between the adaxial and abaxial layers of the epidermis, except K which was somewhat higher in concentration in the adaxial epidermis. In the absence of K alternative solutes such as Na and Mg were accumulated in the vacuole, but in the absence of these, organic solutes could be accumulated (Leigh and Wyn Jones, 1984). Compartmentation of $Na^+$, $Cl^-$ and $K^+$ between different tissues or between different compartments within cells might be responsible for the better growth of *H. vulgare* than of *T. durum* (Gorham, 1990*a*).

#### 1.7.1.2 Intracellular compartmentation

The analysis of sub-cellular compartments is a problem because of the small volumes. At high external salinity osmotic adjustment cannot be achieved with inorganic solutes without substantial and sustained intracellular compartmentation of salt between cytoplasm and vacuole in halophytes (Yeo and Flowers, 1989) because of

the toxicity of inorganic ions to cellular metabolism (Gorham, 1992; Flowers and Dalmond, 1992).

In glandless halophytes the vacuole is the only destination for excess ion storage (Flowers and Yeo, 1986). In dicotyledonous halophytes 80-90 % of the ions within the plant are stored in the vacuoles of the leaves, so compartmentation of the ions within the vacuoles of the leaves is highly effective for salt tolerance (Leach *et al.*, 1990*b*). The vacuole is the largest compartment in mature cells. Vacuoles are important for ion storage because of the low volume of the cytoplasm and apoplast of leaf mesophyll cells (Parkhurst, 1982; Hajibagheri *et al.,* 1984). During the developmental process cell volumes increase between 50-5000 $fm^3$ (Zimmerman and Steudle, 1975), thus accommodating increasing quantities of salts during growth. The concentration of solutes in plant cells is greater than in the medium in which the roots are growing in all but exceptional cases, usually by a considerable margin.

In some dicotyledonous, halophytic plants, especially *Suaeda* species and *Salicornia europaea,* salt was accumulated in the vacuoles. $Na^+$ concentrations exceed 500 mol $m^{-3}$ in the vacuole while cytoplasmic $Na^+$ did not exceed 100-150 mol $m^{-3}$ under optimum salinity in *Suaeda maritima* (Harvey *et al.*, 1981; Hajibagheri and Flowers, 1989). The leaves were succulent due to possession of large vacuoles. In these plants the $K^+$ concentration was low (40 mol $m^{-3}$). On the other hand the NaCl concentrations in the leaves of salt marsh dicotyledonous halophytes can reach 1000 mol $m^{-3}$. This kind of response was not common in monocotyledonous plants and grasses having comparatively rigid and smaller cells, except in *Triglochin maritima* L. (Wyn Jones, 1981; Gorham *et al.*, 1980; Gorham *et al.*, 1985*a*). In the leaves of spinach exposed to 300 mol $m^{-3}$ NaCl, $Na^+$ and $Cl^-$ are mainly sequestered in the vacuoles and high $K^+$ concentrations were observed in the chloroplasts (Yeo and Flowers, 1989).

In some cases organic acids such as malic acid might be present in high concentrations, particularly at the end of the night for those species using Crassulacean Acid Metabolism. All cells compartmentalise ions, and the vacuole is typically about 2 pH units more acid than the cytoplasm (much more in CAM plants).

#### 1.7.1.3 Evidence for the model

There is variation in resolution and in technical complexity in the methods that can be used to analyse the solute concentration within sub-cellular compartments. The

methods are direct sampling, indirect methods and X-ray microanalysis. None of them is universally applicable because all have advantages and disadvantages. For example, X-ray microanalysis cannot reliably measure cytoplasmic concentrations and it cannot be used on living cells (Flowers and Yeo, 1992).

Evidence for the selective distribution of solutes between the vacuolar and cytoplasmic compartments of higher plant cells (Figure 1.4) is derived from X-ray microprobe analysis, analysis of isolated vacuoles (Leigh *et al.*, 1979,1981), flux analysis (Jeschke, 1979), and indirectly from the analysis of tissues of low vacuolation (Jeschke and Stelter, 1976; Munns *et al.*, 1979), phloem constituents (Downing, 1979) or phloem-fed fruits and petals (Gorham *et al.*, 1980). It is obvious from this work that in the cytoplasm $K^+$ is usually high while $Na^+$ is low, while the opposite is true for the vacuole.

Protection of cytoplasmic enzymes from salt inhibition by sequestering most of the salt in vacuole (Gorham, 1992) is shown in Figure 1.4. Indirect evidence for compartmentation arising from the solute sensitivity of enzymes was provided by Flowers (1975) and Osmond (1976). Enzymes that require $K^+$ or $Na^+$ are particularly important for an understanding of salt tolerance (Jennings, 1976). Enzymes of salt-tolerant plants are not resistant to inhibition by high salt concentrations, except enzymes of certain halophilic bacteria (Greenway and Osmond, 1972). Pyruvate kinase from cotton seed assayed at 45 mol $m^{-3}$ $K^+$ showed $Na^+$-inhibition of 40% at a ratio of $Na^+/K^+$ of 1 (Duggleby and Dennis, 1973).

Concentrations of 140 mol $m^{-3}$ KCl or 200 mol $m^{-3}$ K acetate completely inhibited the initiation-dependent *in vitro* translation on wheat germ ribosomes regardless of the salt tolerance of the source of the mRNA (Gibson *et al.*, 1984). Polysomal stability was inversely proportional to salt concentrations in various species when KCl concentrations were higher than 125 mol $m^{-3}$ (Brady *et al.*, 1984). Protein synthesis is sensitive to high $Na^+/ K^+$ ratio and inhibition occurred when $Na^+/K^+$ ratio was 1 or higher (Wyn Jones, 1981). According to Flowers and Dalmond (1992) NaCl can partially substitute the role of K in polysomal preparations from halophytes in a better way than in glycophytes. The incorporation of radioactive amino acid ($^{35}$S-methionine) in an *in vitro* wheat germ translation system was maximum in 80-125 mol $m^{-3}$ K acetate with 2-4 mol $m^{-3}$ Mg acetate but 200 mol $m^{-3}$ of K acetate inhibited incorporation of $^{35}$S-methionine completely. In non-halophilic bacteria also incorporation of aminoacids into acid-

insoluble compounds was markedly inhibited by addition of 175 mol $m^{-3}$ $Na^+$ (Lubin and Ennis, 1964).

To summarise, the inability of most organisms to maintain enzyme activity and protein synthesis in the presence of excessive concentrations of $Na^+$ and $K^+$ provides strong evidence for the intracellular compartmentation model shown in Fig. 1.4.

### 1.7.1.4 Description of the model

As cytoplasmic $Na^+$ is toxic above a threshold level it is extruded by the plasma membrane $Na^+/H^+$ anti-ports that are energised by proton gradients generated by the plasma membrane ATPase (Blumwald *et al.*, 2000). Cytoplasmic $Na^+$ may also be removed by vacuolar $Na^+/H^+$ anti-ports.

$Na^+$ and $Cl^-$ are preferably accumulated in the vacuole (Figure 1.4). Cytoplasmic concentrations in mol $m^{-3}$ are; $K^+$ 100 - 120; Na < 20; Cl < 20-30 and Mg 1-4, under non-saline conditions (Wyn Jones 1979). Estimates of cytoplasmic concentrations in cells of halophytes growing in saline conditions are 20-200 mol $m^{-3}$ $K^+$ and 100-200 mol $m^{-3}$ $Na^+$, even when cations reach 500 mol $m^{-3}$ in the vacuole. Cytoplasmic concentrations of $Na^+$ plus $K^+$ in the cells of halophytes could be as high as 300-450 (Flowers *et al.*, 1986), but more usually 250 mol $m^{-3}$ (Hajibagheri and Flowers, 1989).

Figure 1.4 shows that water potential equilibrium is maintained due to the accumulation of non-toxic, compatible solutes in the cytoplasm (see next section). Other solutes including sugars, organic acids and aminoacids (Wyn Jones, 1981) also accumulate in the vacuoles, as shown in Figure 1.4.

It is evident that cytosolic accumulation of glycinebetaine influences tonoplast $Na^+$ fluxes to increase the vacuolar $Na^+$ content (Ahmad, 1978; Ahmad and Wyn Jones, 1978). Thus an integrated regulation of cytosolic and vacuolar events allows both the maintenance of $\Delta\Psi = 0$ across the tonoplast and the steeper solute gradients in response to increased salt stress (Wyn Jones, 1981). High concentrations of solutes in the vacuole lead to low osmotic potential and as a result water enters into the vacuole and cells become turgid. The black arrows show the turgor pressure on the cell wall in Figure 1.4.

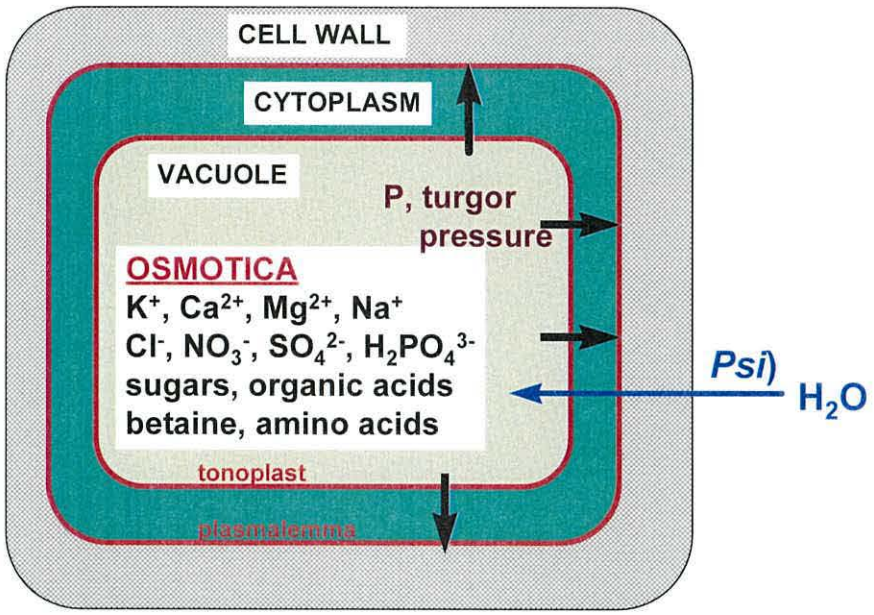


**Figure 1.4 Compartmentation model of a plant cell (Gorham, 1995*b*).**

### 1.7.1.5 Compatible solutes

The term compatible solute was introduced by Brown and Haward (1956) for the description of non-inhibitory substances accumulated in the cytoplasms of cells at low external water potentials. For osmotic adjustment of the cytoplasm and its organelles solutes such as glycinebetaine accumulate. Glycinebetaine is non-toxic up to 300-400 mol $m^{-3}$. Compatible solutes are mostly derivatives of polyols or derived from nitrogen dipoles, however different solutes accumulate in different taxonomic groups. Glycinebetaine is an important cytoplasmic component for osmotic adjustment in *Suaeda maritima* (Hall *et al.*, 1978) and other chenopods. Pollen samples of the grass *Leymus giganteus*, and the Chenopod, *Spinacia oleracea* had higher concentrations of glycinebetaine (142 mmol $kg^{-1}$ FW and 85 mmol $kg^{-1}$ FW respectively) than those of the leaves (30 mmol $kg^{-1}$ FW and 2 mmol $kg^{-1}$ FW respectively) (Gorham *et al.*, 1981; Gorham *et al.*, 1984). High levels of proline in the pollens of other species have also been found (Bathurst, 1954). In *Elytrigia juncea* and *Leymus sabulosus* glycinebetaine and sucrose accumulated in the leaves, but the proline concentration was not increased above 1 mmol $kg^{-1}$ FW by salinity. It was concluded that sucrose and glycinebetaine were produced for osmotic adjustment in response to salinity, while proline accumulated only as a result of severe water stress (Gorham *et al.*, 1985). Glycinebetaine and proline concentrations increased in leaves of salt-treated wheat plants. Trigonelline (a possible

compatible solute) concentration decreased in response to salinity in all cases (Gorham *et al.,* 1986). In *Leptochloa fusca* (Kallar grass) glycinebetaine, but not proline, accumulated as a result of salinity, but for *Eragrostis tef,* which was salt sensitive, the opposite was true (Gorham and Hardy, 1990). High concentrations of glycinebetaine do not lessen enzyme or organelle function or activity and have some protective capability (Shkedy-Vinkler and Avi-Dor, 1975; Larkum and Wyn Jones, 1979; Pollard and Wyn Jones, 1979).

In many salt marsh plants amino acids other than proline were found, but these may not be compatible solutes. Glycinebetaine and β-dimethyl-sulphoniopropionate accumulated in *Spartina* species (Gorham *et al.*, 1980). There was an excellent correlation between glycinebetaine concentrations and soil salinity. Salt stress in sorghum could be measured in terms of betaine accumulation while salinity treatments had no effect on choline concentrations (Grieve and Maas, 1984).

For osmotic adjustment high concentrations of compatible solutes accumulated in the chloroplasts of salt stressed leaves of barley (Greenway, 1962; Rozema, 1978) and spinach (Robinson *et al.*, 1983; Gupta and Berkowitz, 1988). At least 30-40% of the total leaf glycinebetaine was located in the chloroplast of the salt-stressed plants, most of the rest was in the cytosol, while in the vacuoles the concentration was very low. In barley grown on saline substrates, sugars play an insignificant role compared with $Na^+$ and $Cl^-$ which contribute more than 20% to osmotic adjustment in mature leaves (DeLane *et al.*, 1982).

### 1.7.2 Ion exclusion

When plants are subjected to high salinity they exclude salts, although the degree of exclusion is variable. Plants are often grouped into salt includers and salt excluders although all plants exclude salt from their transpiration streams (Gorham *et al.*, 1985). High quantities of salts may also be secreted to the leaf surface by salt glands. Secretion is an active process and is highly temperature-dependent (Gorham, 1987). Usually approximately equal amounts of $Na^+$ and $Cl^-$ are secreted (Gorham, 1987; Ball, 1988).

According to Forster *et al.* (1997) thirty lines of wild barley were tested for salt tolerance. The lines were grown for four weeks under 100 mol $m^{-3}$ NaCl in an hydroponics system. Tolerant lines were better able to exclude sodium from their shoots. *H. vulgaris* was salt tolerant due to having the ability of $Na^+$ and $Cl^-$ exclusion

from the shoots (Storey and Wyn Jones, 1978; Gorham, 1990). $Na^+$ exclusion was observed in a complete diallel cross of nine rice parents including susceptible (IR28, IR29, and M1-48), moderately tolerant (IR4595-4-1-13, IR9884-54-3-1E-P1, and IR10206-29-2-1) and tolerant (Nona Bokra, Pokkali and SR26B) lines (Gregorio and Senadhira, 1993). The leaves of soybean grown under saline conditions exclude $Na^+$ (Abel and Mackenzie, 1964; Abel, 1969; Grattan and Maas, 1984). Nassery *et al.* (1978) reported evidence that $Na^+$ exclusion from leaves was crucial in salt resistance of sesame (*Sesamum indicum* L.).

There was variable exclusion of $Na^+$ and $Cl^-$ ions from the shoot of wheat under salinity (Rashid *et al.*, 1999). According to Gorham *et al.* (1985) high concentrations of $Cl^-$ were accumulated in both vegetative tissue and grain in *Triticum aestivum* cv. Kharchia. The exclusion of ions such as $Na^+$ and $Cl^-$ from young leaves to such an extent that the increase in leaf inorganic ions might not fully compensate osmotically for the change in external salinity was a typical character of *Thinopyrum bessarabicum* and *Thinopyrum scirpeum* (Gorham *et al.*, 1985, 1986). The degree of salt tolerance in an amphiploid wheat derived from a hybrid between *Triticum aestivum* cv. Chinese Spring and *Thinopyrum bessarabicum* was determined by the ability to exclude Na and Cl from the shoots, especially from the young leaves, developing inflorescence and grain. The amphiploid had the highest $K^+/Na^+$ ratio, while the lowest was found in Chinese Spring (Gorham, *et al.*, 1986).

Selection and breeding programmes designed to improve the adaptation of crop plants to saline soils have to consider the various mechanisms for salt tolerance and sensitivity. However, effective excluders of both $Na^+$ and $Cl^-$ will probably not become very productive crops in highly saline soils because water deficit and large photosynthate requirements for osmotic adjustment seriously restrict their growth. In genotypes in which salt exclusion is the principal mechanism of salt tolerance, either the synthesis of organic solutes such as sugars and amino acids or the uptake rate of, *e.g.* $K^+$ and $Ca^{2+}$ must be increased. In terms of energy demand this is most expensive and growth rates of such genotypes are naturally very low (Marschner, 1995). Crop plants of the includer type have a much greater potential for better adaptation coupled with sufficient productivity when grown in highly saline soils.

### 1.7.3 Enhanced $K^+/Na^+$ discrimination

A preference in the uptake of $K^+$ over that of $Na^+$ may be an important genetic character in salt tolerance (Flowers *et al.*, 1977). Salt tolerance is associated with $K^+/Na^+$ selectivity in tomato shoots (Sacher *et al.*, 1982). $K^+/Na^+$ discrimination is mediated by genetic factors (Shannon 1997).

A model has been proposed in yeast for $Na^+$ and $K^+$ fluxes and two genes involved in $K^+$ and $Na^+$ transport systems have been cloned (Haro *et al.*, 1993). The *TRK1* gene is required for the expression of the high affinity mode of $K^+$ uptake. It determines the ratio between $K^+$ and $Na^+$ Km values (Ramos *et al.*, 1985).

During a study of ion accumulation in the ancestors of modern bread wheat, the enhanced $K^+/Na^+$ discrimination trait was observed. At 100 mol $m^3$ NaCl tetraploid wheat accumulated more $Na^+$ and less $K^+$ than hexaploid wheat or the D genome ancestor, *Aegilops squarrosa* (Wyn Jones *et al.*, 1984). In *Aegilops squarrosa* and *T. dicoccoides*, variation was noticed for this character (Gorham and Wyn Jones, 1990; Nevo *et al.*, 1992). The enhanced $K^+/Na^+$ discrimination trait was also found in synthetic hexaploid wheat that was obtained by the combination of the D genome of *Ae. Squarrosa* and the B and A genomes of tetraploid wheat (Shah *et al.,* 1987).

The enhanced $K^+/Na^+$ discrimination character was also studied in aneuploid lines that were ditelosomic for the long arm of chromosome 4D and that were derived from the hexaploid wheat variety Chinese Spring. The gene responsible for enhancement of $K^+/Na^+$ discrimination was located on the long arm of the 4D chromosome (Gorham, 1987). It was also shown by physiological investigations that the $K^+/Na^+$ discrimination character could affect the transport of $K^+$ and $Na^+$ from root to shoot, but had no effect on leaf anion concentrations and root anion or cation concentrations (Gorham *et al.*, 1990). It was also reported that the gene responsible for the enhancement of $K^+/Na^+$ discrimination was located on chromosome 4A of diploid wheat, although it was not expressed in durum wheat (Gorham, 1990; Gorham *et al.*, 1991). The $K^+/Na^+$ discrimination character was thought to be partly responsible for salt tolerance, but it was not observed in the cultivated barley which is the most salt tolerant of the cultivated cereals (Gorham, 1992). The enhanced $K^+/Na^+$ discrimination character was present in many *Aegilops* species having the D and U genomes, and in rye and triticale (Gorham, 1990*a,b*).

A number of recombinant lines based on the tetraploid wheat variety Cappelli were produced in which small pieces of the long arm of 4D were present. This 4D chromosome was derived from the hexaploid wheat variety Chinese Spring (Dvorak and Gorham, 1992). Gorham *et al.* (1997) confirmed that gene *Kna1* was responsible for the enhancement of $K^+/Na^+$ discrimination trait in these recombinant lines. *Kna1* was located on the 4B/4D chromosome in the recombinant lines R3, R23, R112 and R165. The recombinant line, R112 showed high $K^+/Na^+$ discrimination. In the case of recombinant line R146, although the concentration of $K^+$ ion was extremely high, the concentration of $Na^+$ ion was not low. The $K^+/Na^+$ trait partially controlled yield in saline conditions (Gorham, *et al.*, 1997).

Inhibition by $Ca^{2+}$ of negative membrane potential $\Delta\Psi$-dependent uptake of $Na^+$ was greater in hexaploid wheat (*Triticum aestivum* cv. Tory) than in tetraploid wheat (*Triticum turgidum* cv. Langdon). Antiport of $Na^+$ / $H^+$ was $Na^+$-dependent. Antiport activity was not increased in the Langdon 4D chromosome substitution line possessing the trait for enhanced $K^+/Na^+$ discrimination. It was concluded that neither of the transport processes measured was responsible for the $K^+/Na^+$ discrimination trait located on the 4D chromosome of the wheat (Allen *et al.*, 1995).

### 1.7.4 Osmotic adjustment

Osmotic adjustment is defined as the regulation of osmotic potential within a cell by the addition or removal of solutes to restore the difference between intracellular osmotic potential and the potential of the medium (Zhang *et al.*, 1999; Flowers and Yeo, 1992; Turner and Jones, 1981). This is the strict definition. Generally speaking, the addition and removal of water could also contribute to the adjustment of osmotic potential. With a sudden increase in salinity osmotic adjustment is achieved first by a decrease in tissue water content (partial dehydration). Salt tolerance and further growth in saline substrate, however, require a net increase in the quantity of osmotically active solutes in the tissue (Gorham *et al.*, 1985). $Na^+$, $K^+$ and $Cl^-$ are important ions concerned with osmotic adjustment in halophytes (Flowers, 1975). For osmotic adjustment, halophytic Chenopodiaceae (e.g. *Salicornia* spp.) accumulated $Na^+$ and $Cl^-$ in high concentrations in the shoots. Glycinebetaine, which was considered to be a compatible compound, was also synthesised in high amounts (142 mmol $kg^{-1}$ FW)

(Coughlan and Wyn Jones, 1980). In sorghum, glycinebetaine is important for osmotic adjustment only in the leaf blades, not in the leaf sheath (Grieve and Maas 1984). More NaCl was accumulated at low external salinity than was necessary to provide osmotic adjustment (over-adjustment) in rice (Yeo and Flowers, 1986).

Osmotically active concentrations are described in terms of osmolality. This is represented by the following equation (Wyn Jones and Gorham, 1983).

Osmolality = $\Phi$ x n x molality, where

$\Phi$ is the osmotic coefficient (a measure of the deviation of the solution in water from ideality). It is concentration- and temperature-dependent.

n is the number of particles into which a molecule dissociates (Wyn Jones and Gorham, 1983).

Plant cells use $K^+$, $Na^+$ and $NO_3^-$ as osmotica (Leigh and Tomos, 1993), but an osmotic role can be fulfilled by various solutes (Flowers and Läuchli, 1983). A high concentration of osmotically active solutes is needed to provide the driving force for the uptake of water and to generate turgor pressure. Moreover many proteins require about 100 mol $m^{-3}$ of ions for their stability and activation. Ions and organic solutes involved in osmotic adjustment are mainly found in the vacuole (Flowers *et al.*, 1977; Wyn Jones *et al.*, 1977; Munns *et al.*, 1983; Stewart and Ahmad 1983). The major osmotic solutes are sugars and amino acids in barley leaves and they have an important role in salt tolerance in mesophyll cells (Winter *et al.*, 1993; Shannon, 1997).

## 1.8 Membrane transport

In most mature plant cells the vacuole comprises more than 80-90% of the cell volume (Leigh and Wyn Jones, 1986; Wink, 1993) acting as a central storage compartment for ions and also for other solutes. Despite the importance of the tonoplast, little was known of its properties because it was difficult to obtain pure samples for analysis (Leach *et al.*, 1990*b*). Methods for the study of membranes are density gradient centrifugation (Leach *et al.*, 1990*a*), protoplasm isolation, and vacuole isolation. Using chromatographic techniques (for phospholipids and glycolipids) and gas chromatography the lipid components of the membranes in *Suaeda maritima* were identified as fatty acyl saturates ($C_{14}$ to $C_{18}$), phytosterols (cholesterol, campesterol, stigmasterol, β-sitosterol), phospholipids (phosphatidylcholine, phosphatidyl-

ethanolamine, phosphatidylserine and phosphatidylinositol) and glycolipids (digalactosyldiacylglycerol, mono-galactosyldiacylglycerol) (Leach *et al.*, 1990*b*).

The tonoplast plays an important role in ion compartmentation, which is a major characteristic of halophytes. The ability to generate and maintain the $Na^+$ and $Cl^-$ gradients between vacuole and cytoplasm is also conferred by the properties of the tonoplast in halophytes (Leach *et al.,* 1990*a*). There is a direct correlation between the degree of saturation of fatty acids and salt tolerance, (Leach *et al.*, 1990*b*). The pure tonoplast exhibited both vanadate-insensitive ATPase and pyrophosphatase activities (Leach *et al.*, 1990*a*). Fatty acids of tonoplast lipids from *S. maritima* were highly saturated (58%) compared with protoplast (30%) and microsomal membranes (36%) from this species and with *Beta vulgaris* tonoplast (33%) or with plasmalemma and tonoplast from etiolated mung bean seedlings (Yushida and Uemura, 1986). Passive permeability of saturated lipids is lower than that of unsaturated ones (Radda 1975). Changes in membrane chemical components (phospholipids, sterols and glycolipids) were correlated with $Cl^-$ accumulation under salinity (Kuiper, 1968; Flower and Yeo, 1992).

Cholesterol can dramatically reduce the permeability of membranes to water and small molecules such as $K^+$, $Na^+$, $Ca^{2+}$, glucose and glycerol (Houslay and Stanley, 1982). Low passive ionic permeability is an essential feature of the tonoplast of an halophyte, and the usually high proportion of cholesterol that was observed may well have a functional significance (Leach *et al.*, 1990*b*). Some salt-tolerant varieties of fruits such as grape and citrus have higher levels of free sterol than sensitive varieties (Shannon, 1997). Salinity increased free sterol composition and decreased levels of phospholipids and phosphatidylcholine in wheat (Mansour *et al.*, 1994).

Protein passages for movement of ions across membranes are termed channels. Ionic channels are intrinsic proteins that continuously change conformational state. Net movement of ions may be due to an electrochemical potential gradient or energy dependent. However pumps are involved in active transport. Carriers are membrane proteins that bind covalently to the substance being transported.

The ion channels of higher plant tonoplasts appear to have large and poorly specific conductance at the voltages at which they are gated open. There is no evidence yet that the channels of halophytes differ from those of glycophytes in this respect. These channels remain almost closed in a mature halophyte cell, because significant

efflux of NaCl from the vacuole is toxic (Flowers and Yeo, 1992). Solutes diffuse according to Fick's Law of Diffusion, *i.e.* flux of a substance is the amount of solute passing through a unit area in a unit time related to its concentration gradient. Patch clamping and the use of fluorescent probes are valuable techniques that can be used to study transport through membranes (Flowers and Yeo, 1992). An example is the use of PTS tracer (trisodium 3-hydroxy-5,8,10-pyrenetrisulphonic acid) for the determination of transpirational by pass flow of water related to $Na^+$ uptake (Yadav *et al.*, 1996).

P-type ATPases or the plasma membrane ATPases are sensitive to vanadate. This anion interferes with the formation of a phosphorylated intermediate necessary for the function of the enzyme. Activity is optimal around pH 6.5 and stimulated by cations ($K^+ > NH_4^+ > Rb^+ > Na^+$), but not by anions. Activity requires $Mg^{2+}$ with Mg-ATP being the substrate for the enzyme (Briskin, 1990). V-type ATPases or tonoplast or vacuolar ATPases are also present in the golgi and not inhibited by vanadate (Flowers and Yeo, 1992).

ATP is the most common type of energy used by the active transporters. These active transporters including $Na^+$-$K^+$ ATPase and $Ca^{2+}$ ATPase are the gateways for the movements and maintenance of ion concentration gradients across the plasma membrane and across the membranes of internal organelles (Horton *et al.*, 1996).

## 1.9 Tissue culture

Tissue culture can be used as a strategy for adaptation of plants to higher salinity. Rice cells in tissue culture are not inherently sensitive to salinities that inhibit the whole plant (Yeo and Flowers, 1986) and cells can survive under high salinity (Dix and Street, 1975; Gale and Boll, 1979). Variation for survival between the varieties Amber (tolerant) and IR 2153-26-3-5-2 (sensitive) of rice was, however, observed at 600 mol $m^{-3}$ salinity in tissue culture (Flowers *et al.*, 1985*a*) and no reduction in respiration was observed at 200 mol $m^{-3}$ (Flowers *et al.*, 1985*b*).

On the other hand, there was a direct correlation between salt tolerance of the whole plants of cotton and callus tissues obtained from them (Kerimov *et al.*, 1993). In cultivars 133 and INEBR-85 (resistant to chloride salinity), callus and suspension cultures were characterised by the same constitutive tolerance as the whole plants. After prolonged culturing of calluses of both varieties on medium containing 1% NaCl it was

observed that Variety 133 grew successfully while the INEBR-85 strain exhibited inhibition of growth and ultimate death.

A high degree of polymorphism exists in the regenerated rice plants from rice callus cultures. Regenerated plants from callus cultures show significant levels of DNA polymorphism compared to control plants that are derived from normal rice plants.

### 1.9.1 Regeneration of salt tolerant plants from tissue culture

The regeneration frequency in NaCl stressed callus of rice was lower compared with the controls in six rice varieties (Pusa Basmati 1, Basmati 370, Type III, Pant Dhan 4, CSR 10 and Pokkalli) (Shankhdhar *et al.*, 2000). Lutts *et al.* (1999) reported similar results in two japonica (I Kong Pao and Aiwu) and two indica (IR2153 and Nona Bokra) varieties. Recent approaches to study of salinity tolerance in rice have ranged from gene mapping to molecular characterisation of gene products induced by salt stress in tissue culture, followed by regeneration of salt tolerant plants (Winicov and Bastola, 1997; Winicov, 1997). Callus culture of the rice variety, Basmati 370, in MS medium showed 45% decrease in regeneration by NaCl stress. Regeneration was decreased under 85 mol $m^{-3}$ NaCl and inhibited under 128 mol $m^{-3}$ NaCl in R2B5 medium. Regeneration occurred *via* both organogenesis and somatic embryogenesis (Basu *et al.*, 1997).

The salt selection process and regeneration of the embryogenic callus had minimal adverse effects on subsequent plant growth, since the regenerated plants were vigorous, flowered and in most cases were fertile (Winicov, 1996). Heritable improvement in salt tolerance was obtained in R2 seedlings from one plant that had been regenerated after 5 months of selection on salt containing medium. Short term salt selected rice callus contains many embryogenic calluses that do not carry heritable changes in the salt tolerance phenotype and therefore necessitates screening for the tolerance phenotype in the progeny of the regenerated plants. So prolonging the selection process *in vitro* for rice improves the likelihood of regenerating plants with improved salt tolerance.

Tissue culture techniques were used in the production of haploid rice plants from anthers and in the selection of rice lines with salt tolerance and resistance to blast (*Pyricularia oryzae* [*Magnaporthe grisea*]) (Reddy, 1992). The frequencies of callus initiation in rice decreased with the increase of NaCl in the medium (Binh *et al.*, 1990; Kim *et al.*, 1988) and plant regeneration were highly genotype dependent (Kim *et al.*,

1988; Binh *et al.*, 1990; Reddy, 1992). Regeneration rates after salt stress were reduced in indica cultivars of rice (Vajrabhaya *et al.*, 1989). *In vitro* selection for tolerance of NaCl solution was influenced by genotype, ploidy level of the explant, weight of callus inoculum and the subculture state of the callus (Li *et al.*, 1987).

Growth of pre-salt-conditioned calluses in different concentrations of NaCl were inversely proportional to NaCl concentration in the medium and the cells from the tissue culture were physiologically adjusted to high NaCl. The regenerated plants showed aneuploidy, possibly as a result of prolonged exposure of calluses to NaCl in culture (Zapata and Abrigo, 1986).

Tolerant callus did not lose its regeneration potential because the high regeneration capacity in the 4th subculture was retained. The regeneration frequency *in vitro* in the selected lines of rice for salt tolerance was high compared with control lines (Reddy *et al.*, 1986; Reddy and Vaidyanath, 1985). *In vitro* callus culture in different concentrations of NaCl or crude sea salt in the induction and regeneration media was successful in identifying plant lines with improved salt tolerance, assessed by growth response and survival (Woo *et al.*, 1985).

The frequency of callus induction varied from 32% in cv. IR30 to 85% in cv. Reiho. No calluses grew in medium containing 3% NaCl but all grew well in less than 1% NaCl. Plant regeneration from 11-wk-old calluses varied from 0% for cv. IR8, IR26, IR28, IR30 and IR36 to 62% for cv. Taichung 65 (Suenaga, 1982). Marked varietal differences in salt tolerance were observed (Yoshida *et al.*, 1983). Mutations appearing in cultured plant cells of citrus and rice also appear in plants regenerated from these cells and in some cases of rice are passed on through sexual reproduction (Bouharmont, 1994). High concentrations of NaCl reduced callus survival and increased the frequency of albino plants in rice (Krishnaraj and Sreerangasamy, 1993). Most (4050) regenerated plantlets from *in vitro* selections turned brown and died in a nutrient solution with NaCl, but 214 showed normal growth. Of these, 48 produced green shoots when cultured on the regeneration medium. Only 17 regenerated plants survived after 2 weeks of salt stress, while all Mot Bui plants died (Bong *et al.*, 1996). Callus growth and its rate of survival decreased with increase in salinity of NaCl in various genotypes of rice but the regenerative ability of the calluses was not affected by salinity (Peters *et al.*, 1986).

Selected cells from seed-derived callus showed a higher relative growth rate in the presence of NaCl than unselected cells. A wide range of morphological and other variations was observed in plants regenerated from callus culture in an NaCl-containing medium. Field-grown plants displayed variation in both visible and agronomic traits. Plants regenerated from callus cultures showed variation in total number of tillers per plant, number of productive tillers per plant, length of panicle and number of fertile and sterile seed per panicle (Abrigo *et al.,* 1985).

According to Lutts *et al.* (1998), somaclonal variation was dependent on genotype, morphological parameter analysed, NaCl dose and stage of plant at the time of salt application. The extent of somaclonal variation in regenerated plants decreased during the proliferation phase when plants were exposed to NaCl. Somaclonal variation of regenerated mature embryo of rice calluses under NaCl stress was related to physiological characteristics such as a higher $K^+$:$Na^+$ discrimination, preferential $Na^+$ accumulation in the oldest leaves, lower decrease in cell membrane stability or higher tissue tolerance of internal $Na^+$ accumulation. In some cases, these physiological properties were transmitted to R2 progenies (Lutts *et al.*, 1999). Somaclonal variations were different from the parents for yield, grain quality, and biochemical parameters but more or less the same as in the parents for disease and insect pest resistance (Mandal *et al.*, 2000).

In the F2 generation, of 34 R1 somaclones, 28 were salt tolerant at the seedling stage (Bong *et al.*, 1996). Somaclonal variation might be used to increase tolerance of salinity, poor soils, diseases and pests, and the success in regenerating plants from rice protoplasts would allow the use of recombinant DNA technology and somatic cell fusion in rice breeding (Sarma and Rao, 1991)

Several selected plants of rice under NaCl stress showed higher salt tolerance and even a few cell lines survived at a sub-lethal NaCl. Results from the progeny of plants regenerated for the different stresses after field testing in Africa, confirmed the value of somaclonal variation and *in vitro* selection for stress tolerance in breeding (Bouharmont *et al.*, 1991). Regenerants grown to maturity in the greenhouse had variations in their morphology and isoenzyme patterns, and these might have been due to somaclonal variation (Peters *et al.*, 1986).

Somaclonal variation is an important source of genetic diversity. Physical and chemical stresses applied on tissue cultures can select tolerant cell lines from which

improved plants can be regenerated. The growth of rice plants regenerated from these cell lines was improved under saline conditions. Selected cell lines were able to maintain normal contents of $K^+$ and $Ca^{2++}$ in spite of increasing concentrations of $Na^+$ in the medium, and $Na^+$ and $Cl^-$ accumulation was slower in selected cells of rice plants (Bouharmont *et al.,* 1993). In salt tolerance experiments, callus tolerance was found to be enhanced by repeated selections on medium containing NaCl and genotypic differences in callus response were also observed (Kinoshita *et al.*, 1989).

Callus cultures of *Brassica oleracea* L. var. Capitata were grown on Murashige and Skoog agar (Mukhtar and Hasnain, 1994). Calluses that were obtained after the $4^{th}$ subculture were grown on media supplied 0,100, 200, 300, 400 and 500 mol $m^{-3}$ of NaCl up to the $8^{th}$ subculture. Proliferation was less in the calluses in saline medium compared to the control. No callus growth was observed on media with 300 mol $m^{-3}$ NaCl or above. A significant decrease was found in DNA content of calluses grown on salt, while RNA and protein content increased on medium with 100 mol $m^{-3}$ of NaCl.

## 1.10 Transpiration

Transpiration is the loss of water from the plant as water vapour. More than 90% of the transpiration occurs from the leaf surfaces through leaf pores named stomata. Movement of water is driven by differences in vapour pressure between the internal leaf spaces and the ambient air (Hopkins, 1999).

Transpiration rate was reduced by 30% in the salt-tolerant halophyte *Suaeda maritima* 72 h after the transference of plants to 400 mol $m^{-3}$ NaCl and 200 mol $m^{-3}$ NaCl (Clipson, 1987). *Leymus sabulosus* was found to tolerate 200 mol $m^{-3}$ NaCl in solution culture and with the changes in external salinity transpiration rate changed quickly while there was a lag period of several days in the accumulation of $Na^+$ and $Cl^-$ by the leaves (Gorham *et al.*, 1984). In wheat transpiration was reduced relatively more than photosynthesis with salinity stress (Adams *et al.*, 1977). According to Hoffman and Jobes (1978) transpiration of three crops, barley (*Hordeum vulgaris*, cv. CM-67), wheat cv. Siete Cerros, and sweet corn (*Zea mays* L., cv. Bonanza), consistently decreased with the increase of salinity in the root medium. The rate of transpiration was lowered in Pinto bean *(Phaseolus vulgaris* L.) by salinity (Hoffman *et al.*, 1973). Reduction in leaf transpiration was observed with increasing leaf age and with the

increase of $Na^+$ concentrations in *Oryza sativa* L. (Yeo *et al.*, 1985*a*). As salinity increased, water-use efficiency also increased and the rate of transpiration decreased (Hoffman *et al.*, 1973; Hoffman *et al.*, 1975; Gorham *et al.*, 1985; Ziska *et al.*, 1989). The transpiration stream is involved in the translocation of solutes to the leaves (Roger *et al.*, 1993). With increased RH from 45 to 90%, transpiration decreased by about 15% (Hoffman and Jobes, 1978).

Salt stress affected many species osmotically, inducing a water deficit, but some species also showed ionic effects (Bernstein, 1975; Jennings, 1976; Greenway and Munns, 1980). The transpiration rate has major importance for the supply of ions to the shoot, and very effective exclusion of ions was observed from the transpiration stream in rice (Flowers and Yeo, 1986). Rate of transpiration varies in leaves of different ages in dicotyledonous plants (Flowers and Yeo, 1986) and probably it is due to internally regulated changes in stomatal resistance and resistance to water flow to the leaf blades (Neumann and Stein, 1984).

## 1.11 Genetics

Genetics is based on Mendel`s work on peas and hawk-weed, but the significance of his work was not appreciated until 1900. Charles Darwin (1809-1882) was thinking about the development of new individuals in animals, more or less at Gregor Johann Mendel`s time (1822-1884). According to Darwin the hereditary substance was a uniform body, but according to Mendel the hereditary substances were composed of many independent and constant hereditary units. The term gene was firstly introduced in 1909 to describe the unit of inheritance. Discrete units of inheritance are alleles of genes (North 1979). Genetics is the science of heredity and genes are the basic units of biological information and inheritance, and responsible for the transmission of biochemical, physical and behavioural traits from parents to offspring. Alleles of genes are inherited according to Mendel`s law of segregation. The two alleles of each trait separate (segregate) during gamete formation then unite at random, one from each parent, at fertilisation. For each individual inheritance is determined by chance, but within a population this chance operates in a context of statistical probabilities.

Genetic variability within a species is not only a valuable tool for studying mechanisms of salinity tolerance but also an important basis for screening and breeding

for greater salt tolerance. Until recently there were few physiological traits that were found to be under genetic control, for example the genes for the transport of chloride in soybean (Abel and Mackenzie, 1964) and accumulation of $Na^+$ in pepper (*Capsicum annuum*) (Nieman *et al.*, 1988)

Improvement of salinity tolerance of crops through physiological selection is a major objective, but its wide application is limited due to the lengthy process of assessment of parents and large numbers of individuals and families in segregating generations (Flowers *et al.*, 2000). For the use of elite lines, molecular markers would be valuable for the mapping population in backcrossing, but this has to be considered alongside the effort required to develop and map any given population (Flowers *et al.*, 2000). Salt tolerance is a polygenic character, *e.g.* in tomato (Fooled and Chen, 1998), and therefore difficult to manipulate by conventional breeding techniques.

### 1.11.1 Genetics and salt tolerance

Genetics of salt tolerance is based on observations of phenotypic expression in plants under salinity such as growth reduction, visual symptoms of damage, or measurements of ion contents in tissues. The problem with these measurements is that practically it is not possible to measure them in ideal conditions because total control of changing environmental factors is not possible. In most glycophytes salt stress leads to extensive changes in gene expression with increases in some gene products and decreases in others (Gulic and Dvorak, 1987; Hurkman and Tanaka, 1987; Ramagopal, 1987, Singh *et al.*, 1987*a*, 1987*b*; Winicov *et al.*, 1989). Recent approaches to the study of salinity tolerance in crop plants have ranged from gene mapping to molecular characterisation of gene products induced by salt/drought stress (Winicov and Bastola, 1997).

A dominant gene, *Ncl,* for salt tolerance, especially for the accumulation of $Cl^-$, in soybean (*Glycine max* L., cv. Lee) was identified by Abel and McKenzie in 1964. The recessive allele *ncl* in cv. Jackson could not restrict the uptake of $Cl^-$ properly (Abel, 1969). There are differences in ion accumulation and salt tolerance between cultivars of wheat that are correlated and heritable (Salam, 1992).

Adaptation to salinity was observed in sorghum after exposure to 150 mol $m^{-3}$ NaCl salinity at the 5-10 days seedling stage (Amzallag *et al.*, 1990). Furthermore adaptation under salinity of 300 mol $m^{-3}$ NaCl was maintained, as was the growth rate,

under non-saline conditions. After self fertilisation a higher growth rate and lower $Na^+$ concentrations were maintained than in un-adapted controls suggesting Lamarkism - inheritance of environmentally-induced modifications in the next generation. The effects of overexpression of specific plant genes that are known to be up-regulated by salt /drought stress can be tested in transgenic plants (Winicov and Bastola, 1997).

### 1.11.2 Transgenics

The methodology to produce transgenic plants is readily available but the isolation of salt tolerant (halotolerant) genes is the limiting factor.

Transgenic plants have been developed with increased salt tolerance. NaCl tolerance in yeast was improved recently by a gene *HAL1* from *Zea mays* and *Arabidopsis thaliana*, and its expression is induced by osmotic stress. The growth phenotype conferred by *HAL1* overexpression and gene disruption is specific for high concentrations of NaCl (Gaxiola *et al.*, 1992). A transgene (*mtlD*, mannitol-1-phosphate dehydrogenase) from bacteria increased the level of mannitol in roots and leaves of transgenic tobacco and salt tolerance was also improved (Tarczynski *et al.*, 1993). There is, however, a contradiction between the loss of salt tolerance of selected (transgenic) cellular lines during regeneration and maintenance of salt tolerance *in vitro* (Winicov, 1994). In regenerated plants, heritability was expressed as a semi-dominant character (Shannon, 1997).

Several transformation methods, including *Agrobacterium* infection, biolistics and DNA uptake by protoplasts, have been employed to produce transgenic rice. The application of transformation technology to engineer resistance to insect pests, fungal and bacterial diseases plus abiotic stresses (salinity and drought), and to improve nutritional quality (accumulation of provitamin A and essential amino acids in endosperm in Basmati rice) were reviewed by Jain *et al.* (2000) and Reddy *et al.* (1999).

There is a direct relationship between the extent of increased stress tolerance and the amount of HVA1 protein accumulated in transgenic rice plants, and they maintained higher growth rates. Expression of the barley *HVA1* gene regulated by the rice *actin1* gene promoter led to high-level accumulation of the HVA1 protein in both leaves and roots of transgenic rice plants under water deficit and salt stressed conditions.

Significantly increased tolerance to water deficit and salinity was exhibited by transgenic rice plants in the second generation (Xu-DePing *et al.*, 1996).

### 1.11.3 Molecular markers

Molecular markers have significant value in breeding programmes to characterise and evaluate genetic variability in germplasm and to identify varieties (Zhou and Gustafson, 1995). The availability of molecular markers facilitates selection of desired characters in a breeding programme and provide the foundation for map-based gene (Koh *et al.*, 1996) isolation. Molecular markers are helpful in the identification of chromosomal regions associated with many complex traits in various crops particularly rice. Markers can provide essential and clear-cut information on an individual rather than a population,. Construction of genetic maps in various species of plants including rice became possible due to advancement in the molecular marker technology. The use of molecular markers in the mapping of traits of agronomic importance can speed up the development of improved plant varieties and increasing our understanding of the physiological or molecular mechanisms behind biological phenomena (Xu *et al.*, 1999).

Restriction Fragment Length Polymorphism (RFLP) involves cutting DNA at specific regions with restriction enzymes into different fragments, and labelling specific sequences with complementary DNA probes. RFLP analysis using single copy probes does not always depend on the presence or absence of a restriction enzyme site due to a mutation, but can also be performed using differences between individuals. RFLP are mostly co-dominant but are restricted to regions with low or single copy sequences. RFLP analysis of genomic DNA supports and confirms the classification of genotypes of cultivated rice *Oryza sativa* L. into two major groups japonica and indica.. RFLP, VNTR, or minisatellites are classical Southern blot hybridisation-based markers, involve cutting of genomic DNA with restriction endonucleases. However PCR-based markers involve the amplification of single locus markers, microsatellites and SNP, and multi-locus markers, AFLP and RAPD (Mackill *et al.*, 1996).

Randomly Amplified Polymorphic Deoxyribonucleic acid (RAPD) analysis is a quick method compared with RFLP and AFLP for generating genetic maps and analysing populations (Mackill *et al.*, 1996; Ashikawa *et al.*, 1992). The selective enzymic amplification of small DNA fragments using PCR is at random sites complementary to short (usually 10 base) primers (Ashikawa *et al.*, 1992).

Microsatellites are the markers used when there is a very tiny amount of DNA. Microsatellite DNA has repeats of very short sequences (simple sequence repeats). Microsatellite-derived DNA fingerprints are ideally suited for the identification of rice genotypes and, as the majority of the markers detected a high level of polymorphism, they are potentially very useful in monitoring and aiding gene introgression from wild rice into cultivated species (Ramakrishina *et al.*, 1994). Microsatellites genetic markers are valuable because they are co-dominant, detect high levels of allelic diversity and are easily and economically assayed by PCR (McCouch *et al.*, 1997). Microsatellite loci are usually highly polymorphic in the species in which they are first identified. Microsatellite markers detect a large number of alleles and are able to discriminate between even closely related individuals efficiently (Olufowote *et al.*, 1997) Being variable, microsatellites are easy to assay by PCR and by using microsatellite DNA markers it is easy to select plants carrying desired chromosome regions due to their distribution on all chromosomes. Microsatellites and minisatellites are the most informative molecular genetic markers which are dispersed throughout the genome and are considered as key points of recombination (Ramakrishina *et al.*, 1999).

AFLP is the abbreviation for Amplified Fragment Length Polymorphism.. AFLP markers from a limited number of primers are not confined to any particular regions or chromosomes in the rice genome. Amplified fragment length polymorphism (AFLP) has been proposed as a valuable tool for gene mapping in plant species, particularly in rice (Zhu *et al.*, 1998; Chen *et al.*, 1999). The AFLP markers are highly polymorphic in rice and follow Mendelian segregation. AFLP are useful for marker-assisted backcrossing because a linkage map of rice can be generated rapidly with AFLP markers. The ability of two cloned AFLP bands to serve as heritable genetic markers represent single-copy DNA at unique loci in the rice genome. DNA samples from rice are digested with restriction enzymes for amplified fragment length polymorphism (AFLP), as in the case of RFLP (Mackill *et al.,* 1996). The Map maker distribution of AFLP markers is not even. However not much work has been conducted yet in this area so not many AFLP and microsatellites markers in rice were found under salinity.

SNP (Single nucleotide polymorphisms) is the most recent marker technique (Jiahui Zhu, personal communication). SNP can be applied preferably to large population studies because scoring the SNP is not as laborious as in case of RFLP and

RAPD molecular methods for taxa differentiation. Comparisons of the coding sequences for the accumulation of low $Na^+$ and high $Cl^-$ might be revealed by SNP in various genotypes (Jiahui Zhu, personal communication). By using markers RAPD, microsatellite markers, AFLP and SNP, information on individuals at multiple genetic loci across the genome might be obtained. Microsatellite analysis, single nucleotide polymorphism (SNP) markers are important in plant genotype analysis in many crops including barley and wheat and these markers could be used for the research like analysis of wild plant populations, germplasm collections, plant breeding and advance systematic (Henry, 2001).

A programme was set up to sequence the complete rice genome up to 2004-2007, however the complete sequence of *Arabidopsis thaliana* is already available. Gene function identification, systematic analysis of gene expression and evaluation of SNP (Single Nucleotide Polymorphism) using DNA chips are the major expectations for further progress (Delseny, 1999). From the results of 8 genotypes of *Zea mays* representing 90 % of allelic diversity within a test population showed that out of 311 loci for which SNP information has been obtained, 164 could be easily mapped (Bhattramakki *et al.*, 2000).

Rapid and efficient procedures for the detection of sequence polymorphisms are essential for chromosomal walking and mutation detection analysis. Heteroduplex analysis on high resolution of gel matrices efficiently detects sequence polymorphism differing as little as a single base pair for example single nucleotide polymorphism (SNP) with standard laboratory equipment. Furthermore the matrices also discerned differences between homoduplexes a prerequisite for co-dominant markers (Hauser *et al.*, 1998).

### 1.11.4 QTL for salt tolerance

QTL is the abbreviation of Quantitative Trait Locus/Loci and means the markers or the specific points or regions located on the chromosomes related to differences in quantitative traits. QTL analysis is necessary because of the lack of enough knowledge about salt tolerance genes. Markers for ion transport and selectivity were identified from AFLP analysis in a custom-made mapping population of rice (Flowers *et al.*, 2000).

According to Mackill *et al.* (1999) a locus for dehydration tolerance in Co39 was detected and mapped on chromosome 8. MAS (marker assisted selection) can be used to transfer important QTL into cultivars with the broadest range of adaptation. Molecular marker technology will enable the identification of specific genes conferring increased yield as well as resistance to biotic and abiotic stresses in the future (Mackill *et al.*, 1999).

A rice RFLP linkage map was constructed in rice (Tesanai 2 x CB) and marker locus RG13 on chromosome 5 was found to be associated with salt tolerance (HongXuan *et al.*, 1997, 1998). RG13 accounted for 11.6% of the observed phenotypic variance in seedling survival time at 12 dS $m^{-1}$ in $F_7$ Recombinant Inbred Lines (RILs). Transgressive segregations were observed for salt tolerance in the RIL population (HongXuan *et al.*, 1998). Loci on chromosome 3, 4 and 6 for salt-induced genes *REF1A, SAMDC1* and salt repressed gene *SRG1* were detected by RFLP analysis in ZYQ8 x JX17 rice (ZiYin *et al.*, 1999). It was found by RFLP analysis that loci RG4, RG711 and Rab16 might be correlated with salt tolerance in 5 NaCl-tolerant lines (15, 16, 17, 19 and 20) and 4 mutants ($P_2$, $r_3$, $R_2$ and YCR) from rice varieties 98 and 77-170 (Zhang, 1994).

In the $F_2$ generation obtained from M-20 (salt-tolerant rice mutant) x 77-170 (salt-sensitive parent), segregation of traits for RFLP genotypes was normal under NaCl salinity,. Linkage between RFLP probe RG4 and salt tolerance was verified (Zhang *et al.*, 1995). One copy of the *OSA3* gene, a coding DNA fragment corresponding to the PM $H^+$-ATPase was mapped on chromosome 12 to a position where a QTL supposed to be responsible for salt tolerance was located in rice. Sequence analysis of *OSA3*showed high homology with the previously published PM H+-ATPase genes, *OSA1* and *OSA2* (Zhang *et al.*, 1999). *PM $H^+$-ATPase* genes play an important role in the establishment and maintenance of ion homeostasis. The relative abundance of *PM $H^+$-ATPase* gene transcripts in M-20 roots might indicate its active role in the strict control of $Na^+$ and $Cl^-$ uptake into root symplast and apoplast, and further translocation into the shoot (Zhang *et al.*, 1999).

Directed amplification of minisatellite DNA (DAMD analysis) was carried out with primer sequences based on heterologous variable number tandem repeat (VNTR) loci. Simple sequence repeat PCR (SSR-PCR) was employed using a range of anchored

microsatellite primers (Finch *et al.*, 1997). Large numbers of markers were shown by the production of fingerprints on the basis of co-segregation occurring between specific banding patterns and salt tolerance in rice (Erikson *et al.*, 1995).

QTL analysis was used by Dvorak *et al.* (1994) for the enhanced $K^+/Na^+$ discrimination trait in young leaves of wheat plants grown in hydroponic medium in a glasshouse and in a saline field. The recombinants showed that $Na^+$ exclusion and enhanced $K^+/Na^+$ ratios in the shoots were controlled by a single gene, *Kna1*, in the long arm of chromosome 4D. *Kna1* was completely linked to markers Xwg199, Xabc305, Xbcd402, Xpsr567, and Xpsr375 (Dubcovsky *et al.*, 1996).

From RAPD analysis of DNA from individual plants of wheat genotypes (LU26S x Rohtas 90) $F_3$, it was found that a polymorphic DNA fragment of 680 bp amplified by primer OPZ10 was associated with salt tolerance. The OPZ10680DNA marker is suggested for molecular breeding studies for salt tolerance in wheat (Rahman *et al.*, 1998).

Dehydrin *dhn* loci occurred in clusters on more than one chromosome and tended to be multigenic and might be key genetic determinants that control significant physiological processes in barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.) (Campbell and Close, 1997). Physiological traits were evaluated in barley seedlings grown in an hydroponic system under salt stress treatment. Using an AFLP map for cv. Lina x *Hordeum spontaneum* 92 associations between AFLP and quantitative traits were detected by multiple regression (Ellis *et al.*, 1997). The most effective QTL were found at different loci on chromosome 7. At the seedling stage, QTL for salt tolerance were detected on chromosome 7 in doubled haploid lines of Steptoe x Morex and Harrington x TR 306 under 500 and 1000 mol $m^{-3}$ NaCl (Mano and Takeda, 1997). According to Forster *et al.* (1997) 12 polymorphic AFLP from the extremes of salt tolerant and salt sensitive genotypes of barley were found to be significantly associated with shoot $Na^+$ and $\delta^{13}C$.

In tomato, eight genomic regions, five with favourable QTL alleles from *LA716* (*L. pennellii*, salt tolerant) and three with favourable alleles from *UCT5* (*Lycopersicon esculentum*, salt sensitive) were associated with QTL affecting salt tolerance at the germination stage under salinity (Fooled *et al.*, 1997). Fooled and Chen (1998) confirmed the work of Fooled *et al.* (1997) and found that salt tolerance is polygenically controlled in tomato at germination. Polymorphisms for the salt tolerance QTL were

also observed between *Lycopersicon esculentum* and *L. pimpinellifolium.* Many QTL for various traits were detected with internal variation and segregation in $F_2$, $F_3$ and $F_4$ families of tomato (Monforte *et al.*, 1996, 1997*a,b*).

