Physiological and Anatomical Implications of Salinity on Rice as a Semi-Aquatic Species

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By

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PREFACE

Only when I sat down to write this book did I realise that I needed to choose an appropriate title for it. I initially chose *Physiological and Anatomical Responses in Rice Roots under Combined Waterlogging and Salinity Stress*. However, at the back of my mind was the fact that rice is a semi-aquatic plant and needs waterlogged conditions for its survival; since rice is not a dryland cereal crop, it therefore isn't appropriate to refer to waterlogging as "stress." As a result of this, the title was changed to more accurately reflect the study's findings.

The book itself discusses salt stress as an issue of survival for rice plants, a well-established salt-sensitive crop. Taking into account waterlogging as a part and way of life for rice plants, it seeks to discover how this influences the effect of salinity in lab conditions. The book will examine whether salt affects the adaptation features of waterlogging, and also the manner in which salt tolerance of the plant is influenced by these adaptations. This book aims to provide answers to the majority of questions regarding the complex ecophysiological issues of salinity and waterlogging interaction in the rice field. It is believed that beginners and research scholars interested in pursuing this subject to its advanced stages will benefit from the vast information discussed in this issue. Chapter Two, *Overview of Literature on Salinity and Waterlogging Interactions*, discusses the complexity of the problem from various angles, thereby providing a good starting point. The book is best read in the format in which it was intended—chronologically; however, for those who have prior knowledge of the area, it may be advisable to begin reading from Chapter Three onwards.

The work discussed in this book comprises part of my doctoral degree programme, and I am grateful to CSP for inviting me to write it as an extension of their agricultural series. Despite my initial hesitation at taking on the task, I later decided that it would be a missed opportunity not to tackle this complex subject.

Before concluding, I would like to thank those who have made this task possible for me. My heartfelt gratitude goes to Jorge E Mayer, Grains Research and Development Corporation, Australia, for his expertise and critical comments not only on the content but also the writing style of various sections and chapters of this book. I would like to express my sincere thanks to my research supervisors, Dr Timothy D Colmer and Dr Martha Ludwig, University of Western Australia, for their valuable guidance, inspiration and encouragement. This project would not have been possible without financial support for my research from the School of Plant Biology, Faculty of Natural and Agricultural Sciences, University of Western Australia, and funding from the Australian Leadership Awards Scholarships given by AusAID which enabled me to complete my tenure in Australia. I would have failed in my duty were I not to thank my friends and colleagues for helping me in a number of small ways in order to make this dream come true. Finally, I would like to thank my family and the Lord Almighty for his bounteous blessings in this endeavour.

INTRODUCTION

Salinity has been an omnipresent problem faced by farmers almost since the beginning of agriculture, thousands of years ago. During the Mesopotamian civilization approximately 4,200 years ago, Sumerian farm managers, in an effort to counter saline soils, rejected emmer wheat in favour of the intensive cultivation of more salt-tolerant forms of barley. Ever since, efforts to select salt-tolerant crops have met with limited success owing to the complexity of the traits involved. Despite the availability of modern molecular techniques and transgenesis, this continues to hold true today (FAO 2005).

As stated by Flowers (2004):

> It is surprising that, in spite of the complexity of the salt tolerance, there are commonly claims in the literature that the transfer of single or a few genes can increase the tolerance of the plant to saline conditions. After 10 years of research, using transgenic plants to alter salt tolerance, the value of the approach has yet to be established in the field.

Therefore, a more realistic approach is to investigate a number of avenues in order to increase understanding regarding the most complex salt tolerance mechanisms in model crops such as rice and barley. Moreover, the developers of salt-tolerant lines via transgenic and non-transgenic approaches should endeavour to make the best use of existing knowledge and understanding of salt tolerance mechanism in plants. In addition, they should also be ready to absorb new knowledge synthesised using novel techniques including molecular genetics and functional genomics (Munns & Tester 2008).

One interesting and encouraging success story reported in India recently was the transfer of salt tolerance genes from mangroves to cultivated rice varieties, which was subsequently trialled at the laboratory level (FAO 2011). It is important to note here that mangroves are aquatic plants, while rice is a semi-aquatic but salt-sensitive plant (Yeo et al. 1990). Nevertheless, some of the most well-known salt-tolerant rice cultivars, such as Pokkali and Nona Bokra, are also waterlogging tolerant; however, they are wild varieties and have poor agronomic performance

(Gregorio et al. 2002). Ultimately, given the complexity of the salt tolerance trait, the reality of this recent success at the field level remains speculative.