### 1.11.5 Salt tolerant genes in rice

One group of genes is involved in salinity tolerance and $Na^+$-$K^+$ ratio and 2 groups in $K^+$ uptake in rice (Mishra *et al.*, 1996). LEA genes are important for the improvement of stress tolerance due to holding considerable potential for this trait. ABA-inducible (rab) genes are also supposed to play an important role in the mechanism of salt tolerance. Transcript levels of alcohol dehydrogenase were increased in response to stresses including salt stress in rice cv. IR54 seedlings. Pyruvate decarboxylase transcript levels increased in response to anoxia. There were increases in levels of triose phosphate isomerase, aldolase [fructose-bisphosphate aldolase], glyceraldehyde phosphate dehydrogenase and pyruvate kinase increase in response to salt stress (Minhas *et al.*, 1999). Genetic component analysis revealed that a low $Na^+/K^+$ ratio is governed by both additive and dominance gene effects under salinity tolerance in rice (Gregorio and Senadhira 1993).

Direct knowledge about the involvement of genes in physiological mechanisms and their elucidation as candidate genes for breeding is required (Flowers *et al.*, 2000). The interaction of genes for salt tolerance is still unknown in the whole plant and this lack of knowledge is the biggest obstacle in the use of modern technologies. Moreover the effects of these genes on plant physiology and development are also still a mystery (Shannon, 1997). Segregating populations involving contrasting parents, *i.e.* tolerant (CSR10) and sensitive (Bas 370) rice showed continuous variation for salt tolerance, suggesting the quantitative inheritance of the trait. Salinity tolerance is polygenic in nature and lacks maternal influence (Shannon, 1985; Yeo and Flower, 1985; Mishra *et al.*, 1998). Rice varieties with more additive genes for grain yield would perform better in saline soils (Narayanan and Rangasamy, 1991).

Expression of plasma membrane (PM) $H^+$-ATPase was investigated in rice (salt tolerant mutant M-20 and the original variety 77-170) during salt stress. A cDNA fragment corresponding to the *PM $H^+$-ATPase* gene was obtained by PCR from rice variety 77-170 and designated as *OSA3* (Table 1.3).

Two salt-inducible and one salt-repressed cDNA fragment were isolated from rice cv. ZYQ8 by using the PCR technique. The three cDNA fragments were detected, respectively, as a partial sequence of the rice S-adenosylmethionine decarboxylase (*SAMDC*) gene, a new translation elongation factor 1A gene (named *REF1A*, Table 1.3) and a novel gene of unknown function (designated *SRG1*).

**Table 1.3 Plant genes associated with salinity responses in *Oryza sativa* L. (rice)**

| Gene | Gene Product | Description | References |
|---|---|---|---|
| *AlfinI* | | Salt/drought responsive | Winicov and Bastola, 1997 |
| *DELTA1* | $\Delta^1$-pyrroline-5-carboxylate synthetase | Adaptive response by increasing proline level | Lutts *et al.*, 1998 |
| *dhn* | Dehydrin proteins | Produce specific antibodies | Close *et al.*, 1993 |
| *OSA3* | PM $H^+$ ATPase | Salt tolerance | Zhang *et al.*, 1999 |
| *OSR40, OSR40C1,* | Proteins | Dehydration tolerance, Declined after salt shock | Moons *et al.*, 1997 |
| *Rab family, RAB21, Rab16a* | Proteincontaining various glycine-rich repeats | NaCl and ABA responsive, | Mundy and Chua, 1988; Mundy *et al.*, 1990 |
| *rbcL, rbcS* | Ribulase-1,5-bisphosphate carboxylase / oxygenase (Rubisco) | Increases chlorophyll binding proteins, salt stress responsive | Winicov and Seeman, 1990; Winicov, 1994; Zhang *et al.*, 1995 |
| *REF1A, SAMDC1* | S-adenosyl methionine decarboxylase | Salt induction | ZiYin *et al.*, 1999 |
| *SalT* | Salt induced protein | Induces $Na^+$ accumulation, salt and osmotic stresses | Claes *et al.*, 1990 |

Single copy genes *SAMDC1* and *SRG1* were present in the rice genome, while the rice *REF1A* gene was organised as a gene family. The full-length cDNA of the *SAMDC* gene (named *SAMDC1*, Table 1.3), was further isolated by RT-PCR, and the deduced polypeptide was found to be homologous to other plant, yeast and human *SAMDC* proteins (ZiYin *et al.*, 1999). Salinity stress induced the expression of *SAMDC1* and *REF1A* but *SRG1* was repressed (ZiYin *et al.*, 1999). Expression of the *SAMDC1* gene in seedlings of rice is positively correlated with the salt tolerance (Li and Chen, 2000).

According to Claes *et al.*(1990) the *SalT* gene with an open reading frame coding for a protein of 145 amino acid residues is rapidly induced in sheath tissues by both salinity and related osmotic stresses in rice (Taichung native 1). *SalT* mRNA accumulation is very rapid in sheaths and roots of mature rice plants and their seedlings

when they are treated with excess salts and other stresses (Murashige and Skoog salts, air drying, ABA, polyethylene glycol, KCl and NaCl), but no induction was observed in the leaf lamina. $Na^+$ accumulation was organ-specific in rice. Sequence homology of the *SalT* genes of IR36 (Indica) and T309 (Japonica) DNA was demonstrated and homology of *SalT* with other plants (wheat, barley, oil palm and tomato) was observed (Roy *et al.*, 1993).

The ribulose-1,5-bisphosphate carboxylase oxygenase large-subunit was induced by salt in *Oryza sativa* (Zhang *et al.*, 1995) and was attributed to both nuclear (*rbcS*) and cytoplasmic (*rbcL*) encoded genes (Winicov, 1994). Increased expression of ribulose-1,5-bisphosphate carboxylase/oxygenase activity was associated with salt tolerance in selected lines (Shannon, 1997).

A novel gene, *Alfin1,* was isolated from salt-tolerant cells in alfalfa (*Medicago sativa*) and rice (*Oryza sativa*). It is important in gene regulation in roots in response to salt and an important marker for salt tolerance in crop plants (Winicov and Bastola, 1997).

A previously identified salt tolerance-associated, abscisic acid (ABA)-responsive cDNA clone, osr40c1, was isolated from roots of rice seedlings. Exogenous application of ABA and salt shock induced a marked increase in osr40c1 transcript level in roots of seedlings, whereas constant osr40c1 mRNA levels were found in the shoot. OSR40 (Table 1.3) proteins were accumulated in roots upon exposure to salt stress. OSR40c1, as shown in Table 1.3, plays a role in the adaptive response of roots to an hyper-osmotic environment and belongs to a novel plant protein family that most probably has structural functions (Moons *et al.*, 1997). Dehydrins (Table 1.3) are distinguished by the consensus KIKEKLPG amino acid sequence with many repeats within the proteins. They are key components of dehydration tolerance and were produced in response to various stresses, including salinity.

A cDNA (cOsP5CS) for delta-1-pyrroline-5-carboxylate (P5C) synthetase, an enzyme involved in the biosynthesis of proline, was isolated and characterised from a cDNA library prepared from 14-day-old seedlings of *Oryza sativa* cv. Akibare (Lutts *et al.*, 1998). The deduced amino acid sequence of the P5CS protein from *O. sativa* exhibited 74.2% and 75.5% homology to that of the P5CS from *Arabidopsis thaliana* and *Vigna aconitifolia*, respectively. The gene for P5CS (*OsP5CS*) was induced by high

salt, dehydration, abscisic acid treatment and chilling. P5CS gene expression and accumulation of proline in DGWG increased steadily as a result of high salt treatment, while in IR28 a slight increase was observed.

From the *Rab* gene family, both *RAB21* (Mundy and Chua, 1988) and *RAB16a* (Mundy *et al.*, 1990) from rice encoding a basic, glycine-rich protein, were induced when plants were subjected to water stress and NaCl. Rab21 mRNA and protein accumulate in rice embryos, leaves, roots and callus-derived suspension cells under treatment with NaCl or ABA. Furthermore it was observed that instead of cumulative effects of both NaCl and ABA, they share a common response pathway. Salt-induced genes of wheat *ESI18* and *ESI35* have sequence homology to the *Rab16* gene of rice (Mundy and Chua, 1988). $Na^+$ and $K^+$ uptake in rice are controlled by different genes that segregate independently (Garcia *et al.*, 1997).

## 1.12 Purpose of current studies

The initial purpose of the experiments reported here was to compare the accumulation of $Na^+$ and $Cl^-$ in rice leaves with that in other cereals in response to salt treatment of the roots or foliar application of salt spray (Chapter 3). These experiments raised a number of questions about the physiological responses of rice to salinity, including the relative toxicity of $Na^+$ and $Cl^-$, the effects of different growth media and the contribution of bypass flow of the transpiration stream to delivery of salts to the shoots. These questions are addressed in Chapter 4. During these experiments it was observed that the varieties Co39 and Moroberekan differed in the accumulation of $Na^+$ in their leaves. Since a mapping population derived from a cross between these varieties was available in Bangor, further experiments were designed to detect QTL for traits associated with salt responses, and particularly for $Na^+$ accumulation. These experiments are reported in Chapter 5.

# Chapter 2

# Materials and Methods

## 2.1 Plant material

Eight varieties of *Oryza sativa* (rice) were used in my experiments. They were Azucena, Bala, Co39, IR-64, KS-282, Marzhan, Moroberekan, Maratelli and F2 (Moroberekan x Maratelli), F8 (Co39 x Moroberekan) and F9 (Co39 x Moroberekan) recombinant lines. Azucena is a Japonica upland race from the Philippines and is drought resistant. Bala is an improved, drought-resistant upland Indica variety from the East Indies. Co39 is also an improved Indica variety from India. IR-64 is an improved Indica paddy variety from IRRI. KS-282 is from Faisalabad, Pakistan. Moroberekan is a drought-resistant, African Japonica land race, while Marzhan is from Russia. Seeds of Maratelli were obtained from Dr. M. L. Giudici, Centro di Ricerche Sul Riso, Ente Nazionale Risi, Castello d'Agogna, Pavia, Italy.

Four varieties of *Hordeum vulgare* (barley) were used in Chapter 3 (Chevron, CM-67, Kaya and Quantum). Chevron is a Swiss, tall and winter variety. CM-67 is a Californian variety derived from the Egyptian variety, Mariout, a short spring variety. Kaya is Turkish.

## 2.2 Flood bench system

This was a system in which water was flooded to the plants once a day for at least 15 min, twice on hot days and also twice when plants were fully grown in size. It was comprised of ten tanks each containing 20 pots of 2 l capacity. The system consisted of 200 pots in total. There was soil in the pots used as the growth medium for the plants. There were ten water reservoirs each having a capacity of more than 200 l of water, provided with ten submersible electric pumps. Tanks (80 cm x 56.25 cm x 32.5 cm) containing plant pots were placed on an iron frame, approximately 1 m high from the floor. Water reservoirs were placed on the floor. Electric pumps were required to pump the water to the tanks to a

height of 1 m. Seed trays (36.25 cm x 21.25 cm) were placed upside down, at the bottom of each tank.

**Fig. 2.1. Photo of the flood bench system**

Then P-576 plug trays were placed upside down on the seed trays to improve drainage before putting the plant pots into the tanks. There were two water connections to each tank, one for water coming into the tank from the reservoir and the other, which controlled the level of water in the tank, for water going out from the tank to the reservoir. Except in the controls, salts were added to the water reservoirs to the required concentrations. Salt and water levels were maintained by determining the electrical conductivity (EC) of the reservoirs from time to time and adding water up to the fixed level. Supplementary 400 W high pressure sodium vapour lamps were used in House 2 of Pen-y-Ffridd. The minimum temperature was maintained at 25°C and 20°C during the day and night respectively. The photoperiod was at least 16 h of light with 8 h of darkness

## 2.3 Hydroponics system

In another experiment half of the flood bench was used as an hydroponics system with some modifications. Instead of putting plant pots directly into the tanks two small tanks were placed in each of the five big tanks selected for hydroponics. For hydroponics, these big tanks had no connection with the big reservoir of water. Instead each small tank was supplied with seven litres of water and this level was maintained throughout the experiment. To avoid waterlogging conditions, the small tanks were provided with aeration for one hour daily. The salt level was also maintained at a fixed level by noting the electrical conductivity (EC) of the small tanks from time to time and adding some water up to the fixed level or by adding salt. Plastic P-84 plug-trays were cut to fit on the surface of the small tanks. These were then covered with black polythene sheets. One-week-old seedlings, which were grown in P-84 plug-trays in rockwool medium, were transferred to the hydroponics system trays with the rockwool.

## 2.4 Two and six section systems

The two section system was a bench that was divided into two parts by a partition of plastic curtains to avoid the contamination of other treatments with salt spray. Two aspirators of 50 l capacity were placed above the bench to irrigate the plants. Fifty l of solution was added to each aspirator (one per treatment solution) and this was delivered by pipes and then by drip tubes to each double pot assembly (see 2.4.1 below) through a hole in a foam plug lid. Each lid was fitted in the hole of the pot around the plant to check the entry of either the sprayed salty water or fresh water to the soil in the pots. One tank was provided with water of required salinity and the other tank was provided with fresh water to irrigate the roots of the plants in different sections of the system.

The six section system was a modification of the two section system. Due to drawbacks of the two section system, *e.g.* that there was no room for control treatments or replicates, there was a need to convert the two section system to a six section system. The only difference was the number of sections. The plant bench was divided into six sections,

two for controls, two for foliar and two for root treatments. Fifty l and 100 l of water or salt solution were placed in aspirators connected to two sections and four sections respectively.

### 2.4.1 Double pot assembly

The Double pot assembly was used to avoid spray coming into contact with the soil in the pots. Two pots each having 250 ml capacity were filled with John Innes potting compost No. 1 and fitted in such a way that a strong plastic pot was underneath while a pot with flexible plastic was fitted upside down as shown in Figure 2.2. The small central drainage hole of the upper pot was plugged with waterproof foam (*i.e.* not exactly as shown in Figure 2.2).

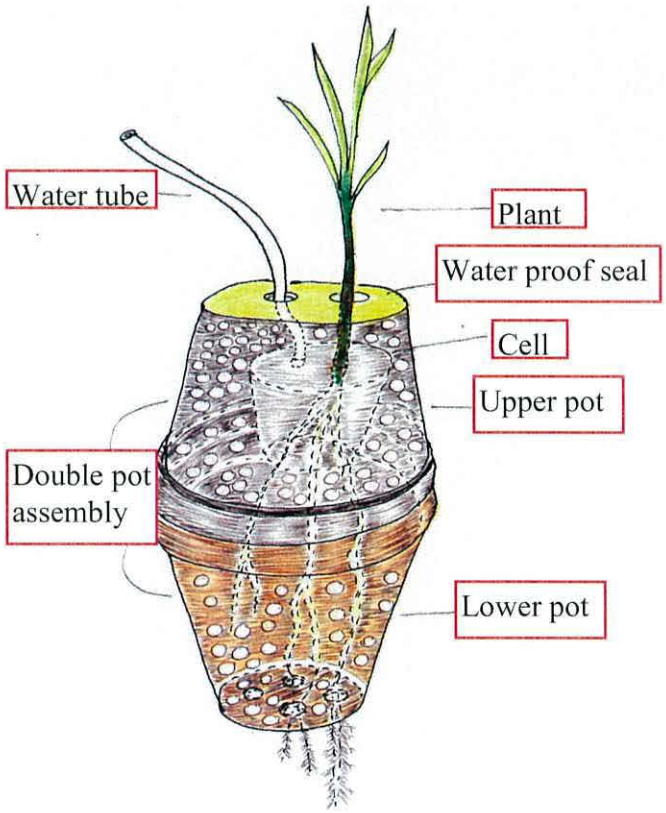


**Figure 2.2. Double pot assembly (modified from Syed, 2000)**

The foam plug had two small holes, one in the centre for the plant and the other on the side of the foam plug for the drip tube that supplied water, nutrients and salt to the roots.

The foam plug was used to avoid contamination of the root medium with foliar spray. The total capacity of the double pot assembly was 500 ml.

## 2.5 Extraction of leaf solutes

Leaf solutes were extracted by the following methods.

### 2.5.1 Sap extraction

The required plant tissues were surface dried with tissue paper and stored in sealed polypropylene 1.5 $cm^3$ microcentrifuge tubes. The tubes were frozen temporarily in a commercial freezer at approximately -18°C for at least 24 h. The tubes were taken out of the freezer for defrosting. After that the tissues were crushed with the help of a steel rod with a pointed end. Two holes were made with the help of a pin, one at the base and another at the top in the cap of the tube. Each tube was then placed in another microcentrifuge tube having the same dimensions and centrifuged at 8000 rpm for 10 min. The sap was collected into the lower tube and remaining tissues was left behind in the upper tube. The sap was then ready for analysis or for storage in the freezer for later analysis (Gorham *et al.*, 1997).

### 2.5.2 Hot water extracts

The required plant material, either dry or fresh depending on the experimental requirement, was weighed and placed in a glass tube. The material was weighed so that the amount of water used per gram dry weight could be calculated. In the case of dry material (either green parts of the leaves or injured parts of the leaves) 5ml of water was used per gram of dry leaf material. The tubes were heated in a water-bath at 80°C for at least three hours, or overnight. The tubes were allowed to cool to room temperature. Then they were centrifuged in an MSE Mistral 3000i centrifuge for 15 min at 2500 rpm at 15°C. The mixture was filtered through Whatman glass microfibre filters (GF/C 7.0 cm) and the filtrate was used for cation analyses or fluorescence measurements.

### 2.5.3 Cold water extracts

Plants were dried in an oven (70°C) and then green and necrotic parts of the leaves were ground separately with a pestle and mortar. Some liquid nitrogen was added to the material and it was ground again until a fine powder was obtained. One gram of plant powder from each plant was suspended in 5 ml of deionized water individually in 15 ml of plastic tubes and then kept in the freezer at -18°C overnight. The samples were then defrosted and centrifuged in a microcentrifuge for 15 min at 2500 rpm.

The material was filtered with 40 mm Whatman filter paper. The filtrate was used for ion analyses after preparation of diluted samples. Both hot and cold water techniques were tried because they are different techniques and they might have had some effect on the results.

## 2.6 Flame Photometer

Leaf sap was used for cation analysis by the flame photometer (Jenway PFP7). The method used for sap extraction was as mentioned above. Samples of various concentrations were prepared from the leaf sap. Only $K^+$ and $Na^+$ could be analysed with the flame photometer. For the preparation of standard curves of $K^+$ and $Na^+$ for flame photometer, 0.5, 0.4, 0.3, 0.2, 0.1, 0.05, 0.025 and 0.0 mol $m^{-3}$ of NaCl and 1.0, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05 and 0.0 mol $m^{-3}$ of KCl concentrations were used.

### 2.6.1 Working principle of flame photometer

When elements are in their stable form, their electrons are in fixed orbitals and they are at their lowest energy levels. In the flame photometer the cations are heated up and hence their electrons become excited due to absorption of energy from the flame. When they return to their low energy levels, energy is emitted as light of specific wavelengths for specific elements. The total energy emitted depends on the concentration of K or Na in the solution that is being fed to the flame photometer. The flame photometer is a digital instrument that can directly read the intensity of emitted light. The desired emission lines can be isolated by interference filters.

## 2.7 Dionex Ion chromatography

Both anions as well as cations were analysed with a Dionex ion chromatograph (Dionex 2000i, Dionex (UK) Ltd, Camberley, Surrey). With the Dionex $Na^+$, $K^+$, $Ca^{2+}$ and $Mg^{2+}$ and $Cl^-$, $NO_3^-$, Malate$^{2-}$ $SO_4^{2-}$, *etc.* could be analysed. Ion analysis was based on the attraction between particles of opposite charges. Ion-exchange separations were carried out in columns packed with an ion exchanger. There were two types of ion-exchanger, cation and anion exchanger. Cation exchangers consist of negatively charged groups and positively charged cations are attracted by them. Anion exchangers have positively charged groups and negatively charged anions are attracted by them. The system was automated by coupling to a Spark-Holland 'Marathon' auto-sampler fitted with a 5 $mm^3$ PEEK sample loop, and a Shimadzu CR5A plotting integrator linked to an Atari 1040 computer. Ions were quantified by measuring peak heights and external standards.

### 2.7.1 Anions

For anion analysis, 20 µl of sap samples was diluted in an auto-injector vial with 1.5 ml of anion eluant (2.5 mol $m^{-3}$ $Na_2CO_3$ + 2.4 mol $m^{-3}$ $NaHCO_3$ in 2.5% propan-2-ol) and analysed with the Dionex ion chromatography fitted with an AS4A anion exchange column and an Anion Micro-Membrane Suppresser at 50°C (Gorham and Bridges, 1995).

### 2.7.2 Cations

For cation analysis 20 µl of sap was diluted in an auto-injector vial with 1.5 ml of cation eluant (20 mol $m^{-3}$ methane sulphonic acid in deionized water, > 18 MOhms) and analysed by ion-exchange HPLC, fitted with a CS12 cation exchange column and a Self-Regenerating Cation Suppresser operated in auto-regeneration model. The column was operated at 50°C.

### 2.7.3 Preparation of standards for the Dionex

Preparation of standards for the Dionex was similar to the sample preparation, but instead of leaf sap, the same amount (20 µl) of anion standard solution, (250 mol $m^{-3}$ $Cl^-$, 100 mol $m^{-3}$ $NO_3^-$, 100 mol $m^{-3}$ malate$^{2-}$, 100 mol $m^{-3}$ $SO_4^{2-}$) was used with 1.5 ml of anion eluant. 20 µl of cation standard (250 mol $m^{-3}$ $Na^+$, 250 mol $m^{-3}$ $K^+$, 100 mol $m^{-3}$ $Ca^{2+}$ and 100 mol $m^{-3}$ $Mg^{2+}$) was used with 1.5 ml cation eluant.

## 2.8 Chlorophyll measurements with a SPAD meter

The chlorophyll meter (Minolta SPAD-502) was developed to measure the nitrogen status of crops. Two specific wavelengths are important for the chlorophyll meter, 650 and 940 nm. At 650 nm wavelength chlorophyll absorbs light, but at 940 nm there is no light absorption by chlorophyll. Soil Plant Analysis Development (SPAD) values, which are highly related with chlorophyll amounts, are calculated on the basis of the above mentioned two transmissions. The SPAD measures transmission of red light at 650 nm and transmission of infrared light at 940 nm. The Minolta SPAD-502 was calibrated and then used to measure the amount of chlorophyll. Fully expanded leaves number 1, 2, and 3 of the plant, starting from the youngest to the oldest respectively, were used for SPAD measurements. Each SPAD observation was the average of 9 values from leaves of the three of the biggest tillers. Three readings from each leaf were taken at approximately the same positions in each leaf. The first reading was taken from ¼ of the whole leaf length, the second was taken from the ½ and the third was measured from the ¾ of the whole leaf length, starting from the base. An average of nine observations was used to determine chlorophyll SPAD readings at the time of plant harvest.

## 2.9 Measurement of stomatal conductance with a porometer

In general, there are two types of porometers, dynamic and steady state. Both of them have some advantages and some disadvantages. These two groups are further subdivided into ventilated and non-ventilated groups. The AP4 model porometer falls in the dynamic category.

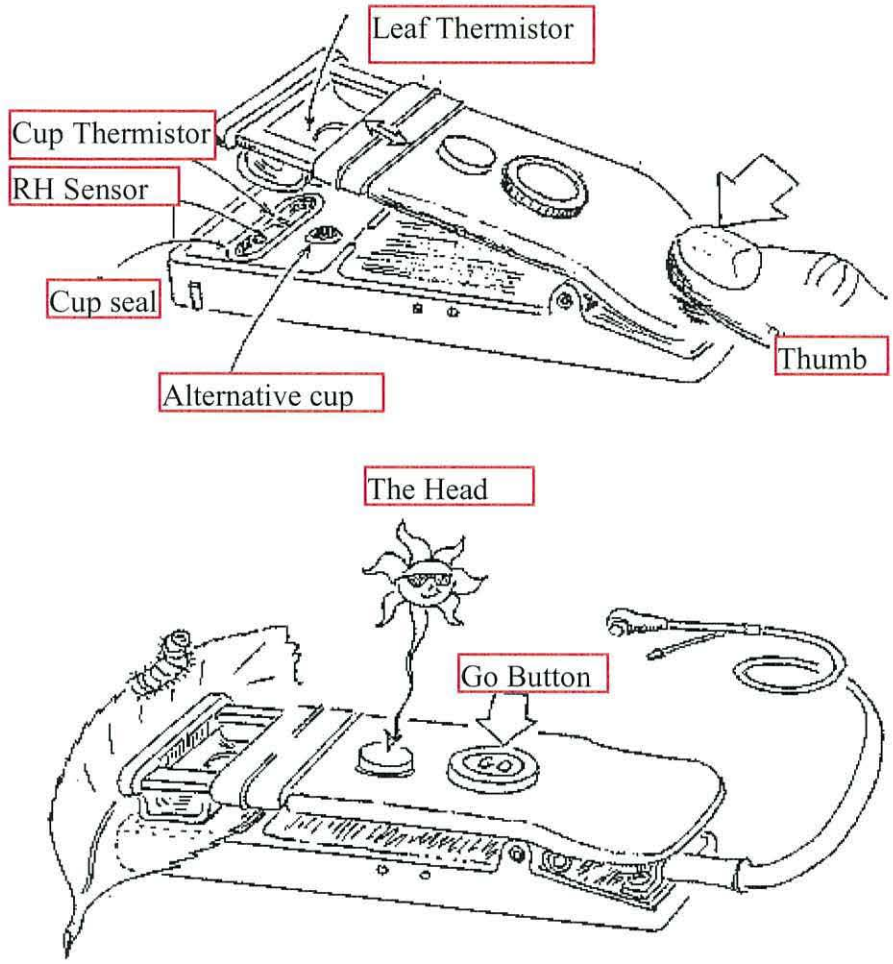


**Figure 2.3. Porometer for the measurement of stomatal conductance (modified from Instrument Manual)**

The principle is based on enclosing the leaf in a small air chamber provided with a humidity sensor, and measuring the time taken for a leaf to release enough water to change the relative humidity in this chamber by a fixed quantity. The cycle is repeated and is compared with a calibration plate of known resistance to give the stomatal resistance of the leaf. This porometer converts the readings into calibrated values of conductance and resistance automatically (Verhoef 1997).

Measurements were taken from the central position of the leaf avoiding the midrib. Four readings from each position (of equal distances along the leaf (length-wise), dividing the leaf into approximately 5 equal parts) were measured from both upper and lower sides

and the actual values were saved and stored in the porometer. Care was taken to ensure that the whole area of the porometer chamber was covered with leaf tissue.

## 2.10 Use of an Infra-Red Gas Analyser (IRGA) for photosynthesis and transpiration measurements.

For photosynthesis, the IRGA works on the principle of measuring the difference of concentration of $CO_2$ going in and coming out of a leaf chamber. The difference between the amount of $CO_2$ inside and outside the chamber, multiplied by the flow rate, is a direct measure of net photosynthesis.

The IRGA has an infrared detector that measures the $CO_2$ concentration in the air leaving the chamber. The IRGA has a relative humidity sensor and a light sensor because light intensity is also an important factor for net photosynthesis (Pn), stomatal conductance (gs) and transpiration (E).

Water use efficiency = Net photosynthesis/Transpiration

An LCA2 with one analyser was used as the IRGA for the measurements of Pn, gs, and E. The second youngest leaf of the biggest tiller of each plant was clamped in a Parkinson narrow leaf chamber. The leaf chamber was provided with a seal to keep the chamber air-tight. After putting the leaf in the chamber it was pressed gently to make it air-tight, but not harshly to avoid crushing the leaf. A Qbasic programme on a PC was used to download the data from the LCA2 data logger as an ASCII file into a spreadsheet (EXCEL).

The ADC LCA2 system (Analytical Development Co. Ltd., Hoddesdon, Herts., UK) contains the following four basic units.

1. IRGA.
2. Parkinson narrow-leaf chamber
3. Air supply unit (ASU) with mass flow meter.
4. Data logger.

The leaf being measured was adaxial side up and supplementary light was used to take measurements under saturating light intensity (> 1000 μmol $m^{-2}$ $s^{-1}$ PAR). The IRGA and Parkinson narrow leaf chamber were connected to an air supply unit taking air from outside the greenhouse. A 10 litre bottle acted as a buffer to minimise variations in

$CO_2$ supply. The air was dried by passing it through two columns of blue silica gel (dry air is supplied and the RH of the air leaving the chamber is measured). Leaves were placed in the chamber promptly and observations were recorded within 60 seconds. Initially when a leaf was clamped in the chamber, RH changed quickly due to the establishment of an initial equilibrium. Readings of $CO_2$ depletion could be taken at the time of slowly changing RH values (From Parkinson leaf chamber manual, Analytical Development Company). Photosynthesis was calculated from the difference in the mole fraction of $CO_2$ between the chamber entrance (reference) and the outlet (sample).

Stomatal conductance and transpiration can also be determined from the data logger information using the equations of von Caemmerer and Farquhar (1981).

**Calculation and analysis of results**

Mass flow of air can be converted to mole flow of air with the help of the following equation.

$$f=(f_v/1000)*(1/22.4)*(273.15)/(273.15+T)*(p/101.3)*(1/60)$$

where

f = mole flow of air (mol $s^{-1}$)

$f_v$ = volumetric flow of air ($cm^3$ $min^{-1}$)

22.4 = volume in $dm^3$ of one mole of air at S.T.P.

T = temperature recorded during measurement (°C)

p = atmospheric pressure during measurement (kPa)

Other parameters can be calculated by the following equations:-

**a. Transpiration rate**

Transpiration rate E could be calculated by the following equation.

$$E = (f/sa)*\{(x_0 - x_e)/(1-x_0)\}$$

where

E = transpiration rate (mol $m^{-2}$ $s^{-1}$)

sa = surface area

$x_0$ = mole fraction of water vapour at leaf chamber outlet (mol $mol^{-1}$)

$x_e$ = mole fraction of water vapour at leaf inlet (mol $mol^{-1}$)

$x_0$ and $x_e$ are calculated from saturated vapour pressure ($x_s$)

At the measured leaf temperature and the given RH:

$x_0 = (x_s)*(RH/100)$

**b. Assimilation rate**

Assimilation rate, A, could be calculated by the following formula

$A = (f/sa)*(\Delta c)*\{(1-x_e)/(1-x_0)\}$

where

$\Delta c$ = $CO_2$ differential between reference and analysis streams (mol $mol^{-1}$)

$(1-x_e)/(1-x_0)$ correction for water vapour

**c. Stomatal conductance**

Stomatal conductance, gs could be calculated by using the following equation:

$gs = (E/x_{s,T1}-x_{0)}$

where

gs = stomatal conductance (mol $m^{-2}$ $s^{-1}$)

$x_s$ = mole fraction of water vapour at saturation. It is assumed here that the leaf is saturated with water at the actual leaf temperature T1.

**d. Internal $CO_2$ concentration, ci:**

Internal $CO_2$ concentration, ci, can be calculated with the equation given as follows:

$ci = (c_0-A)*(1.6/gs)$

where

$c_0$ = mole fraction of $CO_2$ in outlet air from leaf chamber given by ($c_e$- $\Delta_c$) from reference and differential measurements (μmol $mol^{-1}$), and 1.6 = ratio of diffusion of $CO_2$ and water in air.

## 2.11 Measurements of water potential and osmotic pressure with Thermocouple Psychrometer Meter Model 85

Leaf disks were taken with a cork borer from the centre of fully expanded leaves. They were given the leaf numbers 1, 2 and 3 from each plant, from its biggest tiller starting from youngest to old respectively, and immediately placed in the psychrometer chamber. Then the psychrometer chamber was closed tightly. The psychrometer chambers were numbered and the list of plant disks with their respective psychrometer chamber number was made. After the completion of this process the psychrometer chambers were transferred to a water bath previously adjusted at 30$^{O}$C. After at least two hours, the psychrometer leads were connected to the Morgan Model 85 meter. Temperature was noted down by switching the microvolt/temperature switch to the temperature position. Then the switch was returned to the microvolt position. The psychrometers with broken leads were not used. In this position the meter displayed the output of the selected sensor in microvolts. Zero adjustment was rotated until a zero reading was obtained but the zero adjustment was done with the select switch turned to an open channel. Although the meter was ready to begin operation within a few seconds, it was however a good idea to allow it to warm up for a few minutes to stabilise it internally. It was necessary to reset the meter before continuing to the next step and after that start was pressed. Most readings were taken with the same settings, if possible. The next sensor was selected by rotating the select switch. If new settings were required for a sensor then there was the need to make adjustments by repeating the previous process. After the completion of 12 samples that set of psychrometers was disconnected and the other set having numbers 13 to 24 was connected. These numbers were connected with 1 to 12 numbers in ascending order with the connector. Water potential was calculated in microvolts by subtracting the offsets from the actual values because psychrometer readings for both offset and actual values were in

microvolts. Then with the help of a calibration equation the microvolts were converted to MPa. After measuring all the samples the psychrometer chambers were taken out of the water-bath and immersed in liquid nitrogen for a few minutes and then again transferred to the water-bath. After an hour or more the previous process was repeated to measure the new offset values and new microvolt values. From these values osmotic potentials were determined in MPa. A calibration curve was made as outlined below.

### 2.11.1 Calibration curve

A solution of NaCl (1500 mosmol $kg^{-1}$) at a constant temperature was made and standards were prepared from this solution. A thermocouple psychrometer was calibrated by suspending the solution on a filter disk in a sealed chamber containing water vapour source of 0, 300, 600, 900, 1200 mosmol $kg^{-1}$ of NaCl. The calibration curve was produced by using the above mentioned values and the following curve equation was obtained from these values:

$$Y = 0.2275x - 0.66$$

Each psychrometer was zeroed individually to avoid variability among units that could affect their calibration characteristics. With thermocouple psychrometers the following four primary variables could be identified as influencing estimates of water potential and overall performance of psychrometers:

1. The microvolt output of the psychrometer
2. The equilibrium temperature of the sample and the psychrometer
3. The Peltier cooling current and length of cooling time
4. The influence of temperature gradients on psychrometer output.

### 2.11.2 Basic theory of thermocouple psychrometers

According to Brown and Bartos (1982) psychrometers are used to infer the water potential of soils, plant tissues and other media from measurements of equilibrium vapour pressure. Instruments are designed for use as closed systems within a sealed chamber or for *in situ* use in the field. Thermocouple psychrometers are comprised of a thermocouple constructed of 0.00254 cm (0.001 inch) diameter chromel and constantan wires welded to

form a sensing junction. A short distance back from the sensing junction these wires are each attached to separate copper lead wires of large diameter forming the reference junctions of the psychrometer. The sensing junction and two reference junctions form the essential constituents of a thermocouple psychrometer. Type of psychrometers depend on the design and material used in the construction of the protective house around the thermocouple. Materials used include ceramic, stainless steel screen and solid stainless steel or Teflon tubing with a screen end window.

When the water potential of the medium and vapour pressure of the air around it are in dynamic equilibrium, that is called the vapour pressure equilibrium state. After the achievement of this state, water potential measurements are taken by using the Peltier effect. A small electric current (approximately 5 mA) is passed through the psychrometer circuit from the constantan to the chromel side of the thermocouple for a short time (15 seconds). This current causes the sensing junction to cool slightly below ambient temperature. During the cooling of the thermocouple below the dew point of its surrounding atmosphere, water vapour in the air condenses on the sensing junction. After a specified cooling time, the condensed water on the junction immediately begins to evaporate back into the surrounding atmosphere due to termination of the current. Meanwhile the thermocouple is cooled again, but now as a function of the rate of evaporation, which is a function of the vapour pressure of the atmosphere and hence the water potential of the medium. Isothermal conditions are necessary because, due to thermal instability, water vapour moves from warmer to cooler regions, and temperature gradients cause errors in estimates of water potential by disrupting the thermal stability between the sensing junction of the psychrometer and the evaporating sample surface or between the sensing and reference junctions (Brown and Bartos, 1982).

## 2.12 Measurements of Fm, Fo, Fv and Fv/Fm with a chlorophyll fluorescence meter

Fully expanded leaf 2 was selected for the chlorophyll fluorescence measurements. Chlorophyll fluorescence dark adaptation clips were fixed on the centre of leaf number 2 of

the biggest tiller of each plant and the clip shutter was closed to prevent the entry of light to the portion of the leaf underneath the clip. After 20 minutes dark adaptation the fibre optic was connected with the upper part of the clip. The clip shutter was opened so that the part of the leaf that was kept in the dark could receive a flash of light. Photosystem two (PSII or PS 680) has maximum photochemical efficiency at 680 nm wavelength while Photosystem I (PSI or PS 700) has maximum photochemical efficiency at 700 nm. Measurements of Fm, Fo, Fv and Fv/Fm were recorded to study the effect of salinity on photochemical efficiency (Fv/Fm) and for this purpose Fm, Fo, and Fv were determined. Fo is the minimal or initial fluorescence, Fm is the maximum fluorescence, Fv is a variable fluorescence and hence Fv is not a direct measurement but it is dependent on Fo and Fm values (Fv = Fm-Fo). Each observation was recorded and saved in the Hansatech Fluorescence Monitoring System (FMS)

## 2.13 Measurements of fluorescence with a fluorescence spectrometer

PTS tracer (Trisodium, 8-hydroxy-1,3,6-pyrene trisulphonic acid) was measured by fluorescence spectrometry. Plant shoot or leaf extracts, extracted by the above mentioned method, were used for fluorescence measurements. First of all wavelengths were adjusted to the following fixed values:

- λ Excitation = 403 nm
- λ Emission = 510 nm

Then the spectrometer was calibrated to zero with pure water. The samples were transferred to 3 ml cuvettes to measure the fluorescence, and the cuvette was rinsed three times before each sample change. All four sides of the cuvette were cleaned with fine tissue to remove any spots or liquid on them. Fine white tissues were used to avoid scratching the surface of the cuvette.

## 2.14 Shoot fresh weight

Shoot fresh weight was recorded. Firstly the leaf fresh weight was recorded for samples that were stored in the microcentrifuge tubes for ion analysis. Then the rest of the shoot was cut with the help of seceteurs at the point of root and shoot junction and then weighed to record the shoot fresh weight. Total shoot fresh weight was calculated by the addition of individual leaf weights to the rest of the shoot fresh weight.

## 2.15 Root fresh weight

Root fresh weight was recorded only in the case of hydroponics. The water was removed from the tubs and a short time was given to drain off suplus water around the roots. The roots were then dried gently with tissue paper to remove all traces of water to record the actual root fresh weight.

## 2.16 Shoot dry weight

After taking fresh weight the plants were kept in labelled paper bags individually and then dried in an oven at 70°C for at least three days and weighed.

## 2.17 Statistical analyses

All data were analysed by the MINITAB 11 or 12 statistical packages, using descriptive ANOVA, as well as General Linear Model (GLM.) and one way analysis of variance functions to assess significant differences ($P \leq 0.05$), and Tukey's test to make pairwise comparisons. EXCEL and SPSS programs were also used for graphs, correlation and comparisons. The data were compared by SPSS, one way ANOVA, Post Hoc, "Duncan's multiple comparisons" test and the letters a,b, c, and d were used to denote the different groups. Anderson-Darling normality test from the statistical package of basic statistics was applied to the data to look for the distribution either symmetrical or skewed ($P \geq 0.05$ = normal distribution). The level of significance for all figures and tables were ≤ 0.05=*, 0.01=** and non significance =NS.

## 2.18 QTL analysis

An $F_8$ mapping population derived from the cross Co39 x Moroberekan was used to map quantitative traits. The RFLP data of Champoux *et al.* (1995) were used to generate a linkage map using the software MAPMAKER/EXP. The genetic map made by Champoux was used with exception of one of the markers. Data for QTL were prepared using MAPMAKER/EXP version 3.0 b, and QTL were identified using MAPMAKER/QTL version 1.1b. The heights of the peaks in the graphical output were considered to select the level of significance (LOD score) and high peaks were chosen. The MAPMAKER programme could not handle all of the data at one time. Chromosomes 1 to 11 were analysed in one step *i.e.*, 281 lines, 117 markers and 14 traits were studied in that step. In the second step chromosome 12 was studied separately for the same number of lines and traits but the markers were only 9. Data were log transformed only if the distribution was skewed (see Chapter 5)..

### 2.18.1 MAPMAKER

According to Lander *et al.* (1987) MAPMAKER is an interactive computer package for constructing genetic linkage maps, and for mapping genes underlying complex traits using those linkage maps. MAPMAKER/EXP is an experimental-cross only successor to the original MAPMAKER package. MAPMAKER/EXP understands more types of experimental crosses and incorporates an algorithm for detecting potential genotyping errors. A new three-point analysis feature helps to allow automatic analysis of very large data sets faster. A "join haplotypes" feature helps efficiently handle recombinationally unseparated markers. Multi-point linkage analysis considers all of the raw (genotypic) data available simultaneously in each computation to find map orders and map distances. The availability of high-resolution molecular genetic maps has made it feasible to understand the underlying genetic and biochemical basis of many traits displaying complex modes of inheritance. To accomplish this, a maximum likelihood algorithm for mapping the genes underlying quantitative traits segregating in experimental populations. MAPMAKER/QTL is a companion program to MAPMAKER/EXP which allows one to map genes controlling

polygenic quantitative traits in F2 intercrosses and BC1 backcrosses relative to a genetic linkage map (Lander *et al.* 1987).

The raw data were prepared in the EXCEL program for use by MAPMAKER. 'A' and 'B' represented the alleles from parental strain a and b for each marker respectively, while '-' was the representation for missing data for the individual at that particular locus. After the initial linkage map had been created, and checked with the map of Champoux *et al.* (1995), the quantitative data were added to the data files, and MAPMAKER/QTL used to determine the location of probable QTL. QTL were identified as regions with high (see Chapter 5 for definition) LOD scores.

According to Lander *et al.* (1987) inter crosses between inbred lines are more useful than backcrosses, but their analysis is more complicated than backcrosses because inter crosses can provide double information for the construction of a linkage map than backcrosses. For experimental organisms such as rice or maize in which inbred lines are available and large crosses can be arranged easily, construction of a linkage map involves the inheritance of RFLPs (or other markers) in appropriate pedigrees. The scope of MAPMAKER is limited to the construction of primary genetic linkage maps using co-dominant, dominant or recessive traits in F2 type pedigrees. .

# Chapter 3

# Basic Salinity Studies-Foliar versus Root Treatments

## 3.1 Introduction

Salinity is the major parameter that could reduce the yield of rice in irrigated rice production systems, including coastal and arid regions. In the field rice seeds are germinated in small plots, or sometimes in pots, depending on the requirements of the location. The seedlings are then transplanted to the flooded field. These plants are, for a short while, under totally or partially submerged conditions. During this period the plants can absorb different ions present in the water *via* roots as well as *via* leaves. If water is saline then salts in the soil or irrigation water can be absorbed directly by the shoots. Furthermore foliar uptake is relevant to salt spray in coastal areas, *e.g.* in the case of Sardinian land races of barley (Gorham *et al.*, 1994).

Aragues *et al.* (1994) in a field experiment at Zaragoza, Spain, applied salty water irrigation treatments to barley cvs. Georgie, Hassan, Patty and Trait d'Union in a triple line source sprinkler system as follows:

1. Plants were covered to avoid salt spray on leaves and saline water was applied directly to the soil (root treatment);
2. Plants were pre-irrigated for 3 min with fresh water in addition to a regular 30 min irrigation with saline sprinkler water and a 3 min post-washing with fresh water (pre and post-washing );
3. As 2 but without 3 min pre-irrigation with fresh water (post-washing).

Exposure to saline spray plus soil salinity (pre-washing and non-pre-washing treatments) decreased grain yields more than soil salinity only (root treatment). Increased salinity decreased grain yields substantially less in the pre-washing treatment than in the non-pre-washing treatment. 50% reduction of grain yield in barley was observed at 12.9 dS/m of NaCl in treatment 2 and at 10.8 dS/m in treatment 3. Differences in grain yield were also observed and were associated with lower

concentrations of salt in leaves from the pre-washing treatment. The first several minutes of sprinkler irrigation were considered critical in the foliar absorption of salts (Aragues *et al.*, 1992,1994).

Grattan *et al.* (1994) in a similar field experiment at Zaragoza, Spain, irrigated barley with saline water using a triple-line-source sprinkler system. Young leaves of barley were more sensitive to $Cl^-$ accumulation under foliar treatment regardless of whether the plants were uncovered or covered to avoid wetting. The distinction between the foliar and the root treatments for the accumulation of $Cl^-$ was reduced with increased leaf age and reduced salinity of the irrigation water. However the concentration of accumulated $Cl^-$ was doubled in case of uncovered plants due to transpiration and dehydration. Moreover a direct relation was observed between the $Cl^-$ concentrations in young leaves and the $Cl^-$ concentration of the irrigation water. Salt concentration of the medium, leaf age, method of salt application, and their interactions were important in $Cl^-$ accumulation in barley. There was more accumulation of $Cl^-$ at high salinity than low salinity, more accumulation of $Cl^-$ in young leaves than older leaves and more accumulation of $Cl^-$ under foliar treatments compared to root treatment at high salinity and young leaf age. However the difference between foliar and root treatment for the accumulation of $Cl^-$ was reduced at low salinity and in older leaves. Foliar treatment was more injurious than root treatment at early stages because $Cl^-$ ions were so quickly absorbed initially by foliar tissues under foliar saline sprinkler treatment that further absorption of $Cl^-$ ions *via* root treatments was checked due to presence of high concentrations of $Cl^-$ in the leaf tissue (Grattan *et al.*, 1994).

High amounts of $Na^+$ and $Cl^-$ ions were found in the foliage of white cedar (*Thuja occidentalis*) sprayed with de-icing salt (Foster and Maun, 1980). According to Cole *et al.*, (1977) foliar absorption of sodium and chloride occurred during periods of high salinity in the irrigation water in *Citrus* grown on deep sandy soils and irrigated by fixed overhead sprinklers in South Australia. It was thought that foliar $Na^+$ and $Cl^-$ concentrations could be the most important factors causing poor tree health, and consequently low yields and possibly low fruit quality. A micro-sprinkler method could be a more suitable alternative method, but furrow irrigation was not satisfactory (Cole *et al.*, 1977).

Gorham *et al.* (1994) observed that $Na^+$ uptake in barley varieties grown in soil was different under foliar and root treatments of salinity. Malakondaiah and Rao (1971) observed that uptake of $^{32}P$ was lower through the roots than through the leaves and suggested that foliar absorption is an active process coupled with the metabolic activity of leaf cells. Franke (1975) found that ectodesmata are hollow spaces in the secondary cell wall that functioned as pathways for aqueous solution in foliar absorption and excretion in spinach and *Viola tricolor* leaves. Dirr (1990) observed that soil applications were more injurious than sprays when 0.15 M NaCl solutions were applied 3 times/week to established container-grown plants of Leyland cypress, X *Cupressocyparis leylandii,* and red-tip *Photinia.* Leyland cypress did not develop necrosis but *Photinia* leaves were necrotic in both salt treatments, however DW of plants were reduced only by soil salinity treatment (Dirr, 1990).

Fomishina *et al.* (1980) found that foliar sprays of NaCl produced greater adverse effects on sugar beet plants than soil treatment. Verlodt and Boesman (1977) observed that calcium chloride had no effect on yield of tomato cultivars, Ventura, Campbell 1327, Super Roma, Cal J, VF 198 and Petomech when sprayed at 0, 2.5 or 5% at full bloom on leaves.

Wagenvoort (1976) observed the effects of salt sprays (10 g NaCl $m^{-2}$) on 11 ornamental shrub species applied to the soil or over the foliage at various frequencies from December to March to simulate splashing from roadside salt treatment. More severe leaf damage was observed in June in the case of foliar rather than soil treatment. Leaf injury caused by foliar sprays was not permanent, but appeared more severe in June than did damage due to root uptake of salt. The expression of symptoms in the fairly tolerant species *Potentilla fruticosa* showed no differences between foliar and soil treatments, whereas for the highly susceptible *Pachysandra terminalis* a foliar spray was lethal (Wagenvoort, 1976).

Sorour *et al.* (1975) observed that foliar spraying of cotton with trace elements containing various levels of $CaCl_2$ + NaCl did not affect flowering. However a positive relationship was observed between salinity and the number of flowers per plant, bolls per plant and yield per plant with irrigation water up to 2500 ppm salt content.

Dirr (1975) observed higher concentrations of $Na^+$ in the shoot from foliar applied $Na^+$ salts compared to soil treatments, while shoot $Cl^-$ was greater in soil treated

plants compared with spray treatments. However, shoot $Cl^-$ generally corresponded with the severity of plant injury whereas shoot $Na^+$ did not, when $Na_2SO_4$, $K_2SO_4$, NaCl, KCl or $CaCl_2$ at concentrations of 0.25 N were applied daily as soil drenches or foliar spray to plants of *Hedera helix*. Leaf necrosis was visible after only 7 days and the injury was more severe on plants sprayed with $Cl^-$ salts. Shoot dry weights of plants treated with $Cl^-$ salts were significantly less than those of control plants or those sprayed with $SO_4^{--}$ salts. Marginal leaf necrosis developed on plants growing in the soil drenched with $Cl^-$ salts after 28 days (Dirr, 1975).

According to Franke (1975) apoplastic regions for transport were identified in the cell wall. The presence of ectodesmata, ectocythodes or ectoteichodes in the cell wall was determined and their involvement in foliar transport of inorganic ions was also confirmed. Lyon (1973) observed ectodesmata in *Phaseolus vulgaris* by freeze etching as shallow, hemispherical structures with granular contents underneath the cuticle in replicas of transversely fractured leaves after fixation with sublimate fixative, its acid components or mercuric chloride alone.

. Franke and Alexander (1986) suggested that it could be quite possible that absorption or extraction or exclusion of aqueous solutions and the passage of ions across the plasmalemma were not through the whole cuticle but through punctiform areas that connected with the ectoteichodes, and then diffusion through the tubular cavity systems of the ectoteichodes (Franke and Alexander, 1986).

According to Franke (1967) direct stomatal penetration does not account for much foliar uptake. Absorption by the leaves was a multi-step process involving passive penetration through the cuticle and active absorption through the plasma membrane by the leaf cells beneath the cuticle. Ectodesmata were fine structures in the outer walls of epidermal cells. Initially they were thought to be homologous to the plasmodesmata that perforate the inner walls of tissues and connect neighbouring protoplasts. They were named outerwall plasmodesmata due to having similar shapes to plasmodesmata and their location in the outer wall, but they were later called ectodesmata. They seemed to provide a direct connection of the protoplasts with the outside medium. Ectodesmata were chiefly found along the anticlinal walls, in some hairs, in the basal cells of hairs, in epidermal cells surrounding hairs; above, beneath and on both sides of the veins, guard cells and subsidiary cells (Franke, 1967).

The current foliar salt application work (Table 3.1) is related to saline overhead sprinkler irrigation and to areas where seawater sprays onto the foliar parts of plants in coastal areas (Gorham *et al.*, 1994). Salty spray in coastal areas of Pakistan is also important for plants. Experiment 1 is not considered here because the roof of the greenhouse at Pen-y-Ffridd, right above the plants, was blown away resulting in damage to the plants. Experiment 4 was not included in the manuscript because the results were affected by poor growing conditions over the winter. Experiments 2, 3, 5 and 6 were conducted to study the physiological changes occurring in the plants during absorption of saline water foliar versus roots. These experiments were designed to test the following hypothesis:

"Ion uptake in *Oryza sativa* is affected by both method of application and variety".

## 3.2 Experimental design

### 3.2.1 Experiment 2

The objective of this experiment was to test the following hypothesis: "Ion uptake in *Oryza sativa* is affected by both method of application and variety".

There were ten replications for the experiment, ten lines in total, comprising four lines of barley and six lines of rice as shown in Table 3.1. The experiment was started on 10$^{th}$ December 1997 when rice seeds were sown. Barley seeds were sown on 6/01/98. Rice and barley seeds were germinated in single cells in P 84 plug trays. Rice seedlings were transferred 28 days after sowing and barley 8 days after sowing to double pot assemblies (section 2.4.1) which were then transferred to the "two section" system 36 and 16 days after sowing respectively. The nutrients (Phostrogen 1g $l^{-1}$ + 0.5 ml $l^{-1}$ micronutrients + 0.1 ml $l^{-1}$ potassium silicate) were given to all plants through the roots.

**Table 3.1 Summary of experimental designs**

| Expt No. | NaCl (mol $m^{-3}$) | $CaCl_2$ (mol $m^{-3}$) | Pot | Variety | Treat. | Section | Replicate |
|---|---|---|---|---|---|---|---|
| 2 | 100 | 50 | Double 500 ml | **Barley** Chevron, CM-67, Kaya, Quantum **Rice** Azucena, Bala, Co39 IR-64, Marzhan, Moroberekan | Foliar Root | 1 1 | 10 |
| 3 | 100 | 10 | Double 500 ml | Azucena,Bala,Co39 IR-64,Maratelli, Marzhan, Moroberekan | Control Foliar Root | 2 2 2 | 12 |
| 5 | 150 | 15 | Single 2 litre | Co39,IR-64, Maratelli, Moroberekan | Control Foliar Root | 2 2 2 | 8 |
| 6 | 200 | 20 | Single 2 litre | IR-64,KS-282, Maratelli, Moroberekan | Control Foliar Root | 2 2 2 | 8 |

Plants of barley and rice were more or less of the same sizes at the time of salt stress. Salt stress was begun 55 days and 28 days after sowing for rice and barley respectively. Stress was applied to the plants in daily increments of 25 mol $m^{-3}$ NaCl + 12.5 mol $m^{-3}$ $CaCl_2$. Stress was completed 58 days after sowing in the case of rice and 31 days after sowing in the case of barley. After the completion of stress increments, full strength salt (100 mol $m^{-3}$ NaCl + 50 mol $m^{-3}$ $CaCl_2$) was applied on alternate days up to harvest. Rice and barley were harvested 77 days and 50 days after sowing respectively. Treatments were;

T1: NaCl 100 mol $m^{-3}$ + $CaCl_2$ 50 mol $m^{-3}$ in the root medium + foliar spray with water and T2: Foliar spray with NaCl 100 mol $m^{-3}$ + $CaCl_2$ 50 mol $m^{-3}$.

Foliar washing was done to remove the salts from the surface of the leaf prior to harvest to avoid contamination of the surface of the leaf with external salt spray.

Leaf samples were frozen until used for leaf sap extraction (section 2.5.1). Dilutions were prepared for "Dionex" cation analysis as described in section 2.7.3. SPAD meter readings to measure the amounts of chlorophyll were taken as averages of five leaves per plant from 5 cm above the base of the leaf.

### 3.2.2 Experiment 3

The objective of this experiment was the same as experiment 2, but with reduced $CaCl_2$ (from 50 to 10 mol $m^{-3}$ of $CaCl_2$) and the same (100 mol $m^{-3}$) NaCl concentrations. The level of $CaCl_2$ was reduced because of poor survival of plants in Experiment 2, which might have been caused by high $Cl^-$ concentrations.

There were 12 replications for the experiment. Seven lines of rice, as shown in Table 3.1, 12 plants/line and 3 treatments (Table 3.1) were used, making a total of 252 plants. Rice seeds were soaked on 25/3/98, that is one day prior to sowing. Rice seeds were sown on 26/3/98 and were germinated directly in the double pot assemblies. Plants were transferred to the "Six section" system 26 days after sowing. Nutrients (Phostrogen 1g $l^{-1}$ + 0.5 ml $l^{-1}$ micronutrients + 0.1 ml $l^{-1}$ potassium silicate) were given to all plants through the roots. Stress was applied to the plants 38 days after sowing through the roots as well as by foliar spray in daily increments of 25 mol $m^{-3}$ NaCl + 2.5 mol $m^{-3}$ $CaCl_2$. After completion of the increments, full stress (100 mol $m^{-3}$ NaCl + 10 mol $m^{-3}$ $CaCl_2$) was applied on alternate days up to harvest. Treatments were:

T1; control with fresh water foliar spray and fresh water through roots,

T2; foliar spray with 100 mol $m^{-3}$ NaCl + 10 mol $m^{-3}$ $CaCl_2$ + fresh water through roots,

T3; 100 mol $m^{-3}$ NaCl + 10 mol $m^{-3}$ $CaCl_2$ through roots + foliar fresh water spray.

Foliar washing was done to make the leaf surface free of any traces of salt due to foliar salt spray. The youngest leaf was selected from the main stem (shoot) of each plant for leaf sap extraction at 66 days after sowing. Leaf samples were prepared and analysed by "Dionex" as above. Growth parameters were studied at the time of final harvest.

### 3.2.3 Experiment 5

#### 3.2.3.1 Experimental changes for the 5th and 6th experiments

The drawbacks of the third experiment were as follows.

1. Pot size was small for the rice plants, especially at later stages.
2. Water and nutrient supply was not enough to cope with the requirement of the plants.

To remove these constraints the following changes were essential for the next experiment and therefore these modifications were applied in the fifth experiment. Pot size was increased to 2 l to cope with the growth of the rice plants at later stages. The aspirator was bigger (200 l) and the number of pots was reduced.

#### 3.2.3.2 Protocol for Experiment 5

The objective of this experiment was the same as in Experiments 2 and 3, but with a higher concentration of NaCl. The drawbacks of the previous experiments (pot size, nutrient supply) were also removed to obtain greater accuracy of the work.

There were 8 replications for the experiment. Four lines of rice as shown in Table 3.1, 8 plants/line and 3 treatments were used making a total 96 plants. To study the effect of increased concentration of NaCl salinity on foliar and root treatments a higher salinity was used in this experiment Treatments were:

T1, Control [Fresh water in the root medium + fresh water foliar spray],

T2 [Foliar spray with NaCl 150 mol $m^{-3}$ + $CaCl_2$ 15 mol $m^{-3}$],

T3 [NaCl 150 mol $m^{-3}$ + $CaCl_2$ 15 mol $m^{-3}$ in the root medium + foliar spray with fresh water].

The experiment was started on 22nd of July 1998, but the rice seeds were soaked on 21st July. Rice seeds of four varieties, (Co39, IR-64, Maratelli and Moroberekan) were germinated singly, directly in two litre pots. The substrate was John Innes Compost No. 1. Prior to the start of salt stress the pots were provided with foam lids to avoid the entry of foliar spray into the root medium. The plants were transferred to the six section system 28 days after sowing. Nutrients, (Phostrogen 1g $l^{-1}$ + 0.5 ml $l^{-1}$ micronutrients + 0.1 ml $l^{-1}$ potassium silicate) were given through the roots to all plants starting 35 days after sowing and throughout the rest of the experiment. For the details of micronutrients please see the paper of Hoagland and Arnon (1950).

Stress was started 46 days after sowing and full stress was achieved 51 days after sowing. Salts were applied in increments of 25 mol $m^{-3}$ and 2.5 mol $m^{-3}$ NaCl and $CaCl_2$ respectively per day. Full stress of 150 mol $m^{-3}$ of NaCl and 15 mol $m^{-3}$ of $CaCl_2$ was given until harvest. Foliar washing was done to remove salts from the surface of the leaf prior to harvest. The first harvest was taken 70 days after sowing for cation analyses (section 2.7.2). Leaf samples were prepared in the same way as described in chapter 2 for leaf sap preparation. Plants were harvested finally 95 days after sowing for cation analysis and for the study of growth parameters including leaf fresh weight and shoot fresh weight. At the final harvest, shoot fresh weights and shoot dry weights were recorded.

### 3.2.4 Experiment 6

Replications were 4 lines, 8 plants/line and 3 treatments, giving a total 96 plants. Experiment 6 was different in the concentrations of both salts, NaCl and $CaCl_2$, although the ratio of the two salts remained the same. Furthermore Co39 was replaced with KS-282 because it was supposed to be more salt resistant than Co39. Treatments were:

T1, Control [Fresh water in the root medium + fresh water foliar spray],

T2 [Foliar spray with NaCl 200 mol $m^{-3}$ + $CaCl_2$ 20 mol $m^{-3}$] and

T3 [NaCl 200 mol $m^{-3}$ + $CaCl_2$ 20 mol $m^{-3}$ in the root medium + foliar spray with fresh water].

The ratio of NaCl: $CaCl_2$ was 10: 1. Seeds of four varieties of *Oryza sativa* (IR-64, KS-282, Maratelli and Moroberekan) were soaked on 3/11/98. Seeds were sown in 2 l pots directly on 4/11/98. John Innes Compost No 1 was used as the growth medium. Waterproof foam lids were provided 30 days after sowing to the pots to check the entry of foliar sprayed water to the water in the root medium. Plants were transferred to the six section system on the same day. Nutrients [Phostrogen 1g $l^{-1}$ + 0.5 ml $l^{-1}$ micronutrients + 0.1ml $l^{-1}$ potassium silicate] were supplied through roots 31 days after sowing and then throughout the experiment. Full stress was started 63 days after sowing, both in the root medium as well as by foliar spray. Stress was completed 71 days after sowing. Salts were applied in increments of 25 mol $m^{-3}$ and 2.5 mol $m^{-3}$ NaCl and $CaCl_2$ respectively per day.

Full salt stress was applied in the root medium as well as *via* foliar spray daily up to harvest. Leaves were washed before harvesting as in previous experiments. Plants were harvested 88 days after sowing. The biggest leaf of the biggest tiller from each plant was frozen in a labelled 1.5 ml microcentrifuge tube for cation analysis. Shoot fresh weight was recorded at the same day. Shoot dry weight was also measured..

## 3.3 Results

### 3.3.1 Experiment 2

#### 3.3.1.1 $Na^+$ in leaf sap of barley under salinity

Four barley varieties as described in Table 3.1 are presented in Figure 3.1, were grown under 100 mol $m^{-3}$ of NaCl + 50 mol $m^{-3}$ of $CaCl_2$ salinity for comparison with the work already done on these barley varieties by Gorham *et al.* (1994).

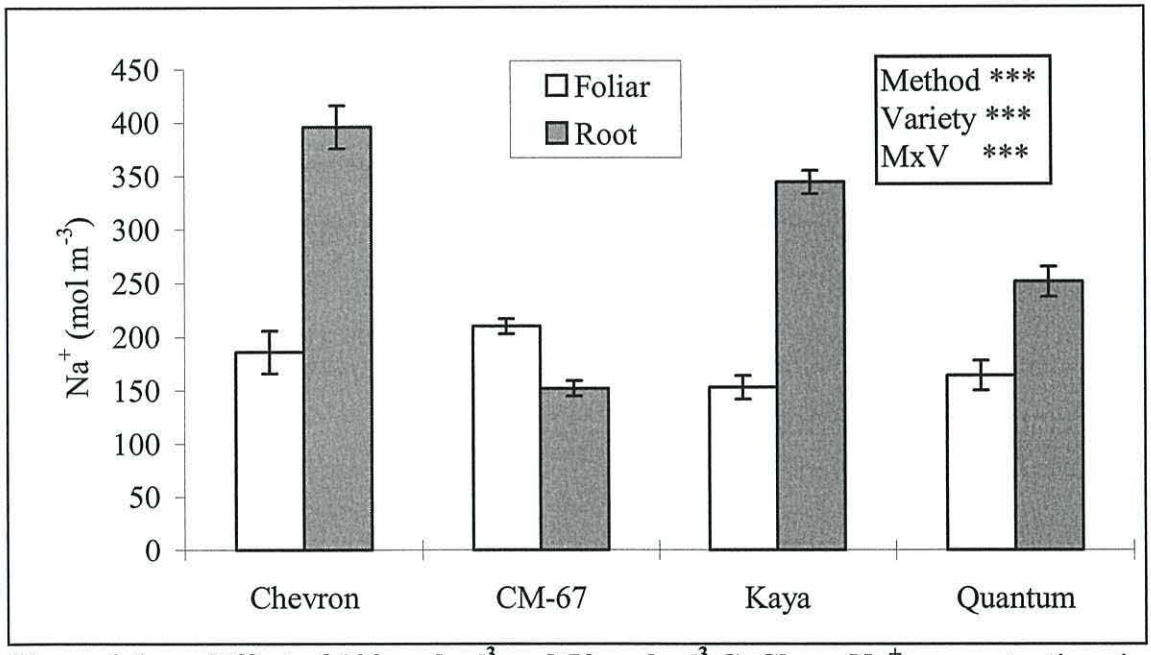


**Figure 3.1. Effect of 100 mol $m^{-3}$ and 50 mol $m^{-3}$ $CaCl_2$ on $Na^+$ concentrations in leaf sap of barley. (experiment 2). The data were analysed by GLM test. Levels of significance ≤ 0.001=***.**

As the data for rice and barley were analysed separately by analysis of variance by applying GLM test so the standard errors which were denoted by vertical bars are for 1 standard error each side of the mean as shown in Figure 3.1. The ≤ 0.001 level of significance was denoted by ***. Varieties, method of salt application and method x

variety interaction had significantly different (P=0.000) effects and had highly significant effects on $Na^+$ concentration in barley (Figure 3.1) . However pair-wise comparisons from the Tukey's test of one way analysis showed that under foliar treatments CM-67 was significantly different (P=0.000) from the other varieties and accumulated the highest $Na^+$ concentration compared with Kaya and Quantum, while under root treatment CM-67 accumulated the lowest concentration of $Na^+$ compared with the other varieties (Tukey's test). Chevron accumulated the highest concentration of $Na^+$ when salt was applied to the roots (Tukey's test). Similar results were obtained by Gorham *et al.* (1994). Both the varieties and methods of salt application had significant effects (p=0.000).

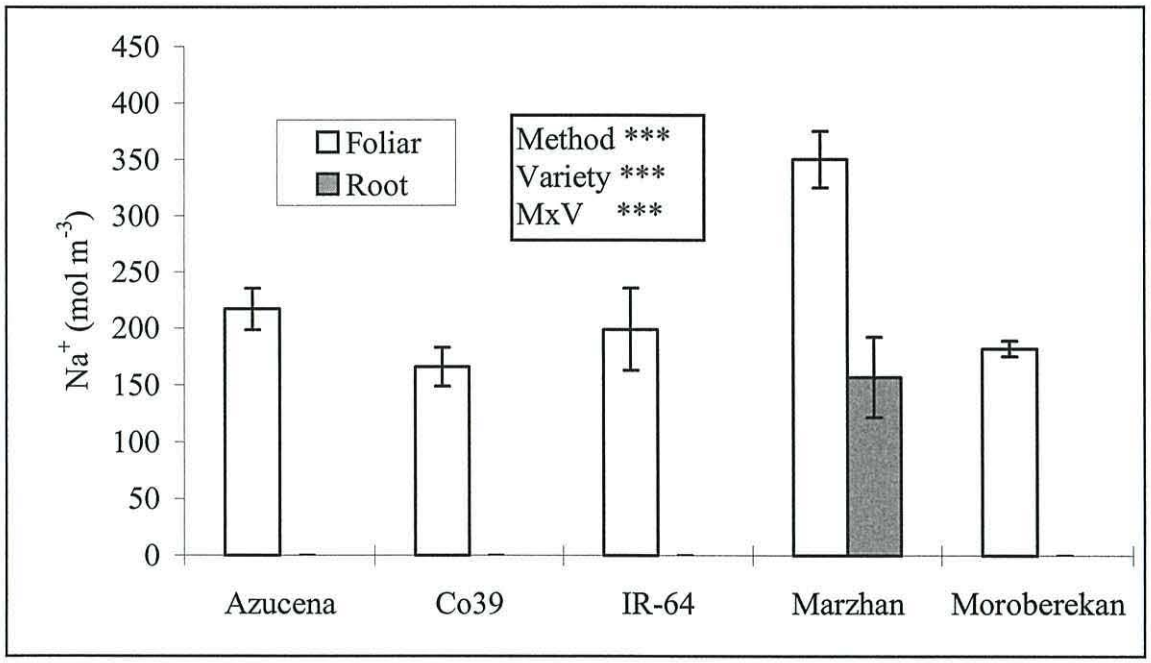


**Figure 3.2. Effect of 100 mol $m^{-3}$ NaCl and 50 mol $m^{-3}$ $CaCl_2$ on $Na^+$ concentrations in leaf sap of rice. (Experiment 2). The data were analysed by analysis of variance by applying one-way analysis. The level of significance ≤ 0.05=*, ≤0.01=**, ≤ 0.001=***.**

### 3.3.1.2 $Na^+$ in leaf sap of rice under salinity

Six varieties of rice as described in Table 3.1 were grown, but no data were obtained in the root treatment except for Marzhan, because the other varieties were completely dead so could not be presented in Figure 3.2. One-way ANOVA was used to analyse the rice data separately. There were no data for Bala under both foliar and

root treatments because it could not survive in the given salt concentrations. Under foliar treatment the $Na^+$ concentration in the leaf sap was the highest in Marzhan, while the lowest accumulation of $Na^+$ was found in the leaf sap of Co39. SPAD data from rice and barley are presented in Table 3.2. Barley has high values for SPAD data compared with rice under both foliar and root treatments. The lowest value for SPAD (14) was recorded in Bala under foliar treatment and the highest value for SPAD (34) was recorded in Marzhan under root treatment in case of rice. Low variation in SPAD readings was observed in most of the rice varieties under foliar treatment as is obvious from Table 3.2. Under foliar treatments in barley, the lowest SPAD value (24) and the highest (38) were recorded in Chevron and Quantum respectively. Under root treatment in barley, the lowest SPAD value (33) was recorded in Chevron and the highest (47) was recorded in CM-67. Data analysed by GLM test and analysis of variance showed that method of salt application and variety had significant (P=0.000 and 0.007 respectively) effects in rice. The method and variety also had significant effects in barley (P=0.001, P=0.025 respectively). However the method x variety interaction was not significant in barley or rice.

**Table 3.2. Effect of 100 mol $m^{-3}$ NaCl and 50 mol $m^{-3}$ $CaCl_2$ on SPAD measurements of leaf chlorophyll content. Values are means ± standard errors of 10 replicates (Experiment 2).**

| Treatment | Rice variety | SPAD reading | Barley variety | SPAD reading |
|---|---|---|---|---|
| **Foliar** | Azucena | 19 ± 1 | Chevron | 24 ± 1 |
| **Root** | | 22 ± 5 | | 33 ± 1 |
| **Foliar** | Bala | 14 ± 3 | CM-67 | 32 ±10 |
| **Root** | | 21 ± 2 | | 47 ± 2 |
| **Foliar** | Co39 | 17 ± 1 | Kaya | 30 ± 1 |
| **Root** | | 24 | | 42 ± 1 |
| **Foliar** | IR-64 | 21 ± 1 | Quantum | 38 ±10 |
| **Root** | | 26 ± 5 | | 40 ± 1 |
| **Foliar** | Moroberekan | 20 ± 2 | | |
| **Root** | | 27 ± 0 | | |
| **Foliar** | Marzhan | 20 ± 2 | | |
| **Root** | | 34 ± 3 | | |

### 3.3.2 Experiment 3

Data was analysed by GLM test. Methods of salt application, varieties, and their interaction had significant effects on $Na^+$ concentrations in the leaf sap and their respective P values are P=0.000, 0.036 and 0.032 as shown in figure 3.3. There was a significant increase in $Na^+$ concentrations in the leaf sap of all the varieties of rice via foliar and root treatments compared with their controls. $Na^+$ concentrations were less in experiment 3 than in experiment 2. Presumably this less accumulation of $Na^+$ could be the result of 50 l more irrigation water daily in experiment 3 than experiment 2. So plants in experiment 3 were not so severely dehydrated as in experiment 2. Furthermore the presence of low concentrations (10 mol $m^{-3}$) of $CaCl_2$ in experiment 3 compared to high concentrations (50 mol $m^{-3}$) of $CaCl_2$ in experiment 2 could be responsible for the low accumulation of $Na^+$ in experiment 3. In experiment 3 plants were more vigorous than in experiment 2, so the concentration of $Na^+$ was diluted to low levels because of greater growth.

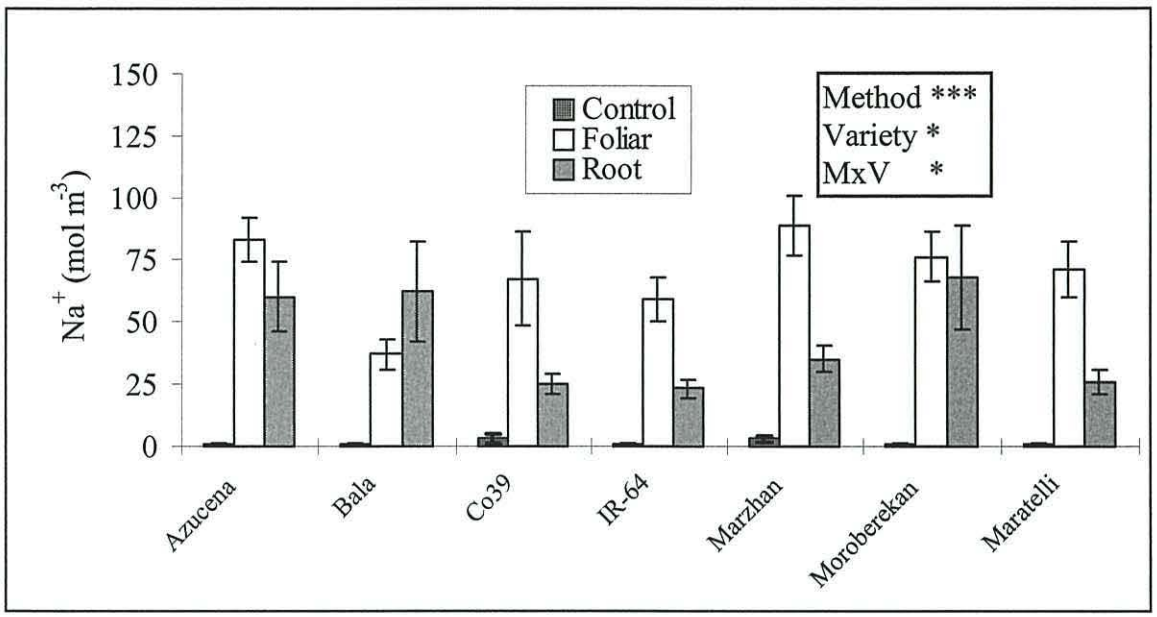


**Figure. 3.3. Effect of 100 mol $m^{-3}$ NaCl and 10 mol $m^{-3}$ $CaCl_2$ on $Na^+$ concentrations in leaf sap of rice (Experiment 3). The data were analysed by the GLM test (excluding control). Levels of significance ≤ 0.05 =*, ≤0.01=**, ≤ 0.001=***.**

Under foliar treatment Bala accumulated the lowest and Marzhan the highest concentration of $Na^+$ in the leaf sap (Figure 3.3) Under root treatment two sets of varieties were distinguished on the basis of high and low $Na^+$ accumulation in their leaf saps. Azucena, Bala and Moroberekan were in the high $Na^+$ accumulation group and the highest $Na^+$ was found in Moroberekan (68 mol $m^{-3}$), while Co39, IR-64, Marzhan and Maratelli fell into the low $Na^+$ accumulation group (< 35 mol $m^{-3}$ $Na^+$).

Table 3.3 shows the growth data from experiment 3. Under root treatment the reduction in shoot fresh weight and shoot dry weight was observed in all the varieties. The lowest SFW (18 g) and the lowest SDW (5.6 g) were recorded in Maratelli under root treatment as shown in Table 3.3. However under foliar treatment no reduction in shoot fresh weight and shoot dry weight was observed, instead, increase in all varieties except Co39 was observed both in shoot fresh weight and dry weight. The highest SFW (60 g) and the highest SDW (16.3 g) were observed in Maratelli under foliar treatment as shown in Table 3.3. The data were analysed by GLM test. Varieties and methods of application had significant effects (P≤0.000) on shoot fresh and dry weights. However shoot fresh and dry weights for method x variety interactions had just significant effects with their respective significance levels P=0.049 and P=0.011.

**Table 3.3. Effect of 100 mol $m^{-3}$ NaCl and 10 mol $m^{-3}$ $CaCl_2$ on growth parameters of rice in g/plant. Values are means ± standard errors of 12 replicates (Experiment 3).**

| Variety | Treatment | Shoot FW (g) | % control | Shoot DW (g) | % control |
|---|---|---|---|---|---|
| **Azucena** | Control | 35.54 ± 3.0 | 100 | 9.62 ± 0.8 | 100 |
| | Foliar | 42.31 ± 5.3 | 119 | 11.55 ± 1.4 | 120 |
| | Root | 21.09 ± 3.7 | 59 | 6.30 ± 1.0 | 65 |
| **Bala** | Control | 31.82 ± 3.3 | 100 | 8.68 ± 0.8 | 100 |
| | Foliar | 39.77 ± 3.2 | 125 | 10.54 ± 1.0 | 121 |
| | Root | 23.56 ± 4.3 | 74 | 6.76 ± 0.9 | 78 |
| **Co39** | Control | 38.45 ± 4.7 | 100 | 10.82 ± 0.9 | 100 |
| | Foliar | 38.41 ± 5.3 | 100 | 10.17 ± 1.2 | 94 |
| | Root | 30.12 ± 2.5 | 78 | 8.56 ± 0.6 | 79 |
| **IR-64** | Control | 31.77 ± 4.6 | 100 | 9.03 ± 1.4 | 100 |
| | Foliar | 37.13 ± 4.6 | 117 | 10.29 ± 1.1 | 114 |
| | Root | 26.50 ± 1.8 | 84 | 7.91 ± 0.5 | 88 |
| **Marzhan** | Control | 53.62 ± 5.1 | 100 | 14.91 ± 1.1 | 100 |
| | Foliar | 56.06 ± 6.2 | 105 | 15.82 ± 1.3 | 106 |
| | Root | 29.14 ± 3.0 | 54 | 8.30 ± 0.8 | 56 |
| **Moroberekan** | Control | 45.94 ± 6.6 | 100 | 11.09 ± 1.5 | 100 |
| | Foliar | 48.97 ± 5.5 | 107 | 12.16 ± 1.4 | 110 |
| | Root | 21.29 ± 3.1 | 46 | 6.01 ± 0.8 | 54 |
| **Maratelli** | Control | 45.03 ± 7.4 | 100 | 13.38 ± 1.6 | 100 |
| | Foliar | 60.34 ± 7.5 | 134 | 16.33 ± 2.0 | 122 |
| | Root | 18.25 ± 3.0 | 41 | 5.58 ± 0.7 | 42 |

### 3.3.3 Experiment 5

In experiment 5, 150 mol $m^{-3}$ of NaCl + 15 mol $m^{-3}$ of $CaCl_2$ salinity were applied to the plants via foliar and root treatments. The data were analysed by one way ANOVA (excluding control). The method of salt application and varieties had significant ($p \leq 0.006, 0.012$) effects on $Na^+$ concentrations (Figure 3.4).

$Na^+$ concentrations in leaf sap increased under both the treatments compared with their controls. Under root treatment the highest concentration of $Na^+$ was found in the leaf sap of Moroberekan. Co39, IR-64 and Maratelli fell into a low $Na^+$ accumulation group (< 30 mol $m^{-3}$ $Na^+$). On comparison of these results with experiments 2 and 3 it is obvious that shortage of water (dehydration) might be the cause of high accumulation of $Na^+$ in the case of experiment 2. However, in the case of experiment 5 these constraints were removed with some experimental modifications. So the results of experiment 5 for $Na^+$ accumulation were more reliable. As the draw backs of experiments 2 and 3 were removed and enough water for irrigation was supplied to the plants so more vigorous plants compared to experiments 2, and 3 were obtained in this experiment. So plant growth is responsible for diluting the concentrations of $Na^+$ ions in the case of experiment 5, and resulted in low $Na^+$ concentrations (Figure 3.4) and high shoot fresh and dry weights (Table 3.4) compared with experiments 2 and 3.

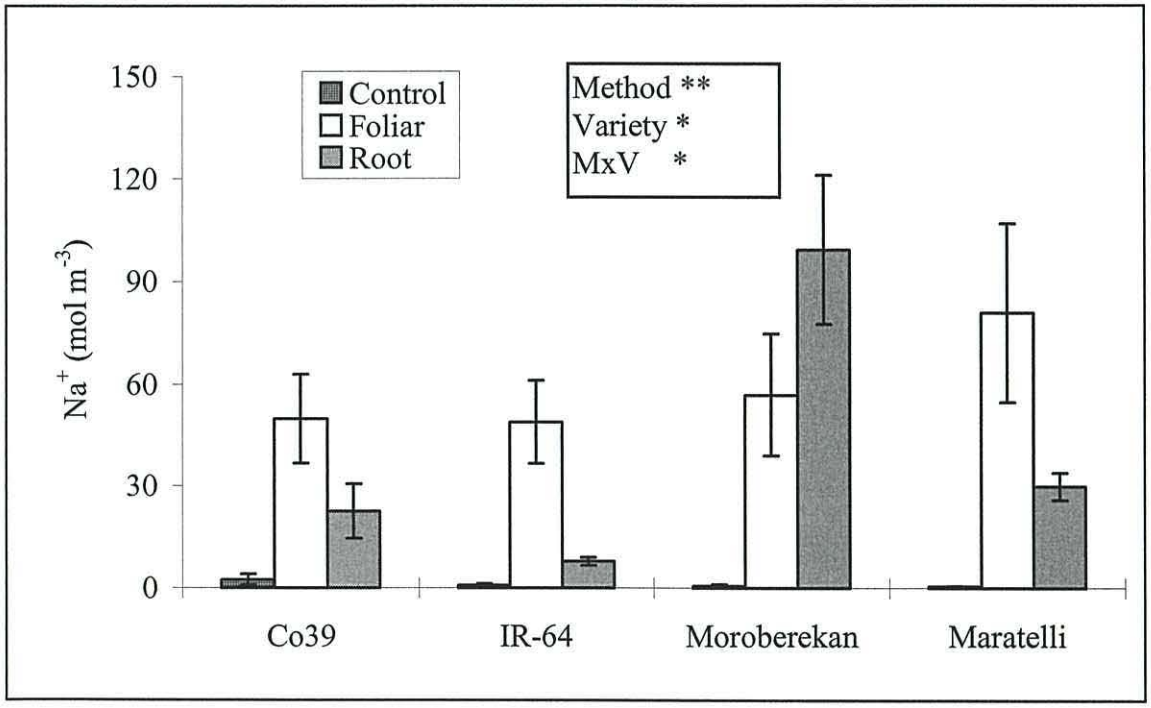


**Figure. 3.4. Effect of 150 mol $m^{-3}$ NaCl and 15 mol $m^{-3}$ $CaCl_2$ on $Na^+$ concentrations in leaf sap of rice (Experiment 5). The data were analysed by GLM test (excluding control) level of significance ≤ 0.05=*, ≤0.01=**, ≤ 0.001=***.**

Data for growth parameters from experiment 5 are illustrated in Table 3.4. The reduction in shoot fresh weight and shoot dry weight was observed in all varieties under

foliar and root treatments. However the reduction in SFW and SDW was more pronounced under root treatment. The lowest percentages for SFW (35 %) and SDW (44 %) were recorded in Moroberekan under root treatment. However under foliar treatment the lowest values for SFW (39 g) and SDW (12 g) were recorded in Maratelli. The highest SFW (81 g) in Moroberekan and the highest SDW (21 g) in IR-64 were observed under foliar treatment. Under root treatment, the highest SFW (75 g) and SDW (18 g) were recorded in IR-64. The data were analysed by GLM test. Varieties had significant effects on shoot fresh weight (P=0.001) and shoot dry weight (P=0.005) while method had highly significant (P=0.000) effects on shoot fresh and dry weights. Method x variety interaction had a significant (P=0.020) effect on SFW but not on SDW.

**Table 3.4. Effect of 150 mol $m^{-3}$ NaCl and 15 mol $m^{-3}$ $CaCl_2$ on growth parameters of rice in g/plant. Values are means ± standard errors of 8 replicates (Experiment 5).**

| Variety | Treatment | Shoot FW (g) | % Control | Shoot DW (g) | % Control |
|---|---|---|---|---|---|
| **Co39** | Control | 112 ± 8 | 100 | 25 ± 3 | 100 |
| | Foliar | 72 ± 5 | 64 | 19 ± 1 | 76 |
| | Root | 71 ± 6 | 63 | 17 ± 2 | 68 |
| **IR-64** | Control | 93 ± 7 | 100 | 23 ± 2 | 100 |
| | Foliar | 63 ± 9 | 68 | 21 ± 2 | 91 |
| | Root | 75 ± 6 | 81 | 18 ± 2 | 78 |
| **Moroberekan** | Control | 114 ± 12 | 100 | 27 ± 3 | 100 |
| | Foliar | 81 ± 8 | 71 | 19 ± 2 | 70 |
| | Root | 40 ± 4 | 35 | 12 ± 1 | 44 |
| **Maratelli** | Control | 81 ± 19 | 100 | 21 ± 6 | 100 |
| | Foliar | 39 ± 8 | 48 | 12 ± 3 | 57 |
| | Root | 42 ± 8 | 52 | 10 ± 2 | 48 |

### 3.3.4 Experiment 6

#### 3.3.4.1 $Na^+$ in leaf sap of rice

Under 200 mol $m^{-3}$ of NaCl + 20 mol $m^{-3}$ of $CaCl_2$ salinity (experiment 6) varieties had significant (P = 0.000) effects on leaf $Na^+$ concentrations in a GLM test applied to the data (excluding control). The method of salt application and method and variety interaction had no significant effects (Figure 3.5). However from a one -way analysis of variance the method of salt application (not including the control) had significant effects in the case of IR-64 (P=0.007).

There were significant increases in $Na^+$ concentrations in the leaf sap of all varieties under foliar and root treatments, compared with their controls as shown in Figure 3.5. Under foliar treatment $Na^+$ accumulation in leaf sap of rice was highest in Maratelli. Under root treatment $Na^+$ accumulation was low in IR-64 and KS-282 but in Moroberekan and Maratelli the $Na^+$ accumulation was high (>200 mol $m^{-3}$) and the highest $Na^+$ concentration was observed in Maratelli._

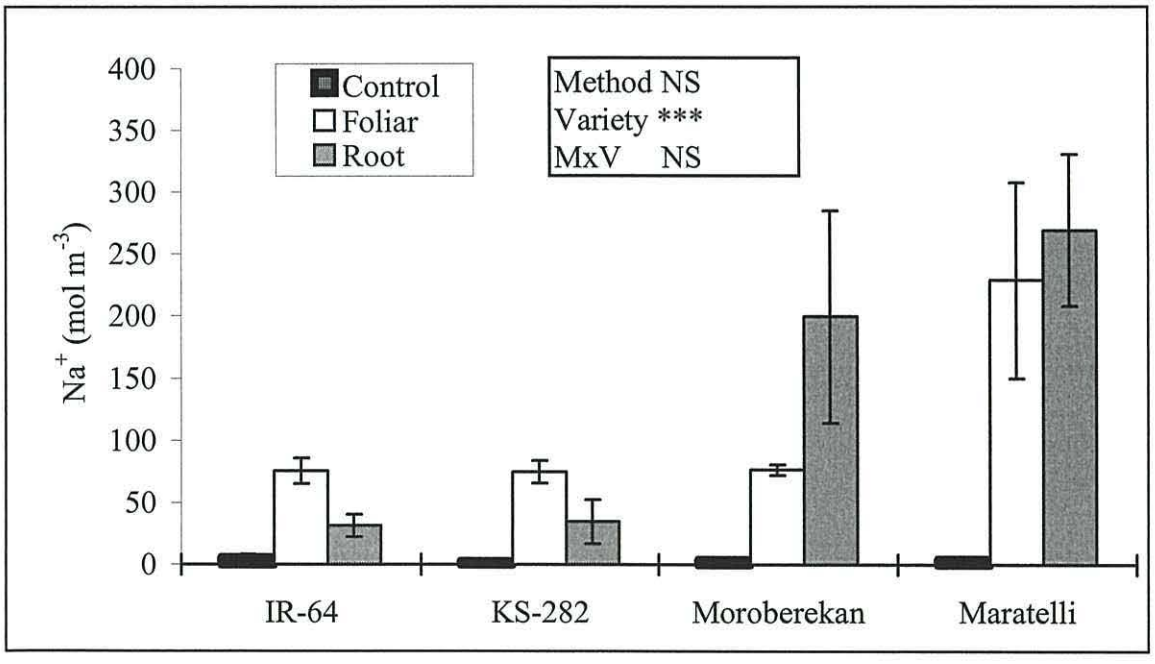


**Figure. 3.5. Effect of 200 mol $m^{-3}$ NaCl and 20 mol $m^{-3}$ $CaCl_2$ on $Na^+$ concentrations in leaf sap of rice (Experiment 6). The data were analysed by GLM test (excluding control). The level of significance ≤ 0.001 =*** and non significance = NS.**

Data for growth parameters from experiment 6 are presented in Table 3.5. The reduction in shoot fresh weight and shoot dry weight was observed in all varieties under foliar and root treatments except IR-64 under foliar treatment. However the reduction in SFW and SDW was more pronounced under root treatment. The lowest percentages for SDW (35 %) and SFW (30 %) were recorded in Maratelli under foliar and root treatment respectively. GLM test was used for analysis of variance and showed that varieties had significant effect for shoot fresh weight (P=0.033) and shoot dry weight (P=0.018) while the method of salt application had highly significant effects on shoot fresh and dry weights (P=0.000). However method and variety interactions had no significant effects on shoot fresh and dry weights under both foliar and root treatments. Furthermore the data were analysed by one way analysis and Tukey's test was used for pair-wise comparisons. IR64 and Moroberekan were not significantly different from each other under both foliar and root treatments.

**Table 3.5. Effect of 200 mol $m^{-3}$ NaCl and 20 mol $m^{-3}$ $CaCl_2$ on growth parameters of rice in g/plant. Values are the means ± standard errors of 8 replicates (Experiment 6).**

| Variety | Treatment | Shoot FW (g) | % Control | Shoot DW (g) | % Control |
|---|---|---|---|---|---|
| **IR-64** | Control | 54 ± 10 | 100 | 9 ± 2 | 100 |
| | Foliar | 62 ± 14 | 115 | 10 ± 2 | 109 |
| | Root | 22 ± 5 | 41 | 5 ± 1 | 55 |
| **KS-282** | Control | 77 ± 15 | 100 | 12 ± 2 | 100 |
| | Foliar | 72 ± 13 | 94 | 11 ± 2 | 93 |
| | Root | 30 ± 18 | 39 | 7 ± 2 | 55 |
| **Moroberekan** | Control | 48 ± 12 | 100 | 7 ± 1 | 100 |
| | Foliar | 41 ± 8 | 85 | 6 ± 1 | 79 |
| | Root | 17 ± 4 | 35 | 4 ± 1 | 53 |
| **Maratelli** | Control | 82 ± 15 | 100 | 14 ± 3 | 100 |
| | Foliar | 31 ± 8 | 38 | 4 ± 1 | 35 |
| | Root | 25 ± 5 | 30 | 5 ± 1 | 41 |

### 3.3.4.2 $K^+$ in leaf sap of rice

The data were analysed by GLM test. Figure 3.6 shows that the method of salt application and variety had highly significant (P=0.000) and (0.001) effects but method and variety interactions had just significant (0.020) effects on $K^+$ concentrations.

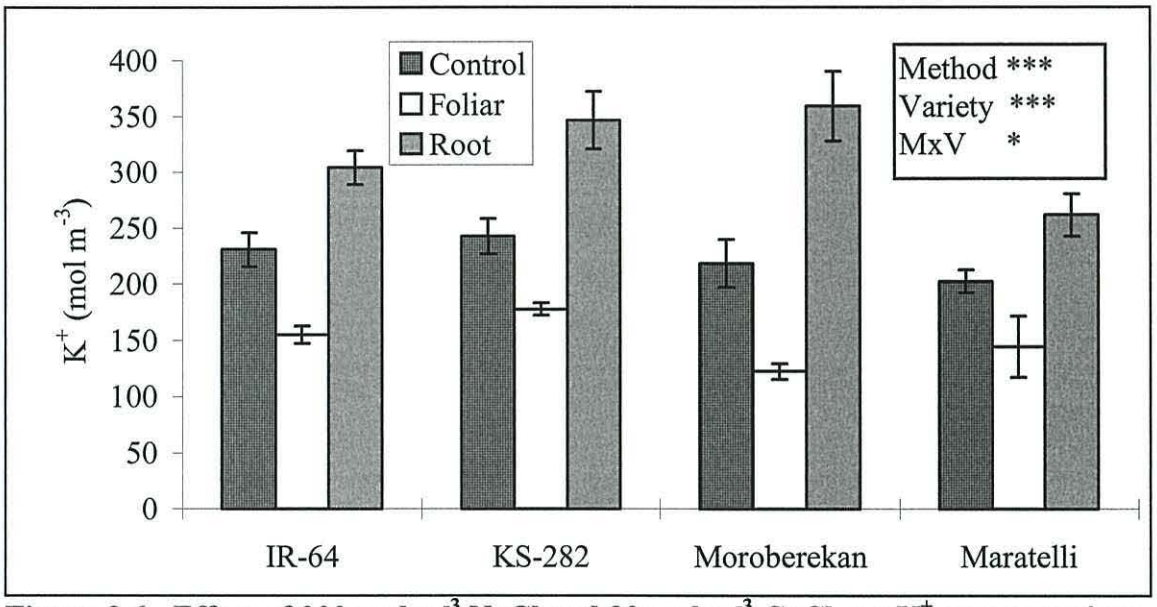


**Figure 3.6. Effect of 200 mol $m^{-3}$ NaCl and 20 mol $m^{-3}$ $CaCl_2$ on $K^+$ concentrations in leaf sap of rice (Experiment 6). The data were analysed by applying GLM test. The levels of significance were ≤ 0.05=*,≤ 0.001=*****

Under foliar treatment, the highest concentration of $K^+$ was found in the leaf sap of KS-282 and the lowest in Moroberekan (Figure 3.6). Under root treatment, Maratelli accumulated the lowest concentration of $K^+$. Under foliar treatment the $K^+$ concentration in the leaf sap of all the varieties decreased compared with the controls, but the opposite was true under root treatment.

### 3.3.4.3 $K^+$ per unit dry weight in rice

The data were analysed by GLM test. The method of salt application and varieties had significant (P=0.000, 0.003) effects on $K^+$ per unit shoot dry weight. However method x variety interaction had no significant effect (Figure 3.7). Under

foliar treatment there was a significant decrease in $K^+$ concentrations per unit dry weight in all the varieties, but under root treatments the $K^+$ was unaffected (Figure 3.7).

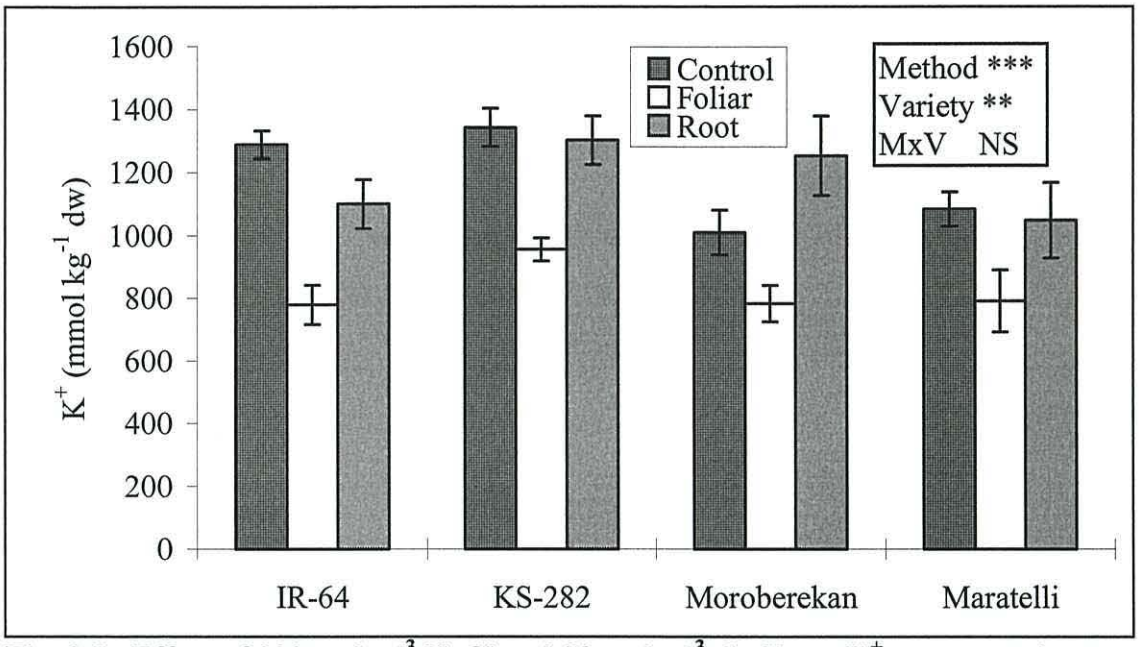


**Fig. 3.7. Effect of 200 mol $m^{-3}$ NaCl and 20 mol $m^{-3}$ $CaCl_2$ on $K^+$ concentrations in per unit shoot dry weight of rice (Experiment 6). The data were analysed by GLM test. The levels of significance were ≤ 0.01=**≤ 0.001=***. and non significance =NS.**

$K^+$ (mmol $kg^{-1}$ dw) per unit shoot dry weight is presented in Figure 3.7. Under foliar treatment the relative $K^+$ concentration per unit shoot dry weight was lowest (60.4% of its control) in IR-64 and the highest (77.5% of its control) in Moroberekan. In the case of Moroberekan, under root treatment, there was an increase in $K^+$ concentrations (123.9 % of its control). The decrease in $K^+$ was greatest in IR-64 under foliar and root treatment. Varieties KS-282 and Maratelli were more or less similar under foliar (71.2 and 72.9 % of their control) and root (97 and 96.7 % of their control) treatments but IR-64 and Moroberekan were different from each other as well as from Maratelli under both the treatments. Under root treatment the lowest percentage for $K^+$ per unit shoot dry weight (85.5 % of its control) was recorded in IR-64 and the highest percentage for $K^+$ per unit shoot dry weight (123.9 % of its control) was recorded in Moroberekan.

### 3.3.4.4 Shoot fresh weight of rice

The method of salt application and varieties had significant (P=0.000, 0.033) effects on shoot fresh weight as shown in Figure 3.8, while the method x variety interaction was not significant (according to GLM).

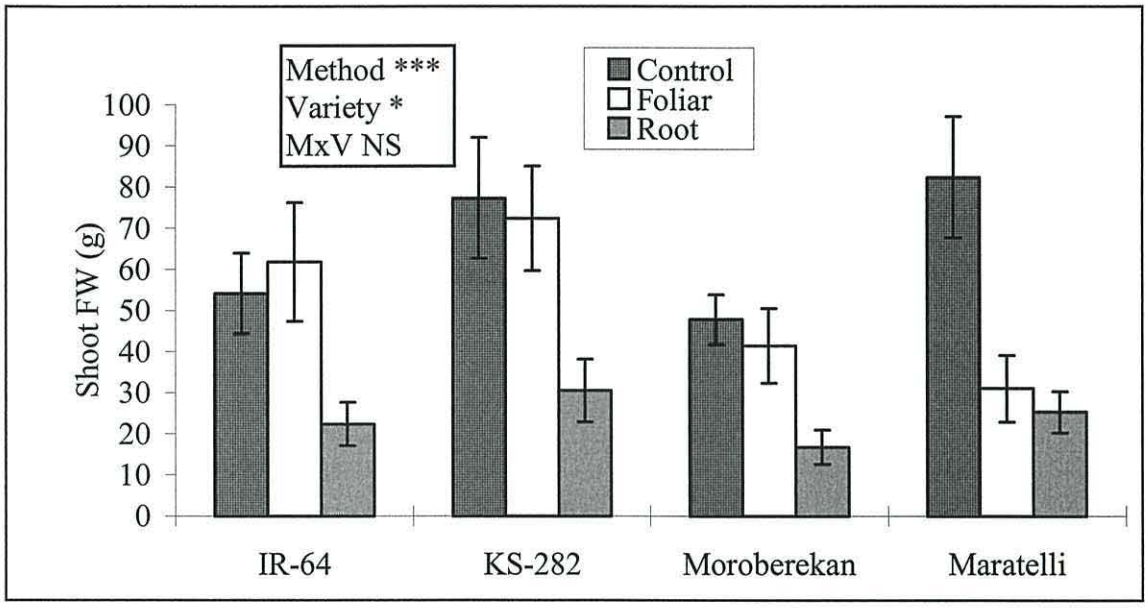


**Fig. 3.8. Effect of 200 mol $m^{-3}$ NaCl and 20 mol $m^{-3}$ $CaCl_2$ on shoot fresh weight (FW) of rice.(Experiment 6). The data were analysed by GLM test. The levels of significance ≤ 0.050=*,≤ 0.001=***, and non significance = NS.**

One-way ANOVA showed that there was a just significant decrease (P=0.033) in shoot fresh weight of all the varieties under foliar and root treatments. The decrease in Maratelli was significant under foliar (37.63 % of its control) and root (30.65 % of its control) treatment. A significant decrease was observed in all the varieties under root treatment. However the lowest shoot fresh weight was found in Maratelli (30.65 % of its control) under root treatment (Figure 3.8).

### 3.3.4.5 Shoot water content in rice

The method of salt application and variety had significant (0.000, 0.026) effects on water content, while the interaction of method x variety was not significant (Figure

3.9). Increase in shoot fresh and dry weights was recorded in IR-64 under foliar treatment compared to its control (Table 3.5)

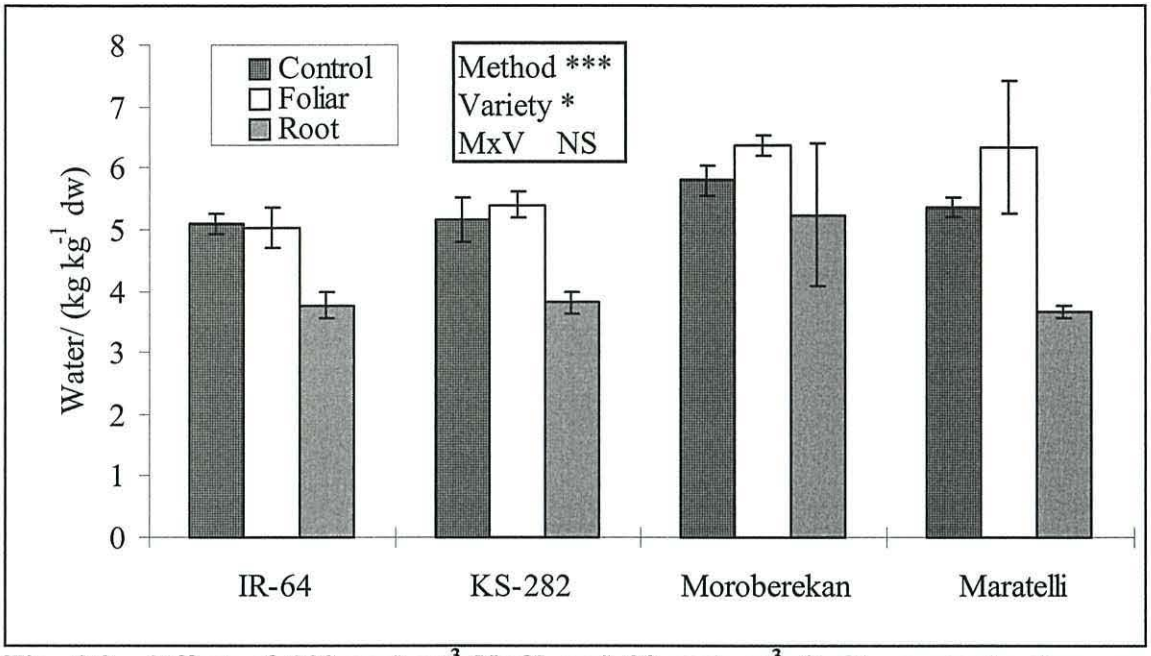


**Fig. 3.9. Effect of 200 mol $m^{-3}$ NaCl and 20 mol $m^{-3}$ $CaCl_2$ concentrations on water/SDW in leaf sap of rice (Experiment 6). The data were analysed by GLM test. The levels of significance ≤ 0.05=*, ≤ 0.000=***. and non significance = NS.**

Water content (kg $kg^{-1}$ SDW) is presented in Figure 3.9. There was non significant increase in water/SDW in all the varieties under foliar treatment except for a slight decrease in IR-64. However the increase and decrease were not significant. Under root treatment there was a decrease in water/SDW in all the varieties. The decrease was least in the case of Moroberekan. Moreover the decrease was significant in all the varieties except Moroberekan under root treatment.

## 3.4 Discussion

$Na^+$ increased significantly under all salinity levels in both rice and barley (Figs. 3.1 - 3.5). Similar findings were observed by Krishnamurthy and Bhagwat for rice (1989).

Ion uptake and growth of barley and rice plants under salinity were significantly influenced by;

Method of salt application

Varieties

Although they were treated at the same salinity the varieties accumulated different $Na^+$ concentrations in the leaf sap when the salt application method was different (Figures 3.1-3.4). Moreover the varieties, under the same salinity application method, accumulated different $Na^+$ concentrations (Figures 3.1-3.5).

When plants were grown under salinity in Experiment 2, leaf $Na^+$ concentrations continued to increase until the rice plants died (Akita and Cabuslay 1990). Rice was somewhat sensitive to salinity relative to other crops (Yeo *et al.*, 1990, Khatun 1995). In experiment 2 high $Na^+$ concentrations might not be the reason for death of the plants because in experiments 5 and 6 plants tolerated 150 and even 200 mol $m^{-3}$ concentrations when supplemented with 15 mol $m^{-3}$ and 20 mol $m^{-3}$ of $CaCl_2$ respectively instead of 50 mol $m^{-3}$ $CaCl_2$. In my opinion it could be $Cl^-$ that was toxic because in experiment 2 50 mol $m^{-3}$ $CaCl_2$ was supplied to the plants with 100 mol $m^{-3}$ NaCl, but unfortunately $Cl^-$ was not measured in these experiments.

In Experiment 6 foliar treatment had only a small effect on water/SDW but $Na^+$ increased with root treatment (Figure 3.9). Moroberekan and Maratelli showed the largest increase in $Na^+$ but had higher water contents than the other two varieties. So low plant vigour contributed towards high $Na^+$ concentrations as in case of Moroberekan and Maratelli (Table 3.5 and Figure 3.9). Koyama *et al.* (2000) also emphasised the relationship of low plant vigour and high ion concentrations. High concentrations of $Na^+$ were partly the consequence of dehydration under root treatment (Fig. 3.5), and similar findings were reported by Flowers *et al.* (1991) and Oertli (1968). According to Oertli (1968) accumulation of solutes in the cell wall would be responsible for the development of low apoplastic solute potential. Moreover the apoplastic ion content was 100 times more toxic than the same protoplastic ionic content because of the smaller apoplastic volume (Oertli 1968). Hence it seemed to be clear that dehydration was the partial cause of high $Na^+$ accumulation. $Na^+$ accumulation was significantly lower in KS-282 and IR-64 at the highest salinity level (200 mol $m^{-3}$ NaCl +20 mol $m^{-3}$ $CaCl_2$) compared with Moroberekan and Maratelli under root treatment (Fig. 3.5). According to Flowers and Yeo (1981) and Basu and Ghosh (1991) there was an inverse relationship between $Na^+$ accumulation in the tissue and salt tolerance. If there were high $Na^+$ concentrations (Figure 3.1), then low shoot fresh and dry weights

were recorded in most of the varieties. The effect was generally more pronounced under root treatments than foliar treatments (Table 3.3- 3.5), particularly in the case of Moroberekan and Maratelli under root treatment. The drastic effects of dehydration in the soil due to the combined effects of water stress and salinity were observed in experiment 2, in the form of low plant vigour. It could be the result of acute dehydration, high concentrations of $Na^+$ or $Cl^-$, or could be the cumulative effect of different ions due to 50 mol $m^{-3}$ $CaCl_2$ accompanied by 100 mol $m^{-3}$ NaCl. Unfortunately $Cl^-$ was not measured in this case. Na+ is known to be a heritable trait (Yeo *et al.*, 1988; Yeo, 1992; Gregorio and Senadhira, 1993; Garcia *et al.*, 1995; Yadav *et al.*, 1996; Garcia *et al*; 1997).

After comparison among the varieties it was determined on the basis of $Na^+$ accumulation that Moroberekan was different from the other varieties under root treatment (Figure 3.4). High levels of ion accumulation ($K^+$, $Na^+$) due to NaCl salinity were most prominent in rice cultivars that were relatively sensitive to salinity (Figures 3.5 and 3.6). Similar findings were observed by Sharma (1986). From observations about cation analysis it has been said that tolerance to NaCl salinity is not simple, but that a large number of physiological variables seem to affect salt resistance in the case of rice (Yeo and Flowers 1984; Chowdhury *et al.*, 1995). $Na^+$ concentrations were highest in Moroberekan and lowest in IR-64, Co39, Marzhan and (in some experiments) Maratelli under root treatment (Figs. 3.2, 3.4, 3.5). Only Maratelli had high accumulation of $Na^+$ in the foliar spray treatment in experiments 5 and 6, accompanied by low relative fresh weights.

The concentration of $K^+$ was reduced in all the varieties by salinity under foliar treatment (Fig. 3.6 and fig 3.7). Lutts *et al.* (1995) observed different responses (growth and development) under NaCl stress in tall indica land races (Nona Bokra, Buhra Rata, Panwell and Pokkali), japonica varieties {I Kong Pao (IKP) and Tainung 67} and superior breeding lines (IR 4630, IR 2153, and IR 31785). Reduction in shoot FW (Fig. 3.8) and shoot dry weight (Table 3.1 - 3.3 ) were observed. Similar results were reported by Krishnamurthy and Bhagwat (1989).

Root NaCl salinity caused substantial decreases in shoot DW in all the varieties of rice (Table 3.1 - 3.3), and in all the five varieties of rice (Pokkali, CSR 1, IR36, IR26, IR2153) examined by Welfare *et al.* (1996). The effect of NaCl is highly disruptive in

the case of Moroberekan (Table 3.3) and it had the lowest shoot dry weight under root treatment (3.8 g) in experiment 6. Varieties IR-64 and Co39, KS-282, and Marzhan seemed to be salt tolerant because of high values of shoot DW at the highest salinity level, while varieties Moroberekan and Maratelli seemed to be salt sensitive (Table 3.2 - 3.5). In general, variations in shoot fresh weight and dry weight under foliar and root treatments were observed in different rice varieties (Table 3.2 - 3.5). Significant variation for salinity tolerance was found between rice cultivars (Akita and Cabuslay, 1990). Inverse relationships were also observed between $Na^+$ concentration in tissues and shoot fresh weight in barley (CM-67 and Quantum) under both foliar and root treatments (Fig 3.1). Varieties did not all show the same response under salinity. In experiment 5 inverse relationships were observed between SFW (Table 3.2) and $Na^+$ accumulation (Fig. 3.4) in Moroberekan and Maratelli under both foliar and root treatments.

Experiments 2 and 3 were similar in having the same small pots (double pot assembly, 500 ml) and 100 mol $m^{-3}$ NaCl salinity, but different due to different varieties and $CaCl_2$ concentrations. Experiments 5 and 6 were similar due to having single 2 l pots and similar ratio of NaCl and $CaCl_2$ (10:1), but different due to actual salt concentrations. Experiment 3 was also similar to experiment 5 and 6 having similar ratio of NaCl and $CaCl_2$ (10:1). Foliar NaCl increased dry weight in the case of IR-64 in Table 3.5, and increases in SFW and SDW were observed in all varieties except Co39 in Table 3.3. The probable reason for the increased SDW in the case of IR-64 in Table 3.5 could be the specific varietal behaviour of IR-64 at high salinity (200 mol $m^{-3}$) in that application of high foliar NaCl checked the further entry of $K^+$ through roots. So it is concluded that reduced growth is not only due to high concentration of $Na^+$ but could be due to the cumulative or combined effect of high concentration of ions like $Na^+$, $K^+$ and $Cl^-$. In subsequent experiments anions were also measured along with the cations. Three parental varieties, Co39, Moroberekan and Maratelli were selected for further work and $Na^+$ accumulation in response to soil salinity was considered an important quantitative trait for further QTL analyses studies (Chapter 5).

## 3.5 Conclusions

- The hypothesis is proved to be true that the effect of salinity in *Oryza sativa* L., as in barley, is influenced by the method of salt application (Figures 3.1, 3.3, 3.6-3.9) and by different varieties (Table 3.5).
- Root treatment differs significantly from foliar treatment in IR64 and Moroberekan (Table 3.5), and IR64 is significantly different from Maratelli under foliar treatment (Figure 3.5) for $Na^+$ accumulation.
- $Na^+$ concentration in CM-67 is significantly different from Kaya and Quantum under foliar and root treatments (Figure 3.1).
- The varieties Azucena, Bala, IR-64, Co39, KS-282 and Moroberekan were relatively salt-tolerant under foliar treatment on shoot fresh and dry weight bases (Tables 3.3 to 3.5).
- IR-64 seems to be the most salt-tolerant under root on relative shoot fresh and dry weight basis (Table 3.3).
- There was no significant difference between indica (IR64) and japonica (Moroberekan) varieties under root treatment on a shoot dry weight basis at 200 mol $m^{-3}$ NaCl + 20 mol $m^{-3}$ $CaCl_2$ (Table 3.5).

# Chapter 4

# Physiological Study and Use of PTS Tracer in Relation to Salinity

## 4.1 Introduction

Net loss of productivity of the leaf was suggested as the cause of reduction in yield (Munns and Termatt, 1986). The salinity response was influenced by the size of the plants and the effect was more pronounced in the case of dwarf plants. Effects of salinity were modified by the salt distribution within the shoot and leaf at the cellular level. Salinity-tolerant and salinity-sensitive varieties were different not only due to the accumulation of different salt concentrations but also in many other respects (Yeo *et al.*, 1990). On this basis Chaubey and Senadhira (1994) said that salinity tolerance was a complex phenomenon, not only on a physiological basis but also on a genetical basis. Welfare *et al.* (1996) observed conspicuous reduction in shoot and root DW in rice varieties (Pokkali, CSR1, IR36, IR26 and IR2153-26-3-5-2) under saline conditions. Shoot $K^+$ concentration was reduced in saline conditions in all 5 varieties. Salinity reduced CO2 assimilation, transpiration and stomatal conductance (Welfare *et al.*, 1996). Growth and photosynthetic gas exchange were reduced under salinity in rice (Yeo *et al.*, 1999).

Asch *et al.* (1995) grew plants of the rice varieties Pokkali, IR 28, IR 50 and IR 31785-58-1-2-3-3 in individual pots and subjected them to low (40/55% day/night) and high (75/90%) air humidity (RH), while soil salinity was gradually increased to 0, 30, 60 or 120 mM NaCl. Bulk root and stem base water potential (SWP) were determined 2 days after each salt application. The SWP decreased throughout the 8 days of treatment. High soil electric conductivity (EC) reduced SWP (Asch *et al.*, 1995). Water potential in callus of the rice cv. SR-26 B (salt-tolerant) decreased more than water potential in Basmati 370 and Gopalbhog (both salt-susceptible) cultivars as salinity increased from 2 to 34.3 dS/m (Subhashini and Reddy 1990). Plants of rice cv. Yamabiko were subjected to 80 mmol $Na^+$ $kg^{-1}$ + 0-50 mmol $Ca^{++}$ $kg^{-1}$ salinity in hydroponics and close

relationships were found between the osmotic potential, cumulative transpiration and top dry weight. Moreover the growth of rice seemed to be dependent on the osmotic potential of the solution (JiQing *et al.*, 1996).

Sultana *et al*, (1999) observed reduction in photosynthesis in rice plants in 25, 50, 100, and 200 mM NaCl salinity, dependent not only on a reduction of available $CO_2$ by stomatal closure, but also on the cumulative effects of leaf water and osmotic potentials, stomatal conductance, transpiration rate, relative leaf water content and biochemical constituents such as photosynthetic pigments, soluble carbohydrates, and protein. The cumulative effects resulted in low concentrations of assimilates in the leaves and their poor translocation from the source resulted in reduced grain dry matter (Sultana *et al.*, 1999). Uptake of $Cl^-$ tended to increase with increasing $CaCl_2$ and the sensitivity of rice to salinity was attributed to changes in the osmotic potential gradient rather than to chloride toxicity (Wilson *et al.*, 1999). Growth inhibition under NaCl salinity was lower in calluses from Nona Bokra (salt-resistant) than in calluses obtained from IKP (salt-sensitive). Na and Cl accumulation as well as internal osmotic potential were lower in Nona Bokra and in Aiwu (moderately resistant), suggesting a cellular component of salt resistance in these genotypes (Lutts *et al.*, 1996).

According to Cho-DongHa *et al.* (1995), variation for salt tolerance was observed in the reduction of relative growth rate (RGR) due to NaCl treatment in seedlings of 6 Korean rice cultivars. The reduction of RGR by NaCl treatment was mainly due to the reduction of the net assimilation rate rather than to the leaf area ratio. Reduction in leaf photosynthesis was observed under NaCl salinity. Moreover, Han-Kang-Chal (salt tolerant) showed a larger decrease in osmotic potential in the NaCl-treated leaves, suggesting that osmotic adjustment developed under salt stress in tolerant cultivars (Cho-DongHa *et al.*, 1995).

Tiwari *et al.*, (1997) worked on solution cultured rice seedlings and gradual decreases in the activity of photosystems I and II as well as in chlorophyll fluorescence transients were observed with an increase in NaCl concentration. These decreases were more prominent in salt-sensitive cultivars (IR 29 and IR 8) than in the tolerant ones (Nona Bokra and Pokkali). A noticeable decrease in net photosynthetic rate was observed in both cultivars (Tiwari *et al.*, 1997). Stomatal conductance and transpiration were reduced under the influence of 11 and 18 days NaCl salinity in salt-

tolerant rice cv. Pokkali and the susceptible cv. Amistad 82, but variation in the varieties was observed for these parameters and for net photosynthesis (Torres, 1996).

NaCl promoted rice leaf senescence, decreased chlorophyll content and increased membrane permeability. The effects were more pronounced in the older leaves of salt-resistant genotypes. NaCl-induced senescence increased basal non-variable chlorophyll fluorescence in all cultivars. After 7 days the alteration in membrane permeability allowed discrimination between salt-resistant and salt-tolerant genotypes (Lutts *et al.*, 1996).

Singh and Dubey (1995) observed that chloroplasts isolated from NaCl stressed (7 and 14 dS $m^{-1}$) seedlings of Ratna and Jaya (sensitive cultivars) showed a 31% reduction in fluorescence at 685 nm in addition to a major decrease in absorption with shifts in peaks in the visible region of the spectrum. A more conspicuous decrease in chlorophyll a and b contents was observed in the salt-sensitive cultivars Ratna and Jaya than in tolerant cv. CSR-1 and CSR-3. At 14 dS $m^{-1}$ salinity about 40 % decreases in both whole chain electron transport and photosystem (PS) II activities were observed in the tolerant cultivars, and about 62-67 % decreases in the sensitive cultivars. No apparent change in PSI activity due to salinity was observed in either set of cultivars (Singh and Dubey, 1995).

Khan *et al.* (1997) found that local coarse grain type cv. Pokkali and Kalobail, and IPK 37011, were able to survive three weeks at 200 mM NaCl. Plant height, green leaf area, leaf weight and shoot and root growth were severely decreased by salinity. However, leaf area decreased more than other growth parameters. Photosynthesis (Pn) was decreased by salinity, and stomatal resistance appeared to be partially responsible for the decreased Pn. Kalijira, an aromatic small grain cultivar, showed considerable reduction in Pn, especially at 150 mol $m^{-3}$ NaCl. $Na^+$ accumulation increased while $K^+$ accumulation decreased in all varieties. Salt-tolerant cultivars of rice accumulated less $Na^+$ and more $K^+$ than susceptible ones. $Ca^{++}$ and $Mg^{++}$ concentrations were decreased by salinity (Khan *et al.*, 1997).

Harinasut *et al.* (1996) noticed excessive decreases in relative water content, chlorophyll and protein content in the leaves of rice cv. Sasanishiki seedlings treated with 150 mM NaCl for 6 days. A 27% reduction was observed in the quantum yield of non-cyclic electron transport under salt stress.

Within the leaves of rice toxic ion concentrations could accumulate by transpirational bypass flow. Bypass flow is the proportion of transpiration that is not subject to membrane control of ion transport at the endodermis. The magnitude of the transpirational bypass flow was approximately 10 times higher in rice than wheat. The average contribution for the bypass flow was 5.47% of the transpirational volume flow in rice. (Garcia *et al.*, 1997). According to Yeo *et al.* (1987) measurement of bypass flow requires a fluorescence compound that can enter the plant quantitatively with the flow of water in the apoplast under the influence of transpirational driving force for water movement and which neither crosses cell membranes nor adheres to cell walls. The apparent bypass flow is represented as a percentage of $J_v$ and is the concentration of PTS in the transpiration stream divided by the concentration of PTS in the external solution. The apparent bypass flow at the time of root cutting was reduced by the decreased water movements to the shoot (Yeo *et al.*, 1987). A median value for the bypass flow of water would be 0.5-1% of the transpirational volume flow. At an estimated contribution of not more than 1% or so of the transpirational volume flow, bypass flow is clearly of minimal importance at low external concentrations. PTS tracer transport is only a measure of bypass flow, not of the relative importance of apoplastic water movement in tissues. Variation in bypass flow is constitutive (Yeo *et al.*, 1987). Excessive transport of all the ions was the cause of damage to rice seedlings. Transpirational bypass flow in $Na^+$ uptake was different in high and low transporting lines of rice developed through intra-varietal selection. The lines did not differ significantly in other physiological traits that are components of salt resistance like compartmentation at the leaf and cellular levels. A strong correlation was observed between $Na^+$ transport and accumulation of a tracer for apoplastic pathways, trisodium 8-hydroxy-1,3,6-pyrenetrisulphonic acid (PTS) (Yadav *et al.*, 1996).

In this chapter data from experiments 8 and 9 are presented to test the following 6 hypothesis for the study of salt tolerance. Experiment 8 was conducted in both flood bench and hydroponic systems. Experiment 9 examined the transport of PTS in different rice varieties. These experiments tested the following six hypotheses.

### 4.1.1 Hypothesis 1

"Salt has highly significant damaging influence due to dehydration of tissues of rice plants".

### 4.1.2 Hypothesis 2

"Systems of salt application (flood bench and hydroponics) have different effects on physiological parameters in rice under salinity". They are different from each other because in flood bench system the growth medium was soil and water was flooded to the plants once a day (see section 2.2) while in hydroponics the plant roots are directly immersed in water so more waterlogged conditions (hypoxia and anoxia) compared to flood bench system. Although the aeration was provided regularly to avoid waterlogging conditions (see section 2.3) but even then worse than flood bench because of the basic difference of the medium of growth.

### 4.1.3 Hypothesis 3

"Time (duration) of exposure of plants to applied salinity has significant effects on physiological parameters in rice".

### 4.1.4 Hypothesis 4

"Leaf position (leaf age) has significant effects on salinity responses in rice".

### 4.1.5 Hypothesis 5

"Rice varieties (Moroberekan, Co39 and Maratelli) differ in their responses to salinity".

### 4.1.6 Hypothesis 6

"The extent of the apoplastic leakage pathway differs between varieties and determines relative $Na^+$ accumulation". To fulfil this aim some plants from experiment 8 were used for PTS tracer application and another experiment (9) was also conducted for this purpose.

## 4.2 Experimental protocols

### 4.2.1 Experiment 8a.

Three varieties of *Oryza sativa* L. were used in this experiment, Co39, Maratelli and Moroberekan. Experiment 8 was conducted in Pen-Y-Ffridd, house 2, with a minimum temperature 25 °C and 12 hours photoperiod, in flood bench and hydroponic systems. In the flood bench system the substrate was John Innes Compost (JI No. 3).

Nutrients (Phostrogen 1g $l^{-1}$, micronutrients (Hoagland and Arnon, 1950) 0.5 ml $l^{-1}$, sodium silicate 0.1 ml $l^{-1}$) were supplied to the flood bench and hydroponics for the whole time of the experiment The concentration of $SiO_2$ was 0.5 mol $m^{-3}$ in the final solution. All plants (except the controls) were given the same salinity (NaCl 100 mol $m^{-3}$ + $CaCl_2$ 10 mol $m^{-3}$) in both systems. Four replications (each of eighteen plants) at the same salinity level as mentioned above were used in each flood bench and hydroponic system. There was one control in each of the flood bench and hydroponics, same in size of their respective populations. There were 18 plants per container. Seeds were soaked in water on 17/6/99 and were sown on 18/6/99. They were germinated directly in 2 litre pots as well as in rockwool in P84 plug trays (the trays each having 84 cells). Plants were transferred to the flood bench and hydroponics 12 days after sowing. Salt stress was started 27 days after sowing and was completed 31 days after sowing.

Data for Fm, $F_o$, Fv, Fm/Fv, LA, LRS (was measured after O' Tool and Moya, 1978), OP, WP, Pn, E, gs, and SPAD readings were recorded from the leaf 2 (the 2nd youngest from the main stem) at the time of five harvests. Data from three plants from each population were recorded at the time of 2nd, 3rd and 4th harvest, from each system. For ion analyses, leaf 0 (not fully emerged), 1 (The youngest fully emerged leaf from the main stem or shoot), 2 (The second youngest from the main stem, and 3 (the third youngest from the main stem) were stored in microcentrifuge tubes from each of the harvested plants Leaves 0, 1, 2, and 3 were harvested in the leaf sequence of the same chronological age regardless of their colour.. However at the time of 1st and 5th harvest data were recorded only from the flood bench system because the plants in the hydroponics were too small to harvest at the time of first harvest, while they were completely injured at the final harvest. 1st, 2nd, 3rd, 4th, and 5th, harvests were taken 7, 14, 21, 28 and 49 days after the application of full stress respectively.

### 4.2.2 Experiment 8b, non-saline PTS tracer study.

Five plants (one of Co39, two of each of Maratelli and Moroberekan) from the control treatment of the flood bench were transferred to the greenhouse on the roof of the Memorial building, University of Wales, Bangor on 6/9/99. Leaving the plant shoots uncovered the pots were covered completely with plastic bags to reduce evaporation from the soil. 50 mg of PTS tracer in 500 ml of water was added to each plant and then the pots were weighed (weight of plant + pot + PTS solution). The plants

were weighed again after five days, and the difference in the total weight was recorded. On the same day the plants were harvested and shoot fresh weight was recorded. Hot water extracts were obtained from these shoots for measurements of PTS fluorescence (Section 2.13).

### 4.2.3 Experiment 9, saline PTS tracer study.

The same varieties of rice (Co39, Moroberekan and Maratelli) were selected for this experiment and it was again conducted in the greenhouse on the roof of the Memorial building, University of Wales, Bangor. Five plants of each variety were grown in each of 2 hydroponic tubs. The measurements for each tub were, length 26.7 cm, width 16.7 cm and height 21.5 cm and 7 $dm^3$ volume. In total 30 plants were grown in this experiment. Rice seeds were immersed in water on 16/7/99 and sown on 17/7/99. Rockwool in a P-84 plug tray was used as a support medium for the plants in the hydroponics. Both trays were covered with black plastic sheet to prevent the penetration of light to the roots. Phostrogen (0.1 g $l^{-1}$) was supplied to the plants 13 and 20 days after sowing. Salts (100 m mol NaCl + 10 m mol $CaCl_2$) and PTS tracer (30 mg $l^{-1}$) were supplied 32 days after sowing, to one tub. All plants were harvested 37 days after sowing and hot water extracts were obtained as described in section 2.5.2.

## 4.3 Results

### 4.3.1 Experiment 8a

#### 4.3.1.1 Visual observations

Table 4.1 lists the visual observations of the rice plants in the flood bench system and hydroponics. All of the control plants were healthy, although at the first harvest Co39 and Maratelli were slightly chlorotic.

##### 4.3.1.1.1 Harvest 1 (7 days salinity)

Under flood bench salinity some plants of Co39 were yellowish-green. In others there was a distinction between the green and chlorotic patches of the leaf, but there was variation between the ratio of green and chlorotic parts from 6:1 to 1:3. In the case of Moroberekan most of the plants were green while one was slightly chlorotic. All plants were green in the case of Maratelli (Table 4.1).

4.3.1.1.2 Harvest 2 (14 days salinity)

In the saline flood bench, necrosis started in Co39 from the tips of almost all leaves, and some old leaves were completely necrotic. However some parts were chlorotic accompanied with that of necrosis. In Moroberekan the necrosis was more pronounced in old leaves. In Maratelli chlorosis started from new leaves and the effect was most prominent in leaf zero. Plant parts were chlorotic initially and then turned to necrotic (Table 4.1).

In saline hydroponics, Co39 plants were smaller in size. Most of the leaf tips were chlorotic and whole plants were showing the symptoms of chlorosis. The greater parts of the leaves were necrotic in some plants. Leaf rolling was observed in some cases. In Moroberekan, leaf tips and old leaves were necrotic. In Maratelli an overall reduction in plant size and leaf necrosis was observed. In most cases the necrosis was clear on young leaves and in some cases on old leaves as well. In general the effect of salt was worse in hydroponics than in the flood bench (Table 4.1).

4.3.1.1.3 Harvest 3 (21 days salinity)

In Co39 the leaf tips were chlorotic, but old leaves were necrotic. In Moroberekan the leaf tips and leaf margins were turning to chlorosis and necrosis and old leaves were showing the same symptoms. In Maratelli new leaves were completely chlorotic and leaf rolling was more prominent in new leaves (Table 4.1).

In saline hydroponics, leaves, especially old ones, were necrotic and there was an overall reduction in the size of the plants. In Moroberekan old leaves were completely necrotic. In Maratelli some of the green parts of the leaf changed to chlorotic and then the young leaf turned necrotic (Table 4.1).

4.3.1.1.4 Harvest 4 (28 days salinity)

In the saline flood bench the leaf tips were necrotic in Co39 including ¼th part of each leaf in most of the leaves. Leaf tips were chlorotic and partly necrotic. Some plants were completely necrotic. In Moroberekan the old leaves were chlorotic and necrotic but healthy parts were even more green than the controls. In Maratelli young leaves L0 and L1 were completely rolled, or in some cases half of the leaf was

completely rolled and chlorotic and the other half was quite healthy and green. Necrosis was rarely visible in the case of old leaves in Maratelli (Table 4.1).

In Co39 in saline hydroponics necrotic dots and streaks were visible. In Moroberekan necrosis at the base of the leaves of weak tillers was more prominent. Old leaves were completely necrotic, young leaves had rolled tips and margins were necrotic. In some leaves chlorotic streaks were visible. On some leaves there were necrotic dots. In Maratelli leaf necrosis was visible on young and old leaves. Necrosis was more pronounced on leaves of weak tillers. In some leaves different combined patches of chlorosis and necrosis were visible on the leaves. In general, salinity had more pronounced effects in hydroponics on all varieties of rice than in the flood bench (Table 4.1).

4.3.1.1.5 Harvest 5 (49 days salinity)

In the saline flood bench most parts of the Co39 plants were necrotic. Some parts were chlorotic mixed with necrotic. However, some plants looked green and only some leaves were necrotic. There was a great variation even in the plants of the same variety. There was an overall reduction in the size of the plants and they appeared very weak. In Moroberekan some plants were green but not quite healthy. Leaves were brittle and easily breakable. Some plants were completely necrotic, including the leaf sheaths. Again great variation was visible among the plants of the same variety. Both chlorosis and necrosis were visible. In Maratelli the green colour of the plants was lighter than in the controls. Leaf chlorosis and necrosis were visible. Veins were more necrotic than the rest of the lamina. Various patches of different grades of chlorosis and necrosis were prominent on the leaf (Table 4.1).

**Table 4.1. Visual observations of rice plants treated with 100 mol $m^{-3}$ NaCl + 10 mol $m^{-3}$ $CaCl_2$**

| Harvest | Variety | Flood Bench | | | Hydroponics | | |
|---|---|---|---|---|---|---|---|
| | | Plant size | Chlorotic | Necrotic | Plant size | Chlorotic | Necrotic |
| 2 | Co39 | | Young leaf | Old leaves | Reduced | Leaf tips severe | Leaf tips slightly |
| 14days salinity | MO | | Young leaf | Old leaves, | Reduced | | Old leaves |
| | Maratelli | | Young leaf Leaf zero | Young leaves, leaf zero | Reduced | Young leaves | Young leaves partly |
| 3 | Co39 | Reduced | Leaf tips | Old leaves | Reduced | Whole plant | Old leaf |
| 21 days salinity | MO | Reduced | Leaf tips and leaf margins | Old leaves | Reduced | | Old leaf |
| | Maratelli | Reduced | leaf (L0) | Young leaf | Reduced | Young leaf | Young leaf partly |
| 4 | Co39 | Reduced | Leaf tips | Complete plant | Reduced | | |
| 28 days salinity | MO | Reduced | Old leave. | Partially | Reduced | Old leaf and few whole plants | |
| | Maratelli | Reduced | | Young leaf | Reduced | Old leaf | Young leaf |
| 5 | Co39 | Reduced | All leaf | Full plants | – | | |
| 49 days salinity | MO | | Stem | Some plants | | | |
| | Maratelli | | Leaf veins | Plants Partly | | | |

### 4.3.2 Analysis related to experiment 8a

#### 4.3.2.1 Hypothesis 1

"Salt has highly significant damaging influence due to dehydration of tissues of rice plants".

Table 4.2, column A for ANOVA, GLM shows that in the salinity (control+salt) shoot fresh weight, shoot dry weight fresh weight dry weight ratio, leaf weight, leaf area, leaf rolling score, water potential, osmotic potential and turgor pressure Pn, E and gs (IRGA), $Na^+$, $K^+$, sum($Na^+$+$K^+$), $Mg^{2+}$, $Ca^{2+}$, sum($Na^+$+$K^+$+$Mg^{2+}$+$Ca^{2+}$), $Cl^-$, $NO_3^-$, malate$^-$, $SO_4^{2-}$ and sum($Cl^-$+$NO_3^-$+malate$^-$+$SO_4^{2-}$) were highly significantly different between the control and 100 mol $m^{-3}$ NaCl salinity at 14, 21 and 28 days harvests. Level of significance were denoted with * and the numbers of * were increasing with the increased level of significance as described as a foot note to Table 4.2.

However ANOVA (GLM) showed that Fv/Fm and SPAD readings were not significantly different as shown in salinity (control + salt) under 100 mol $m^{-3}$ NaCl salinity compared with their respective controls (Table 4.2, column A).

Variety x salinity interactions showed that shoot fresh weight, leaf rolling score, turgor pressure, SPAD readings, $Cl^-$, $NO_3^-$, $SO_4^{-2}$ and sum($Cl^-$+$NO_3^-$+malate$^-$+$SO_4^{2-}$) had significant effect as shown in var x salinity column under 100 mol $m^{-3}$ NaCl salinity compared with their respective controls and all the other parameters in that column had not significant effect for variety x salinity interactions (Table 4.2, column B). The conclusions about main effects are almost invalid in case of significant varietal x salinity interactions so the data were analysed by ANOVA (GLM) for only salt treatment as well as shown in different columns apart from the columns of control and salt (together) for various parameters as shown in Table 4.2.

**Table 4.2. ANOVA (GLM) for various parameters of rice in relation to salinity.**

| Parameter (Treatment) | Salinity (Control +Salt) A | Var x salinity interactions B | (FB+Hy) (Control+ Salt) C | (FB+Hy) (Saline) D | Time (days) (Control+ Salt) E | Time (days) (Saline) F | Leaf position (Control +Salt) G | Leaf Position (Saline) H | Variety (Salt+control) I | Variety (Saline) J |
|---|---|---|---|---|---|---|---|---|---|---|
| **Shoot FW (g/p)** | *** | ** | *** | *** | * | NS | - | - | *** | *** |
| **Shoot DW (g/p)** | ** | NS | *** | *** | *** | * | - | - | *** | *** |
| **FW/DW** | *** | NS | NS | * | *** | *** | - | - | *** | *** |
| **Leaf rolling score** | *** | *** | NS | NS | NS | NS | *** | - | *** | *** |
| **Water potential (Mpa)** | *** | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **Osmotic Potential (Mpa)** | *** | NS | NS | NS | * | ** | NS | NS | NS | NS |
| **Turgor Pressure (Mpa)** | *** | * | ** | ** | NS | * | NS | NS | NS | NS |
| **Fv/Fm** | NS | NS | * | NS | NS | * | - | - | NS | NS |
| **Pn ($\mu$mol $m^{-2}$ $s^{-1}$)** | *** | NS | * | *** | NS | NS | - | - | * | NS |
| **E (mmol $m^{-2}$ $s^{-1}$)** | *** | NS | NS | NS | *** | *** | - | - | NS | NS |
| **gs (IRGA)(mol $m^{-2}$ $s^{-1}$)** | *** | NS | NS | ** | * | * | - | - | NS | NS |
| **SPAD reading** | NS | * | ** | *** | * | *** | - | - | *** | *** |
| **$Na^+$ (mol $m^{-3}$)** | *** | NS | *** | *** | NS | *** | NS | *** | NS | *** |
| **$K^+$ (mol $m^{-3}$)** | *** | NS | *** | *** | NS | *** | *** | NS | *** | *** |
| **Sum ($Na^+$+$K^+$)** | *** | NS | NS | *** | ** | *** | * | *** | *** | *** |
| **$Mg^{2+}$ (mol $m^{-3}$)** | *** | NS | ** | *** | NS | ** | *** | NS | NS | ** |
| **$Ca^{2+}$ (mol $m^{-3}$)** | *** | NS | NS | * | NS | ** | NS | NS | NS | NS |
| **Na+K+Mg+Ca** | *** | NS | *** | * | * | *** | *** | ** | ** | *** |
| **$Cl^-$ (mol $m^{-3}$)** | *** | *** | * | ** | *** | *** | *** | ** | * | *** |
| **$NO_3^-$ (mol $m^{-3}$)** | *** | *** | *** | *** | NS | NS | NS | NS | NS | ** |
| **malate$^-$ (mol $m^{-3}$)** | *** | NS | NS | NS | *** | *** | * | NS | * | ** |
| **$SO_4^{2-}$ (mol $m^{-3}$)** | *** | *** | * | NS | *** | *** | NS | NS | *** | *** |
| **Cl+$NO_3$+malate+$SO_4$** | *** | ** | NS | *** | *** | *** | *** | ** | *** | *** |

**Note:-** * ≤ 0.050, **≤ 0.01, *** ≤ 0.001, NS = none significant; var =variety, FB=Flood bench system, Hy=Hydroponics. A, B, C, D, E, F, G, H, I, and J are columns.

**Table 4.3. Effect of 100 mol $m^{-3}$ NaCl +10 mol $m^{-3}$ $CaCl_2$ on different parameters of rice at various days of salinity (Experiment 8). These data related to column E in Table 4.2.**

| Parameter | 14 days | | 21 days | | 28 days | |
|---|---|---|---|---|---|---|
| | 0 | 100 | 0 | 100 | 0 | 100 |
| Shoot FW (g/p) | 22.3 ± 5.1 | 14.5 ± 1.6 | 33.8 ± 8.6 | 17.9 ± 1.7 | 40.8 ± 8.6 | 15.75 ± 1.9 |
| Shoot DW (g/p | 3.56 ± 0.86 | 2.95 ± 0.34 | 5.96 ± 1.48 | 4.27 ± 0.42 | 8.29 ± 1.77 | 4.24 ± 0.48 |
| FW/DW | 6.59 ± 0.35 | 4.91 ± 0.18 | 5.83 ± 0.34 | 4.18 ± 0.12 | 4.91 ± 0.23 | 3.48 ± 0.18 |
| Leaf rolling score | 1.0 ± 0.0 | 1.5 ± 0.2 | 1.0 ± 0.0 | 1.6 ± 0.3 | 1.1 ± 0.1 | 1.8 ± 0.3 |
| Water potential (MPa) | -0.35 ± 0.21 | -1.35 ± 0.13 | -0.30 ± 0.14 | -1.43 ± 0.38 | -0.30 ± .30 | -1.90 ± 0.15 |
| Osmotic potential (MPa) | -1.37 ± 0.10 | -1.94 ± 0.83 | -1.16 ± 0.12 | -1.67 ± -0.42 | -1.47 ± 0.13 | -2.66 ± 0.10 |
| Turgor pressure (Mpa) | 1.02 ± 0.13 | 0.59 ± 0.13 | 0.86 ± 0.42 | 0.24 ± 0.10 | 1.17 ± 0.30 | 0.76 ± 0.13 |
| Fv/Fm | 0.80 ± 0.01 | 0.79 ± 0.01 | 0.82 ± 0.00 | 0.82 ± 0.00 | 0.79 ± 0.02 | 0.79 ± 0.02 |
| Pn ($\mu$mol $m^{-2}$ $s^{-1}$) | 12.4 ± 2.1 | 5.6 ± 0.6 | 8.3 ± 1.2 | 3.6 ± 0.7 | 8.1 ± 1.2 | 3.9 ± 0.6 |
| E (mmol $m^{-2}$ $s^{-1}$) | 9.6 ± 1.5 | 5.4 ± 0.4 | 11.0 ± 1.3 | 6.0 ± 0.4 | 5.5 ± 0.7 | 3.5 ± 0.3 |
| gs (IRGA) (mol $m^{-2}$ $s^{-1}$) | 361 ± 100 | 130 ± 13 | 364 ± 118 | 179 ± 25 | 276 ± 62 | 108 ± 13 |
| SPAD reading | 37 ± 2 | 36 ± 1 | 35 ± 1 | 39 ± 1 | 34 ± 2 | 34 ± 1 |
| $Na^+$ (mol $m^{-3}$) | 4 ± 1 | 56 ± 8 | 3 ± 0 | 106 ± 13 | 3 ± 0 | 129 ± 16 |
| $K^+$ (mol $m^{-3}$) | 245 ± 12 | 259 ± 8 | 257 ± 13 | 328 ± 15 | 228 ± 14 | 327 ± 17 |
| Sum ($Na^+$+$K^+$) | 250 ± 13 | 315 ± 11 | 260 ± 13 | 434 ± 21 | 231 ± 14 | 456 ± 20 |
| $Mg^{2+}$ (mol $m^{-3}$) | 20 ± 2 | 38 ± 2 | 21 ± 1 | 50 ± 4 | 22 ± 2 | 48 ± 4 |
| $Ca^{2+}$ (mol $m^{-3}$) | 17 ± 4 | 18 ± 2 | 15 ± 5 | 21 ± 2 | 6 ± 3 | 26 ± 2 |
| Na+K+Mg+Ca | 323 ± 18 | 427 ± 14 | 333 ± 18 | 575 ± 27 | 287 ± 17 | 604 ± 24 |
| $Cl^-$ (mol $m^{-3}$) | 68 ± 5 | 286 ± 16 | 47 ± 3 | 412 ± 26 | 81 ± 27 | 437 ± 23 |
| $NO_3^-$ (mol $m^{-3}$) | 10 ± 2 | 6 ± 1 | 13 ± 2 | 6 ± 0 | 14 ± 2 | 7 ± 1 |
| malate (mol $m^{-3}$) | 16 ± 1 | 10 ± 1 | 9 ± 2 | 3 ± 0 | 2 ± 2 | 1 ± 0 |
| $SO_4^{2-}$ (mol $m^{-3}$) | 30 ± 2 | 13 ± 1 | 37 ± 4 | 10 ± 1 | 28 ± 3 | 7 ± 1 |
| Cl+$NO_3$+malate+$SO_4$ | 156 ± 8 | 329 ± 15 | 143 ± 10 | 441 ± 26 | 153 ± 26 | 459 ± 22 |

**Note:-** The means of flood bench and hydroponics are the means of leaf sap of leaf 0, 1, 2 and 3 for ion analyses while the readings for Fv/Fm, Pn, E, gs, SPAD were recorded from the 2nd leaf. Samples for water potential and osmotic potential were taken from leaf 1, 2 and 3. LRS was recorded from leaf 0, 1, 2 and 3.

There were significant reductions in shoot fresh weight and shoot dry weight and fresh weight /dry weight ratio at 14, 21, and 28 days harvests (Table 4.3). However the reductions in shoot fresh weight and shoot dry weight were more pronounced in the 28 days harvest. Leaf rolling score increased significantly at all harvests (14, 21 and 28 days salinity) under the influence of salinity. Pn, and E decreased significantly in all harvests. Reduction in Pn was maximum at 14 days salinity while reduction in E was maximum at 21 days salinity (Table 4.3).

Table 4.3 shows that the values for gs decreased significantly in all harvests. Reduction in gs was maximum after 14 days salinity. There was a decrease in SPAD readings at 14 days and an increase in SPAD values at 21 days, but they remained the same at 28 days salinity. There were increases in $Na^+$, $K^+$, sum ($Na^+ + K^+$), $Mg^{2+}$, $Ca^{2+}$ and sum ($Na^+ + K^+ + Mg^{2+} + Ca^{2+}$) concentrations at all three harvests, but the increase was greatest at 28 days. In the case of $Mg^{2+}$, however, the maximum increase was at 21 days salinity. There was an increase in the accumulation of $Cl^-$ and sum($Cl^- + NO_3^- + malate^- + SO_4^{2-}$) at all harvests, but the accumulation of $Cl^-$ was maximum at 21 days salinity. The sum($Cl^- + NO_3^- + malate^- + SO_4^{2-}$) was maximum at 28 days salinity. On the other hand there was a decrease in the accumulation of $NO_3^-$, $malate^-$ and $SO_4^{2-}$ at all harvests (14, 21 and 28 days salinity) and the decrease was maximum at 21 days salinity (Table 4.3).

Decreased FW/DW data, increased leaf rolling score, decreased gs, lower Pn and increased ion concentrations of $Na^+$, $K^+$ , all sums and $Cl^-$ (Table 4.3) contributed towards toxicity, resulted into dehydration of tissues. So it is concluded that salt has a highly significant damaging influence due to dehydration of tissues of rice plants and due to uptake of NaCl. (Table 4.2).

#### 4.3.2.2 Hypothesis 2

"Systems of salt application (flood bench and hydroponics) have different effects on physiological parameters in rice under salinity". To study this hypothesis the following questions were asked.

Are there differences between system? As in systems (FB +Hy and control + salt) (Table 4.2, column C).

Are the effect of salt different in the 2 systems? As in systems {(FB +Hy) (saline)} (Table 4.2, column D).

ANOVA (GLM) in the system (control+salt) (Table 4.2, column C) shows that shoot fresh weight, shoot dry weight, $Na^+$, $K^+$, sum($Na^+$+ $K^+$ $Mg^{2+}$+$Ca^{2+}$), and $NO_3^-$, were highly significantly different in the two systems (flood bench and hydroponics) at all harvests, even when the control data were included in the analysis. Turgor pressure, photochemical efficiency (Fv/Fm), photosynthetic rate (Pn), SPAD readings, $Mg^{2+}$, $Cl^-$ and $SO_4^{2-}$ were significantly different in the two systems under both treatments (control+salt). FW/DW ratio, water potential, osmotic potential, leaf rolling score, E, gs, sum ($Na^+$+ $K^+$), $Ca^{2+}$, malate$^-$ and sum($Cl^-$+$NO_3^-$+malate$^-$+$SO_4^{2-}$) were not significantly different under both treatments in the two systems (flood bench and hydroponics) as expressed in Table 4.2, column C. The data from column D, including only the saline treatment, showed that the system of salt application (Flood bench and hydroponics) had significant effects on SFW, SDW, Pn, gs, SPAD, $Na^+$, $K^+$, sum($Na^+$+ $K^+$), $Mg^{2+}$ $Ca^{2+}$, sum($Na^+$+ $K^+$+$Mg^{2+}$+$Ca^{2+}$), $Cl^-$, $NO_3^-$, and sum($Cl^-$+$NO_3^-$+malate$^-$+$SO_4^{2-}$). Variety x system interactions were significant under salinity including control for shoot fresh weight, SPAD readings and $NO_3$ and were significant for Pn, E, SPAD readings, $K^+$, sum($Na^+$+ $K^+$), sum($Na^+$+ $K^+$+$Mg^{2+}$+$Ca^{2+}$) only for saline treatment (Table 4.2, column D).

Table 4.4 shows that shoot fresh weight and shoot dry weight were low in hydroponics compared with the flood bench system at all harvests under the same salinity (100 mol $m^{-3}$ NaCl), but the difference was least at 14 days. Larger decreases in Pn were found in hydroponics than in the flood bench in all harvests. The difference in hydroponics compared with the flood bench in Pn was greatest at 14 days salinity while the decrease in E was most at 28 days salinity. The values for E were lower in hydroponics than in the flood bench at all harvests (Table 4.4).

Table 4.4 shows that the decrease in gs in hydroponics compared with the flood bench was greater at 21 days salinity than at 14 and 28 days salinity. The lowest SPAD value (31) was observed after 28 days salinity in hydroponics, but at 21 days harvest the values were more or less the same in the flood bench and hydroponics (Table 4.4).

Concentrations of $Na^+$, $Ca^{2+}$, sum($Na^+$+$K^+$) and sum($Na^+$+$K^+$+$Mg^{2+}$+$Ca^{2+}$) were higher in hydroponics than in the flood bench at all harvests, but the difference was largest at 28 days salinity for $Na^+$ concentration. Increase in concentration of sum ($Na^+$+$K^+$), and sum ($Na^+$+$K^+$+$Mg^{2+}$+$Ca^{2+}$) was maximum at 21 days salinity in

hydroponics as compared to flood bench. $Ca^{2+}$ concentrations were higher in the flood bench than in hydroponics.

$K^+$ and $Mg^{2+}$ concentrations were lower in hydroponics than in the flood bench at 14, 21 and 28 days, but the difference in their concentrations was greatest at 28 days. $Cl^-$ and sum($Cl^-$+$NO_3^-$+malate$^-$+$SO_4^{2-}$) were higher in hydroponics than in the flood bench at 14 and 21 days, but not at 28 days. On the other hand $NO_3^-$ concentrations were lower in hydroponics at 14 days. Concentrations of $SO_4^{2-}$ were not different between the two systems except at the 28 day harvest.

The hypothesis, "Systems of salt application (flood bench and hydroponics) have different effects on physiological parameters in rice under salinity" was found to be correct for many parameters on the basis of the data presented in Table 4.4 as well as in Tables 4.5 and 4.6, discussed below. Effect of salt was more pronounced in hydroponics than in flood bench system.

#### 4.3.2.3 Hypothesis 3

"Time (duration) of exposure of plants to applied salinity has significant effects on physiological parameters in rice".

Time of salt application or duration of salinity is shown in column Time (days) (control + salt) (Table 4.2, column E). Time is expressed in days (14, 21 and 28 days) in Table 4.3. Table 4.2 shows that shoot dry weight, fresh weight: dry weight ratio, E, concentrations of malate$^-$, $SO_4^{2-}$, were highly significant ($p \leq 0.001$) between different harvests (14, 21 or 28 days) in plants grown in the saline flood bench and in saline hydroponics. Photosynthetic rates (Pn) were not significantly different and concentrations of $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, $Cl^-$, $NO_3^-$, and all summed concentrations were significantly different as shown in Table 4.2, column E. Time of salt application under saline treatment were significantly different for all parameters except for shoot fresh weight, leaf rolling score, water potential, Pn and $NO_3^-$ (Table 4.2, column F). However variety x time interactions in both the treatments (control + 100 mol $m^{-3}$ NaCl) were significant for gs and $SO_4^{2-}$ and variety x time were significant for $K^+$, sum($Na^+$+$K^+$), $Cl^-$ and sum($Cl^-$+$NO_3^-$ +malate$^-$+$SO_4^{2-}$) interactions under only saline treatment (Table 4.2, column E). Table 4.3 shows the means of three varieties of rice in the two systems in the control and 100 mol $m^{-3}$ NaCl treatments at different harvests. The following observations apply only to the saline treatment.

**Table 4.4. Effect of growing system (Flood bench and hydroponics) at 100 mol $m^{-3}$ NaCl +10 mol $m^{-3}$ $CaCl_2$ on different parameters of rice at various days of salinity (Experiment 8). These data related to Column D in Table 4.2.**

| Parameter | 14days | | 21days | | 28days | |
|---|---|---|---|---|---|---|
| | Flood Bench | Hydroponics | Flood Bench | Hydroponics | Flood Bench | Hydroponics |
| Shoot FW (g) | 20.2 ± 1.2 | 8.7 ± 1.8 | 24.5 ± 1.3 | 11.4 ± 1.7 | 22.0 ± 1.6 | 9.5 ± 2.2 |
| Shoot DW (g) | 4.1 ± 0.3 | 1.8 ± 0.4 | 5.7 ± 0.4 | 2.8 ± 0.4 | 5.7 ± 0.4 | 2.8 ± 0.6 |
| FW/DW | 5.06 ± 0.2 | 4.8 ± 0.3 | 4.4 ± 0.1 | 4.0 ± 0.2 | 3.9 ± 0.1 | 3.1 ± 0.3 |
| Leaf rolling score | 1.5 ± 0.4 | 1.5 ± 0.3 | 1.6 ± 0.4 | 1.6 ± 0.3 | 1.8 ± 0.4 | 1.8 ± 0.4 |
| Water Potential (Mpa) | -1.29 ± 0.17 | -1.42 ± 0.23 | -2.11 ± * | -1.33 ± 0.43 | -1.78 ± 0.23 | -2.05 ± 0.16 |
| Osmotic Potential (Mpa) | -2.12 ± 0.07 | -1.74 ± 0.11 | -2.21 ± * | -1.59 ± 0.47 | -2.70 ± 0.16 | -2.61 ± 0.11 |
| Turgor Pressure (Mpa) | 0.82 ± 0.15 | 0.32 ± 0.16 | 0.09 ± * | 0.26 ± 0.11 | 0.92 ± 0.16 | 0.56 ± 0.19 |
| Fv/Fm | 0.78 ± 0.01 | 0.80 ± 0.01 | 0.82 ± 0.00 | 0.82 ± 0.01 | 0.78 ± 0.03 | 0.81 ± 0.2 |
| Pn (μmol $m^{-2}$ $s^{-1}$) | 7.5 ± 0.6 | 3.8 ± 0.8 | 5.1 ± 1.1 | 2.1 ± 0.5 | 5.1 ± 0.8 | 2.7 ± 0.8 |
| E (mmol $m^{-2}$ $s^{-1}$) | 5.4 ± 0.3 | 5.3 ± 0.7 | 6.3 ± 0.7 | 5.7 ± 0.4 | 4.2 ± 0.3 | 2.8 ± 0.5 |
| gs (IRGA) (mol $m^{-2}$ $s^{-1}$) | 134 ± 11 | 126 ± 24 | 229 ± 45 | 128 ± 15 | 133 ± 17 | 82 ± 18 |
| SPAD reading | 38 ± 1 | 33 ± 2 | 39 ± 1 | 38 ± 2 | 36 ± 1 | 31 ± 2 |
| $Na^+$ (mol $m^{-3}$) | 17 ± 2 | 114 ± 14 | 58 ± 8 | 171 ± 23 | 75 ± 12 | 227 ± 30 |
| $K^+$ (mol $m^{-3}$) | 281 ± 9 | 225 ± 14 | 337 ± 8 | 318 ± 26 | 392 ± 20 | 209 ± 14 |
| Sum ($Na^+$+$K^+$) | 298 ± 8 | 340 ± 23 | 393 ± 21 | 489 ± 40 | 467 ± 25 | 436 ± 34 |
| $Mg^{2+}$ (mol $m^{-3}$) | 42 ± 3 | 32 ± 3 | 53 ± 5 | 45 ± 6 | 56 ± 5 | 34 ± 3 |
| $Ca^{2+}$ (mol $m^{-3}$) | 15 ± 2 | 23 ± 4 | 18 ± 2 | 26 ± 4 | 25 ± 3 | 29 ± 4 |
| Na+K+Mg+Ca | 412 ± 12 | 449 ± 31 | 535 ± 28 | 630 ± 50 | 628 ± 29 | 561 ± 39 |
| $Cl^-$ (mol $m^{-3}$) | 256 ± 12 | 330 ± 34 | 362 ± 25 | 482 ± 50 | 464 ± 30 | 391 ± 34 |
| $NO_3^-$ (mol $m^{-3}$) | 8 ± 1 | 2 ± 1 | 5 ± 1 | 5 ± 1 | 7 ± 1 | 5 ± 1 |
| malate (mol $m^{-3}$) | 12 ± 1 | 8 ± 1 | 3 ± 1 | 3 ± 1 | 1 ± 0 | 2 ± 1 |
| $SO_4^{2-}$ (mol $m^{-3}$) | 13 ± 1 | 13 ± 1 | 10 ± 1 | 10 ± 1 | 8 ± 1 | 6 ± 1 |
| Cl+$NO_3$+malate+$SO_4$ | 304 ± 11 | 367 ± 33 | 392 ± 24 | 509 ± 50 | 489 ± 29 | 409 ± 32 |

There were increases in shoot fresh weight and shoot dry weight from 14 days to 21 days salinity but there were non-significant decreases in shoot fresh weight and shoot dry weight from 21 to 28 days salinity. There was a trend of reduction in shoot fresh weight dry weight ratio from 14 to 21 and 21 to 28 days salinity.

Table 4.3 shows that osmotic potential was significantly lower (more negative) at 28 days than at 21 or 14 days, but no simple trend was observed in turgor pressure. The maximum Pn value was recorded at 14 days salinity and the minimum value at 21 days salinity. Reduction in E was observed from 21 to 28 days of salinity.

The concentrations of $Na^+$, $Ca^{2+}$ and $Cl^-$ increased from 14 to 28 days salinity (Table 4.3). Those of $K^+$ and $Mg^{2+}$ increased from 14 days to 21 days, but were not different between 21 and 28 days. No change in nitrate concentration with time was observed at this salinity (100 mol $m^{-3}$ NaCl). Concentrations of malate and sulphate decreased in successive harvests. The sums of cations and anions increased with time, but always with an excess of cations over anions.

The hypothesis, "Time (duration) of exposure of plants to applied salinity has significant effects on physiological parameters in rice" proved to be correct for some parameters on the basis of the data presented in Table 4.3. All parameters were measured from the leaf in one way or the other as is mentioned earlier in the foot note of the table 4.3 except for the shoot fresh and dry weights because these two include (shoot = stem + leaves) stem as well other than the leaves. All leaves were not present at the time of salt application so the older leaves showed pronounced effects because of longer exposure to salinity.

#### 4.3.2.4 Hypothesis 4

"Leaf position (leaf age) has significant effects on salinity responses in rice".

Table 4.2, column H, shows that variety x leaf position interactions under salinity were highly significantly ($p \leq 0.001$) affected the effect of leaf position by $Na^+$ and sum($Na^+$ + $K^+$) while the effect of leaf position was only significantly ($p \leq 0.05$) different for sum($Na^+$+ $K^+$ + $Mg^{2+}$ +$Ca^{2+}$), $Cl^-$ and sum($Cl^-$ + $NO_3^-$ + malate$^-$+ $SO_4^{2-}$). The effect of leaf position were not significantly affected by water potential, osmotic potential, turgor pressure, and concentrations of $K^+$, $Mg^{2+}$, $Ca^{2+}$, $NO_3^-$ malate$^-$ and $SO_4^{2-}$. Table 4.2, column G shows that variety x leaf position interactions under salinity including control were highly significant ($p \leq 0.001$) for leaf rolling score and for concentrations of, $K^+$,

$Mg^{2+}$, sum($Na^+ + K^+ + Mg^{2+} + Ca^{2+}$), $Cl^-$ and the sum of anions, while sum($Na^+ + K^+$) and malate$^-$ were only significantly ($p \leq 0.05$) different amongst leaf positions. Water potential, and osmotic potential turgor pressure, and concentrations of $Na^+$, $Ca^{2+}$, $NO_3^-$ and $SO_4^{2-}$ were not significantly affected by leaf position (Table 4.2, column G). No suitable data were available regarding leaf position for the other parameters (Table 4.2, column G and H)..

The data concerning this hypothesis are shown in Table 4.5, which gives mean values at different leaf positions in Co39, Moroberekan and Maratelli only after 21 days of salinity. Results from the saline flood bench and saline hydroponics are shown separately. A few values are not increasing in the sequence of the other values so these could be due to some faulty operation during the process somewhere.

In general, concentrations of $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$ and $Cl^-$, and the sums of cations and anions, increased with leaf position from youngest to oldest. Except for $Mg^{2+}$, the increases were greater in hydroponics than in the flood bench. Values for sum($Na^+ + K^+$) were not significantly affected by leaf position in either system because of the large standard errors of the means. Values for nitrate, malate and sulphate were low and not much different between leaf positions, except for malate in leaves 0 and 1 in the flood bench. The hypothesis "Leaf position (leaf age) has significant effects on salinity responses in rice" was found to be true for the concentrations of $Na^+$, all sums and for $Cl^-$.

#### 4.3.2.5 Hypothesis 5

"Rice varieties (Moroberekan, Co39 and Maratelli) differ in their responses to salinity" (Table 4.6).

Table 4.2, column J for ANOVA (GLM) shows that shoot weights, fresh weight and dry weight ratio leaf rolling score, SPAD values, ion concentrations of $K^+$, and all sums were highly significantly different ($p \leq 0.001$) in the three varieties in the saline treatments. Pn and the concentrations of $Cl^-$ and malate$^-$ were only significantly different at the 0.050 and 0.01 probability levels respectively. Water potential, osmotic potential, turgor pressure, fv/fm ratios, E, gs, $Na^+$ $Mg^{2+}$, $Ca^{2+}$, and $NO_3^-$ were not significantly different between varieties in this analysis which includes data from all harvests.

**Table 4.5. Different leaf positions under 100 mol $m^{-3}$ NaCl +10 mol $m^{-3}$ $CaCl_2$ at 21 days salinity on various ions (mol $m^{-3}$) and their summations of rice in flood bench system and hydroponics (Experiment 8). These data related to column G in Table 4.2.**

| Parameter | Flood Bench | | | | Hydroponics | | | |
|---|---|---|---|---|---|---|---|---|
| | Leaf 0 | Leaf 1 | Leaf 2 | Leaf 3 | Leaf 0 | Leaf 1 | Leaf 2 | Leaf 3 |
| $Na^+$ | 60 ± 15 | 65 ± 18 | 22 ± 7 | 82 ± 20 | 159 ± 42 | 143 ± 29 | 173 ± 34 | 217 ± 84 |
| $K^+$ | 301 ± 35 | 351 ± 29 | 370 ± 29 | 321 ± 52 | 268 ± 15 | 322 ± 50 | 329 ± 49 | 363 ± 93 |
| Sum ($Na^+$+$K^+$) | 361 ± 31 | 416 ± 29 | 392 ± 66 | 403 ± 66 | 427 ± 50 | 465 ± 56 | 502 ± 66 | 580 ±151 |
| $Mg^{2+}$ | 24 ± 3 | 39 ± 5 | 71 ± 9 | 76 ± 12 | 19 ± 2 | 33 ± 6 | 58 ± 10 | 74 ± 18 |
| $Ca^{2+}$ | 14 ± 4 | 19 ± 3 | 10 ± 2 | 27 ± 5 | 8 ± 2 | 23 ± 5 | 34 ± 8 | 42 ± 8 |
| Na+K+Mg+Ca | 437 ± 35 | 532 ± 37 | 555 ± 48 | 610 ± 82 | 481 ± 53 | 576 ± 65 | 685 ± 81 | 813 ±174 |
| $Cl^-$ | 238 ± 36 | 354 ± 35 | 386 ± 43 | 461 ± 58 | 300 ± 46 | 439 ± 68 | 557 ± 74 | 706 ±192 |
| $NO_3^-$ | 5 ± 2 | 6 ± 1 | 7 ± 1 | 5 ± 1 | 8 ± 2 | 6 ± 2 | 3 ± 1 | 5 ± 3 |
| $malate^-$ | 7 ± 2 | 3 ± 1 | 2 ± 1 | 2 ± 2 | 2 ± 2 | 5 ± 4 | 1 ± 1 | 2 ± 2 |
| $SO_4^{2-}$ | 13 ± 2 | 11 ± 2 | 7 ± 1 | 10 ± 1 | 11 ± 2 | 10 ± 3 | 7 ± 1 | 10 ± 3 |
| Cl+$NO_3$+malate+$SO_4$ | 277 ± 35 | 385 ± 33 | 407 ± 43 | 489 ± 57 | 333 ± 47 | 471 ± 68 | 575 ± 73 | 733 ±191 |

**Table 4.6. Effect of 100 mol $m^{-3}$ NaCl +10 mol $m^{-3}$ $CaCl_2$ at 21 days salinity on various parameters of rice in flood bench system and hydroponics, relevance to the rice varieties (Experiment 8). These data related to column J in Table 4.2. Row values with different letters are significantly different.**

| Parameter | Flood Bench | | | Hydroponics | | |
|---|---|---|---|---|---|---|
| | Co39 | MO | Maratelli | Co39 | MO | Maratelli |
| Shoot FW (g/plant) | 21.9 ± 1.8 cd | 23.0 ± 1.9 d | 28.4 ± 1.9 d | 6.2 ± 2.7 a | 11.9 ± 1.6 ab | 16.0 ± 2.6 bc |
| Shoot DW (g/plant) | 5.22 ± 0.58cd | 4.88 ± 0.68 c | 7.08 ± 0.66 d | 1.74 ± 0.72 a | 2.82 ±0.53 ab | 3.88 ±0.56 bc |
| FW/DW | 4.24 ± 0.18 b | 4.82 ± 0.26 b | 4.05 ±0.12 ab | 3.42 ± 0.19 a | 4.44 ± 0.44 b | 4.11 ±0.10 ab |
| Leaf rolling score | 1.12 ± 0.08 | 1.06 ± 0.04 | 2.66 ± 0.67 | 1.25 ± 0.14 | 1.12 ± 0.07 | 2.4 ± 0.95 |
| Pn (µmol $m^{-2}$ $s^{-1}$) | 3.6 ± 0.6 a | 2.4 ± 2.3 a | 9.1 ± 0.5 b | 0.5 ± 0.5 a | 3.5 ± 0.7 a | 2.3 ± 0.6 a |
| E(mmol $m^{-2}$ $s^{-1}$) | 5.3 ± 0.4 a | 5.0 ± 1.4 a | 8.5 ± 0.7 b | 5.0 ± 0.8 a | 6.8 ± 0.7 ab | 5.2 ± 4.9 a |
| gs (IRGA) (mol $m^{-2}$ $s^{-1}$) | 157 ± 17 a | 188 ± 104 ab | 343 ± 67 b | 100 ± 21 a | 175 ± 30 a | 110 ± 9 a |
| SPAD | 34 ± 2 b | 39 ± 1 bc | 44 ± 2 c | 27 ± 2 a | 37 ± 3 b | 44 ± 1 c |
| $Na^+$ (mol $m^{-3}$) | 53 ± 15 a | 76 ± 15 a | 41 ± 11 a | 201 ± 64 b | 218 ± 46 b | 115 ± 14 a |
| $K^+$(mol $m^{-3}$) | 411 ± 30 c | 293 ± 34 ab | 295 ± 23 ab | 370 ± 33 bc | 396 ± 52 bc | 223 ± 16 a |
| Sum ($Na^+$+$K^+$)(mol $m^{-3}$) | 465 ± 30 ab | 368 ± 43 a | 336 ± 25 a | 571 ± 44 bc | 615 ± 82 c | 337 ± 15 a |
| $Mg^{2+}$(mol $m^{-3}$) | 61 ± 10 b | 46 ± 7 ab | 52 ± 9 ab | 25 ± 2 a | 62 ± 13 b | 36 ± 6 ab |
| $Ca^{2+}$(mol $m^{-3}$) | 18 ± 3 | 19 ± 4 | 15 ± 4 | 14 ± 5 | 27 ± 5 | 29 ± 7 |
| Na+K+Mg+Ca (mol $m^{-3}$) | 624 ± 47 ab | 499 ± 51 a | 471 ± 40 a | 651 ± 47 ab | 794 ±107 b | 469 ± 25 a |
| $Cl^-$(mol $m^{-3}$) | 417 ± 25 ab | 411 ± 57 ab | 245 ± 19 a | 528 ± 21 bc | 662 ±102 c | 294 ± 23 a |
| $NO_3^-$(mol $m^{-3}$) | 6 ± 1 ab | 4 ± 0 a | 8 ± 2 b | 7 ± 2 ab | 4 ± 1 ab | 6 ± 1 ab |
| malate$^-$(mol $m^{-3}$) | 5 ± 2 | 2 ± 1 | 4 ± 2 | 5 ± 5 | 1 ± 1 | 3 ± 1 |
| $SO_4^{2-}$(mol $m^{-3}$) | 12 ± 1 c | 7 ± 1 ab | 12 ± 2 bc | 15 ± 3 c | 6 ± 0 a | 11 ± 2 abc |
| Cl+$NO_3$+malate+$SO_4$ | 451 ± 24 ab | 430 ± 56 ab | 281 ± 15 a | 569 ± 25 bc | 680 ±102 c | 325 ± 22 a |

The data relating to this hypothesis are shown in Table 4.6. Plants were harvested at three times (14, 21 and 28 days salinity), but only data from the 21 day harvest of the saline treatments are shown in Table 4.6.

Moroberekan had the highest concentrations of most cations and $Cl^-$ in saline hydroponics. Maratelli generally had lower ion concentrations than the other two varieties except for $Ca^{2+}$.

The hypothesis, "Rice varieties (Moroberekan, Co39 and Maratelli) differ in their responses to salinity" was found to be correct for Moroberekan at least in hydroponics because Moroberekan was significantly different from Co39 and Maratelli for SPAD, on the basis of comparison of the data presented in Table 4.6. The data were compared by SPSS, one way ANOVA, Post Hoc, "Duncan's multiple comparisons" test and the letters a,b, c, and d were used to denote the different groups as shown in Table 4.6.

Furthermore the hypothesis is strongly supported by many parameters in both flood bench and hydroponics as the Maratelli is significantly different from Co39 and Moroberekan for Pn, and E in flood bench system and SPAD, $Na^+$ $K^+$, sum($Na^+$+$K^+$), sum($Cl^-$+$NO_3^-$+malate$^-$+$SO_4^{2-}$), and $Cl^-$ in hydroponics when the data were compared by Duncan's multiple test. So there is enough evidence from the data presented in Table 4.6 to support the hypothesis that rice varieties differ in their responses to salinity.

### 4.3.3 Bypass flow and PTS tracer in rice

"The extent of the apoplastic leakage pathway differs between varieties and determines relative $Na^+$ accumulation". Table 4.7 shows varietal differences in PTS tracer accumulation in non-saline conditions. There were no standard error means in Co39 because there were no replicates in this case. Maratelli had the highest fluorescence per shoot and the highest fluorescence per gram shoot fresh weight after five days exposure of plants to 30 mg $l^{-1}$ PTS tracer. The lowest fluorescence per shoot and the lowest fluorescence per gram shoot fresh weight were observed in Moroberekan (Table 4.7).

**Table 4.7. Fluorescence in rice after 5 days exposure of rice plants to 30 mg $l^{-1}$ PTS tracer to determine the transpirational bypass flow and apoplastic leakage pathway at λ excitation 403, and λ emission 510 (Experiment 8b, non-saline).**

| Treatment | Co39 | Moroberekan | Maratelli |
|---|---|---|---|
| **Fluorescence /Shoot** | 18.5 | 13.3 ± 0.10 | 34.7 ± 14.0 |
| **Fluorescence/g SFW** | 6.0 | 3.9 ± 0.17 | 9.9 ± 4.7 |

### 4.3.4 Bypass flow and Salinity + PTS tracer in rice

The data from experiment 9 are shown in Table 4.8 for the three varieties of rice. The varieties differed significantly under salinity + PTS treatment.

**Table 4.8 Fluorescence per gram shoot dry weight in rice (Transpirational bypass flow and apoplastic leakage pathway in rice) at λ excitation 403, and λ emission 510 under 5 days salinity. (Experiment 9, saline).**

| Treatment | Co39 | Moroberekan | Maratelli |
|---|---|---|---|
| **control** | 19 ± 6 | 10 ± 0 | 17 ± 4 |
| **100 mol $m^{-3}$ NaCl + 30 mg $l^{-1}$ PTS tracer** | 186 ± 12 | 120 ± 22 | 255 ± 44 |

There was a significant increase in fluorescence per gram shoot dry weight under the influence of salinity and PTS tracer in all the varieties under consideration. The highest fluorescence value was observed in the case of Maratelli (255) and the lowest was observed in Moroberekan (120) under salinity with PTS tracer treatment. Moroberekan was significantly different from Co39 and Maratelli under salinity with PTS tracer (Table 4.8).

## 4.4 Discussion

"Salt has highly significant damaging influence due to dehydration of tissues of rice plants".

Leaf rolling may be due to apoplastic salt accumulation caused by dehydration (Table 4.1). These results support the Oertli hypothesis (Oertli 1968; Flowers *et al.*, 1991). Shoot fresh and dry weight decreased with the increase of salinity in rice varieties (Table 4.3). Similar results were found by Welfare *et al.* (1996) and Aslam *et al.* (1993*b*). Rashid *et al.* (1999) observed that shoot fresh weight decreased in different wheat varieties under 100 and 200 mol $m^{-3}$ NaCl salinity. Marked increases in concentrations of $Na^+$ and $Cl^-$ with increased salinity were observed in leaf sap of Co39, Moroberekan and Maratelli (Table 4.3). Aslam *et al.* (1993*a*) also observed conspicuous increases in $Na^+$ and $Cl^-$ in shoot sap of NIAB-6, BG 402-4, Basmati 370, IR 1561. All the varieties of rice under consideration have significantly different concentrations of $Na^+$ (low) and $Cl^-$ (high) in leaf sap of rice (Table 4.3).

High internal (leaf sap) $Cl^-$ and $Na^+$ concentrations were the major cause of dehydration as is obvious from the shoot fresh weight/ dry weight ratio (Table 4.3). This idea was also supported in the case of $Na^+$ but not in the case of $K^+$ (Chapter 3). Apparently high concentration of salts in hydroponics could be due to low plant vigour compared to flood bench system. So inevitably the combined effect of salts accumulated in mmol $kg^{-1}$ dw caused plant chlorosis, necrosis, less vigorous plant growth, plant damage and in some cases complete plant injury (Table 4.1). However salt effect could be modified due to g x e interactions. $Cl^-$ was higher than $Na^+$ in all varieties of rice under salinity (J. Akhtar, personal communication, 1999; Aslam *et al.*, 1993*a,b*; Azhar Naeem, 1994; Noor Muhammad, 1998). These results concord with the results of Welfare *et al.* (1996) for $Na^+$ and $Cl^-$ concentrations in some varieties of rice except IR26 and IR2153. Osmotic potential of the leaf sap decreased under salinity (Table 4.3) as found by Aslam *et al.* (1993*a*). Low turgor was observed under salinity (Table 4.3), and similar results were found by Welfare *et al.* (1996) and Oertli (1968).

Dehydration partly contributed to the increase of different ion concentrations in the plant tissues. High concentrations of ions could be toxic to the plants. Logically it

is right to say that the higher the concentration of the individual ion in the leaf tissue, the more toxic that individual ion. Specifically, $Cl^-$ contributed the major part of the ionic concentrations but it could not be said that $Na^+$ was not toxic because of its smaller contribution. However it is the contributing effect of various ions in the leaf tissue that is responsible for plant injury.

"Systems of salt application (flood bench and hydroponics) have different effects on physiological parameters in rice under salinity" at 2nd (21 days salinity) 3rd (28 days salinity) and 4th (49 days salinity) harvests" (Table 4.4).

Large differences were observed in the shoot fresh and dry weight, and ion concentrations of 3 varieties in the flood bench system and hydroponics and this shows that the effect of salinity is influenced by the system of salt application (Table, 4.2 and 4.4). Similar results were found by Pecetti and Gorham (1997), and they observed that varietal responses to salinity were determined by salt application techniques. Aslam *et al.* (1993) also found differences in shoot fresh and dry weight of rice under salinity in different systems (pot culture and salt affected-field). Probably within the systems the physiology of root could be affected due to different medium of growth or it could be the differences in the development of aerenchyma in different systems of salt application.

"Time (duration) of exposure of plants to applied salinity has significant effects on physiological parameters in rice" (Table 4.3).

The duration of salinity treatment affects shoot fresh weight and shoot dry weight of plants (Tables 4.2 and 4.3), and similar results were recorded by Azhar Naeem (1994) and Noor Muhammad (1998) at different times after salinity treatment. Osmotic potential decreased under salinity in all the varieties with increased time of exposure to salt (Table 4.3). Aslam *et al.* (1993) recorded similar results in rice and Rashid *et al.* (1999) observed similar findings in wheat. Sarg *et al.* (1993) observed similar results in tomato.

Ion concentrations such as $K^+$, $Na^+$, and $Cl^-$ were significantly different under various durations of salinity and their concentrations increased with the increase of salinity duration (Table 4.3). Similar trends were observed by Azhar Naeem (1994).

"Leaf position (leaf age) has significant effects on salinity responses in rice" (Table 4.5).

Leaf to leaf gradients were observed for $Na^+$, $K^+$ and $Cl^-$ accumulation at different levels of applied salinity in rice (Table 4.5) and similar observations were recorded by Rashid *et al.* (1999) in wheat.

"Rice varieties (Moroberekan, Co39 and Maratelli) differ in their responses to salinity (Table 4.2).

Aslam *et al.* (1993) observed that salinity damage was influenced by rice varieties for different parameters like shoot fresh weight, shoot dry weight and mortality. Differences in accumulation of $Na^+$ and $Cl^-$ were observed between rice varieties as Maratelli accumulated lower $Na^+$ and $Cl^-$ compared with Co39 and Moroberekan in both the systems. $Cl^-$ concentrations were much higher in all the varieties than $Na^+$ concentrations in both the systems (Table 4.6). Similar results were found by other authors (Aslam *et al.*, 1993*a*; Azhar Naeem, 1994; Welfare *et al.*, 1996; Noor Muhammad, 1998;).

"The extent of the apoplastic leakage pathway differs between varieties and determines relative $Na^+$ accumulation". Varieties were not significantly different from each other for PTS fluorescence per shoot and fluorescence per gram shoot fresh weight in rice after 5 days exposure of plants to PTS tracer without salinity (Table 4.7). According to Yeo *et al.* (1999) excessive transport is mainly the result of leakage along an apoplastic pathway to the xylem. The low value of fluorescence in Moroberekan indicated low level of leakage of solutes through bypass flow (Table 4.7). According to Yeo *et al.* (1999) transpirational bypass flow was the major pathway for the transport of $Na^+$.

. Varieties were significantly different from each other for the fluorescence per gram shoot dry weight in rice and hence apoplastic leakage of $Na^+$ in rice under 5 days of salinity. Natural fluorescence was very low as compared to the fluorescence under treated (PTS) conditions in all the varieties (Table 4.7). Similar results were also found by Yeo *et al.* (1999) and he observed that natural fluorescence was negligible in the control plants when the plants were not exposed to PTS tracer. This significant variation in the fluorescence with PTS + NaCl did not indicate significant differences in the $Na^+$ transport within the varieties of rice (Table 4.8) because Moroberekan was a

high $Na^+$ accumulator but showed low fluorescence, while Maratelli was a low $Na^+$ accumulator and showed high fluorescence. According to Yeo *et al.* (1999) more PTS was taken up by the high sodium transporting lines and low PTS was taken up by the low $Na^+$ transporting lines contrary to my results. The root endodermis may account here because the plasma membranes of endodermal cells represented the final point at which the root could control the entry of PTS tracer due to presence of the casparian strip in the endodermis. The casparian strip is a specialised local impregnation of the wall, involving the production of material similar to cutin. The casparian strip (zone of corky cells in the endodermis of plant root) runs round all the anticlinal walls and completely encircles the cell. The apoplastic pathway involves movement through the cell wall network as far as the casparian strip is concerned so then due to presence of these physical barrier at endodermis level, movement through the symplasm and at this endodermis level membrane transport is different in Moroberekan from Co39 and Maratelli. Exodermis or rhizodermis (hypodermis with casparian strips) could also restrict the movement of dyes because it is also an important control point that can force selection of external solutes to be absorbed by the plasma membrane of exodermis or rhizodermis in mature cells. So the different membrane transport in rice varieties is responsible for the contrary results from the current study with that of Yadav *et. al.* (1996), Yeo *et. al.* (1999) and Yeo (1998).

It is concluded in the light of my work that differences in transpirational bypass flow or apoplastic leakage pathway are not important for $Na^+$ transport in Co39 and Moroberekan. According to Yadav *et al.* (1996) varietal comparisons are complex because there are differences other than in salt transport. It is concluded that membrane transport is different in rice varieties

## 4.5 Conclusions

- Salt has a highly damaging influence due to dehydration of tissues of rice plants.
- Systems of salt application are significantly different from each other (flood bench and hydroponics) in rice at all harvests.
- Time (duration) of salinity application is important in rice.
- Leaf position (leaf age) has a significant effect on ion accumulation under salinity in rice.
- Rice variety Moroberekan is significantly different from Co39 and Maratelli for SPAD values in hydroponics under salinity.
- Maratelli is significantly different from Co39 and Moroberekan under salinity for Pn and E in the flood bench system and for SPAD, $Na^+$ $K^+$, sum($Na^+$+$K^+$), sum($Cl^-$+$NO_3^-$+malate$^-$+$SO_4^{2-}$), and $Cl^-$ in hydroponics on the basis of the comparison of the data presented in Table 4.6.
- High $Na^+$ accumulation is related to low bypass flow in rice varieties Co39 and Moroberekan.
- The endodermis in the root of rice varieties could be important because membrane transport is different in rice varieties.
- Plant responses to salinity can be affected by salt concentrations, time of exposure of plants to salinity, leaf age, system of salt application and varietal differences.
- Older leaves showed pronounced effects because of longer exposure to salinity.
- The relative performance of rice varieties could be different between the systems.

# Chapter 5

# QTL Analyses for $Na^+$ Accumulation and Other Traits

## 5.1 Introduction

Flowers *et al.* (2000) studied the importance of QTL in rice and their position in engineering tolerance of rice to salinity. It was concluded that selection based on phenotypic characters without genotypic knowledge could be used, but wide application was limited by the assessment of large numbers of individuals in segregating generations. Not many genes for salt tolerance have been identified, therefore a QTL/ marker assisted selection approach should be used. There are problems in devising protocols when genes of interest are variably influenced by the experimental treatments and the environment.

A number of QTL for $Na^+$ accumulation in the shoots of rice were found with AFLP analyses (Flowers *et al.*, 2000). Sixteen QTL were identified in RILs from a cross between parents with extreme phenotypes of $Na^+$ transport (IR4630 x IR15324, designated IR55178). Only one QTL for high $Na^+$ uptake was found on chromosome 1. One QTL for $Na^+/K^+$ discrimination was observed on chromosome 4. With AFLP analyses, QTL were identified in two other populations of rice, the cross IR59462 and the selections for high and low $Na^+$ in IR36. No marker was directly associated with salt tolerance in the mapping population (IR55178). No association was observed with high or low subsets of IR59462. It is hoped that a relatively small number of QTL might control complex physiological characters (Yeo 1998).

Selection of both the extremes of high and low $Na^+$ transporting lines is very important for plant breeding in rice (Yeo and Flowers, 1986; Yeo *et al.*, 1988; Flowers *et al.*, 1990; Flowers *et al.*, 2000). Uptake of $Na^+$ is genetically controlled in rice by different genes that segregated independently (Garcia *et al.*, 1997).

Rice is classified into two major groups, japonica and indica. Indica types were less cold-tolerant than most of the japonica types (Morishima and Oka, 1982). Recombinant lines of *Oryza sativa* L. with improved root traits were developed from the

cross Bala x Azucena by Price *et al.* (1997). It was observed in an $F_2$ population derived from this cross that the existence of RFLP between the parents was 32%. Moreover a molecular map was created. After 28 days growth, one QTL for maximum root length on chromosome 11 was observed. Large variation was observed with the developmental stage in some root length QTL including that on chromosome 11. One QTL was detected for root volume and two QTL were detected for adventitious root thickness (Price and Tomos, 1997). Identification of several genomic regions of potential value for the improvement of drought tolerance in rice became possible by comparison of multiple-season and multiple-site field drought screens in several populations (Price *et al.*, 1999).

QTL for drought tolerance were determined and mapped by Champoux *et al.* (1995) in 203 recombinant inbred lines (Co39 x Moroberekan) of rice that were grown in fields and greenhouses. Most of the identified QTL were associated with root thickness, root/shoot ratio, and root dry weight per tiller, and only a few with deep root weight. Correlation of root parameters in greenhouse experiments with field drought avoidance/ tolerance were significant but not highly predictive. Twelve chromosomal regions containing putative QTL associated with field drought avoidance / tolerance also contained QTL associated with root morphology. An effective strategy for altering the root phenotype of rice towards that commonly associated with drought-resistant cultivars might be the selection for Moroberekan alleles at marker loci associated with the putative root QTL (Champoux *et al.*, 1995).

Fifty two recombinant inbred lines from the same Co39 x Moroberekan cross were grown by Lilley *et al.* (1996) under slowly developed water stress conditions. RILs were studied for QTL identification and for their association with dehydration tolerance, osmotic adjustment, root traits and leaf rolling scores. The single estimated osmotic adjustment locus and 2 out of 5 loci associated with dehydration tolerance were close to chromosomal regions associated with root morphology (linkage between the traits).

Yadav *et al.* (1997) compared QTL locations from populations of recombinant inbred lines of different crosses (IR-64 x Azucena and Co39 x Maratelli). One to three common QTL were found per trait, among which the most significant was in one or other population. From these results it might be possible to derive near-isogenic lines

introgressed with these common segments, separately in indica and japonica backgrounds.

Wang and Paterson (1994) observed that an efficient strategy for the identification of DNA markers closely linked to genes or genomic regions of interest, was the synthesis of DNA pools from segregating populations. QTL having allelic effects of less than 0.75 SD (standard deviation), could not be easily tagged (identifying DNA markers closely linked) in DNA pools. Segregation distortion could have a large effect on the allelic composition of DNA pools. So to minimise the false positive and false negative results, more individuals should be used in the pools. The use of phenotype-based DNA pools is not very good for the comprehensive identification of the majority of QTL affecting a complex trait like salinity .

QTL analyses of $Na^+$ and $Cl^-$ accumulation were carried out by Tozlu *et al.* (1999*a,b*) in an $F_1$ intergeneric hybrid of *Citrus grandis* [*C. maxima*] and *Poncirus trifoliata*, and a $BC_1$ progeny population (*C. grandis* x $F_1$) under 40 mol $m^{-3}$ NaCl. It was observed that for many traits, $BC_1$ progeny segregated transgressively. It was indicated by correlation analysis and locations of potential QTL that fewer genes could control many traits than the actual number of QTL mapped for them. For example, 21 potential QTL mapped for $Na^+$ accumulation and $Cl^-/Na^+$ ratios were located in a cluster at the beginning of one linkage group, while a cluster of 10 potential QTL mapped for $Cl^-$ accumulation and $Cl^-/Na^+$ ratios were located at the beginning of another linkage group. It means fewer genes can control many traits than the actual number of QTL mapped for them because the potential QTL can indicate the region in which the probability to find the QTL is maximum that is why they are called potential or putative

The following hypotheses were formulated to assess the importance of ion accumulation in salt tolerance of rice, based on vegetative growth parameters;

**Hypothesis 1**

Leaf $Na^+$ concentrations are under genetic control and are important in salt tolerance (based on relative vegetative growth rates) in rice .

**Hypothesis 2**

Leaf $Cl^-$ concentrations are under genetic control and are important in salt tolerance (based on relative vegetative growth rates) in rice .

## 5.2 Experimental design

Parental varieties Co39, Moroberekan and Maratelli were selected because some work had already been done on these parental varieties and seeds for their hybrid lines were available. Maratelli was chosen because F2 hybrids of a Maratelli x Moroberekan cross were also available.

**Table 5.1. Experimental design in flood bench system of 10 blocks with 2 litre pots.**

| Expt. No. | NaCl (mol $m^{-3}$) | $CaCl_2$ (mol $m^{-3}$) | Population | Total plants |
|---|---|---|---|---|
| 7.1 | 100 | 10 | Co39, Moroberekan, Maratelli and RILs F8 (Co39 x Moroberekan) | 200 |
| 7.2 | 150 | 15 | | 200 |
| 7.3 | 0 | 0 | | 200 |
| 10 | 150 | 15 | Co39, Moroberekan, and RILs F9 (Co39 x Moroberekan) | 596 |

Pure inbred lines could be selected by repeated selfing over many generations because the quantitative traits ($Na^+$ and $Cl^-$) are heritable in rice and continue to segregate for many generations. The chance of heterozygosity within RILs was reduced to 3% in an $F_8$ generation. Fortunately an $F_8$ generation of RILs from the Co39 x Moroberekan cross was available, and this was used in experiment 7 for the study of the population under salinity (Table 5.1). Experiment 10 was conducted with the same cross from the $F_9$ generation, but with selected inbred lines to obtain reliable data because the QTL are identified better with more genotypes and fewer replicates.

These experiments were conducted in a flood bench system (see Chapter 2). Different salt concentrations (Table 5.1) were applied with the exception of control in experiment 7.3. Initially all plants were under the same salinity level (Table 5.1) in experiment 7 (7.1 and 7.2). In the second part of experiment 7 (7.2) salinity was later increased to 150 mol $m^{-3}$ NaCl.

### 5.2.1 Experiment 7

Ten replicates of three parental varieties (Co39, Moroberekan and Maratelli) and 170 $F_8$ generation (Co39 x Moroberekan) inbred lines were used in this experiment. The location of the experiment was the flood bench system of house 2 at Pen-y-Ffridd. Seeds were sown in John Innes Compost No.1 on 12/3/99 in P-84 plug trays. Four hundred plants were transplanted to 2 litre rose pots and then one set of 200 plants was transferred to the flood bench system 11 days after sowing (23/3/99) for experimental work (Experiment 7.1 and 7.2).

The other set of 200 plants was transferred to a fresh water flood bench with no added salt for seed production. Anthers were covered with paper bags at the time of anthesis to avoid cross pollination among the inbred lines. Date of anthesis was recorded for each plant (Experiment 7.3).

In the flood bench system nutrients (Phostrogen 1g $l^{-1}$ + 0.5 ml $l^{-1}$ micro-nutrients + sodium silicate 0.1 ml $l^{-1}$) were supplied to the plants *via* roots with the irrigation water. Stress 1 was started 24 days after sowing (5/4/99) in daily increments of 25 mol $m^{-3}$ and 2.5 mol $m^{-3}$ for NaCl and $CaCl_2$ respectively and stress was completed on 27 days after sowing (8/4/99). All populations were under the same salinity level (100 mol $m^{-3}$ NaCl + 10 mol $m^{-3}$ $CaCl_2$) in experiment 7.1. In total 200 plants were sampled for ion ($Na^+$, $K^+$ and $Cl^-$) analysis after 14 days salinity / 41 days after sowing (22/4/99) and the youngest fully expanded leaf was selected from each plant for ion analysis.

After the 7.1 harvest the stress was increased in increments of 25 mol $m^{-3}$ and 2.5 mol $m^{-3}$ for NaCl and $CaCl_2$ respectively. Stress of 150 mol $m^{-3}$ NaCl + 15 mol $m^{-3}$ $CaCl_2$ salinity was complete on 24/4/99 and was maintained for 7 days in experiment 7.2. Plants were sampled a second time for ion analysis 21 days after salinity / 49 days after sowing (1/5/99) and the fully expanded youngest leaf was selected for ion analysis. Resistance score was recorded from 0 to 5 depending on the general physical health of the plants, estimated proportion of the green colour in comparison with the dry matter of their leaves and general size of the plants. Score 0 was given to the dead plants and score 5 to the plants in good condition. However no plant had a score of 5 under salinity. Resistance score 1 was recorded 36 days after salinity /65 days after sowing (16/5/99) and resistance score 2 was recorded 49 days after salinity / 78 days after sowing (29/5/99) both under 150 mol $m^{-3}$ NaCl. Plants were finally harvested 63 days

after salinity / 92 days after sowing (14/6/99) for shoot fresh weight and shoot dry weight.

### 5.2.2 Experiment 10

Ten replicates of two parental varieties (Co39 and Moroberekan) and 6 replicates of 96 $F_9$ generation RILs were used in experiment 10 (Table 5.1). On the basis of the accumulation of various ions in their leaf saps in Experiment 7, 76 RILs from the total RILs used in experiment 7, $F_8$ generation, 25 were selected with high $Na^+$, and high $Cl^-$ and 22 RIL were selected with low $Na^+$ and low $Cl^-$ while 29 were chosen on the basis of medium accumulation of $Na^+$ and $Cl^-$. However values for SFW, SDW and $K^+$ concentration were also taken into consideration. Furthermore an additional 20 RIL, not used in Experiment 7, were included in this experiment. The protocol and location of this experiment were the same as in experiment 7.

Experiment 10 was started on 13/7/00. The salt and nutrient solution was changed once during the course of the experiment on 21/8/00. Plants were harvested for ion analysis after 15 days stress of 150 + 15 mol $m^{-3}$ NaCl and $CaCl_2$ respectively. Resistance score 1 was recorded on 8/9/00, 17 days after salt stress and resistance score 2 was recorded on 25/9/00, 34 days after salt stress. Plants were harvested for growth parameters on 7/10/00, after 47 days of salt stress. The means of the 6 plants of each RIL were taken for QTL analysis as described in chapter 2.

## 5.3 Results

### 5.3.1 Experiment 7

Experiment 7 was split into three parts depending on the different concentrations of salts supplied.

#### 5.3.1.1 Experiment 7.1

Rice plants were supplied with 100 mol $m^{-3}$ NaCl + 10 mol $m^{-3}$ $CaCl_2$ salinity. The plants were sampled 36 days after sowing at 14 days of salinity.

5.3.1.1.1 RIL frequency and sodium in experiment 7.1 (Na1)

Figure 5.1 illustrates the frequency distribution of 170 recombinant inbred lines (RILs) with three parental rice varieties (Co39, Moroberekan and Maratelli) shown as ranges (coloured lines). Parental mean values are given in Table 5.5.

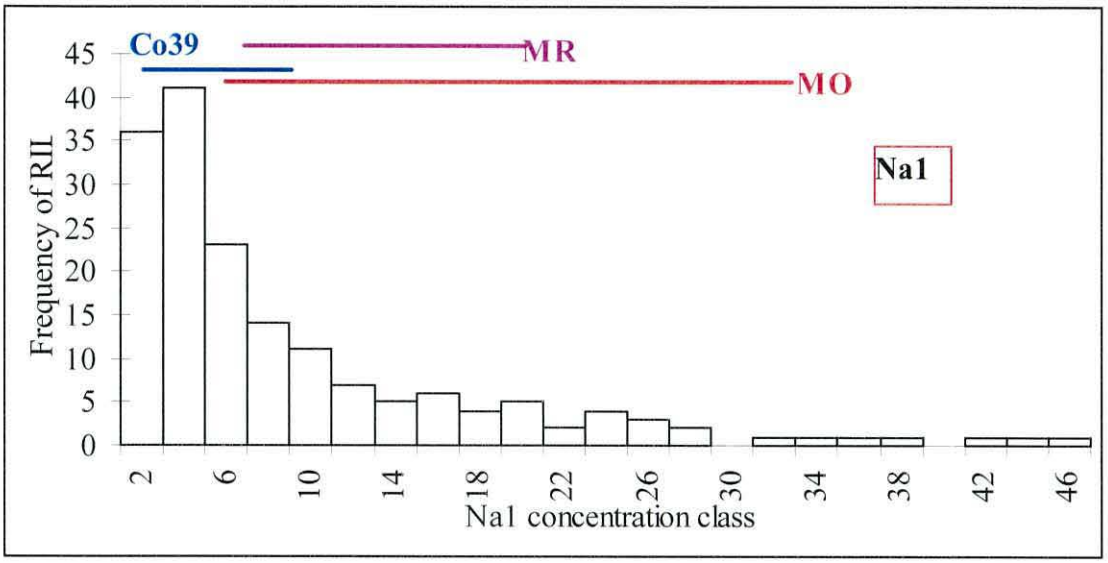


**Figure 5.1. Effect of 100 + 10 mol $m^{-3}$ NaCl and $CaCl_2$ salinity on mol $m^{-3}$ of Na1 (36 das. and 14 days of salinity, Experiment 7.1).**

Na1 stands for leaf $Na^+$ concentrations in experiment 7.1. The mode having , 41 RILs, was recorded for Na1 values between 2.1 and 4 mol $m^{-3}$. In the parental varieties the highest value (31 mol $m^{-3}$) for Na1 was recorded in Moroberekan and the lowest value (2 mol $m^{-3}$) for Na1 was recorded in Co39. The RILs having values for Na1 concentrations above 31 mol $m^{-3}$ fell within the range of transgressive values because they deviated from the extremes of their parental range for Na1 values. The number of RILs decreased with high Na1 values. The Anderson-Darling Normality test was applied and it was found that distribution of Na1 was not normal within 170 inbred lines and instead the distribution was skewed.

5.3.1.1.2 RIL frequency and chloride in experiment 7.1 (Cl1)

Figure 5.2 illustrates the frequency distribution of 170 recombinant inbred lines (RILs) and their parental rice varieties.

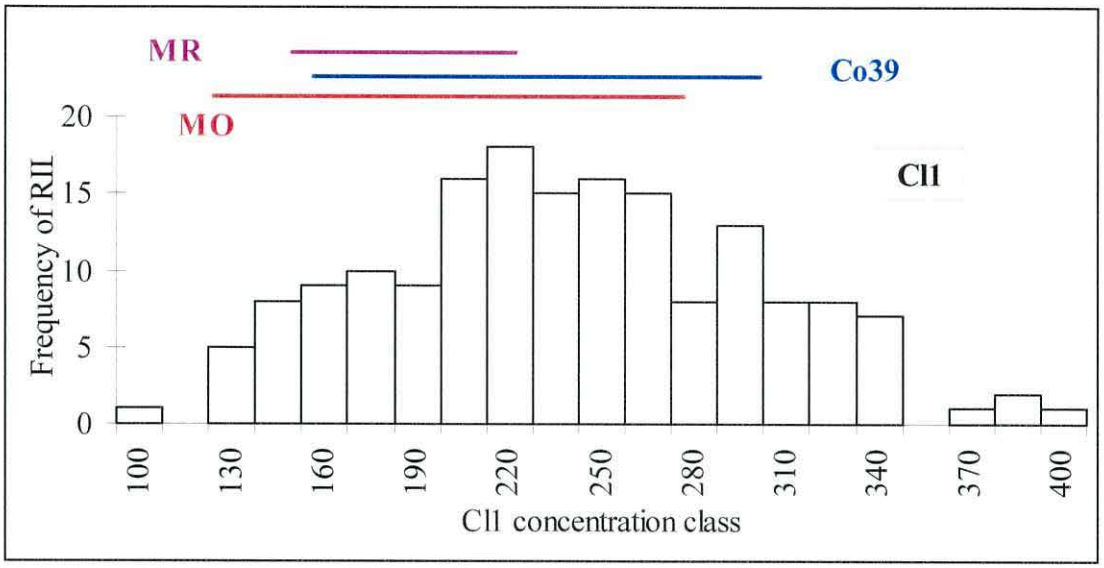


**Figure 5.2. Effect of 100 + 10 mol $m^{-3}$ NaCl + $CaCl_2$ salinity on mol $m^{-3}$ of Cl1 (36 das. and 14 days of salinity, Experiment 7.1).**

Cl1stands for leaf $Cl^-$ in experiment 7.1. The mode, having highest number, 18 RILs, was recorded for Cl1 values between 205 and 220 mol $m^{-3}$. In the parents (Table 5.5), the highest value (289 mol $m^{-3}$) for Cl1 was recorded in Co39 and the lowest value (119 mol $m^{-3}$) for Cl1 concentration was recorded in Moroberekan. The RILs with Cl1 concentration values below 119 mol $m^{-3}$ and above 289 mol $m^{-3}$ fell within the transgressive range. The number of RILs decreased at the extreme limits of $Cl^-$ concentrations (low and high). The distribution of $Cl^-$ within 170 recombinant inbred lines was normal according to the Anderson-Darling Normality test

## 5.3.1.2 Experiment 7.2

Rice plants were harvested at 150 mol $m^{-3}$ NaCl + 15 mol $m^{-3}$ $CaCl_2$ salinity.

5.3.1.2.1 RIL frequency and resistance score1

The frequency distributions of 170 recombinant inbred lines (RILs) of rice with their parental rice varieties are illustrated in fig 5.3 for resistance score 1. The ranges of

resistance scores of the parental varieties Co39, Moroberekan and Maratelli are indicated with lines.

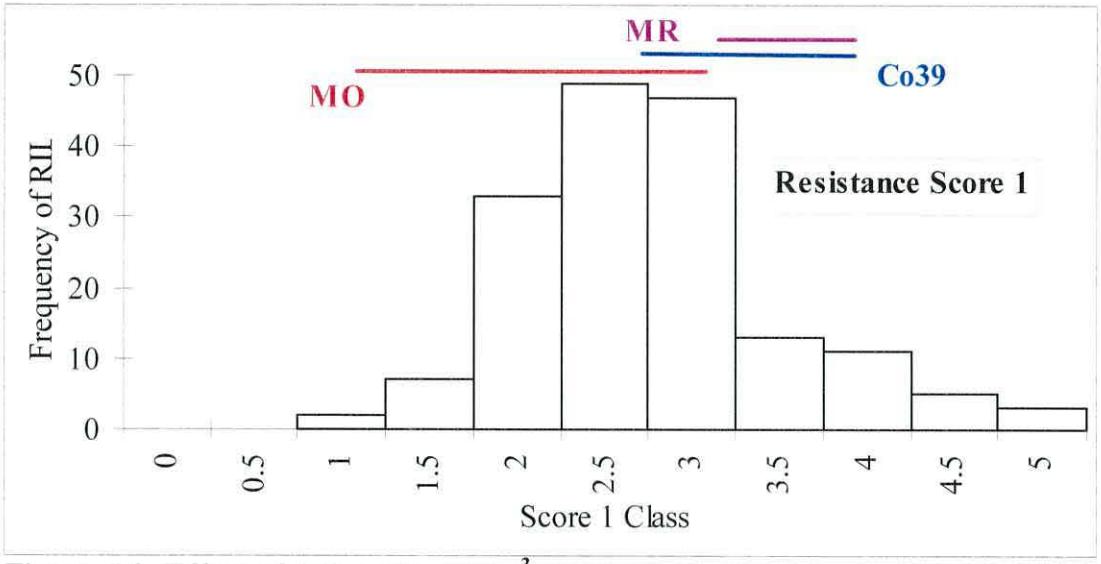


**Figure 5.3. Effect of 150 + 15 mol $m^{-3}$ NaCl + $CaCl_2$ salinity on resistance score 1 (65 das, Experiment 7.2).**

There is no unit for resistance score values because these are the comparative values which were recorded based on plant vigour, chlorosis, necrosis and their general physical health conditions. The scores were given from 0-5 to the plants, 0 for dead or decaying plants and 5 for excellent physical health conditions, so increasing grade points from 0 to 5 dependent on plant vigour, chlorosis, necrosis, and their general physical health conditions. Score 1 stands for the observations recorded for the resistance score under 150 mol $m^{-3}$ NaCl + 15 mol $m^{-3}$ $CaCl_2$ salinity on 65 das in Experiment 7.2.

The mode having 49 RILs was recorded with resistance score 1 values between 2.1 and 2.5. In the parental varieties, the highest individual value (4) for resistance score was recorded in Co39 and the lowest individual value (0.75) for resistance score was noticed in Moroberekan. The parental means are shown in Table 5.5. The RILs with resistance score values below the level of 0.75 and above the level of 3.5 were in the transgressive range. The number of RILs decreased under both the extreme ends (low and high) of resistance score. The distribution of resistance score 1 within 170 recombinant inbred lines was slightly skewed as the probability of the resistance score 1 data being normally distributed was 0.000 from the Anderson-Darling Normality test.

5.3.1.2.2 RIL frequency and resistance score 2 (Experiment 7.2)

The frequency distribution of 170 recombinant inbred lines (RILs) with the three parental rice varieties is shown in Figure 5.4.

Score 2 stands for the observations recorded in Experiment 7.2 under 150 mol $m^{-3}$ NaCl, after 49 days salinity / 78 days after sowing (29/5/99). The mode having 63 RILs had resistance score 2 values between 0.1 and 0.5. The highest resistance score (4.5) was recorded in Maratelli and the lowest resistance score (0) was recorded in Moroberekan. Some values with high resistance score 2 (3.4 and 4) fell in the transgressive range. Distribution of resistance score 2 is skewed (0.5009), (Fig. 5.4) but slightly skewed (0.4075) in case of resistance score 1 (Fig. 5.3). The distribution of resistance score 1 within 170 recombinant inbred lines was asymmetrical as the probability of the resistance score 2 data being normally distributed was 0.5009 from the Anderson-Darling Normality test.

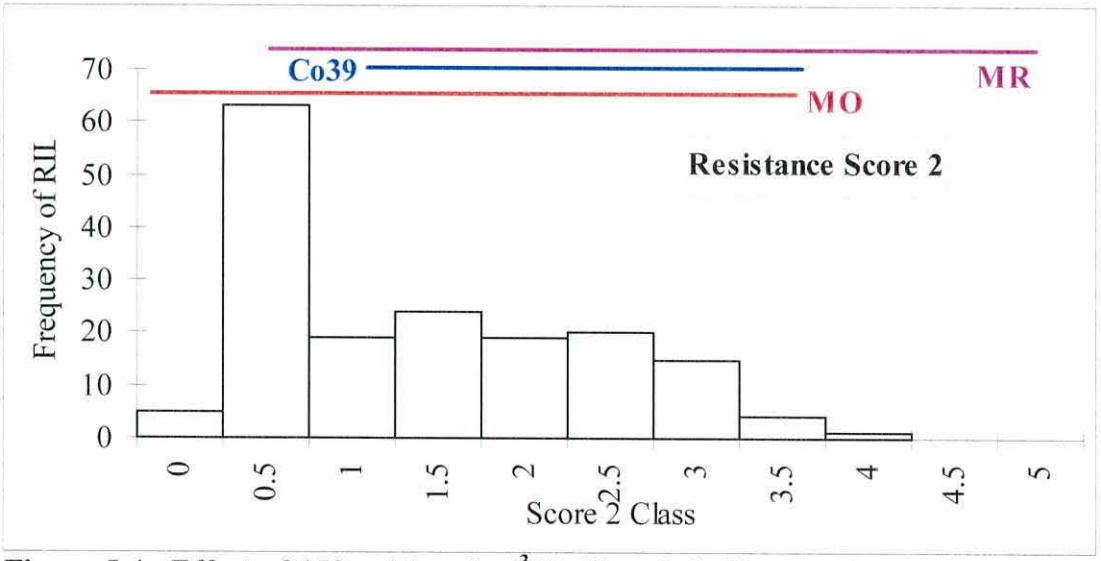


**Figure 5.4. Effect of 150 + 15 mol $m^{-3}$ NaCl and $CaCl_2$ on resistance score 2 (78 days after sowing and 49 days after salinity, Experiment 7.2).**

5.3.1.2.3 RIL frequency and sodium in experiment 7.2 (Na2)

Na2 stands for $Na^+$ in Figure 5.5. The highest number, 41 RILs, were recorded for Na2 between 1 and 5 mol $m^{-3}$. In the parental varieties, the highest value (99 mol $m^{-3}$) for Na2 was noticed in Moroberekan and the lowest value (2 mol $m^{-3}$) for Na2 was observed in Co39. The number of RILs was decreased in the bins with high Na2 and the highest Na2 (120 mol $m^{-3}$) was observed only in 2 recombinant inbred lines.

However the number of RILs with low values for Na2 was high. The distribution of Na2 was not normal (Figure 5.5) but skewed as in Na1 according to the Anderson-Darling Normality test.

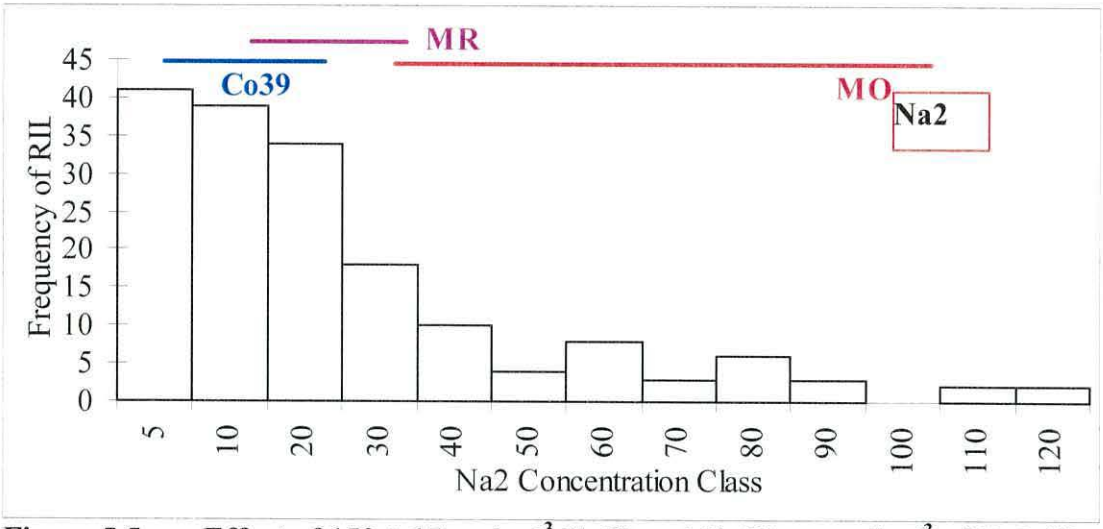


**Figure 5.5. Effect of 150 + 15 mol $m^{-3}$ NaCl and $CaCl_2$ on mol $m^{-3}$ of Na2 (43 das., 21 days of salinity) in RILs and parental varieties of rice (Experiment 7.2).**

5.3.1.2.4 RIL frequency and chloride in experiment 7.2 (Cl2)

The frequency distribution of 170 recombinant inbred lines (RILs) with two parental rice varieties is illustrated in Figure 5.6. The ranges of these varieties for $Cl^-$ 2 concentrations are indicated with line. Cl2 stands leaf Cl2 at the second harvest. The mode having 60 RILs was recorded for Cl2 between 401 and 500 mol $m^{-3}$. The highest Cl2 was recorded in Moroberekan. The number of RILs was decreased in the bins with high $Cl^-$ and low $Cl^-$.

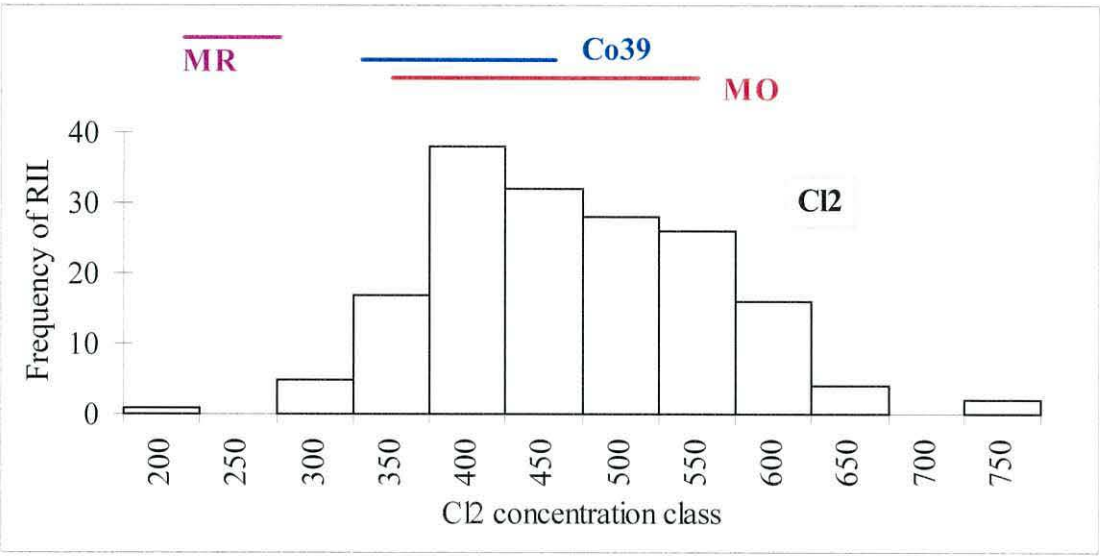


**Figure 5.6. Effect of 150 + 15 mol $m^{-3}$ NaCl and $CaCl_2$ on mol $m^{-3}$ Cl2 (43 das., 21 dos.) in RILs and parental varieties of rice (Experiment 7.2).**

However high $Cl^-$ (738, 744 and 770 mol $m^{-3}$) was observed only in 3 recombinant inbred lines (RILs 76, 54 and 140 respectively). The RILs with Cl2 concentrations below the level of 313 mol $m^{-3}$ (observed in Co39) and above the level of 536 mol $m^{-3}$ (observed in Moroberekan) were within the range of transgressive values for Cl2. The distribution of Cl2 within 170 recombinant inbred lines was normal as the probability of the Cl2 data not being normally distributed was 0.067 from the Anderson-Darling Normality test

5.3.1.2.5 RIL frequency and shoot fresh weight (SFW)

The frequency distribution of 170 recombinant inbred lines (RILs) with the three parental rice varieties is shown in Figure 5.7.

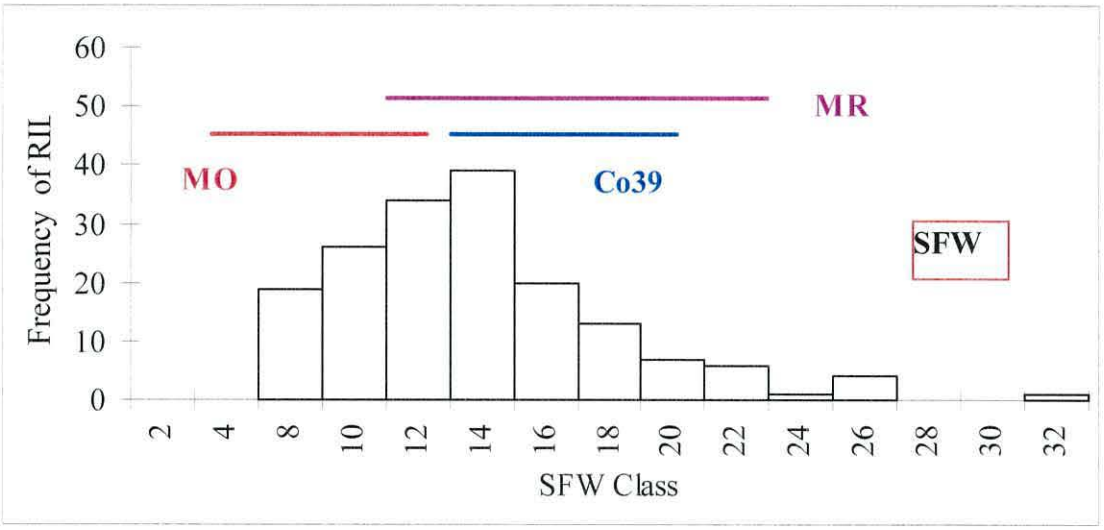


**Figure 5.7. Effect of 150 + 15 mol $m^{-3}$ NaCl and $CaCl_2$ on SFW (g) (92 das., 67 dos.) in RILs and parental varieties of rice (Experiment 7.2).**

The mode having 39 RILs was recorded for SFW values with 12.1 g to 14 g. In the parental varieties, the highest value (22 g) for SFW was recorded in Maratelli and the lowest value for SFW (2 g) was recorded in Co39. The RILs having the values for SFW below 2 and above 22 were in the range of transgressive values. The number of RILs per bin decreased at the high SFW end. The distribution was not normal for shoot fresh weight because the probability of the SFW data being normally distributed was 0.000 from the Anderson-Darling Normality test.

5.3.1.2.6 RIL frequency and shoot dry weight (SDW)

The frequency distribution of 170 recombinant inbred lines (RILs) for SDW with the three parental rice varieties are illustrated in figure 5.8. These varieties are indicated with lines.

The mode having, 59 RILs was recorded for SDW between 6.1 g and 7.0 g. In the parents, the highest value (7.98 g) for SDW was recorded in Maratelli and the lowest value (1.56 g) was noticed in Moroberekan. The number of RILs decreased in the bins with high SDW and with low SDW. However the decrease in the number of RILs was more pronounced with high SDW values. The RILs with SDW values

above 7.98 g fell within the range of transgressive values for SDW. No RIL fell in the transgressive range at the lower SDW end. The distribution was not normal for shoot dry weight because the probability of the SDW data being normally distributed was 0.000 from the Anderson-Darling Normality test.

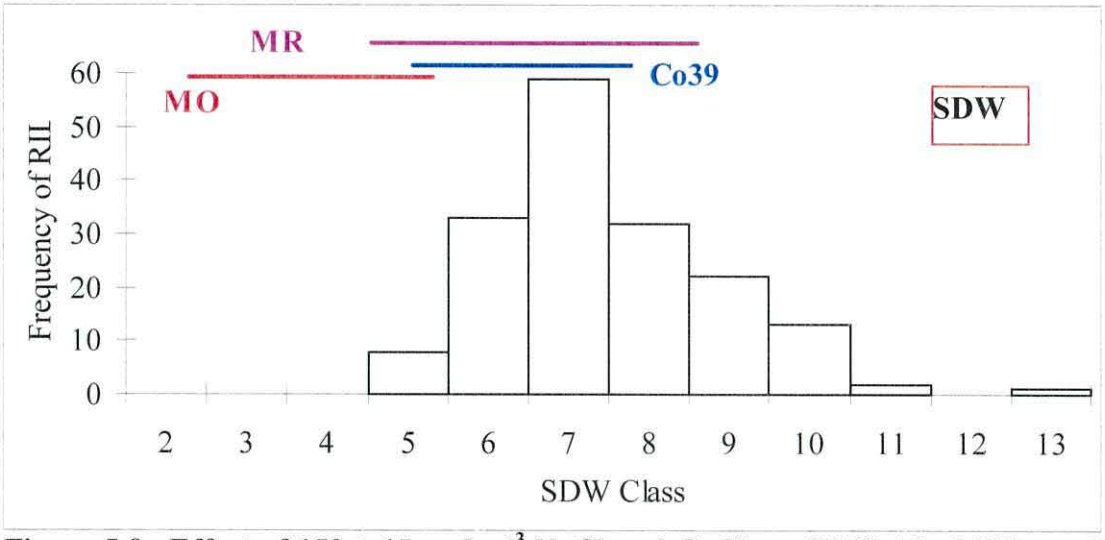


**Figure 5.8. Effect of 150 + 15 mol $m^{-3}$ NaCl and $CaCl_2$ on SDW (g) of RILs and parental varieties of rice (Experiment 7.2).**

### 5.3.1.3 Experiment 7.3

Time to anthesis in days was studied in 193 recombinant inbred lines of rice with three parental rice varieties (Co39, Moroberekan and Maratelli), with no salt.

#### 5.3.1.3.1 RILs frequency and days to anthesis

The frequency distribution of 193 recombinant inbred lines (RILs) with the three parental rice varieties for time to anthesis are shown in Figure 5.9 without added salinity. The parental varieties are indicated with arrows in this figure.

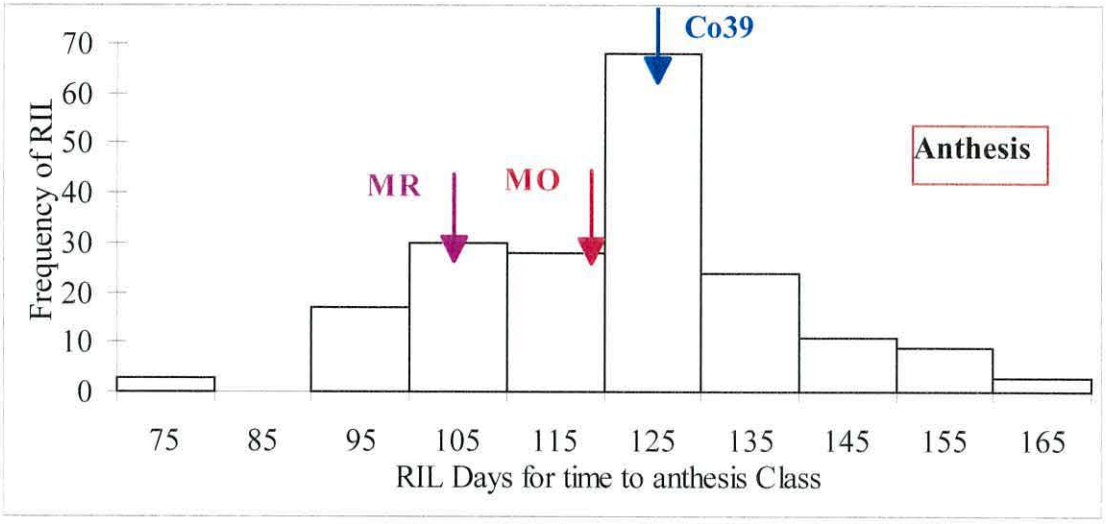


**Figure 5.9. Frequency distribution of 193 recombinant inbred lines (RILs) with the three parental rice varieties for time to anthesis (days) without salinity (Experiment 7.3).**

The mode having 68 RILs was recorded for anthesis time between 116 and 125 days. No RIL fell in the transgressive range. Early anthesis (96 days) was observed in Maratelli and late anthesis (119 days) was recorded in Co39. However in Moroberekan intermediate anthesis (113 days) was noticed. The RILs with anthesis times longer than 125 days and less than 96 days were in the range of transgressive values for anthesis. Number of RILs were decreased at the extreme ends few days and many days to anthesis. The distribution was not normal for days to anthesis because the probability of the days to anthesis data being normally distributed was 0.000 from the Anderson-Darling Normality test.

### 5.3.1.4 Regression analyses (Experiment 7.1 and 7.2)

5.3.1.4.1 Na1, Cl1 from experiment 7.1 versus resistance score 1 in experiment 7.2

A scatter plot of $Na^+$ and $Cl^-$ concentrations versus resistance score 1 is presented in fig. 5.10. Values for resistance score 1 were negatively correlated with concentrations of $Na^+$ and $Cl^-$. The $r^2$ value is the measure of the degree of closeness of the data to the trend line. If all the data points are on the trend line then the $r^2$ value is 1, and if the data is close to the trend line then the $r^2$ value is close to, but

smaller than, 1. The $r^2$ value for $Na^+$ and resistant score 1 was 0.0224 (P≤0.038), while the value for $r^2$ in the case of $Cl^-$ and resistance score1 was 0.1522 (P≤0.000). The concentration of $Cl^-$ was highest (400 mol $m^{-3}$) in the case of RIL 128 while $Na^+$ concentration was highest (45 mol $m^{-3}$) in RIL 64 The concentration of $Cl^-$ was lowest (58 mol $m^{-3}$) in RIL 88 while $Na^+$ concentration was the lowest (0.420 mol $m^{-3}$) in RIL 79. The highest resistance score 4.7 was given by RIL 37. Intermediate value for $Cl^-$ (200 mol $m^{-3}$) was in RIL 105 including the parental varieties with about 200 mol $m^{-3}$ of $Cl^-$ as the actual values were 219, 191 and 180 in Co39, Moroberekan and Maratelli respectively for $Cl^-$. Intermediate values for $Na^+$ were 25 and 24 mol $m^{-3}$ in RILs 16 and 109 respectively.

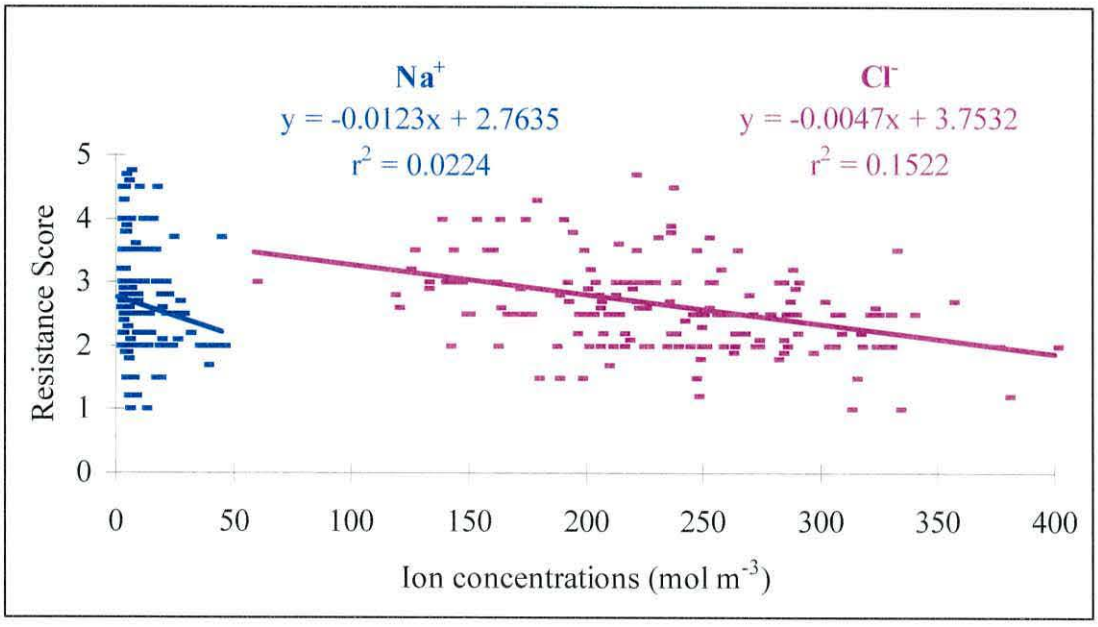


**Fig 5.10. Regression analysis of Na1 and Cl1, at 100 mol $m^{-3}$ NaCl + 10 mol $m^{-3}$ $CaCl_2$ (Experiment 7.1) versus resistance score 1 in rice (Experiment 7.2).**

5.3.1.4.2 Na1 and Cl1 versus shoot fresh weight (SFW) in Experiment 7.1.

The effect of salinity on shoot fresh weight under 100 mol $m^{-3}$ NaCl + 10 mol $m^{-3}$ $CaCl_2$ concentrations in 170 rice inbred lines is shown in Fig. 5.11 A trend was observed in shoot fresh weight reduction, similar to that already observed in the case of resistance score 1. The $r^2$ value for $Na^+$ and fresh weight was 0.0411 while the value for $r^2$ in the case of $Cl^-$ and shoot fresh weight was 0.0197. The regression for SFW and $Na^+$ 1 was significant (P=0.003) but for SFW and $Cl^-$ 1 it was not (P= 0.194). The highest SFW (30.8 g) was observed in RIL 98 while the lowest SFW (4.59 g) was recorded in RIL 30.

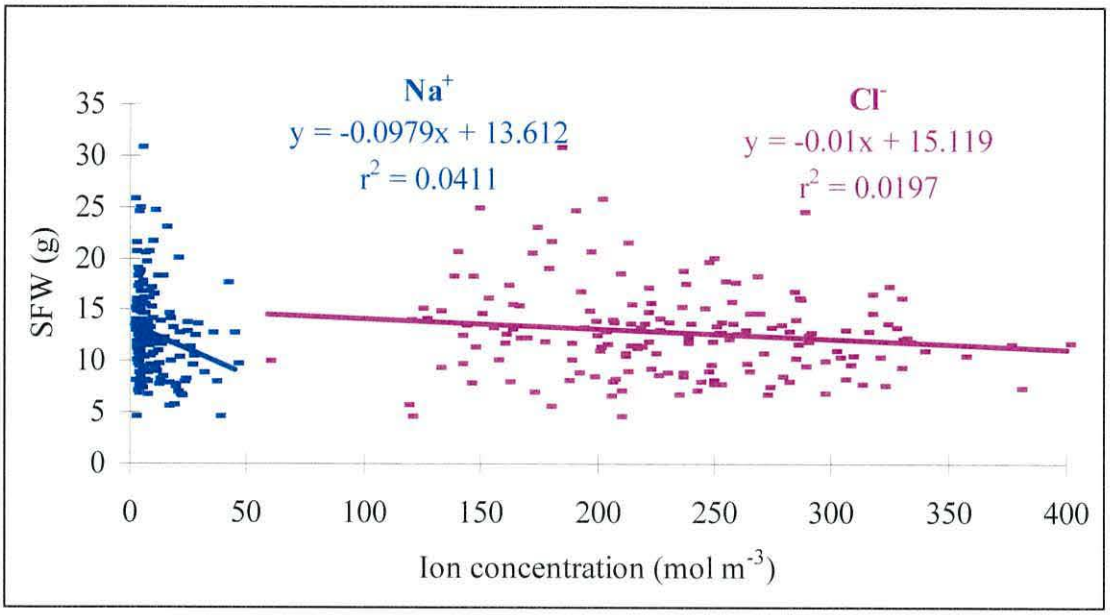


**Fig 5.11. Regression analysis of Cl1 and Na1 at 100 mol $m^{-3}$ NaCl + 10 mol $m^{-3}$ $CaCl_2$ (Experiment 7.1) versus SFW (Experiment 7.2) in rice.**

5.3.1.4.3 Na2 and $Cl2^-$ versus resistance score 2 (Experiment 7.2).

Na2 and Cl2 (49 days after sowing and 21 days of salinity) at the time of the second harvest versus resistance score 2 (78 days after sowing and 49 days of salinity) is presented in Fig. 5.12 (scattered) with regression lines.

Resistance score 2 was negatively correlated with concentration of $Na^+$ and $Cl^-$. The $r^2$ value for Na2 and resistant score 2 was 0.1308 while the value for $r^2$ in the case of Cl2 and resistance score 2 was 0.2652. Both regressions were highly significant (P= 0.000). The concentration of $Cl^-$ was very high compared with $Na^+$ and the highest concentration of $Cl^-$ (770 mol $m^{-3}$) was found in RIL 140 while the highest $Na^+$ concentration (116 mol $m^{-3}$) was observed in RIL 89. The concentration of $Cl^-$ was the lowest (122 mol $m^{-3}$) in the case of RIL 88 while the lowest concentration of $Na^+$ (1 mol $m^{-3}$) was observed in case of RIL 10. Intermediate concentrations of $Cl^-$ were found in RIL 58 (385 mol $m^{-3}$) and the two parental varieties (388 and 400 mol $m^{-3}$ in Co39 and Moroberekan respectively). Intermediate concentrations of $Na^+$ (59 mol $m^{-3}$ and 57 mol $m^{-3}$) were observed in RIL 22 and RIL 68 respectively.

So low $Na^+$ concentrations were always observed compared with $Cl^-$. Even the highest concentration of $Na^+$ (116 mol $m^{-3}$) in RIL 89 was low compared with the

lowest concentration of $Cl^-$ (122 mol $m^{-3}$) in RIL 88. For $Na^+$ the highest value of 116 mol $m^{-3}$ was recorded in RIL 89. The highest resistance scores were given by RILs 98, 62 and 103.

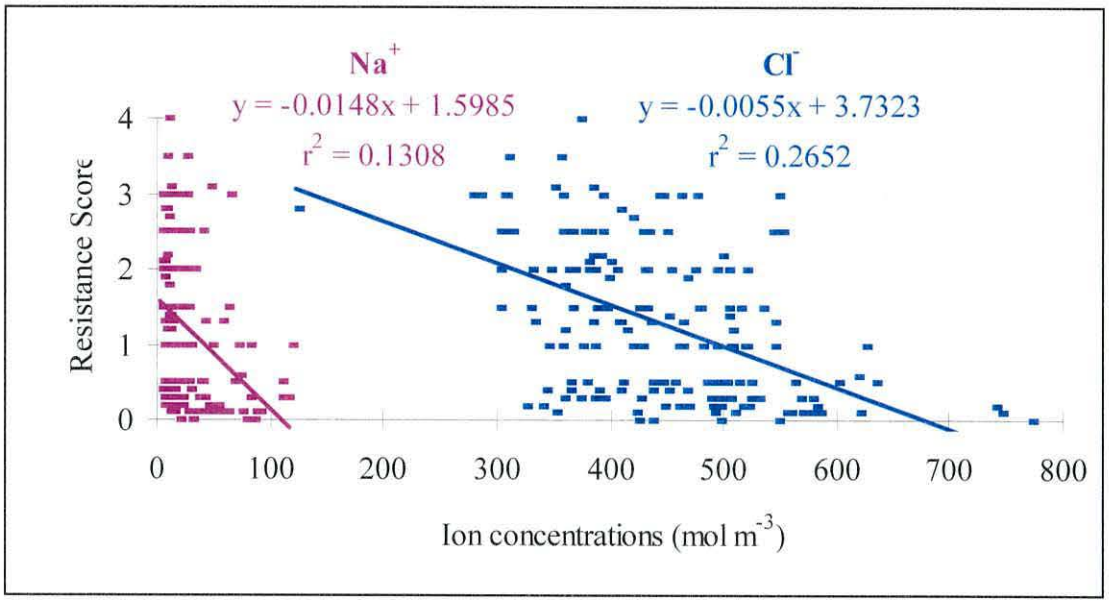


**Fig. 5.12. Regression analysis of Cl2 and Na2 and resistance score 2 at 150 mol $m^{-3}$ NaCl + 15 mol $m^{-3}$ $CaCl_2$ in rice (Experiment 7.2).**

5.3.1.4.4 Na2, Cl2 versus shoot fresh weight (SFW) (Experiment 7.2)

The effect of salinity on shoot fresh weight under 150 mol $m^{-3}$ NaCl + 15 mol $m^{-3}$ $CaCl_2$ concentrations in 170 rice inbred lines is shown in fig. 5.13. A similar trend was observed in shoot fresh weight reduction, as in resistance score2.

The $r^2$ value for $Na^+$ and fresh weight was 0.1587 while the value for $r^2$ in the case of $Cl^-$ and shoot fresh weight was 0.1299. Both regressions were highly significant (P= 0.000). The highest SFW (30.8 g) was observed in RIL 98 while the lowest SFW (4.59) was recorded in RIL 30.

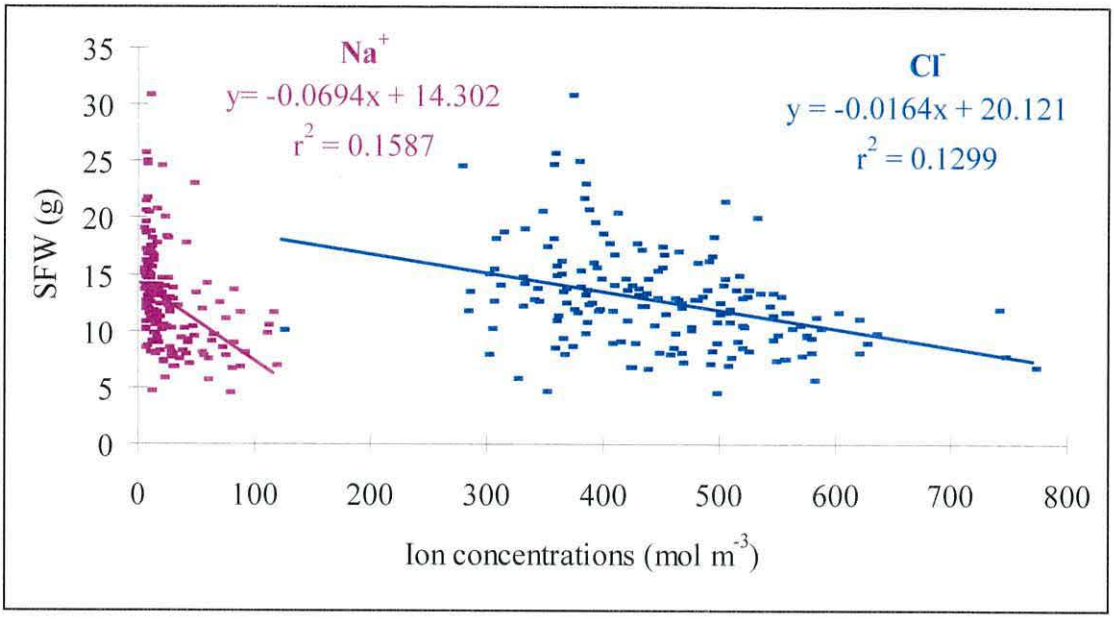


**Fig 5.13. Regression analysis of Cl2 and Na2 versus SFW at 150 mol $m^{-3}$ NaCl + 15 mol $m^{-3}$ $CaCl_2$ in rice (Experiment 7.2).**

### 5.3.1.5 Correlations of different parameters

Correlations of various parameters for experiments 7.1 and 7.2 are shown in Table 5.2. Correlations for experiment 10 are shown in Table 5.3. Comparisons of correlations between the two experiments are presented in Table 5.4. All the correlations were analysed by SPSS programme, version 9.0. Levels of significance were denoted with ** and * when significance levels were 0.01 and 0.05 respectively.

#### 5.3.1.5.1 Experiments 7.1 and 7.2

Correlations between physiological and growth parameters at various harvests (Experiments 7.1 and 7.2) are shown in Table 5.2. For Cl1, Cl2, K1, K2 and Na1 there were significant negative correlations with score 1, score 2, SFW, SDW and water (water content per g dry weight). For Na2 there were significant negative correlations with score 1, score 2, SFW, SDW and water. Both score 1 and score 2 were significantly but negatively correlated with sum1, (Na1+K1) and sum2, (Na2+K2). SFW was significantly negatively correlated with sum2, (Na2 + K2). A negative but significant correlation was observed between sum2 and water (Table 5.2).

5.3.1.5.2 Experiment 10

Correlation between the means of physiological and growth parameters for experiment 10 are shown in table 5.3. Correlations were determined in SPSS programme, version 9.0. The general trends for correlation as shown in table 5.3 were similar to experiment 7. For the means of Na and Mg there were significant negative correlations with score 1, score 2, SFW, SDW and water. However significant positive correlation was observed for water (water content per g dry weight) with score2, SFW, SDW (Table 5.3).

5.3.1.5.3 Correlations between experiments 7 and 10.

Correlation were also determined by SPSS programme, version 9.0 for experiment 7 and 10, and are presented in table 5.4. Significant positive correlation was observed for the means of Na and Mg from experiment 10 with Na1, Na2, Cl1 and Cl 2 from experiment 7. Na1, Na2, Cl1, from experiment 7.1 and 7.2 are significantly negatively correlated with means of ratio2 and score2 from experiment 10. However Na1, and Na2, from experiment 7.1 and 7.2 are only negatively correlated with means of SFW, SDW and water (water content per g dry weight) from experiment 10. Correlation for Cl1 from experiment 7 was significantly negatively correlated with the means of ratio, score 2, SFW and water from experiment 10. Score1, score2 and SFW from experiment 7 were significantly but negatively correlated with the means of Na from experiment 10. However significant negative correlation for water was observed with Na, K, sum, Mg and score 1 (Table 5.4).

**Table 5.2 Pearson's correlation coefficients between various parameters in Experiment 7**

| | Cl2 | K1 | K2 | Na1 | Na2 | Score1 | Score2 | SDW | SFW | sum1 | sum2 | water |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cl1** | 0.539(**) | 0.310(**) | 0.433(**) | 0.120 | 0.175(*) | -0.368(**) | -0.303(**) | 0.214(**) | -0.092 | 0.336(**) | 0.444(**) | -0.259(**) |
| **Cl2** | 1.000 | 0.403(**) | 0.773(**) | 0.160(*) | 0.491(**) | -0.547(**) | -0.563(**) | 0.047 | -0.356(**) | 0.437(**) | 0.836(**) | -0.473(**) |
| **K1** | | 1.000 | 0.533(**) | -0.175(*) | -0.056 | -0.235(**) | -0.215(**) | 0.239(**) | 0.058 | 0.985(**) | 0.475(**) | -0.090 |
| **K2** | | | 1.000 | -0.086 | 0.184(**) | -0.435(**) | -0.378(**) | 0.130 | -0.168(*) | 0.526(**) | 0.969(**) | -0.278(**) |
| **Na1** | | | | 1.000 | 0.507(**) | -0.153(*) | -0.184(**) | -0.036 | -0.206(**) | -0.002 | 0.052 | -0.229(**) |
| **Na2** | | | | | 1.000 | -0.352(**) | -0.341(**) | -0.198(**) | -0.437(**) | 0.032 | 0.422(**) | -0.387(**) |
| **Score1** | | | | | | 1.000 | 0.599(**) | 0.268(**) | 0.458(**) | -0.265(**) | -0.490(**) | 0.343(**) |
| **Score2** | | | | | | | 1.000 | 0.262(**) | 0.532(**) | -0.251(**) | -0.434(**) | 0.435(**) |
| **SDW** | | | | | | | | 1.000 | 0.691(**) | 0.236(**) | 0.070 | 0.022 |
| **SFW** | | | | | | | | | 1.000 | 0.022 | -0.265(**) | 0.696(**) |
| **sum1** | | | | | | | | | | 1.000 | 0.491(**) | -0.132 |
| **sum2** | | | | | | | | | | | 1.000 | -0.354(**) |
| **water** | | | | | | | | | | | | 1.000 |

**Note:-** 1 :- 100 mol $m^{-3}$ NaCl +10 mol $m^{-3}$ $CaCl_2$ salinity, 2:- 150 mol $m^{-3}$ NaCl +15 mol $m^{-3}$ $CaCl_2$ salinity. ** = Correlation is significant at the 0.01 level (2-tailed), * = Correlation is significant at the 0.05 level (2-tailed).

**Table 5.3-Pearson's correlation coefficients between various parameters in experiment 10.**

| | Na | K | Sum | Ratio | Mg | Score1 | Score2 | SFW | SDW | Water |
|---|---|---|---|---|---|---|---|---|---|---|
| **Na** | 1.000 | 0.300(**) | 0.697(**) | -0.538(**) | 0.704(**) | -0.285* | -0.473** | -0.306(**) | -232(*) | -0.594(**) |
| **K** | | 1.000 | 0.893(**) | -0.064 | 0.347(**) | 0.043 | -0.153 | 0.030 | 0.093 | -0.331(**) |
| **Sum** | | | 1.000 | -0.302 | 0.592(**) | -0.102 | -0.338(**) | -0.122 | -0.040 | -0.529(**) |
| **Ratio** | | | | 1.000 | -0.485(**) | 0.349(**) | 0.521(**) | 0.394(**) | 0.348(**) | 0.428(**) |
| **Mg** | | | | | 1.000 | -0.277(*) | -0.495(**) | -0.331(**) | -0.261(*) | -0.597(**) |
| **Score1** | | | | | | 1.000 | 0.755(**) | 0.834(**) | 0.837(**) | -0.467(**) |
| **Score2** | | | | | | | 1.000 | 0.892(**) | 0.843(**) | 0.586(**) |
| **SFW** | | | | | | | | 1.000 | 0.985(**) | 0.520(**) |
| **SDW** | | | | | | | | | 1.000 | 0.405(**) |
| **Water** | | | | | | | | | | 1.000 |

**Note:-** 1 :- 100 mol $m^{-3}$ NaCl +10 mol $m^{-3}$ $CaCl_2$ salinity, 2:- 150 mol $m^{-3}$ NaCl +15 mol $m^{-3}$ $CaCl_2$ salinity. ** = Correlation is significant at the 0.01 level (2-tailed), * = Correlation is significant at the 0.05 level (2-tailed).

**Table 5.4-Pearson's correlation coefficients between various parameters after comparison of experiment 7 and 10.**

| | 10Na | 10K | 10sum | 10ratio | 10Mg | 10sco1 | 10sco2 | 10SFW | 10SDW | 10 water |
|---|---|---|---|---|---|---|---|---|---|---|
| **7Na1** | 0.557(**) | -0.113 | 0.178 | -0.463(**) | 0.429(**) | -0.191 | -0.263(*) | -0.196 | -0.171 | -0.199 |
| **7Na2** | 0.518(**) | 0.011 | 0.253(*) | -0.423(**) | 0.355(**) | -0.140 | -0.298(**) | -0.099 | -0.052 | -0.212 |
| **7K1** | 0.017 | 0.374(**) | 0.290(*) | 0.038 | -0.005 | 0.003 | 0.057 | 0.112 | 0.121 | -0.031 |
| **7K2** | 0.0150 | 0.185 | 0.209 | -0.032 | 0.120 | 0.080 | -0.021 | 0.095 | 0.127 | -0.115 |
| **7Sum1** | 0.137 | 0.354(**) | 0.332(**) | -0.061 | 0.088 | -0.034 | 0.005 | 0.077 | 0.092 | -0.073 |
| **7Sum2** | 0.279(*) | 0.161 | 0.252(*) | -0.150 | 0.206 | 0.028 | -0.105 | 0.053 | 0.093 | -0.160 |
| **7Cl1** | 0.287(*) | 0.149 | 0.247(*) | -0.409(**) | 0.380(**) | -0.175 | -0.328(**) | -0.226(*) | -0.176 | -0.402(**) |
| **7Cl2** | 0.348(**) | 0.152 | 0.279(*) | -0.349(**) | 0.353(**) | 0.027 | -0.200 | -0.026 | 0.019 | -0.201 |
| **7Score1** | -0.252(*) | -0.028 | -0.140 | 0.126 | -0.216 | 0.052 | 0.267(*) | 0.087 | 0.069 | 0.044 |
| **7Score2** | -0.258(*) | -0.033 | -0.096 | 0.185 | -0.337(**) | 0.104 | 0.176 | 0.071 | 0.039 | 0.151 |
| **7SFW** | -0.343(**) | 0.129 | 0.042 | 0.062 | -0.088 | -0.006 | -0.007 | -0.068 | -0.054 | -0.120 |
| **7SDW** | 0.144 | 0.292(**) | 0.288(*) | -0.154 | 0.130 | 0.000 | -0.122 | -0.063 | -0.023 | -0.250 |
| **7water** | -0.210 | 0.056 | -0.057 | 0.152 | -0.164 | 0.013 | 0.048 | -0.041 | -0.039 | -0.047 |

**Note:-** 1 :- 100 mol $m^{-3}$ NaCl +10 mol $m^{-3}$ $CaCl_2$ salinity, 2:- 150 mol $m^{-3}$ NaCl +15 mol $m^{-3}$ $CaCl_2$ salinity**.** ** = Correlation is significant at the 0.01 level (2-tailed), * = Correlation is significant at the 0.05 level (2-tailed), 7= Experiment 7, 10=Experiment 10.

Mean values of several parameters for the parents with their standard errors are given in Table 5.5. Concentrations of $Na^+$, $K^+$, $Cl^-$ and sum of $K^+$ and $Na^+$ were increased with the increase of salt concentrations (From 100 mol $m^{-3}$ NaCl + 10 mol $m^{-3}$ $CaCl_2$ to 150 mol $m^{-3}$ NaCl + 15 mol $m^{-3}$ $CaCl_2$) applied to the plants and time of salt application (see chapter 4) in experiment 7.1 and 7.2 as shown in Table 5.5. One way analyses showed that comparison between the varieties Co39 and Moroberekan were significantly different for $Na^+$ accumulation at low salt (Experiment 7.1) (P=0.001) and high salt (Experiment 7.2) (P=0.000) concentrations but the $Na^+$ μ mol $g^{-1}$ SDW and water g $g^{-1}$ dw were not significantly different in experiment 7.2. From the growth data the varieties Co39 and Moroberekan were significantly different for resistance scores (P=0.004 for both resistance score1 and 2), shoot fresh weight (P=0.000), shoot dry weight (P=0.000), K1+Na1 (P=0.000), K1 (P=0.000), water content (P=0.000), $Na^+$/SDW (P=0.000) and water content g $g^{-1}$ SDW (P=0.008). In experiment 10, $Na^+$ concentrations were higher than in experiment 7.2 (Table 5.5) although the salinity level (150 mol $m^{-3}$ NaCl) was the same. However the concentrations of $K^+$ and sum of $Na^+$ and $K^+$ were lower than in experiment 7.2 (Table 5.5). The concentrations of $Mg^{2+}$ and $Ca^{2+}$ were also measured in this experiment (Table 5.5) but they were not measured in experiment 7. All measured growth parameters in experiment 10 were lower than in experiment 7.2 (Table 5.5). Varietal comparison showed that resistance scores, SFW, SDW of Co39 were lower than Moroberekan in experiment 10. The obvious reason for low growth data in case of experiment 10 is the higher accumulation of $Na^+$ than in experiment 7.2 (Table 5.5). Varietal comparison (one way analyses) showed that Co39 and Moroberekan were significantly different for $Mg^{2+}$ (P=0.000) only but not significant for the rest of the physiological parameters {$Na^+$, $K^+$, sum ($Na^+$+$K^+$), ratio ($K^+$/$Na^+$)} in experiment 10 (Table 5.5). One way analyses showed that varieties Co39 and Moroberekan were significantly different for both the resistance scores, SFW and SDW (Table 5.5). As shoot fresh weight in experiment 10 is lower than in experiment 7 so high concentrations of $Na^+$ in experiment 10 could be the result of low plant vigour. Moroberekan accumulated higher $Na^+$ in experiments 7.1, 7.2 and 10 than Co39 regardless of the plant vigour in experiment 7.2 and 10, so Moroberekan is confirmed to be a high $Na^+$ accumulator.

**Table 5.5. Parental mean values ± s.e. for various physiological and growth parameters.**

| Parameter | Co39 | | | Moroberekan | | | Probability |
|---|---|---|---|---|---|---|---|
| **Experiment 7.1** | | | | | | | |
| **Na1** (mol $m^{-3}$) | 4 | ± | 0 | 13 | ± | 2 | 0.001 |
| **K1** (mol $m^{-3}$) | 267 | ± | 3 | 210 | ± | 5 | 0.000 |
| **Cl1** (mol $m^{-3}$) | 219 | ± | 13 | 191 | ± | 11 | 0.120 |
| **K1+Na1** (mol $m^{-3}$) | 272 | ± | 3 | 223 | ± | 5 | 0.000 |
| **Experiment 7.2** | | | | | | | |
| **Na2** (mol $m^{-3}$) | 6 | ± | 1 | 48 | ± | 8 | 0.000 |
| **K2** (mol $m^{-3}$) | 372 | ± | 11 | 338 | ± | 25 | 0.229 |
| **Cl2** (mol $m^{-3}$) | 388 | ± | 13 | 400 | ± | 21 | 0.618 |
| **K2+Na2** (mol $m^{-3}$) | 378 | ± | 11 | 386 | ± | 11 | 0.801 |
| **Score1** | 3.2 | ± | 0.1 | 2.3 | ± | 0.2 | 0.004 |
| **Score2** | 1.9 | ± | 0.2 | 0.7 | ± | 0.3 | 0.004 |
| **SFW** (g) | 15.9 | ± | 0.7 | 6.0 | ± | 0.7 | 0.000 |
| **SDW** (g) | 6.5 | ± | 0.3 | 3.6 | ± | 0.4 | 0.000 |
| **Water content** (g) | 9.4 | ± | 0.6 | 2.4 | ± | 0.7 | 0.000 |
| **Water** (g $g^{-1}$ DW) | 1.5 | ± | 0.1 | 0.9 | ± | 0.3 | 0.092 |
| **Na2** µmol $g^{-1}$ **SDW** | 9.24 | ± | 1.5 | 37.8 | ± | 15 | 0.080 |
| **Experiment 10** | | | | | | | |
| **Na** (mol $m^{-3}$) | 46 | ± | 12 | 63 | ± | 20 | 0.579 |
| **K+Na** (mol $m^{-3}$) | 216 | ± | 27 | 317 | ± | 54 | 0.237 |
| **K** (mol $m^{-3}$) | 170 | ± | 18 | 254 | ± | 35 | 0.140 |
| **Mg** (mol $m^{-3}$) | 109 | ± | 4 | 48 | ± | 7 | 0.000 |
| **Ca** (mol $m^{-3}$) | 21 | ± | 7 | 9 | ± | 3 | 0.090 |
| **Ratio** (K/Na) | 5.2 | ± | 1.9 | 6.2 | ± | 1.3 | 0.687 |
| **Score1** | 0.6 | ± | 0.1 | 1.9 | ± | 0.4 | 0.003 |
| **Score2** | 0.0 | ± | 0.0 | 0.5 | ± | 0.1 | 0.011 |
| **SFW** (g) | 0.6 | ± | 0.3 | 4.4 | ± | 1.3 | 0.009 |
| **SDW** (g) | 0.3 | ± | 0.1 | 1.3 | ± | 0.3 | 0.016 |

### 5.3.1.6 Number of QTL (Quantitative Trait Loci) in rice under salinity

The map with A and B alleles already constructed by Champoux *et al.* (1995) for 123 markers of rice on 12 chromosomes was used with the programs MAPMAKER and MAPMAKER QTL. The number of QTL (Quantitative Trait Loci) for various traits associated with salinity in 12 chromosomes of rice at two harvests is shown in Table 5.6. Log transformations of Na1, Na2, K1, sum1, ratio1, ratio2, score2, anthesis and water were used because data were not normally distributed for these traits.

Lengths of the bars in Figures 5.14 to 5.21 indicate the lengths of the chromosomes. The darkened and cross-hatched areas on the chromosomal bars are the presentation of the potential QTL for drought avoidance that were previously determined in the three and two field experiments respectively by Champoux *et al.* in 1995 (Figures 5.14-5.21). The coloured bars represent areas of potential QTL for salinity for various traits

LOD is the $\log_{10}$ of an odds ratio of maximum likelihood estimate (MLE) for the presence of a QTL and MLE without linked QTL. Actually MLE without linked QTL are assuming no QTL effects to avoid false positives. So the LOD score indicates how much more likely the data are to have arisen assuming the presence of a QTL than in its absence. The LOD threshhold value for avoiding a false positive with a given confidence, say 95 %, depends on the number of markers and the length of the genome. LOD > 5 was selected because there were several high peaks for $\log Na^+$ at both salinity levels (100 mol $m^{-3}$ NaCl + 10 mol $m^{-3}$ $CaCl_2$ and 150 mol $m^{-3}$ NaCl + 15 mol $m^{-3}$ $CaCl_2$). For $Cl^-$ at both salinity levels a LOD score greater than 2 was chosen because there was not any peak for selection at high level so the minimal step value 2 was selected. LOD score was greater than 4 for SFW because there were no peaks for selection at a higher level.

**Table 5.6 Number of QTL (Quantitative Trait Loci) in rice under salinity (Co39 x Moroberekan) $F_8$ (Experiment 7).**

| Traits | LOD | Chromosome | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| **logNa1** | >5 | 3 | 0 | 2 | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 0 |
| **logNa2** | >5 | 3 | 0 | 0 | 3 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| **Cl1** | >2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Cl2** | >2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **logK1** | >5 | 0 | 1 | 2 | 1 | 0 | 1 | 2 | 3 | 2 | 0 | 0 | 0 |
| **K2** | >2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| **logsum 1** | >5 | 0 | 0 | 1 | 2 | 0 | 1 | 2 | 1 | 2 | 0 | 0 | 0 |
| **Sum2** | >2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **logratio1** | >5 | 2 | 0 | 2 | 1 | 0 | 1 | 1 | 0 | 3 | 0 | 0 | 0 |
| **logratio2** | >5 | 2 | 1 | 0 | 1 | 0 | 1 | 2 | 1 | 0 | 0 | 1 | 0 |
| **Score1** | >2 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **log score2** | >2 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 2 | 0 | 0 | 0 |
| **loganthesis** | >2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **SFW** | >4 | 0 | 1 | 2 | 2 | 1 | 0 | 0 | 3 | 3 | 0 | 2 | 1 |
| **SDW** | >4 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| **logwater** | >5 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 2 | 0 | 1 | 0 |

**Note:-** Experiment 7.1 = Na 1, Cl 1, K 1, sum1, 14 days 100 mol $m^{-3}$ NaCl and experiment 7.2 = Na 2, Cl 2, K 2, sum 2, 21 days 150 mol $m^{-3}$ NaCl salinity, Anthesis = time to anthesis without added salinity, SFW = shoot fresh weight, SDW = shoot dry weight, Score = Physical health and vigour grade points, Water = shoot water content, Sum = Na + K

Table 5.6 shows that there are 10 QTL for LogNa1. Out of these, 3 QTL were on chromosome 1 (Figure 5.14, Experiment 7), 2 QTL each on chromosomes 3 (Figure 5.14, Experiment 7) and 9, while 1 QTL was found on chromosomes 4, 6 (Figure 5.15, Experiment 7) and 7 (Figure 5.16, Experiment 7). However no QTL were found on chromosomes 2, 5, 8, 10, 11 and 12. QTL were identified for logNa2. Out of them 6 QTL were found on chromosomes number 1 (Figure 5.14, Experiment 7) and 4 (Figure 5.15, Experiment 7), 3 QTL on each of them. 1 QTL was found on each of chromosome numbers 6 (Figure 5.15, Experiment 7), 7 and 8 (Figure 5.17, Experiment 7) and no QTL were found on chromosomes 2, 3, 5, 9, 10, 11 and 12.

No QTL was found for Cl1. However one weak QTL was found on chromosome 1 (Figure 5.14, Experiment 7) for Cl2. For logK1, 12 QTL were identified on chromosomes 2 to 9 (Figure 5.14, Experiment 7 to Figure 5.16, Experiment 7) excluding chromosome 5 (Figure 5.15, Experiment 7) with LOD scores greater than 5. Out of those 12, one QTL for logK1 was identified on each of chromosomes 2 (Figure 5.14, Experiment 7), 4 and 6 (Figure 5.15, Experiment 7) and two QTL for logK1 were found on each of the chromosomes 3 (Figure 5.14, Experiment 7), 7, and 9 (Figure 5.16, Experiment 7) and three QTL were identified on chromosome 8 (Figure 5.16, Experiment 7). However no QTL for logK1 were identified on chromosomes 1 (Figure 5.14, Experiment 7), 5 (Figure 5.15, Experiment 7) and 10 to 12 (Figure 5.17, Experiment 7). Only one weak QTL was identified on chromosome 10 (Figure 5.17, Experiment 7) for K2 with LOD > 2.

Nine QTL were identified for logsum1 with LOD score > 5 on different chromosomes. One QTL was identified on each of the chromosomes 3 (Figure 5.14, Experiment 7), 6 (Figure 5.15, Experiment 7) and 8 (Figure 5.16 Experiment 7). Two QTL for logsum1 were identified on each of the chromosomes 4 (Figure 5.15, Experiment 7) and 7 and 9 (Figure 5.16, Experiment 7). No QTL were identified for sum2 in 170 inbred lines of rice with LOD score > 2. For logratio1, 10 QTL with LOD score > 5 were identified. Nine QTL for logratio2 with LOD score > 5 were identified. Individual locations for these QTL on different chromosomes of rice are shown in Table 5.6.

For score1 only two QTL were identified, one on each of chromosomes 2 (Figure 5.14, Experiment 7) and 7 (Figure 5.16, Experiment 7) with LOD score > 2. Six QTL were identified for logscore2 with LOD score > 2. Out of these, four QTL

were found on chromosomes 4, 6 (Figure 5.15, Experiment 7), 7 and 8 (Figure 5.16, Experiment 7). Two QTL were present on chromosome 9 (Figure 5.16, Experiment 7) for logscore2. Fifteen QTL were identified for SFW with LOD score > 4 on chromosomes of rice. One QTL was identified on each of the chromosomes 1(Figure 5.14, Experiment 7), 5 (Figure 5.16, Experiment 7) and 12 (Figure 5.17, Experiment 7) for SFW. Three QTL were found on chromosomes 8 and 9 (Figure 5.16, Experiment 7), while 2 QTL were found on chromosomes 3 (Figure 5.14, Experiment 7), 4 (Figure 5.15, Experiment 7) and 11 (Figure 5.17, Experiment 7) for SFW. No QTL were found on chromosomes 2, 6, 7 and 10 for SFW.

For log water, 8 QTL were identified with LOD score > 5. Two QTL were identified on chromosomes 7 and 9 (Figure 5.16, Experiment 7) and only one QTL was identified in each of the chromosome 5, 6 (Figure 5.15, Experiment 7), 8 (Figure 5.16, Experiment 7) and 11(Figure 5.17, Experiment 7). For SDW only three QTL were identified with LOD score > 4. Out of these, two were found on chromosome 8 (Figure 5.16, Experiment 7) and one was found on chromosome 4 (Figure 5.15, Experiment 7). No QTL were identified in 193 recombinant inbred lines of rice (Co39 x Moroberekan) for time to anthesis. 7

### 5.3.2 Experiment 10

The results for experiment 10 were similar to the results of experiment 7 for score 1, score 2, SFW and $Na^+$. The general trends for correlation (determined by SPSS programme version 9.0) as shown in table 5.3 and regressions analyses for these parameters were also similar to experiment 7. Comparison of the correlation (determined by SPSS programme version 9.0) for experiment 7 and 10 were also presented in Table 5.4. Hence the $r^2$ values for regressions showed the same trend and the regression for $Na^+$ and score 1 (P = 0.002), $Na^+$ and score 2 (P = 0.000) and $Na^+$ and SFW (0.002) were highly significant.

In experiment 10 there was only one QTL for logNa with LOD score > 5. For K, six weak QTL with LOD score > 2 were identified on different chromosomes, two QTL on chromosomes 1 (Figure 5.18, Experiment 10) and 8 (Figure 5.20, Experiment 10) and one QTL on chromosomes 4 and 6 (Figure 5.19, Experiment 10). No QTL were identified for logsum1 with LOD score > 2. Five QTL for log ratio

with LOD score > 5 were identified. Two QTL for log ratio mean were on chromosomes 1 (Figure 5.18, Experiment 10) and 4 (Figure 5.19, Experiment 10) and only one QTL was detected on chromosome 11 (Figure 5.21, Experiment 10). For score1 only one weak QTL with LOD score > 2 was identified on chromosome 1(Figure 5.18, Experiment 10), while for logscore2 no QTL were identified.

Four QTL were identified for log SFW with LOD score > 2. One QTL was found on chromosomes 1, 3 (Figure 5.18, Experiment 10), 7 (Figure 5.20, Experiment 10) and 11 (Figure 5.21, Experiment 10). For water, two QTL were identified with LOD score > 2 one each on chromosomes 1 (Figure 5.18, Experiment 10) and 9 (Figure 5.21, Experiment 10). For log SDW mean only three QTL were identified with LOD score > 2, 1 QTL on each of the chromosomes 1 (Figure 5.18, Experiment 10), 4 (Figure 5.19, Experiment 10) and 11 (Figure 5.21, Experiment 10).

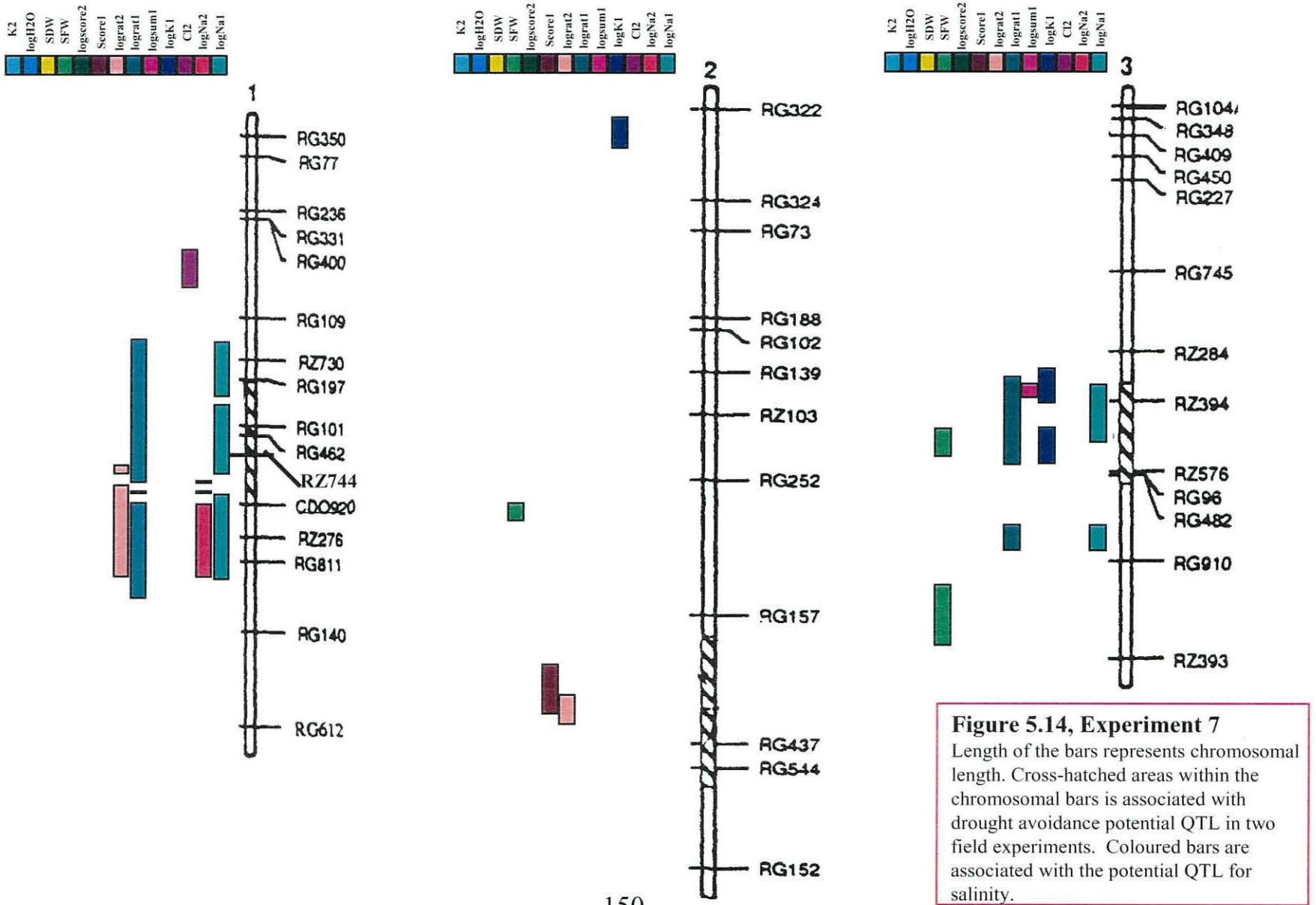


**Figure 5.14, Experiment 7**
Length of the bars represents chromosomal length. Cross-hatched areas within the chromosomal bars is associated with drought avoidance potential QTL in two field experiments. Coloured bars are associated with the potential QTL for salinity.

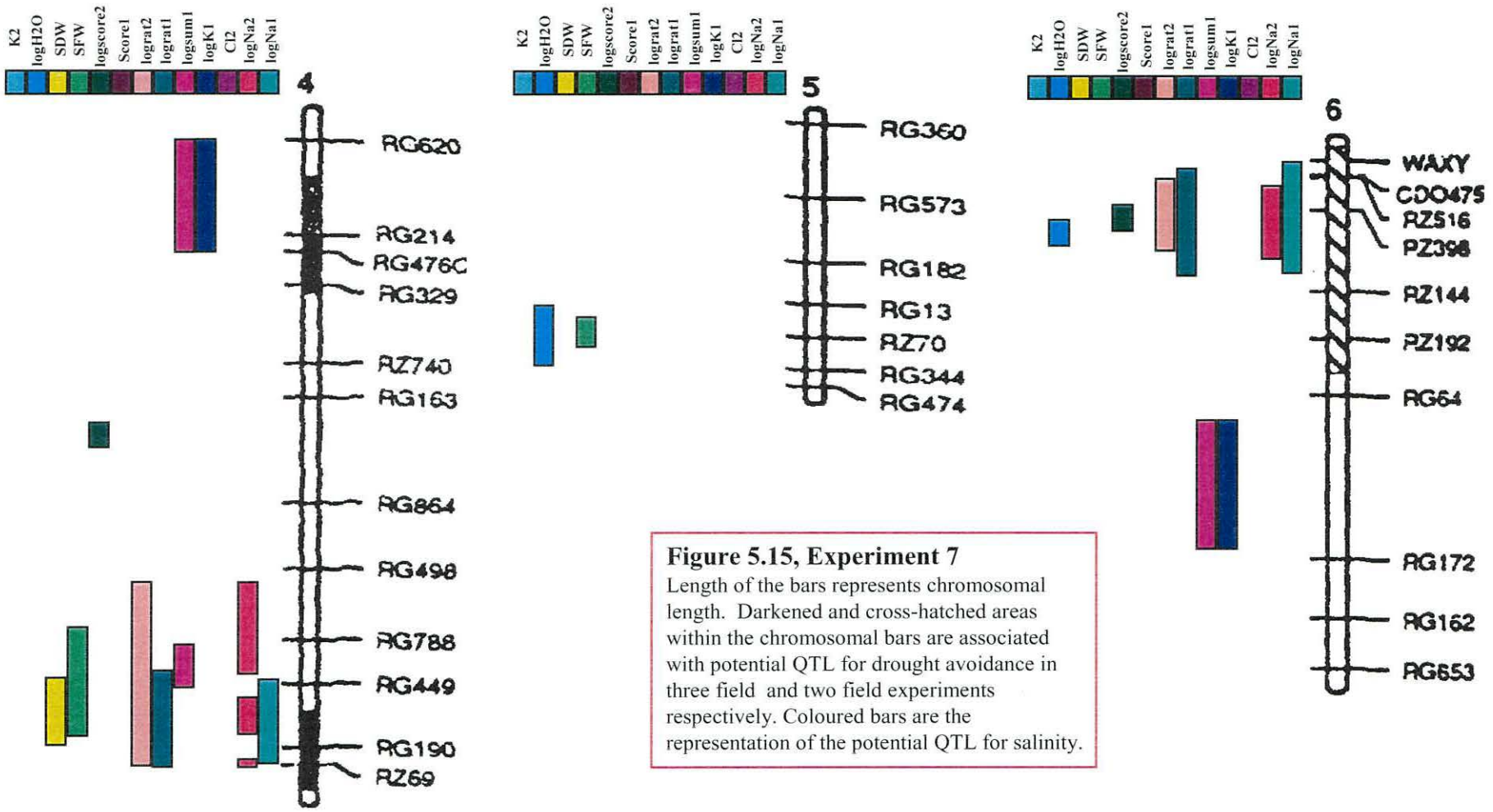


**Figure 5.15, Experiment 7**
Length of the bars represents chromosomal length. Darkened and cross-hatched areas within the chromosomal bars are associated with potential QTL for drought avoidance in three field and two field experiments respectively. Coloured bars are the representation of the potential QTL for salinity.

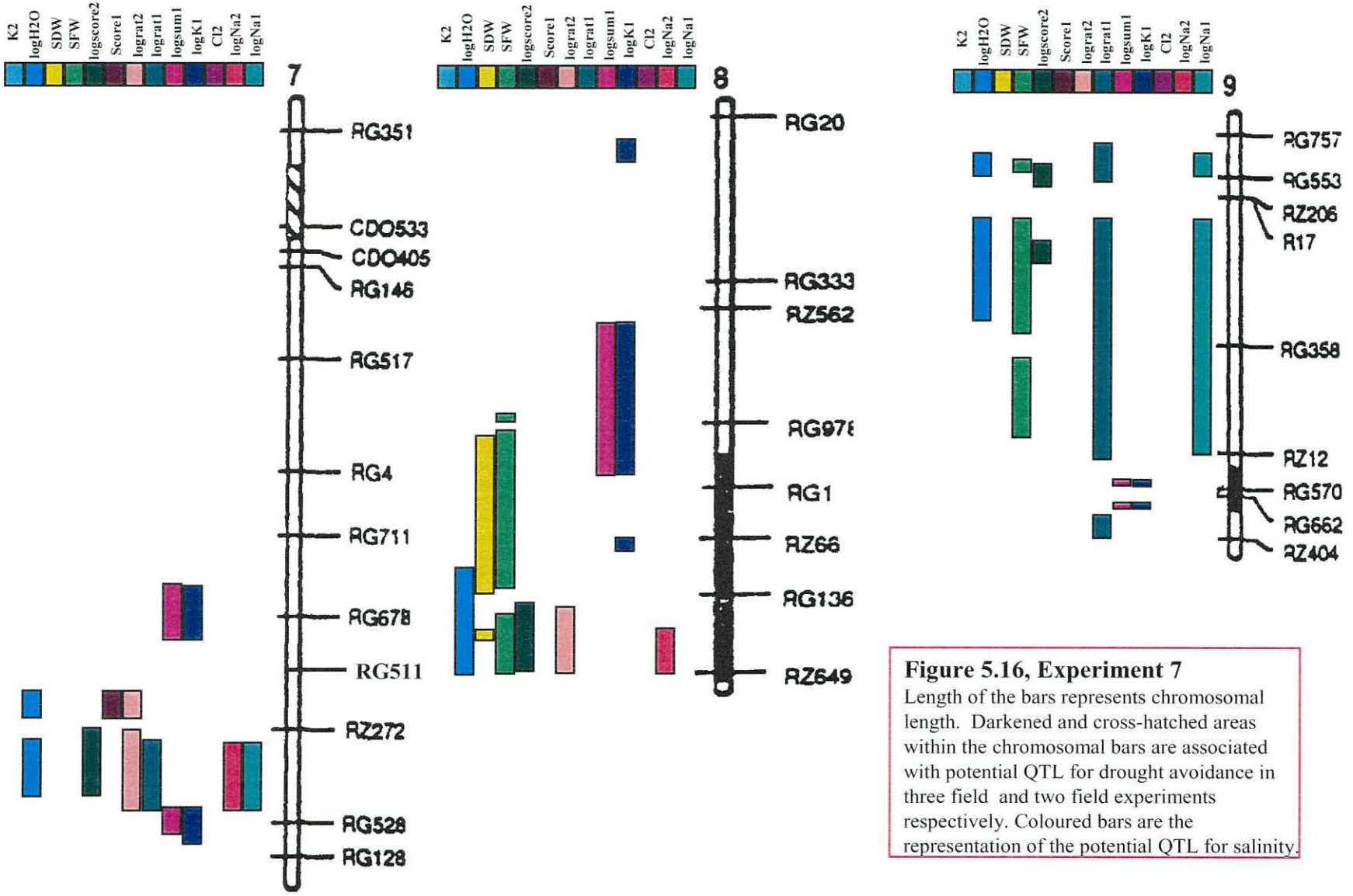


**Figure 5.16, Experiment 7**
Length of the bars represents chromosomal length. Darkened and cross-hatched areas within the chromosomal bars are associated with potential QTL for drought avoidance in three field and two field experiments respectively. Coloured bars are the representation of the potential QTL for salinity.

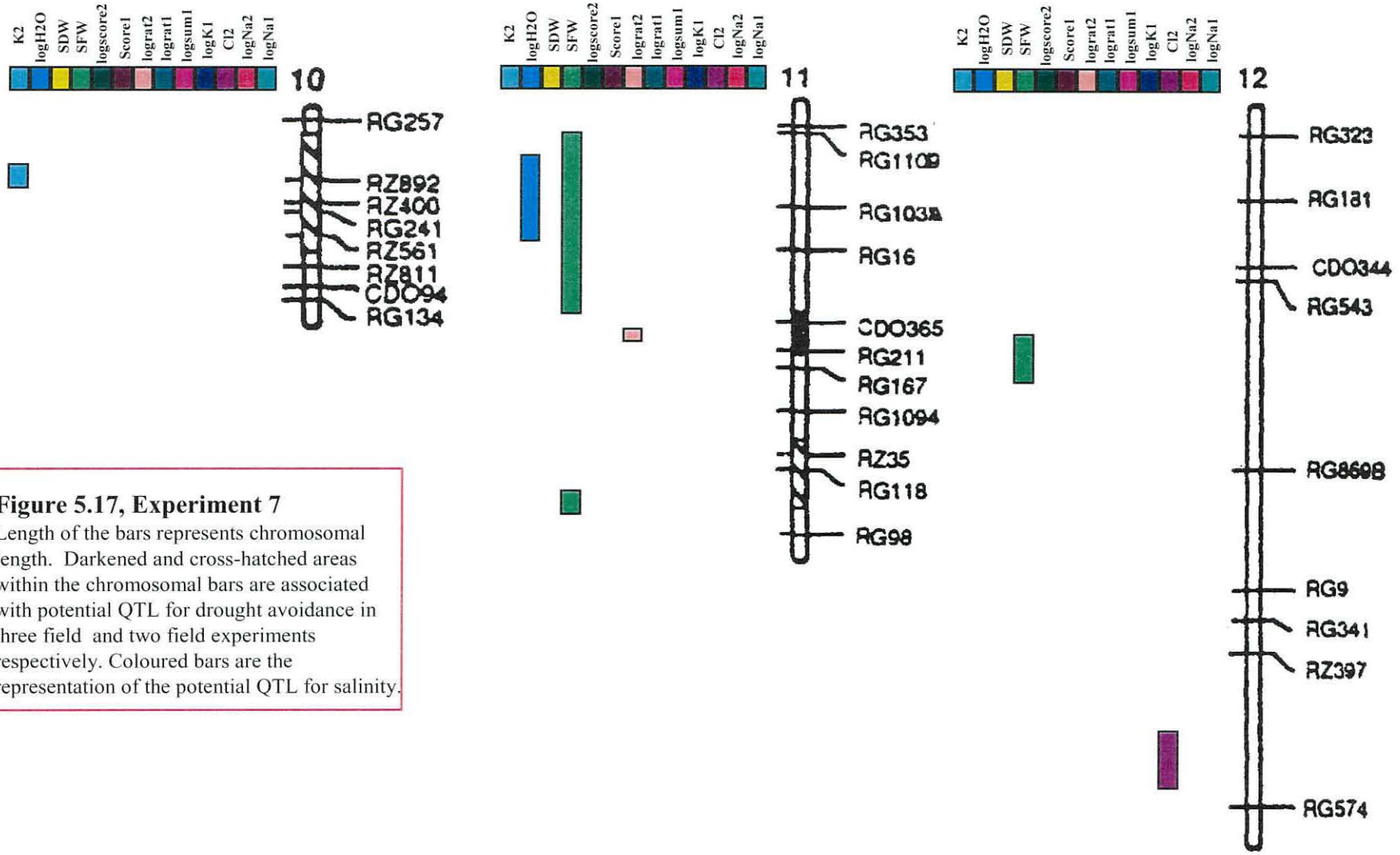


**Figure 5.17, Experiment 7**
Length of the bars represents chromosomal length. Darkened and cross-hatched areas within the chromosomal bars are associated with potential QTL for drought avoidance in three field and two field experiments respectively. Coloured bars are the representation of the potential QTL for salinity.

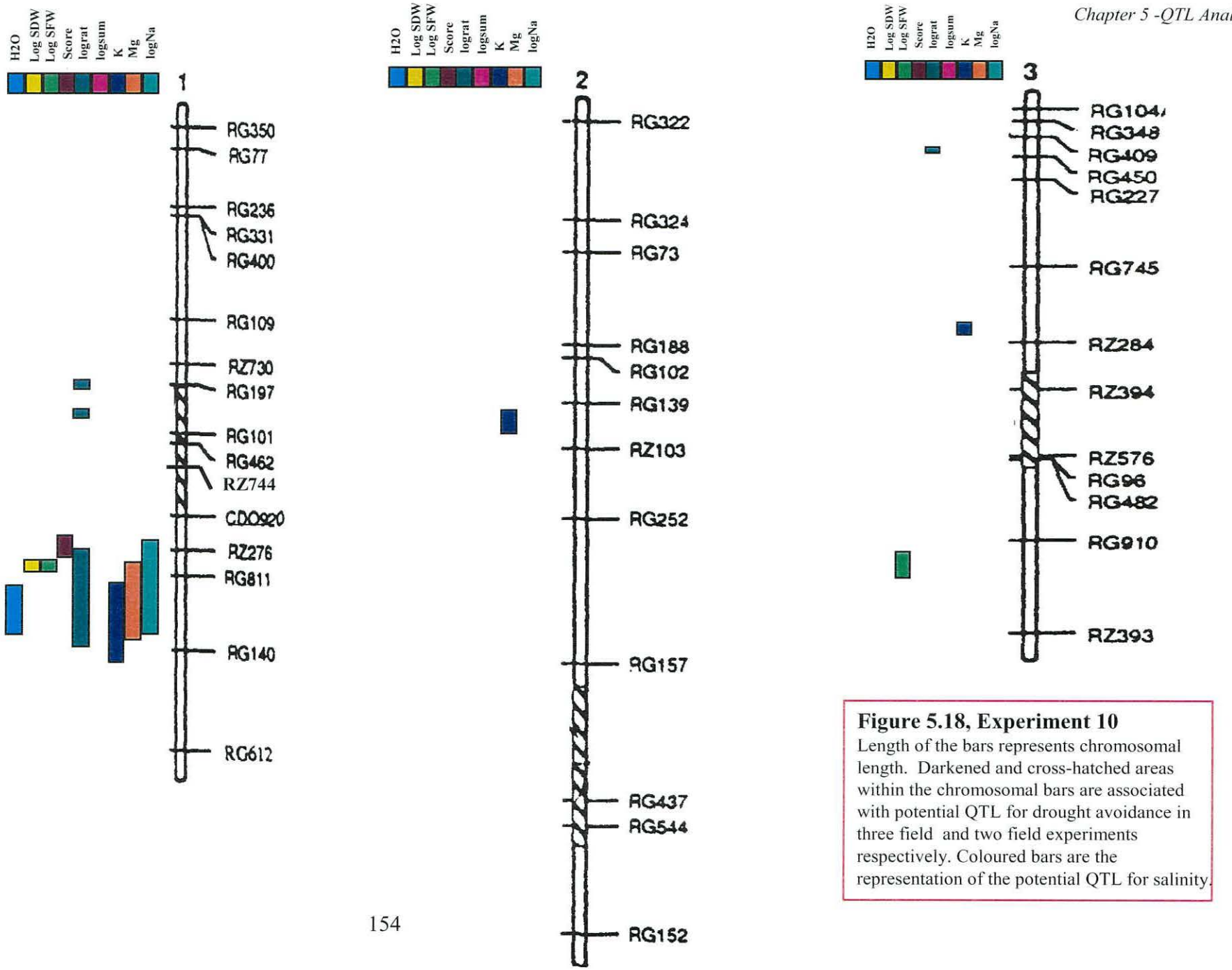


**Figure 5.18, Experiment 10**
Length of the bars represents chromosomal length. Darkened and cross-hatched areas within the chromosomal bars are associated with potential QTL for drought avoidance in three field and two field experiments respectively. Coloured bars are the representation of the potential QTL for salinity.

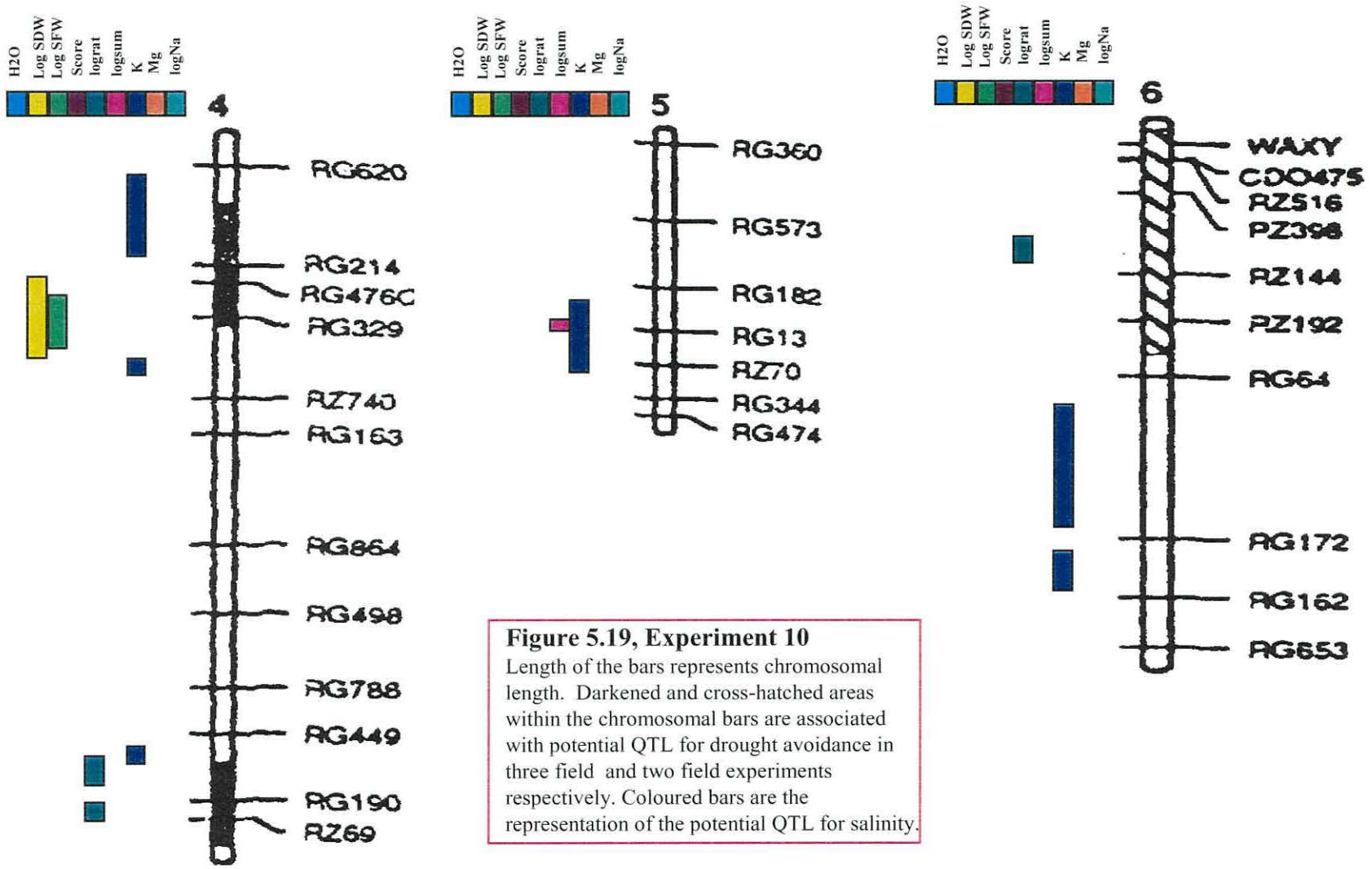


**Figure 5.19, Experiment 10**
Length of the bars represents chromosomal length. Darkened and cross-hatched areas within the chromosomal bars are associated with potential QTL for drought avoidance in three field and two field experiments respectively. Coloured bars are the representation of the potential QTL for salinity.

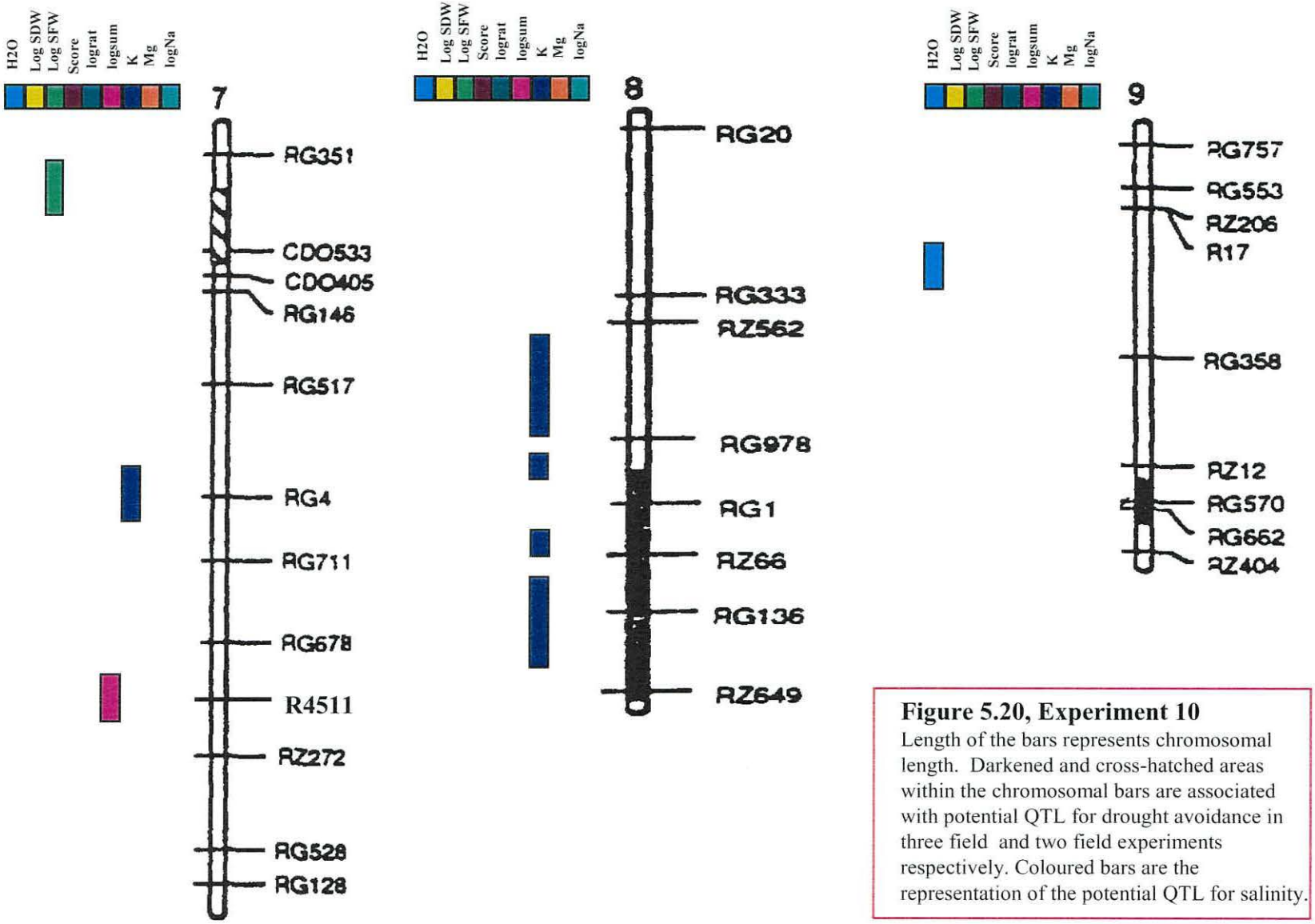


**Figure 5.20, Experiment 10**
Length of the bars represents chromosomal length. Darkened and cross-hatched areas within the chromosomal bars are associated with potential QTL for drought avoidance in three field and two field experiments respectively. Coloured bars are the representation of the potential QTL for salinity.

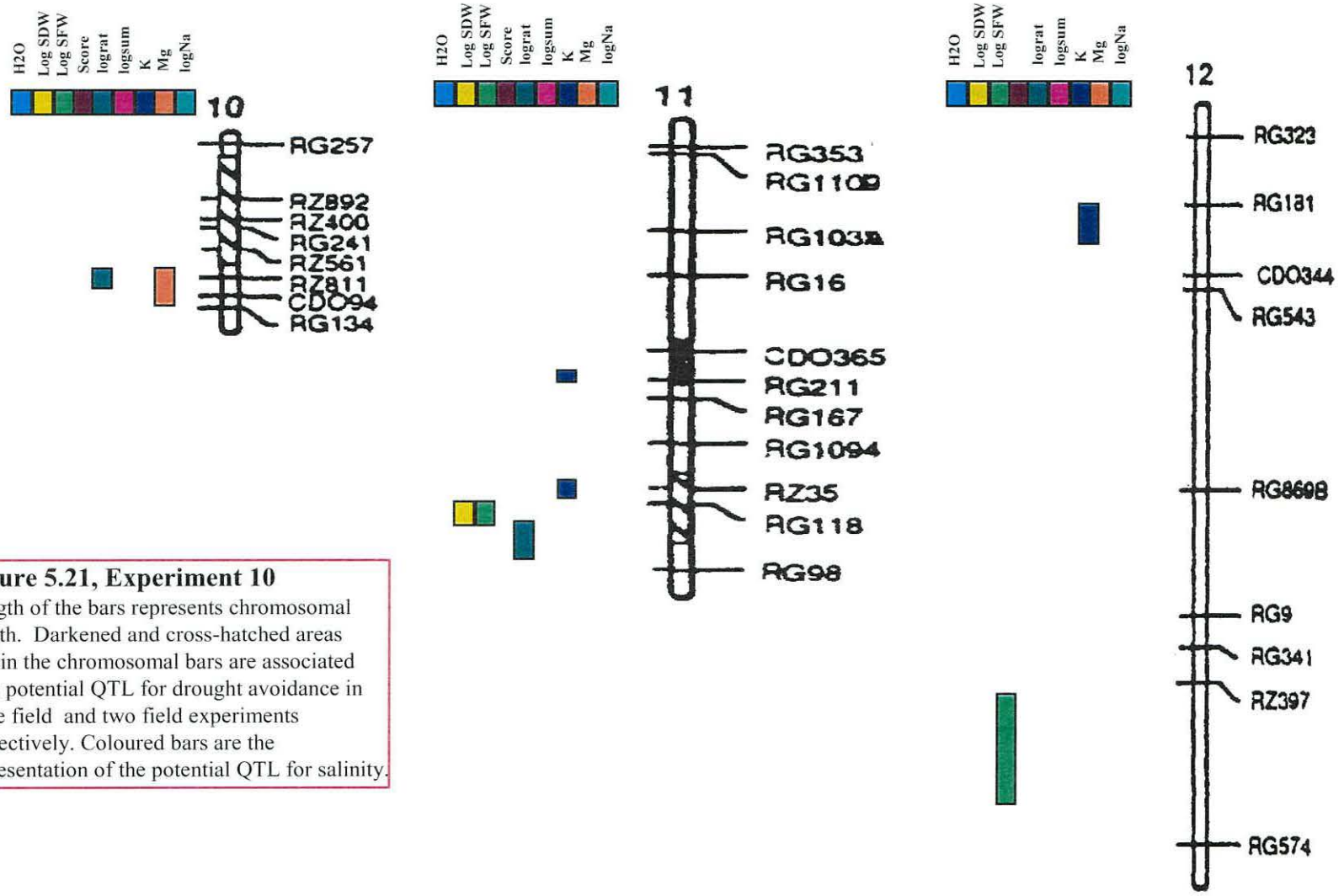


**Figure 5.21, Experiment 10**
Length of the bars represents chromosomal length. Darkened and cross-hatched areas within the chromosomal bars are associated with potential QTL for drought avoidance in three field and two field experiments respectively. Coloured bars are the representation of the potential QTL for salinity.

## 5.3.3 ANOVA for single markers on chromosome 1

The major QTL for log Na1, log Na2, and log Na, were considered to be located on chromosome 1, and markers on this chromosome were subjected individually to 1-way ANOVA (Table 5.7). These QTL are considered as major due to their consistency with the trait (logNa), position (RG118 and RZ276) and number of chromosome (chromosome 1) in both the experiments 7 or 10. P values of ANOVA for some important traits on chromosome 1 (Figure 5.14, Experiment 7) and (Figure 5.18, Experiment 10) are given in the following table.

**Table 5.7. Analysis of variance for individual markers on chromosome 1 (Experiment 7 and 10). The numbers are the P values of ANOVA.**

| | Trait | | | | | | |
|---|---|---|---|---|---|---|---|
| | Experiment 7 | | Expt 10 | Experiment 7 | | Expt 10 | Expt 7 |
| Markers | logNa1 | logNa2 | logNa | logratio1 | logratio 2 | logratio | Cl2 |
| RG350 | 0.810 | 0.129 | 0.002 | 0.173 | 0.611 | 0.012 | 0.779 |
| RG77 | 0.002 | 0.017 | 0.007 | 0.008 | 0.007 | 0.011 | 0.468 |
| RG236 | 0.010 | 0.061 | 0.204 | 0.004 | 0.009 | 0.156 | 0.756 |
| RG331 | 0.112 | 0.005 | 0.365 | 0.429 | 0.003 | 0.321 | 0.994 |
| RG400 | 0.515 | 0.022 | 0.343 | 0.781 | 0.008 | 0.288 | 0.844 |
| RG109 | 0.539 | 0.022 | 0.275 | 0.873 | 0.010 | 0.017 | 0.875 |
| RZ730 | 0.035 | 0.227 | 0.001 | 0.016 | 0.003 | 0.001 | 0.965 |
| RG197 | 0.000 | 0.029 | 0.010 | 0.000 | 0.005 | 0.001 | 0.912 |
| RG101 | 0.000 | 0.085 | 0.048 | 0.000 | 0.004 | 0.003 | 0.291 |
| RG462 | 0.000 | 0.004 | 0.116 | 0.001 | 0.072 | 0.003 | 0.770 |
| CDO920 | 0.000 | 0.045 | 0.006 | 0.000 | 0.040 | 0.129 | 0.659 |
| RZ744 | 0.000 | 0.000 | 0.015 | 0.001 | 0.011 | 0.127 | 0.322 |
| RZ276 | 0.000 | 0.000 | 0.000 | 0.616 | 0.321 | 0.000 | 0.186 |
| RG811 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.841 |
| RG140 | 0.000 | 0.000 | 0.093 | 0.000 | 0.002 | 0.428 | 0.396 |
| RG612 | 0.000 | 0.000 | 0.447 | 0.001 | 0.001 | 0.157 | 0.748 |

Note:- Cut off level for significance is 0.010

Some variations between the observations from the ANOVA (Table 5.5) and linkage map (Figure 5.14, Figure 5.19) were observed, but both analyses revealed the importance of markers RZ276 and RG811 for $Na^+$ accumulation in leaves (highlighted in blue). Chapters 4 and 5 showed that growth is inversely related to $Na^+$ concentrations, so these markers (RZ276 and RG811) are important in both experiments 7 and 10. Marker RG811 is also associated with K/Na ratio in both

experiments 7 and 10. As far as ANOVA is concerned it gave information for a particular marker for the trait under consideration while MAP MAKER gave information in relation to the other markers.

## 5.4 Discussion

### 5.4.1. Variation between parents

The two parental varieties, Co39 and Moroberekan, differed in their accumulation of leaf $Na^+$ under salinity (Figures 5.1 and 5.5, and Appendix 2), whereas the ranges for leaf $Cl^-$ concentration in experiment 7 overlapped considerably (Figures 5.2 and 5.6). Thus it is reasonable to expect that QTL would be detected for $Na^+$, but not for $Cl^-$, in this particular mapping population. Concentrations of $Cl^-$ were not measured in experiment 10, partly because of the limited variation in the parents and also because of lack of time. Co39 accumulated the lowest $Na^+$ and Moroberekan accumulated the highest $Na^+$ in leaf sap in experiment 7 (Figures 5.1 and 5.5). The highest concentration of $Na^+$ was recorded for Moroberekan in experiment 10 (Appendix 2).

In experiment 7 shoot fresh weights (SFW) and dry weights (SDW) differed between the parents, with Co39 producing the heavier plants (Figures 5.7 and 5.8). Similar observations were recorded in experiment 10 (Appendix 3), although there was more overlap in the ranges. Thus QTL for plant weight under salinity could be expected.

In experiment 7 Co39 had a higher resistance score1 than Moroberekan (Figure 5.3), but in experiment 10 Moroberekan had the higher score1 values (Appendix 2). As the stress developed the resistance scores (score2) for the two parents overlapped in both experiments (Figure 5.4 and Appendix 2). The varieties (Co39 and Moroberekan) were significantly different for both the resistance scores in both the experiment 7 and 10, but Co39 had higher scores than Moroberekan in Experiment 7 and lower scores than Moroberekan in Experiment 10.

## 5.4.2. Variation among the RILs

The distribution of $Na^+$ concentrations in the RILs was not normal but was skewed (Figures 5.1 and 5.5) and the same trend was followed in experiment 10 (Appendix 2). Similar observations were recorded by Flowers *et al.* (2000) for the distribution of $Na^+$ concentrations in rice. Zhang *et al.* (1999) found that the gene *SOA3* could play an important role in salt tolerance in rice and it might have an active role in the strict control of $Na^+$ and $Cl^-$ uptake into root symplast and apoplast, and hence translocation into the shoot.

Higher concentrations of $K^+$ were observed in different varieties of rice in comparison with $Na^+$ concentrations at all salinity levels (Table 5.5). Similar observations in the literature have already been discussed in chapter 4.

Transgressive segregation values for Na1 and Na2 were observed (Figure 5.1 and 5.5). These values deviated from the limits of parental varieties but the transgressive segregation was observed in Na1 only at the high end. However no transgressive segregation was observed for $Na^+$ concentrations in experiment 10 (Appendix 2). RILs with values outside the range of the parents may be due to the bigger populations of RILs than the parents, or there may have been real transgressive segregation due to reshuffling of genes or heterosis. Transgressive segregation was observed by Tozlu *et al.* (1999*b*) in many morphological traits under saline condition in BC1 progeny plants in citrus.

There was variation in the growth parameters score1, score2, SFW and SDW within the RILs (Figure 5.3 - 5.8). Some apparently transgressive values were observed for these parameters in both experiments 7 and 10. A large range in the time to anthesis in RILs was recorded (75-165 days) without added salinity (Figure 5.9).

## 5.4.3. Relationships between parameters

There were reductions in SFW and resistance scores with the increased concentration of $Na^+$ (Experiment 7) and $Cl^-$ in the RILs (Figures 5.11 and 5.13), but the accumulation of $Na^+$ was very low compared to $Cl^-$ accumulation (Figures 5.10 and 5.12). High concentrations of $Cl^-$ or $Na^+$ could be responsible for the reduction

in SFW. Similar findings were recorded in experiment 10 (Appendix 2) for $Na^+$ in 96 RILs. An inverse relationship between growth and $Na^+$ concentration was also observed by Flowers *et al.* (1985). Aslam *et al.* (1993*b*) had similar results for different rice varieties under salinity.

There were significant correlations between many of the measured parameters in experiment 7 (Table 5.2) and experiment 10 (appendix 2), but these correlations are of limited value in determining cause and effect. Both $Na^+$ and $Cl^-$ concentrations were correlated with shoot fresh and dry weights, but to similar extents. Thus this analysis does not allow an evaluation of the relative toxicities of $Na^+$ and $Cl^-$.

Investigations of rice prior to 1972 showed no relationship between any morphological character and resistance to salinity in rice varieties (Akbar *et al.*, 1972). Responses to salinity are multigenic (controlled by several genes) so morphological characters are not considered so important. However, physiological characters, especially $Na^+$ and $Cl^-$ concentrations in the leaf sap, are thought to be important for salinity tolerance (Flowers and Yeo 1985, Leach *et al.*, 1990; Zhang *et al.*, 1999). Significant negative correlations were observed for SFW with $Na^+$ and $Cl^-$ (Aslam *et al.*, 1993*b*).

### 5.4.4. QTL

Only one QTL for $Cl^-$ was found, on chromosome 1 in experiment 7 (Table 5.4). This is not surprising since there was little variation for $Cl^-$ in the parents (see above). In a citrus mapping population Tozlu *et al.* (1999*a*) found 31 potential QTL for $Na^+$ and $Cl^-$ accumulation and $Cl^-/Na^+$ ratios, so it is still possible that $Cl^-$ QTL could be found in rice if parents differing in $Cl^-$ accumulation could be identified.

Although a large number of potential QTL were identified in experiments 7 and 10, there are problems with evaluating their importance. Firstly there is the question of the reliability of the analyses. The statistical analyses can only be as good as the trait data. Experiment 10 should, therefore, be more reliable than experiment 7 since the former is based on the mean of 5 replicates per RIL, while there was only one replicate (but of more RILS) in experiment 7. It is known that environmental interactions can complicate QTL analysis, so that comparison of experiments 7 and 10 (and also of the two parts of experiment 7) may not be simple. It is also possible

that different genes may be more or less important at different stages in the development of salt stress. Given these constraints, it is still valid to look for consistency between experiments. Secondly there is possible linkage between quantitative traits. A gene might, for example, directly affect $Na^+$ accumulation, and this might indirectly affect solute potential or $K^+$ accumulation etc. Thus a cluster of apparent QTL at a particular chromosomal location might indicate the action of one or a few genes.

From my experiments the following major clusters can be identified;

**Cluster 1.** QTL for Na1, Na2, ratio1 and ratio2 were associated with the region between markers RZ276 and RG140 on chromosome 1 (Figure 5.14, Experiment 7). This region also contained QTL for Na, Mg, K, ratio, score, SFW, SDW and water in experiment 10 (Figure 5.18, Experiment 10).

**Cluster 2.** QTL for Na1, SFW, K1, sum1 and ratio1 are associated with the marker RZ284 on chromosome 3 (Figure 5.14). These QTL were not found in experiment 10.

**Cluster 3.** QTL for Na1, Na2, , sum1, ratio1, ratio2, SFW and SDW are associated with the region between markers RZ69 and RG498 on chromosome 4 (Figure 5.15, Experiment 7). Only QTL for K and (K/Na) ratio were found in this region in experiment 10.

**Cluster 4.** QTL for Na1, Na2, water, ratio1, and ratio2 were found in the region from markers WAXY to RZ144 on chromosome 6 (Figure 5.15, Experiment 7). A QTL for ratio was also found here in experiment 10.

**Cluster 5.** On chromosome 7, QTL for Na1, Na2, ratio1, ratio2, score2 and water were found between RZ272 and RG128 (Figure 5.16, Experiment 7). In experiment 10 no QTL were found in this region.

**Cluster 6.** On chromosome 8, QTL for Na2, ratio2, score2, SFW, SDW and water were found between RG136 and RZ649 (Figure 5.16, Experiment 7). In experiment 10 only one QTL for K was found more or less in this region (Figure 5.20, Experiment 10).

**Cluster 7.** QTL for Na1, ratio1, score 2, SFW and water were found in the region from markers RG757 to RZ12 on chromosome 9 in two groups (Figure 5.16, Experiment 7). A QTL for water was also found here in experiment 10 (Figure 5.20, Experiment 10).

Leaf $Na^+$ and $K^+/Na^+$ ratio QTL are roughly at the same place on chromosome 1 (around RZ276) in both experiments 7 and 10, and this is the only region where these QTL are consistent between both experiments. QTL for ion traits are coincident with those for growth traits on chromosome 3, 4, 8, and 9 as already explained in clusters 2, 3, 6 and 7 respectively in experiment 7 (Figures 5.14 and 5.16). Another association for Na, Mg, ratio, SFW and SDW was observed on chromosome 1 (Fig 5.18, Experiment 10) around the RG811 marker. On chromosome 11 an association for ratio, SFW and SDW was observed around RG98 and RG118 (Figure 5.21, Experiment 10).

The experiments were designed to study $Na^+$ uptake and not specifically for growth. As there was no control treatment (because of lack of space in the flood benches), relative growth (of the salt-treated plants compared with controls) could not be calculated. Therefore the effect of the vigour of the individual RILs cannot be assessed.

Other workers have also found $Na^+$ QTL on chromosome 1. The position of the QTL near marker RZ276 coincides with the estimated positions of QTL for K concentration, Na uptake and Na:K ratio identified by Koyama *et al.* (2001) on chromosome 1 in an *indica* x *indica* rice population (IR55178, derived from IR4630 and IR15324). Furthermore the *SalT1* gene is close to RZ276 (Rice Genes database) confirming the importance of this region for salt tolerance in rice. In another *indica* cross (IR66946, IR29 x Pokkali) the salinity tolerance gene *saltol* is also found on chromosome 1 and may be equivalent to *SalT1* (Gregorio *et al.*, 1998). *Saltol* had major effects on leaf Na and K contents and Na/K ratios. In an *indica* x *japonica* doubled haploid population (ZJDH, Zhaiyeqing8 x Jingxi17) Gong *et al.* (1999) identified a QTL for seedling salt tolerance (Std) on chromosome 1, but between markers RG612 and C131 at the end of the chromosome, *i.e.* at a different location to Cluster 1.

Another marker for salt tolerance, SalT2, was located on chromosome 6 (Zhang *et al.*, 1995), as were *OsZFP1,* a zinc-finger protein down-regulated by salt stress (Li and Chen, 2001) and qrSLT-6-1, a QTL for salt tolerance in the cross Tesanai2 x CB (Lin *et al.*, 1998). Other salt tolerance QTL were located on chromosomes 3, 4 and 5 (Lin *et al.*, 1998). The position for cluster 4 matches the position for the QTL for K uptake, dry mass and Na concentration on chromosome 6 identified by Koyama *et al.*

(2001). QTL associated with seedling salt tolerance were also detected on chromosome 6 in a doubled haploid population derived from IR64 x Azucena (Prasad *et al.*, 2000).

One marker locus was significantly associated with salt tolerance on chromosome 5 in Tesanai 2/CB after the construction of a linkage map of RFLP markers (Lin-Hongxuan *et al.*, 1997). It might be the same as the locus from my results for SFW and log water traits for Co39 x Moroberekan (Fig 5.15, Experiment 7).

On chromosome 12 only one QTL for SFW was mapped near RG869B (Figure 5.17, Experiment 7), and it might be the same as the locus for salt tolerance ($Na^+$ and $Cl^-$ uptake) in roots of rice that was observed after mapping the *OSA3* gene (Zhang *et al.*, 1999). The QTL for water content on chromosome 8 seems to be similar to a locus conferring dehydration tolerance in Co39 that was mapped on chromosome 8 (Mackill *et al.*, 1999).

The position of the cluster 3 markers RG449, RG788 (between RZ69 and RG498) is consistent with the estimated position for the QTL for the traits Na:K ratio, Na concentration and K uptake in IR55178 Koyama *et al.* (2001), and the position of the markers RG476 and RG214 for K1 and sum1 is also consistent with the estimated position of the QTL for K concentration identified by Koyama *et al.* in 2001 on chromosome 4.

QTL mapping showed a cluster of QTL for many traits (Na1, $K^+/Na^+$ ratio1, and SFW) at one locus (RG358) on chromosome 9. This is in agreement with Tozlu *et al.* (1999*a*), who pointed out that fewer genes could control many traits than the actual number of QTL mapped for them. Presence of clusters of QTL for many traits could be due to pleiotropic effects, where one gene can affect several physiological or developmental processes, or it could be due to accuracy of mapping. This map is not very accurate because few markers on chromosomes were used for mapping. For some traits several peaks were visible that can give estimated or potential QTL, but not the precise QTL for the trait. So more markers are required to improve the accuracy of the mapping and locate QTL with few peaks. QTL for K1 and sum1 were identified in the region RZ12, RG570 and RG662 on chromosome 9, and this region contains a QTL for K uptake identified by Koyama *et al.* (2001) on chromosome 9.

## 5.5. Conclusions

- $Na^+$ concentrations in the leaf sap were low compared with $Cl^-$ in all rice accessions.
- Maratelli was salt tolerant on a SFW basis and resistance score basis and is also a low $Na^+$ accumulator.
- Moroberekan was salt susceptible on SFW and resistant score basis and is a high $Na^+$ accumulator.
- Early anthesis was observed in Maratelli.
- Significant negative correlations were found for $Cl^-$, $Na^+$ with resistance score, SFW, SDW.
- QTL were identified for many traits including logNa1, logNa2, Cl2, logK1, K2, logsum1, logratio1 and logratio2.
- A consistent QTL for $Na^+$ accumulation was associated with markers RG811 and RZ276 on chromosome 1.
- Salt tolerance is under the control of QTL for $Na^+$ trait as QTL for $Na^+$ trait are coincident with QTL for SFW trait on chromosomes 1, 3, 4, 8, 9 and 11.
- QTL marker can be used to pyramid the effects of various traits and can be used in marker assisted selection.

# Chapter 6

# General Discussion

Rice is generally regarded as less tolerant of salinity than other major cereals (Maas and Hoffman 1977). In barley (Gorham *et al.*, 1994) and wheat (Syed, 2000) there are varietal differences in $Na^+$ accumulation both when salt is applied to the roots and when saline solution is sprayed directly on the leaves. Experiment 1 was not considered here because the roof of the Pen-y-Ffridd, right above the plants was blown away and the weather was extremely cold, windy and rainy. Experiment 4 was not included because the results were severely affected by water shortage. Thus my $2^{nd}$,$3^{rd}$,$5^{th}$ and $6^{th}$ experiments compared the accumulation of $Na^+$ in rice leaves with that in barley ($2^{nd}$ experiment) in response to salt treatment of the roots or foliar application of salt spray (Chapter 3). Considerable variation in leaf $Na^+$ accumulation was observed between varieties when salt was applied to the roots, but not as a result of foliar application of salt. These experiments also raised a number of questions about the physiological responses of rice to salinity, including the relative toxicity of $Na^+$ and $Cl^-$, the effects of different growth media, the extent of dehydration and apoplastic solute accumulation, and the contribution of bypass flow to delivery of salts to the shoots. These questions were addressed in Chapters 4 and 5. The varieties Co39 and Moroberekan differed in the accumulation of $Na^+$ in their leaves, and since a mapping population derived from a cross between these varieties was available, further experiments (Chapter 5) were designed to detect QTL for $Na^+$ accumulation and other traits associated with salt responses.

## 6.1 $Na^+$ accumulation in rice and barley leaves in response to foliar or root salt treatment.

In both barley and rice, ion accumulation in leaves was influenced by salt application method (foliar spray or *via* root). Furthermore, under the same application method, different varieties responded differently to salinity (Chapter 3). In barley the varieties

CM-67 and Chevron responded as previously reported by Gorham *et al.* (1994) in that $Na^+$ concentrations were higher with root than with foliar application in Chevron, but the opposite was true for CM-67. In experiment 2 the leaf $Na^+$ concentrations were similar in rice and barley, contrary to the expectation (Flowers, 1985; Gorham, 1992) that the more sensitive species (rice) would have higher $Na^+$ concentrations. Moreover plants are categorised on the basis of their responses to salinity. The group of plants that like sweet (non-saline) water are called glycophytes (Gorham, 1985) and includes rice. In rice, foliar application resulted in higher $Na^+$ concentrations than root application in all varieties except Bala and Moroberekan (and Maratelli in some experiments). In response to root salinity, the varieties Moroberekan, Azucena and Bala accumulated higher leaf $Na^+$ concentrations than Maratelli, Marzhan, Co39, IR64 and KS282. In experiments 3, 5 and 6 the concentrations of $Na^+$ in the latter varieties were low (<50 mol $m^{-3}$) for a reputedly salt-sensitive species at salinities from 100 to 200 mol $m^{-3}$ NaCl. The implications of this finding are discussed in section 6.3.

## 6.2 Dehydration and the Oertli effect

There was an increase in $K^+$ concentrations in leaf sap under salinity, but also a decrease in water content per unit shoot dry weight (water/SDW). The $K^+$ concentration per unit shoot dry weight was unaffected by salinity, so the increase in $K^+$ concentration in leaf sap was due to dehydration. Dehydration would also account for part of the increases in leaf sap $Na^+$ and $Cl^-$ concentrations.

Low turgor was also noticed under salinity, but this low turgor was not due to good osmotic adjustment because over-adjustment was observed (Chapter 4). So symplastic dehydration could be due to very high apoplastic ion concentrations compared with vacuolar ion concentrations - a phenomenon known as the Oertli effect (Oertli, 1968; Flowers *et al.*, 1991). It was suggested that low turgor is due to non-accumulation of solutes in the protoplast or the accumulation of solutes in the apoplast or cell wall. Low turgor combined with highly negative osmotic potentials were recorded in mature leaves of *Suaeda maritima* by Clipson *et al.* (1988) and in other halophytes (A.D. Tomos and J. Gorham, personal communications).

## 6.3 Relative toxicity of $Na^+$ and $Cl^-$

High $Cl^-$ concentrations relative to those of $Na^+$ were recorded irrespective of the variety and growth conditions in Chapters 4 and 5. $Cl^-$ might be the main cause of injury to rice plants because its concentration was very high compared with $Na^+$ concentrations (Chapter 5). The literature contains reports of higher $Cl^-$ concentrations in rice regardless of the medium of growth, experimental conditions and other environmental differences (Aslam *et al.*, 1993ab; Azhar Naeem, 1994; Noor Muhammad, 1998; Welfare, 1995). The reason for low sodium and high chloride as observed in chapters 4 and 5 might be due to efficient $Na^+$ exclusion and low $Cl^-$ exclusion from the leaf (Hajibagheri *et al.*, 1987; Subbarao *et al.*, 1990; Jacoby, 1965; Rains, 1969; Kramer, 1977). Welfare *et al.* (1995) reported higher concentrations of $Na^+$ than of $Cl^-$, but there appears to be an ionic imbalance with $K^+ + Na^+$ amounting to 1.5 mmol $g^{-1}$ DW while $Cl^-$ was only 0.68 mmol $g^{-1}$ DW. It should be noted that these authors used different analytical methods to those employed in my study (Chapter 2).

Highly significant negative correlations were recorded between both $Na^+$ and $Cl^-$ concentrations and growth (shoot fresh and dry weights) in Chapter 5. This does not, however, resolve the question of which ion is most toxic. The much higher concentrations of $Cl^-$ suggest that this ion cannot be ignored, but at present QTL have only been found for $Na^+$ accumulation (see section 6.7).

## 6.4 The effects of different growth media

Systems of salt application (Flood Bench and Hydroponics), had significantly different effects on growth and ion accumulation in rice under salinity (Chapter 4). Similar differences were reported by Semikhodskii *et al.* (1997) for wheat grown either in hydroponic culture or in rockwool blocks. In hydroponic culture leaf $Na^+$ concentrations were much higher than in rockwool, even though the applied salt concentration was higher in rockwool (200 mol $m^{-3}$) than in hydroponics (160 mol $m^{-3}$ NaCl). Pecetti and Gorham (1997) also reported differences in ion concentrations and shoot and root weight in wheat between different techniques (sand culture and hydroponics). Ion concentrations in wheat and barley were much lower in field studies than in hydroponics (Gorham, unpublished observations; Aloy, 1995), and the ranking of varieties could be affected by the growth medium. Care should be taken, therefore, when extrapolating results from hydroponics to field conditions.

## 6.5 The effects of leaf age and duration of exposure to salinity

In my work there was variation in the concentrations of $K^+$ and $Na^+$ in different leaves and an increase in $Cl^-$ concentrations in the flood bench system in all the varieties with increased leaf age under salinity (Chapter 4). Such gradients have been reported by other authors. According to Khatan and Flowers (1995) there was a leaf to leaf gradient in $K^+$ concentrations in leaves of different ages and an opposite gradient in the case of $Na^+$ and $Cl^-$. $K^+$ concentration was maximum in the flag leaf and $Na^+$ and $Cl^-$ were minimal in flag leaves under all salinity levels (Khatan and Flowers 1995). Sarg *et al.* (1993) measured high accumulation of $Na^+$ and $Cl^-$, but less $K^+$, in mature leaves of tomato compared to young leaves. High concentrations of $Na^+$ and low concentrations of $K^+$ were observed in the flag leaf sap of wheat plants (Kharchia) grown in saline soil (Rajpur and Wright 1999).

Leaf $Na^+$ and $Cl^-$ concentrations increase with time of exposure to salinity (Chapter 4, Table 4.3). Visual symptoms of salt damage took several weeks to develop in my experiments, starting with leaf rolling and dehydration and proceeding to chlorosis and finally necrosis. Thus lower ion concentrations in younger, developing leaves could partly be explained by shorter exposure to salinity and partly by more rapid expansion growth (diluting the concentrations of ions delivered to the leaves). However, dehydration and leaf rolling were observed first in the youngest leaves.

## 6.6 The contribution of bypass flow to delivery of salts to the shoots

Transpirational bypass flow did not account for differences in sodium accumulation by different rice varieties in my experiments, in disagreement with Garcia *et al.* (1997) and Yeo *et al.* (1991,1999). Moroberekan, the high $Na^+$ variety, had lower PTS tracer accumulation than Co39 or Maratelli (Chapter 4). There was no correlation between $Na^+$ and $K^+$ transport into the shoot, and they have different mechanisms of uptake (Garcia *et al.*, 1997; Yeo, 1998).

## 6.7 QTL for traits associated with salt responses

$Na^+$ and $K^+$ uptake was different in rice from wheat and was controlled by different genes that segregated independently (Garcia *et al.*, 1997) and this is confirmed by the presence of various $Na^+$ and $K^+$ QTL on different chromosomes of rice (Chapter 5).

Eleven different traits were studied for QTL analysis in rice in $F_8$ and $F_9$ generation RILs from the Co39 x Moroberekan cross. Of these, 6 were physiological traits and the rest were growth traits. Variation was found for all traits by one way ANOVA QTL analyses. A QTL map was generated for these physiological and growth traits on all 12 chromosomes of rice using Mapmaker QTL (Chapter 5). Chromosome 1 was shown to contain the most important QTL for $Na^+$ concentration and $K^+/Na^+$ ratio in experiments 7 and 10. The designs of experiments 7 and 10 were different. In experiment 7 one replicate each of 170 RILs was used, but in experiment 10 the mean values of 6 plants each of 90 RILs were analysed. The data were log transformed for Na and K/Na ratio in experiments 7 and 10 because the data were not normally distributed but skewed towards low $Na^+$ concentrations. $Na^+$ concentrations and enhanced $K^+/Na^+$ ratio in the shoots were controlled by the region around the locus RZ276. Agreement for the presence of QTL for $Na^+$ and ratio ($K^+/Na^+$) at marker RZ276 in both experiments was observed (Chapter 5).

Other workers have also found $Na^+$ QTL at the same locus on chromosome 1 in different rice mapping populations (Gregorio *et al.*, 1998; Koyama *et al.,* 2001) Furthermore the salt tolerance gene, *SalT1,* is close to RZ276 (Clase *et al.*, 1990; Rice Genes database) confirming the importance of this region for salt tolerance in rice. Gong *et al.* (1999) identified a QTL for seedling salt tolerance (Std) on chromosome 1, but between markers RG612 and C131 at the end of the chromosome. In wheat, $Na^+$ exclusion and enhanced $K^+/Na^+$ ratio in shoots were controlled by a single locus, *Kna1,* on the long arm of chromosome 4D (Dvorak *et al.*, 1994). QTL affecting $Na^+$ and $K^+$ concentrations (and hence $K^+/Na^+$ ratios) were found on chromosome 5A of wheat by Semikhodskii *et al.* (1997).

The position for cluster 4 (Chapter 5) matches the position for the QTL for K uptake, dry mass and $Na^+$ concentration on chromosome 6 identified by Koyama *et al.*

(2001). Another marker for salt tolerance, *SalT2*, was also located on chromosome 6 (Zhang *et al.*, 1995), as were *OsZFP1* (Li and Chen, 2001) and the QTL qrSLT-6-1 (Lin *et al.*, 1998). Other salt tolerance QTL were located on chromosomes 3, 4 and 5 (Lin *et al.*, 1998). QTL associated with seedling salt tolerance were detected on chromosome 6 (Prasad *et al.*, 2000). Three regions carrying QTLs for various growth and physiological traits were identified on chromosomes 1, 4 and 8 (Gong *et al.*, 2001).

The interpretation of QTL affecting the concentrations of ions in the shoot is dependent on plant vigour because net water uptake of the most vigorous plants was high, so diluting the concentration of ions entering the shoot. Hence high concentrations of different ions ($Na^+$ and $Cl^-$) can be explained partly in terms low vigour. Koyama *et al.* (2001) emphasise the difference between ion concentration (amount per unit dry weight, and hence related to vigour expressed as total dry weight) and uptake (amount per shoot). This is, to some extent, supported by my results. In experiment 7 Co39 had higher fresh and dry weights than Moroberekan and here the differences in $Na^+$ concentration were high (*i.e.* the $Na^+$ was diluted by the greater vigour of Co39). In experiments 8a and 10, however, Moroberekan was more vigorous, but the $Na^+$ concentrations were not lower than in Co39. So vigour is an important factor for the comparison of ion concentrations in plants and cannot be ignored.

Such differences between experiments raise the question of genotype by environment (g x e) interactions. G x e interactions are commonly observed in QTL studies (Monforte *et al.,* 1997*b*,1999; Gong *et al.*, 2001). Genotype by environment interactions were observed in sunflower (Uma *et al.*, 1995), safflower (Uma and Patil, 1994), wheat (Uma and Patil, 1995; Sastry and Muralia, 1994; Singh and Chatrath, 1995), black gram (*Vigna mungo*) (Kandaswami 1995) and rice (Moreno and Gonzalez, 1997).

Complexities of salinity x environment interactions (together constituting the 'environment' in g x e interactions) change with plant development and differentiation. Salt tolerance also varies with the changes in concentrations of major and minor nutrients in the root zone. Plant growth models might provide a method to integrate the complexities of plant responses to salinity stress with the relevant environmental variables that interact with the measurement of tolerance (Chapter 1). Models responsive to salinity stress would provide insights for breeders and aid in developing

more practical research on the physiological mechanisms of plant salt tolerance (Shannon 1996).

Genotype x salinity level interaction is commonly large. Thus, breeding for saline areas can be compared to wide adaptation. The target environment in breeding for saline soils is actually a population of many possible environments, for which there exist significant genotype x environment interactions. Thus, it is possible to study the merit of potential strategies for breeding for salinity tolerance using the tools that have been developed for the study of breeding for wide adaptation. The evidence from selection and breeding experiments for wide adaptation seems to favour testing on a representative subset of environments, including stress and non-stress locations; but the choice of these locations is complicated by the multidimensional nature of g x e interactions (Igartua *et al.*, 1995). Stable QTL (identified in several different environments) are the most useful in marker-assisted selection.

Not only may some QTL only be detectable in specific environments, but there are also a number of possible interactions between apparent QTL. One of these is pleiotropy, where one gene can affect several physiological or developmental processes (*e.g.* a gene for vigour might affect ion concentrations, as discussed above). Thus it is possible that a cluster of apparent QTL (as found in Chapter 5) could result from only one or a few genes.

Another interaction is epistasis, the interaction of different non-allelic genes or QTL. For example, the suppression of a gene by a different gene (epistatic dominance, in contrast to allelic dominance). Segregation of epistatic genes in a cross could modify phenotypic characters in progeny for traits that they affect. Detection of QTL that are affected by epistasis requires comparison in different genetic backgrounds, *i.e.* different mapping populations (not available in this study). Epistasis was not studied due to shortage of time. Examples of studies of g x e interactions and epistasis affecting various parameters in rice are those of Liao *et al.* (2001), Cao *et al.* (2001), Shen *et al.* (2001) and Zhang *et al.* (2001).

## 6.8 Possible further studies

For this purpose natural habitat (coastal areas) for foliar salt application can be used. Ion content rather than concentrations (affected by vigour) could be used for QTL analysis. Ion content per plant or per leaf could be compared with the data of Koyama

*et al.* (2001), but not completely because data for the old leaves are not available. Epistasis could also be studied. Different analytical methods can be tried for ion analysis like single cell sampling techniques (SiCSA). Measurements of the extent of dehydration and apoplastic solute accumulation could be made.

QTL mapping in relation to g x e interaction could also be studied in various environments such as controlled environments (greenhouse, growth cabinets) and natural habitats or field conditions (saline soils, saline sodic or sodic soils). RILs from crosses of other rice varieties could also be used for QTL mapping of salt tolerance parameters. The information from QTL mapping can be used widely in rice breeding if the QTL are common in a range of varieties, otherwise they are only applicable to the particular mapping population in which they were detected. The finding of similar QTL for $Na^+$ concentrations and salt tolerance at the same locus (near RZ276) on chromosome 1,in different mapping populations of rice might, therefore, allow wider use of this marker in marker-assisted selection.

QTL for ion traits are coincident with those for growth traits on chromosome 3, 4, 8, and 9 as already explained for clusters 2, 3, 6 and 7 respectively (Chapter 5). QTL for Na, , sum1, ratio SFW and SDW are associated with the region between markers RZ69 and RG498 on chromosome 4 in experiment 7 and is coincident with QTL for K and (K/Na) ratio in this region in experiment 10. QTL for $Na^+$ accumulation were coincident with QTL for SFW on chromosomes 1, 3, 4, 8, 9 and 11 (see details in Chapter 5). So it is important to study QTL for $Na^+$ and ratio (K/Na) in rice, and using this information to develop markers for marker assisted selection for salinity tolerance. Such studies are particularly important in rice because it is a diploid with a small genome, and because of the synteny between rice and other cereals such as oats, wheat, maize, pearl millet, sorghum, sugar cane and foxtail millet (Gale and Devos, 1998).

# Chapter 7

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## Appendix 1. Data for experiment 7

| RIL/var | Cl1 | Cl2 | Na1 | Na2 | Score1 | Score2 | SFW | SDW | K1+Na1 | K2+Na2 | Water | w/gdw | K1 | K2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RIL 1 | 168.26 | 383.84 | 7.64 | 14.79 | 2.50 | 1.00 | 12.34 | 6.44 | 290.50 | 348.00 | 5.90 | 0.92 | 282.86 | 333.21 |
| RIL 2 | 326.03 | 541.91 | 16.96 | 45.76 | 2.00 | 1.00 | 13.32 | 9.05 | 308.40 | 594.40 | 4.27 | 0.47 | 291.44 | 548.64 |
| RIL 3 | 220.93 | 542.41 | 4.36 | 37.99 | 3.00 | 1.30 | 9.29 | 5.34 | 286.00 | 532.10 | 3.95 | 0.74 | 281.64 | 494.11 |
| RIL 4 | 301.88 | 517.77 | 4.64 | 42.48 | 2.00 | 0.20 | 10.39 | 7.37 | 275.70 | 502.40 | 3.02 | 0.41 | 271.06 | 459.92 |
| RIL 5 | 251.09 | 364.71 | 5.87 | 10.56 | 3.00 | 2.00 | 13.77 | 8.80 | 295.00 | 418.70 | 4.97 | 0.56 | 289.13 | 408.14 |
| RIL 6 | 228.99 | 340.18 | 23.05 | 26.17 | 3.70 | 0.40 | 13.80 | 6.95 | 275.70 | 396.20 | 6.85 | 0.99 | 252.65 | 370.03 |
| RIL 7 | 269.42 | 504.71 | 1.92 | 6.01 | 2.50 | 1.20 | 10.75 | 6.10 | 308.50 | 520.70 | 4.65 | 0.76 | 306.58 | 514.69 |
| RIL 8 | 260.08 | 386.89 | 1.78 | 4.19 | 2.50 | 0.40 | 12.59 | 6.05 | 270.30 | 389.20 | 6.54 | 1.08 | 268.52 | 385.01 |
| RIL 9 | 210.18 | 382.49 | 1.74 | 3.26 | 2.50 | 0.30 | 10.78 | 5.80 | 262.80 | 402.20 | 4.98 | 0.86 | 261.06 | 398.94 |
| RIL 10 | 123.64 | 299.36 | 0.82 | 1.36 | 3.20 | 2.50 | 15.13 | 7.48 | 313.60 | 446.00 | 7.65 | 1.02 | 312.78 | 444.64 |
| RIL 11 | 142.03 | 281.96 | 5.41 | 10.00 | 3.50 | 3.00 | 13.49 | 5.32 | 281.10 | 376.80 | 8.17 | 1.54 | 275.69 | 366.80 |
| RIL 12 | 156.21 | 301.03 | 1.29 | 3.16 | 3.50 | 2.00 | 10.20 | 6.10 | 227.90 | 384.90 | 4.10 | 0.67 | 226.61 | 381.74 |
| RIL 13 | 266.49 | 491.43 | 12.37 | 20.43 | 2.20 | 2.00 | 18.31 | 9.25 | 341.10 | 572.80 | 9.06 | 0.98 | 328.73 | 552.37 |
| RIL 14 | 234.67 | 311.61 | 2.51 | 6.28 | 3.90 | 2.50 | 18.83 | 7.74 | 258.50 | 336.40 | 11.09 | 1.43 | 255.99 | 330.12 |
| RIL 15 | 323.10 | 429.16 | 1.74 | 2.14 | 2.50 | 1.50 | 17.28 | 8.08 | 320.80 | 458.10 | 9.20 | 1.14 | 319.06 | 455.96 |
| RIL 16 | 306.59 | 571.29 | 25.39 | 59.94 | 2.50 | 0.10 | 9.65 | 8.06 | 281.30 | 556.40 | 1.59 | 0.20 | 255.91 | 496.46 |
| RIL 17 | 247.96 | 506.25 | 3.08 | 24.56 | 2.30 | 0.10 | 7.73 | 6.45 | 242.30 | 538.90 | 1.28 | 0.20 | 239.22 | 514.34 |
| RIL 18 | 261.52 | 458.19 | 3.30 | 3.74 | 1.90 | 0.20 | 12.99 | 6.95 | 308.90 | 484.60 | 6.04 | 0.87 | 305.60 | 480.86 |
| RIL 19 | 220.04 | 372.24 | 0.98 | 3.25 | 2.70 | 1.00 | 11.90 | 6.43 | 278.10 | 489.50 | 5.47 | 0.85 | 277.12 | 486.25 |
| RIL 20 | 159.09 | 303.42 | 2.54 | 4.24 | 3.00 | 2.50 | 12.71 | 6.34 | 241.50 | 353.30 | 6.37 | 1.00 | 238.96 | 349.06 |
| RIL 21 | 207.92 | 397.00 | 2.24 | 3.44 | 2.50 | 2.10 | 18.62 | 8.07 | 296.10 | 460.50 | 10.55 | 1.31 | 293.86 | 457.06 |
| RIL 22 | 263.28 | 512.54 | 9.99 | 59.45 | 2.50 | 1.50 | 9.07 | 6.45 | 286.50 | 573.80 | 2.62 | 0.41 | 276.51 | 514.35 |
| RIL 23 | 282.91 | 559.63 | 2.34 | 24.52 | 1.90 | 0.50 | 10.25 | 6.50 | 256.80 | 514.80 | 3.75 | 0.58 | 254.46 | 490.28 |
| RIL 24 | 236.76 | 429.37 | 13.01 | 30.37 | 2.20 | 2.00 | 12.28 | 5.38 | 279.80 | 443.30 | 6.90 | 1.28 | 266.79 | 412.93 |
| RIL 25 | 267.78 | 434.77 | 5.03 | 16.16 | 2.80 | 0.50 | 14.62 | 7.20 | 316.50 | 513.00 | 7.42 | 1.03 | 311.47 | 496.84 |
| RIL 26 | 283.93 | 501.15 | 9.90 | 6.98 | 2.50 | 1.40 | 11.69 | 6.18 | 334.80 | 453.20 | 5.51 | 0.89 | 324.90 | 446.22 |
| RIL 27 | 218.40 | 382.48 | 1.52 | 1.79 | 2.90 | 2.00 | 13.62 | 6.54 | 278.50 | 392.30 | 7.08 | 1.08 | 276.98 | 390.51 |
| RIL 29 | 287.40 | 571.60 | 2.21 | 43.41 | 2.90 | 0.10 | 9.56 | 7.45 | 356.10 | 602.20 | 2.11 | 0.28 | 353.89 | 558.79 |
| RIL 30 | 208.62 | 494.19 | 37.43 | 75.95 | 1.70 | 0.00 | 4.59 | 4.02 | 256.30 | 490.10 | 0.57 | 0.14 | 218.87 | 414.15 |
| RIL 31 | 204.06 | 434.98 | 20.63 | 76.83 | 2.80 | 0.00 | 6.71 | 5.86 | 248.50 | 410.10 | 0.85 | 0.15 | 227.87 | 333.27 |
| RIL 32 | 136.94 | 304.98 | 1.91 | 8.54 | 4.00 | 3.00 | 18.26 | 7.15 | 282.40 | 414.20 | 11.11 | 1.55 | 280.49 | 405.66 |
| RIL 33 | 238.12 | 416.78 | 3.54 | 9.44 | 2.00 | 1.00 | 13.63 | 7.34 | 284.40 | 426.70 | 6.29 | 0.86 | 280.86 | 417.26 |
| RIL 34 | 282.79 | 499.34 | 4.24 | 12.85 | 2.10 | 0.50 | 14.04 | 7.46 | 292.30 | 463.70 | 6.58 | 0.88 | 288.06 | 450.85 |
| RIL 35 | 282.61 | 406.21 | 6.75 | 4.67 | 3.00 | 2.80 | 16.77 | 8.08 | 273.50 | 351.80 | 8.69 | 1.08 | 266.75 | 347.13 |
| RIL 36 | 254.55 | 464.07 | 2.28 | 2.97 | 2.50 | 1.90 | 12.15 | 8.40 | 328.30 | 476.10 | 3.75 | 0.45 | 326.02 | 473.13 |
| RIL 37 | 220.12 | 328.05 | 2.63 | 2.76 | 4.70 | 2.00 | 14.75 | 7.24 | 278.80 | 393.20 | 7.51 | 1.04 | 276.17 | 390.44 |
| RIL 38 | 228.33 | 404.21 | 2.28 | 10.88 | 3.00 | 1.30 | 8.94 | 7.38 | 305.70 | 470.30 | 1.56 | 0.21 | 303.42 | 459.42 |
| RIL 39 | 276.79 | 362.13 | 3.07 | 6.44 | 2.50 | 0.40 | 13.53 | 7.58 | 324.70 | 427.00 | 5.95 | 0.78 | 321.63 | 420.56 |
| RIL 40 | 224.17 | 370.01 | 2.66 | 4.48 | 2.60 | 0.30 | 8.63 | 5.83 | 309.00 | 336.60 | 2.80 | 0.48 | 306.34 | 332.12 |
| RIL 41 | 190.37 | 423.49 | 6.18 | 13.43 | 3.00 | 0.30 | 8.90 | 6.92 | 278.80 | 441.90 | 1.98 | 0.29 | 272.62 | 428.47 |
| RIL 42 | 245.54 | 389.91 | 5.74 | 3.17 | 3.50 | 2.50 | 19.70 | 7.65 | 268.00 | 346.00 | 12.05 | 1.58 | 262.26 | 342.83 |
| RIL 43 | 244.00 | 360.91 | 2.69 | 5.30 | 2.50 | 2.00 | 15.15 | 6.48 | 312.90 | 435.90 | 8.67 | 1.34 | 310.21 | 430.60 |
| RIL 44 | 211.13 | 411.04 | 7.80 | 8.80 | 2.80 | 1.20 | 9.09 | 6.56 | 303.10 | 499.00 | 2.53 | 0.39 | 295.30 | 490.20 |
| RIL 45 | 315.62 | 490.17 | 8.82 | 9.23 | 2.20 | 0.50 | 16.62 | 9.20 | 630.00 | 527.60 | 7.42 | 0.81 | 621.18 | 518.37 |
| RIL 46 | 328.25 | 575.57 | 9.30 | 33.48 | 2.00 | 0.30 | 9.45 | 6.72 | 408.40 | 592.00 | 2.73 | 0.41 | 399.10 | 558.52 |
| RIL 47 | 234.48 | 355.58 | 2.66 | 2.96 | 3.80 | 1.00 | 8.48 | 6.08 | 273.90 | 262.70 | 2.40 | 0.39 | 271.24 | 259.74 |
| RIL 48 | 288.59 | 463.86 | 7.01 | 6.84 | 2.20 | 0.40 | 12.53 | 6.98 | 295.00 | 442.00 | 5.55 | 0.80 | 287.99 | 435.16 |
| RIL 49 | 247.41 | 392.70 | 3.55 | 9.50 | 1.80 | 0.30 | 11.86 | 6.57 | 361.00 | 522.60 | 5.29 | 0.81 | 357.45 | 513.10 |
| RIL 50 | 147.58 | 375.35 | 2.70 | 3.70 | 2.50 | 0.50 | 25.00 | 5.82 | 286.10 | 423.30 | 19.18 | 3.30 | 283.40 | 419.60 |
| RIL 51 | 200.11 | 355.47 | 0.73 | 3.26 | 3.20 | 2.50 | 25.80 | 9.81 | 1.40 | 406.80 | 15.99 | 1.63 | * | 403.54 |
| RIL 52 | 158.76 | 280.99 | 8.17 | 22.77 | 3.50 | 3.00 | 11.82 | 5.16 | 291.70 | 283.10 | 6.66 | 1.29 | 283.53 | 260.33 |
| RIL 53 | 165.30 | 327.03 | 2.03 | 2.10 | 2.50 | 1.50 | 12.27 | 6.23 | 296.60 | 430.90 | 6.04 | 0.97 | 294.57 | 428.80 |
| RIL 54 | 311.36 | 743.86 | 11.21 | 71.81 | 1.00 | 0.10 | 7.77 | 6.34 | 381.90 | 768.40 | 1.43 | 0.23 | 370.69 | 696.59 |
| RIL 55 | 225.14 | 520.04 | 0.77 | 14.49 | 2.00 | 0.20 | 13.05 | 8.15 | 299.10 | 517.30 | 4.90 | 0.60 | 298.33 | 502.81 |
| RIL 56 | 219.95 | 461.13 | 7.46 | 7.35 | 3.50 | 1.30 | 17.14 | 8.50 | 327.20 | 482.20 | 8.64 | 1.02 | 319.74 | 474.85 |
| RIL 57 | 251.23 | 475.40 | 43.03 | 20.86 | 3.70 | 1.50 | 12.78 | 8.35 | 313.50 | 422.30 | 4.43 | 0.53 | 270.47 | 401.44 |
| RIL 58 | 195.38 | 384.55 | 6.53 | 12.39 | 2.50 | 1.50 | 20.73 | 9.35 | 284.80 | 400.60 | 11.38 | 1.22 | 278.27 | 388.21 |
| RIL 59 | 201.81 | 443.43 | 22.90 | 18.87 | 2.50 | 0.50 | 12.89 | 8.34 | 319.10 | 495.10 | 4.55 | 0.55 | 296.20 | 476.23 |
| RIL 60 | 204.71 | 464.12 | 35.82 | 50.40 | 2.00 | 0.20 | 8.07 | 5.89 | 302.40 | 493.30 | 2.18 | 0.37 | 266.58 | 442.90 |
| RIL 61 | 119.10 | 349.01 | 1.13 | 8.80 | 2.60 | 0.10 | 4.69 | 4.05 | 248.00 | 268.60 | 0.64 | 0.16 | 246.87 | 259.80 |
| RIL 62 | 118.91 | 307.83 | 5.03 | 5.56 | 4.75 | 3.50 | 14.10 | 6.63 | 279.20 | 325.30 | 7.47 | 1.13 | 274.17 | 319.74 |
| RIL 63 | 145.94 | 357.35 | 2.84 | 6.22 | 3.00 | 1.20 | 11.37 | 6.92 | 280.00 | 426.90 | 4.45 | 0.64 | 277.16 | 420.68 |
| RIL 64 | 262.13 | 632.36 | 44.96 | 107.28 | 2.00 | 0.50 | 9.75 | 6.07 | 365.30 | 665.50 | 3.68 | 0.61 | 320.34 | 558.22 |
| RIL 65 | 144.25 | 299.37 | 15.39 | 10.85 | 3.00 | 1.50 | 7.91 | 5.68 | 241.60 | 281.30 | 2.23 | 0.39 | 226.21 | 270.45 |
| RIL 66 | 161.75 | 303.42 | 1.12 | 1.71 | 4.00 | 3.00 | 15.52 | 7.10 | 303.70 | 371.90 | 8.42 | 1.19 | 302.58 | 370.19 |
| RIL 67 | 178.30 | 379.40 | 8.05 | 3.66 | 4.50 | 2.50 | 21.74 | 9.52 | 264.60 | 286.10 | 12.22 | 1.28 | 256.55 | 282.44 |

| RIL/var | Cl1 | Cl2 | Na1 | Na2 | Score1 | Score2 | SFW | SDW | K1+Na1 | K2+Na2 | Water | w/gdw | K1 | K2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RIL 68 | 178.24 | 578.14 | 15.08 | 57.11 | 1.50 | 0.10 | 5.68 | 4.27 | 287.60 | 623.80 | 1.41 | 0.33 | 272.52 | 566.69 |
| RIL 69 | 214.88 | 553.96 | 1.15 | 12.42 | 2.00 | 0.10 | 11.60 | 7.50 | 288.80 | 569.90 | 4.10 | 0.55 | 287.65 | 557.48 |
| RIL 70 | 208.75 | 489.37 | 2.31 | 39.97 | 2.50 | 0.10 | 7.15 | 4.96 | 287.70 | 489.90 | 2.19 | 0.44 | 285.39 | 449.93 |
| RIL 71 | 117.38 | 323.81 | 17.44 | 18.63 | 2.80 | 0.20 | 5.83 | 4.73 | 248.10 | 321.40 | 1.10 | 0.23 | 230.66 | 302.77 |
| RIL 72 | 246.89 | 546.55 | 7.05 | 61.92 | 1.20 | 3.00 | 9.68 | 7.38 | 293.40 | 534.50 | 2.30 | 0.31 | 286.35 | 472.58 |
| RIL 73 | 125.38 | 421.23 | 8.40 | 13.57 | 3.50 | 1.50 | 14.13 | 5.69 | 297.50 | 449.40 | 8.44 | 1.48 | 289.10 | 435.83 |
| RIL 74 | 153.68 | 381.73 | 1.27 | 6.04 | 4.50 | 2.20 | 13.28 | 6.79 | 361.00 | 544.50 | 6.49 | 0.96 | 359.73 | 538.46 |
| RIL75 | 160.95 | 453.92 | 22.15 | 37.58 | 2.00 | 0.50 | 8.01 | 5.59 | 280.30 | 461.60 | 2.42 | 0.43 | 258.15 | 424.02 |
| RIL76 | 288.23 | 738.17 | 12.32 | 50.89 | 2.50 | 0.20 | 11.94 | 9.10 | 324.30 | 674.00 | 2.84 | 0.31 | 311.98 | 623.11 |
| RIL 78 | 247.79 | 575.43 | 4.83 | 88.04 | 2.50 | 0.10 | 8.13 | 6.57 | 280.30 | 541.60 | 1.56 | 0.24 | 275.47 | 453.56 |
| RIL 79 | 185.90 | 617.22 | 0.42 | 24.42 | 2.00 | 0.10 | 8.10 | 5.93 | 1.20 | 661.20 | 2.17 | 0.37 | * | 636.78 |
| RIL 80 | 149.00 | 395.04 | 4.22 | 4.38 | 2.50 | 1.90 | 14.66 | 6.41 | 335.50 | 447.00 | 8.25 | 1.29 | 331.28 | 442.62 |
| RIL 81 | 215.28 | 579.09 | 1.17 | 15.83 | 2.20 | 0.20 | 11.30 | 5.20 | 4.50 | 665.40 | 6.10 | 1.17 | * | 649.57 |
| RIL 82 | 170.13 | 408.78 | 4.88 | 4.86 | 2.90 | 0.50 | 20.55 | 9.24 | 318.10 | 481.60 | 11.31 | 1.22 | 313.22 | 476.74 |
| RIL 83 | 160.35 | 447.28 | 3.63 | 6.64 | 3.00 | 2.50 | 17.45 | 8.16 | 316.50 | 496.30 | 9.29 | 1.14 | 312.87 | 489.66 |
| RIL 84 | 196.91 | 457.69 | 2.85 | 68.29 | 1.50 | 1.00 | 8.50 | 6.90 | 248.70 | 574.70 | 1.60 | 0.23 | 245.85 | 506.41 |
| RIL 85 | 187.25 | 502.65 | 17.34 | 112.78 | 1.50 | 0.30 | 11.73 | 6.58 | 312.40 | 495.10 | 5.15 | 0.78 | 295.06 | 382.32 |
| RIL 86 | 192.85 | 431.18 | 1.92 | 15.99 | 3.80 | 2.50 | 13.25 | 8.01 | 206.40 | 416.40 | 5.24 | 0.65 | 204.48 | 400.41 |
| RIL 87 | 131.25 | 362.15 | 3.86 | 12.50 | 2.90 | 0.50 | 9.45 | 7.08 | 250.60 | 359.40 | 2.37 | 0.33 | 246.74 | 346.90 |
| RIL 88 | 58.45 | 122.37 | 2.42 | 3.22 | 3.00 | 2.80 | 10.04 | 4.11 | 233.00 | 330.20 | 5.93 | 1.44 | 230.58 | 326.98 |
| RIL 89 | 171.44 | 503.84 | 19.42 | 115.97 | 2.50 | 1.00 | 7.03 | 5.48 | 255.50 | 512.00 | 1.55 | 0.28 | 236.08 | 396.03 |
| RIL 90 | 194.96 | 513.41 | 1.10 | 6.29 | 2.20 | 0.20 | 14.96 | 6.89 | 279.80 | 487.80 | 8.07 | 1.17 | 278.70 | 481.51 |
| RIL 91 | 140.84 | 364.31 | 2.50 | 3.02 | 3.00 | 2.50 | 12.51 | 5.75 | 284.50 | 431.80 | 6.76 | 1.18 | 282.00 | 428.78 |
| RIL 92 | 131.54 | 356.55 | 2.98 | 7.03 | 3.00 | 1.80 | 14.99 | 6.35 | 270.60 | 430.00 | 8.64 | 1.36 | 267.62 | 422.97 |
| RIL 93 | 175.39 | 393.46 | 11.27 | 4.10 | 2.50 | 1.50 | 11.92 | 5.53 | 258.20 | 420.10 | 6.39 | 1.16 | 246.93 | 416.00 |
| RIL 94 | 140.68 | 381.41 | 11.10 | 11.12 | 2.00 | 1.30 | 9.77 | 4.84 | 233.40 | 343.60 | 4.93 | 1.02 | 222.30 | 332.48 |
| RIL 95 | 205.74 | 490.86 | 11.54 | 15.66 | 2.20 | 1.00 | 8.30 | 6.27 | 271.20 | 433.30 | 2.03 | 0.32 | 259.66 | 417.64 |
| RIL 96 | 172.23 | 381.45 | 14.02 | 43.88 | 4.00 | 3.10 | 23.03 | 10.25 | 226.60 | 360.50 | 12.78 | 1.25 | 212.58 | 316.62 |
| RIL 97 | 198.13 | 496.50 | 26.36 | 65.90 | 2.50 | 0.50 | 12.57 | 9.02 | 301.70 | 490.80 | 3.55 | 0.39 | 275.34 | 424.90 |
| RIL 98 | 182.76 | 370.72 | 3.88 | 7.13 | 4.60 | 4.00 | 30.83 | 12.73 | 237.70 | 405.90 | 18.10 | 1.42 | 233.82 | 398.77 |
| RIL 99 | 234.08 | 443.04 | 7.55 | 4.63 | 2.70 | 3.00 | 15.35 | 9.31 | 319.20 | 488.60 | 6.04 | 0.65 | 311.65 | 483.97 |
| RIL 100 | 216.45 | 434.11 | 6.97 | 12.27 | 3.00 | 0.40 | 13.34 | 8.08 | 325.90 | 500.30 | 5.26 | 0.65 | 318.93 | 488.03 |
| RIL 101 | 151.78 | 360.43 | 4.27 | 3.42 | 4.00 | 2.50 | 16.20 | 7.41 | 258.50 | 361.20 | 8.79 | 1.19 | 254.23 | 357.78 |
| RIL 102 | 138.65 | 344.81 | 1.29 | 3.14 | 3.00 | 2.00 | 20.70 | 8.61 | 255.30 | 313.40 | 12.09 | 1.40 | 254.01 | 310.26 |
| RIL 103 | 145.05 | 353.78 | 10.44 | 22.34 | 3.00 | 3.50 | 18.26 | 7.08 | 262.10 | 346.10 | 11.18 | 1.58 | 251.66 | 323.76 |
| RIL 104 | 212.63 | 447.83 | 4.80 | 8.75 | 2.50 | 1.40 | 15.61 | 7.13 | 314.40 | 450.90 | 8.48 | 1.19 | 309.60 | 442.15 |
| RIL 105 | 199.81 | 542.07 | 1.62 | 11.25 | 2.00 | 1.00 | 11.43 | 5.67 | 263.50 | 581.90 | 5.76 | 1.02 | 261.88 | 570.65 |
| RIL 106 | 249.80 | 337.91 | 33.79 | 5.07 | 2.00 | 0.20 | 12.76 | 8.15 | 332.50 | 336.60 | 4.61 | 0.57 | 298.71 | 331.53 |
| RIL 107 | 211.40 | 500.78 | 0.99 | 2.50 | 3.00 | 2.00 | 21.54 | 9.87 | 280.20 | 458.60 | 11.67 | 1.18 | 279.21 | 456.10 |
| RIL 108 | 188.59 | 354.55 | 9.55 | 3.58 | 4.00 | 3.00 | 24.74 | 10.18 | 297.60 | 279.20 | 14.56 | 1.43 | 288.05 | 275.62 |
| RIL 109 | 217.40 | 449.92 | 24.33 | 12.43 | 2.10 | 2.00 | 11.47 | 6.47 | 285.50 | 434.30 | 5.00 | 0.77 | 261.17 | 421.87 |
| RIL 110 | 234.84 | 494.71 | 30.21 | 39.05 | 2.20 | 0.30 | 8.90 | 6.58 | 297.30 | 459.00 | 2.32 | 0.35 | 267.09 | 419.95 |
| RIL 111 | 198.96 | 355.96 | 2.70 | 7.11 | 2.90 | 0.30 | 10.99 | 6.32 | 238.00 | 297.40 | 4.67 | 0.74 | 235.30 | 290.29 |
| RIL 112 | 191.05 | 449.42 | 2.84 | 8.18 | 2.70 | 0.40 | 16.84 | 7.35 | 312.00 | 429.10 | 9.49 | 1.29 | 309.16 | 420.92 |
| RIL 113 | 164.88 | 377.69 | 2.08 | 2.64 | 2.50 | 2.10 | 15.40 | 6.30 | 326.60 | 412.50 | 9.10 | 1.44 | 324.52 | 409.86 |
| RIL 115 | 245.60 | 624.25 | 4.97 | 78.71 | 1.50 | 1.00 | 9.01 | 5.68 | 557.10 | 619.50 | 3.33 | 0.59 | 552.13 | 540.79 |
| RIL 116 | 210.01 | 516.54 | 4.36 | 10.64 | 2.00 | 1.00 | 10.54 | 6.06 | 296.70 | 457.00 | 4.48 | 0.74 | 292.34 | 446.36 |
| RIL 117 | 212.06 | 496.40 | 6.79 | 5.72 | 3.60 | 2.20 | 11.58 | 6.98 | 330.20 | 549.50 | 4.60 | 0.66 | 323.41 | 543.78 |
| RIL 118 | 223.11 | 494.20 | 8.30 | 3.93 | 2.00 | 0.20 | 10.80 | 6.58 | 309.70 | 492.80 | 4.22 | 0.64 | 301.40 | 488.87 |
| RIL 119 | 247.64 | 528.91 | 19.09 | 19.65 | 2.00 | 0.30 | 20.05 | 9.84 | 326.10 | 497.20 | 10.21 | 1.04 | 307.01 | 477.55 |
| RIL 120 | 212.51 | 510.68 | 2.69 | 5.07 | 3.00 | 0.50 | 13.68 | 6.74 | 309.30 | 499.60 | 6.94 | 1.03 | 306.61 | 494.53 |
| RIL 121 | 250.95 | 566.88 | 17.98 | 52.87 | 2.60 | 0.10 | 7.77 | 5.87 | 351.20 | 543.80 | 1.90 | 0.32 | 333.22 | 490.93 |
| RIL 122 | 187.19 | 583.25 | 1.64 | 35.85 | 2.80 | 0.10 | 10.22 | 5.03 | 292.90 | 538.50 | 5.19 | 1.03 | 291.26 | 502.65 |
| RIL 123 | 161.90 | 550.07 | 1.27 | 3.54 | 2.60 | 2.50 | 13.15 | 8.17 | 263.50 | 530.50 | 4.98 | 0.61 | 262.23 | 526.96 |
| RIL 124 | 256.93 | 425.65 | 40.65 | 36.88 | 2.00 | 2.50 | 17.74 | 7.15 | 293.90 | 408.80 | 10.59 | 1.48 | 253.25 | 371.92 |
| RIL 125 | 304.48 | 481.85 | 2.52 | 11.06 | 2.00 | 0.50 | 13.09 | 6.07 | 322.50 | 615.70 | 7.02 | 1.16 | 319.98 | 604.64 |
| RIL 126 | 300.24 | 417.44 | 2.66 | 12.68 | 2.70 | 1.00 | 11.00 | 6.01 | 264.30 | 431.70 | 4.99 | 0.83 | 261.64 | 419.02 |
| RIL 127 | 304.41 | 521.68 | 22.39 | 34.47 | 2.50 | 0.50 | 8.26 | 5.67 | 270.10 | 462.40 | 2.59 | 0.46 | 247.71 | 427.93 |
| RIL 128 | 399.74 | 556.69 | 9.04 | 84.55 | 2.00 | 0.10 | 11.73 | 8.32 | 320.00 | 527.10 | 3.41 | 0.41 | 310.96 | 442.55 |
| RIL 129 | 289.38 | 461.45 | 19.60 | 26.47 | 3.00 | 1.00 | 12.80 | 7.57 | 288.70 | 458.10 | 5.23 | 0.69 | 269.10 | 431.63 |
| RIL 130 | 338.43 | 616.66 | 10.29 | 71.41 | 2.50 | 0.60 | 11.07 | 7.71 | 248.20 | 591.50 | 3.36 | 0.44 | 237.91 | 520.09 |
| RIL 131 | 240.70 | 465.70 | 1.55 | 18.03 | 2.40 | 0.20 | 7.19 | 5.25 | 261.30 | 423.10 | 1.94 | 0.37 | 259.75 | 405.07 |
| RIL 132 | 263.09 | 416.35 | 15.55 | 6.67 | 3.50 | 2.70 | 14.66 | 6.20 | 249.50 | 380.50 | 8.46 | 1.36 | 233.95 | 373.83 |
| RIL 133 | 204.59 | 405.71 | 0.67 | 2.35 | 2.60 | 0.40 | 14.07 | 6.67 | 291.30 | 444.90 | 7.40 | 1.11 | 290.63 | 442.55 |
| RIL 134 | 248.97 | 531.55 | 4.21 | 16.27 | 2.50 | 1.50 | 13.38 | 6.17 | 274.60 | 552.30 | 7.21 | 1.17 | 270.39 | 536.03 |
| RIL 135 | 332.38 | 504.78 | 4.48 | 5.30 | 1.00 | 1.00 | 11.93 | 7.30 | 308.60 | 614.10 | 4.63 | 0.63 | 304.12 | 608.80 |
| RIL 136 | 205.32 | 426.27 | 1.91 | 6.45 | 2.70 | 1.00 | 13.79 | 6.32 | 280.60 | 469.90 | 7.47 | 1.18 | 278.69 | 463.45 |

| RIL/var | Cl1 | Cl2 | Na1 | Na2 | Score1 | Score2 | SFW | SDW | K1+Na1 | K2+Na2 | Water | w/gdw | K1 | K2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RIL 137 | 330.64 | 539.93 | 4.63 | 21.52 | 3.50 | 2.50 | 12.24 | 5.79 | 246.90 | 551.30 | 6.45 | 1.11 | 242.27 | 529.78 |
| RIL 138 | 322.08 | 521.72 | 1.38 | 13.92 | 2.00 | 0.30 | 13.62 | 7.03 | 312.80 | 554.30 | 6.59 | 0.94 | 311.42 | 540.38 |
| RIL 139 | 328.11 | 487.88 | 6.80 | 9.51 | 2.50 | 0.20 | 16.17 | 8.28 | 333.10 | 553.00 | 7.89 | 0.95 | 326.30 | 543.49 |
| RIL 140 | 233.25 | 770.21 | 5.98 | 83.78 | 2.00 | 0.00 | 6.81 | 5.40 | 257.80 | 703.30 | 1.41 | 0.26 | 251.82 | 619.52 |
| RIL 141 | 280.24 | 444.28 | 3.88 | 6.42 | 1.80 | 0.20 | 8.08 | 4.50 | 295.90 | 420.90 | 3.58 | 0.80 | 292.02 | 414.48 |
| RIL 142 | 197.58 | 377.80 | 11.75 | 19.26 | 3.50 | 2.00 | 13.92 | 6.59 | 233.60 | 399.10 | 7.33 | 1.11 | 221.85 | 379.84 |
| RIL 143 | 262.02 | 458.69 | 1.53 | 9.43 | 2.20 | 3.00 | 9.83 | 6.71 | 280.00 | 431.50 | 3.12 | 0.46 | 278.47 | 422.07 |
| RIL 144 | 284.49 | 477.90 | 4.69 | 5.20 | 2.00 | 1.00 | 16.13 | 7.78 | 280.50 | 456.80 | 8.35 | 1.07 | 275.81 | 451.60 |
| RIL 145 | 272.50 | 427.37 | 2.69 | 8.05 | 2.00 | 2.00 | 13.30 | 5.95 | 308.30 | 507.50 | 7.35 | 1.24 | 305.61 | 499.45 |
| RIL 146 | 295.32 | 471.61 | 15.80 | 15.94 | 4.50 | 2.00 | 10.05 | 6.94 | 276.00 | 474.50 | 3.11 | 0.45 | 260.20 | 458.56 |
| RIL 147 | 272.07 | 553.07 | 18.07 | 56.33 | 2.10 | 0.10 | 7.51 | 6.27 | 220.20 | 445.00 | 1.24 | 0.20 | 202.13 | 388.67 |
| RIL 148 | 379.07 | 545.87 | 3.87 | 17.45 | 1.20 | 0.00 | 7.34 | 5.80 | 334.30 | 507.70 | 1.54 | 0.27 | 330.43 | 490.25 |
| RIL 150 | 355.46 | 565.59 | 25.80 | 108.38 | 2.70 | 0.30 | 10.48 | 7.64 | 305.30 | 596.70 | 2.84 | 0.37 | 279.50 | 488.32 |
| RIL 151 | 277.37 | 516.80 | 12.71 | 23.51 | 2.50 | 1.00 | 8.52 | 6.00 | 293.20 | 496.00 | 2.52 | 0.42 | 280.49 | 472.49 |
| RIL 152 | 321.18 | 552.64 | 1.94 | 31.12 | 2.60 | 0.10 | 7.60 | 6.54 | 304.60 | 587.50 | 1.06 | 0.16 | 302.66 | 556.38 |
| RIL 153 | 285.18 | 387.77 | 0.98 | 4.52 | 2.70 | 2.20 | 16.05 | 7.10 | 246.30 | 366.40 | 8.95 | 1.26 | 245.32 | 361.88 |
| RIL 154 | 319.06 | 472.80 | 19.94 | 20.17 | 2.50 | 3.00 | 10.36 | 5.91 | 280.50 | 440.70 | 4.45 | 0.75 | 260.56 | 420.53 |
| RIL 155 | 242.62 | 364.04 | 6.21 | 25.56 | 2.00 | 1.50 | 7.98 | 5.77 | 264.70 | 444.20 | 2.21 | 0.38 | 258.49 | 418.64 |
| RIL 158 | 313.95 | 516.86 | 5.85 | 23.23 | 1.50 | 2.00 | 12.90 | 7.00 | 311.70 | 552.60 | 5.90 | 0.84 | 305.85 | 529.37 |
| RIL 160 | 374.81 | 598.73 | 12.01 | 9.98 | 2.00 | 0.50 | 11.63 | 7.98 | 258.60 | 557.30 | 3.65 | 0.46 | 246.59 | 547.32 |
| RIL 161 | 252.85 | 401.94 | 4.04 | 12.75 | 3.00 | 2.00 | 17.76 | 8.01 | 269.00 | 421.80 | 9.75 | 1.22 | 264.96 | 409.05 |
| RIL 162 | 270.83 | 420.06 | 20.50 | 25.73 | 2.00 | 0.10 | 6.82 | 5.23 | 292.00 | 440.80 | 1.59 | 0.30 | 271.50 | 415.07 |
| RIL 163 | 255.95 | 486.20 | 27.52 | 79.02 | 2.50 | 0.30 | 13.68 | 8.46 | 302.00 | 471.10 | 5.22 | 0.62 | 274.48 | 392.08 |
| RIL 164 | 307.27 | 440.51 | 7.00 | 17.34 | 2.20 | 3.00 | 10.53 | 6.61 | 285.60 | 459.30 | 3.92 | 0.59 | 278.60 | 441.96 |
| RIL 165 | 286.70 | 275.14 | 1.93 | 16.86 | 3.20 | 3.00 | 24.55 | 9.90 | 269.00 | 248.20 | 14.65 | 1.48 | 267.07 | 231.34 |
| RIL 166 | 255.72 | 356.26 | 1.55 | 3.71 | 3.20 | 2.50 | 15.75 | 6.35 | 309.50 | 428.20 | 9.40 | 1.48 | 307.95 | 424.49 |
| RIL 167 | 177.31 | 328.80 | 1.56 | 1.88 | 4.30 | 2.00 | 19.05 | 7.51 | 251.80 | 386.20 | 11.54 | 1.54 | 250.24 | 384.32 |
| RIL 168 | 235.71 | 348.76 | 2.30 | 8.85 | 4.50 | 3.10 | 17.53 | 8.52 | 248.40 | 415.00 | 9.01 | 1.06 | 246.10 | 406.15 |
| RIL 169 | 315.79 | 500.71 | 3.87 | 21.86 | 2.00 | 1.50 | 14.63 | 6.51 | 307.10 | 569.70 | 8.12 | 1.25 | 303.23 | 547.84 |
| RIL 170 | 257.87 | 342.04 | 2.30 | 2.97 | 2.50 | 1.00 | 12.65 | 6.08 | 241.70 | 361.90 | 6.57 | 1.08 | 239.40 | 358.93 |
| RIL 171 | 225.09 | 329.59 | 15.89 | 55.43 | 3.00 | 1.30 | 14.19 | 6.24 | 207.40 | 359.30 | 7.95 | 1.27 | 191.51 | 303.87 |
| RIL 172 | 221.05 | 391.12 | 3.71 | 4.46 | 2.60 | 3.00 | 15.72 | 6.79 | 296.60 | 517.40 | 8.93 | 1.32 | 292.89 | 512.94 |
| RIL 173 | 328.66 | 432.59 | 9.15 | 3.55 | 2.50 | 1.30 | 12.18 | 7.09 | 283.00 | 300.10 | 5.09 | 0.72 | 273.85 | 296.55 |
| RIL 174 | 280.29 | 477.27 | 1.93 | 5.54 | 3.00 | 1.50 | 12.86 | 8.51 | 334.80 | 510.90 | 4.35 | 0.51 | 332.87 | 505.36 |
| RIL 175 | 295.32 | 421.94 | 2.05 | 28.06 | 1.90 | 0.00 | 6.90 | 5.93 | 263.40 | 493.50 | 0.97 | 0.16 | 261.35 | 465.44 |
| RIL 176 | 236.93 | 405.30 | 1.52 | 6.29 | 3.00 | 1.30 | 11.77 | 7.06 | 264.70 | 458.10 | 4.71 | 0.67 | 263.18 | 451.81 |
| RIL 177 | 202.39 | 373.97 | 4.64 | 24.04 | 3.00 | 2.50 | 11.63 | 5.72 | 255.20 | 413.90 | 5.91 | 1.03 | 250.56 | 389.86 |
| CO-39 | 289.27 | 400.07 | 4.27 | 12.02 | 3.50 | 2.00 | 17.92 | 7.18 | 266.70 | 444.60 | 10.74 | 1.50 | 262.43 | 432.58 |
| CO-39 | 187.36 | 351.94 | 5.04 | 7.76 | 4.00 | 2.90 | 16.46 | 6.29 | 281.90 | 368.50 | 10.17 | 1.62 | 276.86 | 360.74 |
| CO-39 | 204.07 | 410.68 | 6.75 | 6.42 | 3.00 | 1.50 | 17.65 | 8.24 | 252.00 | 372.20 | 9.41 | 1.14 | 245.25 | 365.78 |
| CO-39 | 159.86 | 456.31 | 3.64 | 7.87 | 3.00 | 2.00 | 12.38 | 6.44 | 263.20 | 428.90 | 5.94 | 0.92 | 259.56 | 421.03 |
| CO-39 | 211.07 | 401.34 | 2.30 | 5.99 | 3.00 | 1.80 | 13.83 | 6.50 | 270.70 | 397.70 | 7.33 | 1.13 | 268.40 | 391.71 |
| CO-39 | 174.64 | 313.54 | 3.71 | 3.92 | 3.50 | 2.50 | 13.35 | 5.69 | 279.80 | 347.70 | 7.66 | 1.35 | 276.09 | 343.78 |
| CO-39 | 228.78 | 416.61 | 3.30 | 6.03 | 2.50 | 1.00 | 16.50 | 6.66 | 268.50 | 352.90 | 9.84 | 1.48 | 265.20 | 346.87 |
| CO-39 | 251.79 | 381.46 | 3.46 | 7.82 | 3.20 | 1.50 | 17.13 | 5.53 | 270.40 | 364.20 | 11.60 | 2.10 | 266.94 | 356.38 |
| CO-39 | 261.48 | 402.74 | 5.01 | 2.03 | 3.20 | 2.00 | 15.33 | 5.48 | 291.70 | 377.10 | 9.85 | 1.80 | 286.69 | 375.07 |
| CO-39 | 219.49 | 342.95 | 4.52 | 3.89 | 2.90 | 2.00 | 18.72 | 7.12 | 271.20 | 328.60 | 11.60 | 1.63 | 266.68 | 324.71 |
| MO | 183.53 | 428.87 | 12.70 | 44.27 | 2.00 | 0.20 | 2.99 | 2.13 | 223.20 | 345.00 | 0.86 | 0.29 | 210.50 | 300.73 |
| MO | 170.43 | 417.74 | 11.92 | 98.83 | 3.00 | 3.00 | 4.08 | 3.53 | 236.70 | 424.80 | 0.55 | 0.13 | 224.78 | 325.97 |
| MO | 195.40 | 481.59 | 10.25 | 84.47 | 2.50 | 0.20 | 5.19 | 4.68 | 207.60 | 544.70 | 0.51 | 0.11 | 197.35 | 460.23 |
| MO | 192.38 | 536.25 | 9.10 | 66.10 | 0.75 | 0.00 | 11.22 | 3.93 | 245.90 | 464.30 | 7.29 | 1.85 | 236.80 | 398.20 |
| MO | 200.38 | 336.92 | 16.03 | 24.52 | 2.60 | 0.20 | 4.72 | 2.58 | 225.10 | 268.10 | 2.14 | 0.83 | 209.07 | 243.58 |
| MO | 265.91 | 356.09 | 31.15 | 24.52 | 2.50 | 0.10 | 7.32 | 5.56 | 213.70 | 268.10 | 1.76 | 0.32 | 182.55 | 243.58 |
| MO | 211.32 | 364.83 | 13.93 | 38.46 | 2.80 | 1.00 | 5.75 | 4.08 | 241.30 | 274.80 | 1.67 | 0.41 | 227.37 | 236.34 |
| MO | 192.30 | 383.81 | 11.83 | 36.77 | 1.50 | 0.10 | 4.40 | 2.95 | 208.80 | 419.00 | 1.45 | 0.33 | 196.97 | 382.23 |
| MO | 176.34 | 354.19 | 9.20 | 39.84 | 3.00 | 2.00 | 6.88 | 1.56 | 227.90 | 437.00 | 5.32 | 3.41 | 218.70 | 397.16 |
| MO | 119.45 | 342.37 | 4.92 | 26.86 | 2.50 | 0.50 | 7.15 | 4.57 | 199.70 | 417.80 | 2.58 | 0.56 | 194.78 | 390.94 |
| MR | 163.53 | 238.47 | 6.83 | 7.71 | 3.00 | 3.00 | 10.48 | 6.44 | 210.10 | 244.30 | 4.04 | 0.63 | 203.27 | 236.59 |
| MR | 218.83 | 214.45 | 15.06 | 12.35 | 3.50 | 3.00 | 16.99 | 7.98 | 192.90 | 340.10 | 9.01 | 1.13 | 177.84 | 327.75 |
| MR | 153.03 | 231.44 | 5.29 | 10.30 | 3.50 | 3.00 | 14.26 | 6.53 | 226.80 | 270.10 | 7.73 | 1.18 | 221.51 | 259.80 |
| MR | 202.88 | 265.24 | 11.02 | 12.44 | 3.50 | 3.30 | 21.76 | 7.01 | 204.60 | 285.70 | 14.75 | 2.10 | 193.58 | 273.26 |
| MR | 181.18 | 264.86 | 15.34 | 24.38 | 4.00 | 4.00 | 18.18 | 7.90 | 202.60 | 397.30 | 10.28 | 1.30 | 187.26 | 372.92 |
| MR | 152.31 | 257.37 | 8.86 | 13.15 | 2.90 | 3.50 | 13.01 | 6.34 | 223.20 | 260.40 | 6.67 | 1.05 | 214.34 | 247.25 |
| MR | 170.65 | 215.00 | 8.24 | 17.03 | 2.80 | 0.50 | 20.00 | 3.83 | 233.60 | 253.20 | 16.17 | 4.22 | 225.36 | 236.17 |
| MR | 211.00 | 199.53 | 18.89 | 26.96 | 3.00 | 3.00 | 22.45 | 7.52 | 189.10 | 464.30 | 14.93 | 1.99 | 170.21 | 437.34 |
| MR | 185.90 | 216.79 | 7.18 | 9.00 | 3.50 | 4.00 | 16.77 | 7.75 | 220.30 | 260.00 | 9.02 | 1.16 | 213.12 | 251.00 |
| MR | 162.57 | 209.69 | 4.71 | 9.87 | 3.50 | 4.50 | 18.93 | 6.43 | 214.30 | 277.00 | 12.50 | 1.94 | 209.59 | 267.13 |

**Appendix 2. Means for experiment 10**

| RIL | Score1 | Score2 | SFW | SDW | w/gdw | Na | K | Mg | Sum | Ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 2.52 | 0.15 | 5.75 | 2.26 | 1.76 | 113.22 | 480.08 | 95.61 | 593.30 | 4.57 |
| 5 | 2.65 | 1.30 | 12.23 | 2.73 | 6.35 | 14.08 | 250.25 | 32.51 | 264.35 | 34.18 |
| 6 | 0.42 | 0.05 | 0.85 | 0.38 | 2.02 | 117.78 | 308.80 | 97.02 | 426.55 | 2.78 |
| 9 | 0.85 | 0.18 | 2.33 | 0.88 | 1.34 | 14.19 | 367.73 | 66.84 | 381.90 | 26.66 |
| 10 | 1.62 | 0.62 | 3.35 | 1.16 | 1.64 | 15.07 | 373.82 | 30.75 | 388.90 | 37.90 |
| 11 | 1.02 | 0.28 | 2.22 | 0.60 | 2.67 | 18.65 | 260.76 | 33.58 | 279.40 | 15.36 |
| 13 | 0.78 | 0.12 | 1.93 | 0.84 | 1.64 | 115.99 | 178.21 | 57.38 | 294.20 | 1.04 |
| 14 | 1.25 | 0.10 | 2.43 | 0.97 | 1.55 | 27.21 | 309.10 | 64.00 | 336.30 | 13.13 |
| 15 | 1.88 | 0.18 | 3.53 | 1.28 | 1.77 | 20.87 | 273.91 | 66.92 | 294.78 | 14.01 |
| 16 | 0.87 | 0.07 | 0.78 | 0.40 | 0.94 | 84.43 | 264.15 | 51.02 | 348.55 | 3.22 |
| 20 | 1.64 | 0.82 | 4.66 | 1.31 | 2.57 | 6.06 | 254.96 | 19.04 | 261.03 | 44.90 |
| 22 | 1.72 | 0.08 | 0.28 | 0.16 | 0.81 | 117.28 | 280.93 | 84.33 | 398.20 | 2.52 |
| 27 | 1.68 | 0.50 | 4.47 | 1.27 | 2.52 | 7.27 | 330.12 | 40.46 | 337.40 | 59.00 |
| 30 | 2.13 | 0.90 | 8.13 | 1.85 | 2.80 | 77.99 | 193.91 | 38.50 | 271.88 | 4.11 |
| 31 | 0.58 | 0.37 | 1.68 | 0.45 | 2.81 | 25.87 | 168.60 | 23.87 | 194.45 | 6.58 |
| 32 | 1.53 | 0.53 | 3.68 | 1.19 | 1.55 | 32.29 | 203.54 | 49.14 | 235.83 | 9.77 |
| 35 | 3.78 | 1.92 | 15.80 | 4.49 | 2.48 | 4.59 | 281.36 | 25.60 | 285.95 | 64.77 |
| 37 | 2.97 | 2.05 | 13.53 | 3.88 | 2.48 | 14.85 | 314.36 | 41.29 | 329.22 | 43.62 |
| 42 | 2.47 | 0.63 | 7.72 | 2.73 | 1.64 | 27.38 | 299.34 | 42.99 | 326.72 | 26.16 |
| 46 | 1.67 | 0.92 | 5.98 | 1.71 | 1.97 | 7.62 | 258.97 | 40.51 | 266.58 | 42.59 |
| 47 | 1.82 | 1.12 | 6.20 | 1.68 | 2.64 | 19.74 | 235.18 | 61.08 | 254.94 | 32.13 |
| 51 | 2.93 | 1.63 | 12.30 | 3.57 | 2.28 | 4.91 | 240.03 | 24.59 | 244.94 | 59.55 |
| 52 | 3.97 | 3.07 | 24.73 | 6.17 | 3.06 | 10.68 | 262.54 | 32.86 | 273.23 | 28.06 |
| 53 | 2.53 | 0.85 | 7.63 | 2.33 | 2.10 | 8.30 | 266.90 | 41.10 | 275.18 | 50.43 |
| 54 | 1.22 | 0.28 | 3.93 | 1.31 | 1.79 | 24.00 | 329.70 | 39.76 | 353.73 | 23.94 |
| 58 | 1.37 | 0.55 | 4.80 | 1.53 | 2.09 | 48.14 | 307.41 | 77.93 | 355.55 | 11.13 |
| 60 | 2.87 | 0.87 | 6.82 | 2.13 | 2.68 | 56.74 | 263.94 | 56.16 | 320.70 | 6.31 |
| 62 | 2.84 | 1.24 | 9.84 | 2.60 | 3.77 | 9.82 | 295.72 | 42.65 | 305.52 | 40.89 |
| 64 | 1.53 | 0.37 | 2.63 | 0.93 | 1.66 | 56.62 | 300.51 | 85.11 | 357.13 | 9.44 |
| 66 | 1.27 | 0.35 | 2.07 | 0.78 | 1.40 | 5.16 | 325.54 | 44.43 | 330.70 | 65.92 |
| 67 | 1.77 | 0.23 | 2.50 | 0.94 | 1.63 | 42.33 | 351.93 | 64.74 | 394.27 | 11.05 |
| 68 | 3.23 | 0.85 | 6.42 | 1.84 | 2.30 | 17.85 | 263.68 | 48.99 | 281.52 | 23.67 |
| 70 | 2.62 | 0.82 | 11.75 | 3.84 | 1.84 | 23.85 | 279.76 | 48.79 | 303.62 | 22.79 |
| 72 | 3.63 | 1.17 | 16.03 | 4.47 | 2.51 | 31.87 | 308.53 | 51.88 | 340.40 | 55.49 |
| 74 | 4.20 | 3.10 | 28.07 | 7.94 | 3.79 | 6.02 | 348.03 | 20.75 | 354.03 | 69.03 |
| 76 | 2.13 | 0.35 | 4.72 | 1.70 | 1.77 | 101.73 | 386.82 | 60.75 | 488.55 | 14.35 |
| 79 | 3.65 | 1.42 | 14.80 | 4.40 | 2.33 | 35.02 | 320.19 | 58.71 | 355.22 | 40.88 |
| 82 | 3.72 | 1.22 | 13.97 | 4.08 | 2.30 | 15.35 | 310.17 | 37.95 | 325.52 | 85.33 |
| 83 | 3.53 | 2.02 | 14.13 | 3.89 | 2.63 | 6.20 | 324.83 | 37.82 | 331.03 | 116.61 |
| 84 | 2.18 | 0.28 | 9.73 | 2.65 | 2.59 | 38.73 | 394.00 | 47.83 | 432.73 | 13.09 |
| 85 | 1.88 | 0.50 | 8.60 | 2.64 | 2.14 | 54.57 | 317.00 | 51.88 | 371.57 | 7.90 |
| 88 | 1.52 | 1.04 | 6.38 | 1.49 | 3.33 | 6.89 | 240.60 | 24.80 | 247.49 | 194.08 |
| 89 | 3.15 | 0.53 | 7.77 | 3.02 | 1.55 | 157.83 | 271.17 | 60.17 | 429.00 | 1.79 |
| 91 | 3.72 | 1.90 | 12.60 | 3.42 | 2.69 | 9.50 | 288.50 | 34.87 | 298.00 | 113.48 |
| 96 | 3.77 | 1.10 | 13.42 | 4.20 | 2.06 | 45.06 | 304.65 | 67.54 | 349.70 | 10.66 |
| 97 | 3.78 | 1.17 | 16.05 | 4.84 | 2.26 | 116.72 | 358.28 | 70.23 | 475.00 | 4.33 |
| 98 | 1.34 | 0.32 | 2.18 | 0.65 | 2.82 | 20.16 | 252.26 | 40.77 | 272.43 | 14.02 |
| 99 | 3.13 | 1.42 | 11.88 | 3.61 | 2.21 | 32.37 | 337.88 | 38.98 | 370.27 | 15.59 |

| RIL | score1 | score2 | SFW | SDW | w/gdw | Na | K | Mg | sum | ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| 101 | 2.90 | 0.93 | 7.83 | 2.10 | 2.61 | 16.86 | 259.34 | 29.94 | 276.20 | 18.92 |
| 102 | 3.15 | 0.82 | 6.68 | 1.75 | 2.79 | 10.45 | 241.81 | 34.06 | 252.27 | 28.62 |
| 103 | 2.15 | 0.50 | 3.07 | 0.85 | 2.49 | 25.88 | 231.59 | 33.18 | 257.45 | 10.16 |
| 107 | 2.58 | 0.72 | 4.70 | 1.49 | 1.84 | 10.86 | 292.02 | 36.17 | 302.87 | 52.55 |
| 108 | 0.82 | 0.07 | 1.47 | 0.59 | 1.50 | 132.15 | 489.22 | 86.12 | 621.37 | 6.47 |
| 110 | 2.38 | 0.58 | 5.95 | 1.54 | 2.61 | 22.52 | 269.96 | 41.77 | 292.50 | 12.37 |
| 113 | 2.13 | 0.55 | 5.43 | 1.70 | 2.08 | 10.40 | 292.57 | 42.42 | 302.95 | 34.03 |
| 115 | 1.85 | 0.15 | 4.95 | 1.61 | 2.01 | 87.00 | 428.79 | 62.98 | 515.78 | 8.57 |
| 117 | 2.65 | 1.25 | 11.77 | 3.34 | 2.42 | 35.89 | 308.40 | 41.49 | 344.32 | 11.64 |
| 120 | 3.70 | 1.90 | 12.37 | 3.26 | 2.81 | 8.28 | 259.10 | 51.74 | 267.37 | 37.90 |
| 121 | 1.78 | 1.03 | 6.82 | 1.85 | 2.67 | 19.14 | 240.13 | 38.99 | 259.25 | 18.03 |
| 124 | 1.68 | 0.32 | 5.48 | 1.82 | 1.94 | 112.14 | 278.77 | 79.53 | 390.92 | 2.83 |
| 127 | 1.20 | 0.28 | 2.02 | 0.93 | 1.05 | 75.61 | 300.98 | 67.82 | 376.58 | 5.43 |
| 130 | 2.48 | 0.20 | 3.50 | 1.39 | 1.44 | 78.91 | 376.38 | 68.69 | 455.30 | 5.94 |
| 132 | 2.42 | 1.15 | 7.02 | 1.92 | 2.46 | 17.05 | 247.68 | 51.05 | 264.73 | 19.52 |
| 133 | 3.37 | 1.53 | 14.58 | 4.20 | 2.42 | 3.02 | 278.57 | 36.04 | 281.60 | 128.25 |
| 140 | 2.30 | 0.85 | 10.92 | 2.87 | 2.59 | 8.28 | 246.61 | 54.13 | 254.90 | 41.39 |
| 142 | 3.65 | 1.73 | 10.08 | 2.86 | 2.46 | 5.80 | 261.88 | 24.22 | 267.68 | 51.04 |
| 143 | 2.50 | 0.58 | 5.58 | 1.53 | 2.65 | 29.95 | 252.67 | 47.23 | 282.62 | 15.89 |
| 147 | 1.90 | 0.60 | 4.90 | 1.60 | 1.99 | 44.11 | 184.10 | 74.35 | 228.20 | 5.06 |
| 150 | 2.22 | 0.52 | 8.37 | 2.85 | 1.83 | 42.77 | 294.83 | 53.72 | 337.58 | 7.03 |
| 154 | 2.75 | 0.42 | 5.80 | 1.85 | 2.08 | 34.71 | 326.86 | 38.19 | 361.57 | 15.17 |
| 163 | 1.53 | 0.40 | 4.30 | 1.64 | 1.71 | 89.53 | 301.99 | 87.85 | 391.50 | 4.29 |
| 165 | 1.58 | 0.37 | 2.68 | 0.95 | 1.79 | 19.85 | 351.25 | 20.83 | 371.10 | 21.03 |
| 166 | 1.72 | 0.75 | 4.30 | 1.41 | 1.74 | 29.17 | 309.84 | 31.38 | 339.02 | 23.64 |
| 167 | 3.40 | 0.77 | 7.10 | 2.03 | 2.54 | 13.27 | 269.13 | 41.82 | 282.42 | 35.40 |
| 170 | 2.98 | 1.35 | 8.82 | 2.50 | 2.49 | 3.83 | 263.49 | 38.58 | 267.32 | 93.05 |
| 171 | 1.13 | 0.40 | 3.12 | 1.35 | 1.22 | 32.56 | 201.12 | 50.01 | 233.70 | 6.51 |
| 173 | 3.37 | 0.45 | 8.52 | 2.55 | 2.30 | 25.39 | 323.34 | 77.18 | 348.74 | 18.80 |
| 179 | 3.52 | 1.12 | 8.53 | 2.16 | 2.77 | 12.21 | 295.63 | 32.33 | 307.85 | 34.50 |
| 180 | 2.82 | 1.13 | 9.57 | 2.43 | 2.89 | 4.06 | 247.18 | 36.48 | 251.23 | 99.91 |
| 181 | 3.72 | 1.27 | 12.95 | 3.50 | 2.66 | 2.51 | 276.32 | 36.03 | 278.82 | 125.54 |
| 182 | 4.58 | 3.10 | 17.30 | 4.90 | 2.53 | 4.88 | 307.22 | 26.13 | 312.12 | 78.44 |
| 183 | 4.07 | 1.00 | 9.83 | 2.76 | 2.44 | 5.31 | 244.69 | 36.84 | 250.02 | 58.88 |
| 184 | 3.48 | 1.42 | 14.88 | 4.54 | 2.17 | 35.68 | 287.88 | 31.78 | 323.56 | 11.85 |
| 185 | 1.80 | 1.07 | 5.00 | 1.56 | 2.18 | 9.42 | 279.86 | 44.03 | 289.28 | 33.52 |
| 186 | 3.15 | 0.70 | 14.70 | 4.19 | 2.52 | 50.63 | 385.40 | 60.06 | 436.03 | 11.01 |
| 187 | 3.10 | 1.32 | 10.25 | 3.10 | 2.20 | 48.98 | 213.38 | 48.26 | 262.37 | 8.06 |
| 188 | 2.53 | 1.50 | 9.32 | 2.40 | 2.79 | 4.60 | 269.61 | 21.92 | 274.20 | 64.83 |
| 189 | 3.12 | 1.25 | 6.97 | 2.07 | 2.22 | 17.30 | 293.71 | 26.67 | 311.02 | 29.48 |
| 190 | 2.65 | 0.78 | 7.50 | 2.28 | 2.28 | 14.28 | 286.36 | 32.67 | 300.65 | 37.85 |
| 191 | 2.90 | 1.65 | 8.23 | 2.23 | 2.44 | 6.37 | 283.82 | 28.28 | 290.20 | 66.91 |
| 192 | 0.98 | 0.13 | 1.40 | 0.53 | 1.48 | 143.67 | 208.00 | 62.67 | 351.67 | 1.60 |
| 193 | 3.23 | 0.97 | 9.02 | 2.38 | 2.54 | 68.89 | 253.53 | 41.78 | 322.42 | 5.48 |
| 195 | 2.05 | 0.52 | 5.32 | 1.70 | 2.06 | 43.03 | 304.77 | 61.44 | 347.82 | 8.01 |
| 196 | 3.35 | 1.20 | 14.05 | 4.26 | 2.09 | 61.84 | 313.86 | 50.81 | 375.68 | 6.47 |
| 198 | 1.77 | 1.02 | 6.02 | 1.53 | 2.90 | 6.36 | 265.65 | 24.18 | 272.00 | 56.27 |
| 199 | 2.10 | 0.75 | 5.73 | 1.75 | 1.92 | 123.82 | 320.96 | 69.51 | 444.78 | 8.30 |
| Co39 | 0.56 | 0.03 | 0.62 | 0.29 | 1.14 | 45.60 | 170.20 | 109.47 | 215.70 | 5.21 |
| MO | 1.94 | 0.48 | 4.45 | 1.27 | 2.50 | 63.1 | 253.90 | 47.89 | 316.90 | 6.18 |