India has become a pioneer in this field of research, following its successful release in 2010 of a new salt- and waterlogging-tolerant variety, Vandana, a product of research from a group of rice scientists at the Central Rice Research Institute (CRRI), Orissa. The new variety is expected to perform on a par with Pokkali and Nona Bokra in terms of salt tolerance. In addition, varieties like Ketumbar (Indonesia), Khao Seetha (Thailand) and Soc Nau (Vietnam) are all deep-water rice and have been identified as having salt-tolerant characteristics similar to that of Pokkali.

The information available so far suggests that there is some form of relationship between the salt- and waterlogging-tolerance of the plant. As we know, rice is a semi-aquatic plant possessing innate adaptations to waterlogging, such as:

1. **Morphological adaptations**, including the formation of aerenchyma, production of thicker adventitious white roots and shoot elongation (Colmer et al. 2006; Colmer 2003; Ram et al. 2002; John 1977).
2. **Physiological traits** which involve an increase in sugar concentration for sustained energy, owing to an increase in metabolism and switching to alcoholic fermentation, especially in root tips or in regions where there is a depletion of oxygen supply (Colmer & Pederson 2008; Ram et al. 2002).
3. **Development of anatomical traits** like thickening of the apoplastic barrier in roots in the outer regions of the cortical cell layers, between the cortical region and the exodermis (Colmer et al. 2006[b]; Insalud et al. 2006; Ranathunge et al. 2005; Colmer 2003 [a and b]; Armstrong & Armstrong 2001; Armstrong & Armstrong 1987; John 1977).
4. **Expression of sub1A gene**, which also serves as a molecular marker for screening waterlogging tolerant cultivars (Xu & Mackill 1996).

Generally, rice is a salt-sensitive plant. However, research on salinity tolerance in rice has identified some important salt tolerance traits, including increased shoot length, and a lowering of Na^+ and higher K^+ ions in the shoots, which takes place despite the fact that Na^+ and K^+ ion transport are independent in rice. Other traits include increased dry weight of the shoots and roots and increased tissue tolerance of the plant, expressed as $LC_{50.}$

A recent development in increasing the physiological tolerance of the rice plant to salinity stress was achieved through the benchmarking work of Plett et al. (2010), whereby modern techniques were used to increase salt tolerance via targeted changes in mineral transport. In the study, the cell-type specific expression of the AtHKT1;1 gene (*Arabidopsis* high affinity K^+ transporter 1;1) was induced in the cortical cells of rice using GAL4-GFP trap cell lines. In this case, the AtHKT1;1 gene was responsible for compartmentalisation of Na^+ions into the vacuoles of the root cortical cells.

Despite the major achievements detailed above surrounding the removal of accumulated salt in rice, further research is needed to understand more fully the avoidance of entry of Na^+ ions at the root level itself. This is because the entry of sodium ions is via apoplastic routes, while the entry of potassium ions is via symplastic routes (Singh n.d.).

At this point it is necessary to reflect on some of the well-established knowledge about the apoplastic barrier in rice, which under waterlogged conditions has been presumed to physiologically induce a “Radial Oxygen Loss (ROL) barrier” (Colmer 2006; Insalud et al. 2006; Ranathunge et al. 2005 [a]; Colmer 2003 [a & b] and Armstrong & Armstrong 2001). This barrier has been observed to be the result of thickening of the apoplastic barrier under stagnant, deoxygenated conditions simulating the oxygen-deficient waterlogged conditions in the field (Insalud 2006), as well as a concomitant increase in the area occupied by the aerenchyma. This strategy prevents the radial loss of oxygen into the surrounding anoxic environment, which also helps in retaining the normal glycolytic pathway for respiration and energy synthesis without the need to switch to anoxic fermentation.

The following chapters will focus on the anatomical characterization of the induced ROL barrier, and, from the physiological perspective, the interaction of this adaptation on the salt tolerance of the plant as a whole entity. Firstly though, it is pertinent to present a review of the existing knowledge on salinity and waterlogging, and the combined effects of these factors in rice.

CHAPTER ONE

OVERVIEW OF LITERATURE ON WATERLOGGING AND SALINITY INTERACTIONS

Waterlogging, in combination with dryland salinity, secondary salinization, or groundwater-associated salinization, is a major problem for agriculture and land rehabilitation. Around the world, the combined problem of salinity and waterlogging has affected about 45 million hectares of arable land (Ghassemi et al. 1995). Some of the worst affected areas include 1 million hectares in Pakistan, 16% of arable land in Australia and 2.5 million hectares that are being deserted each year in India (Robertson 1996; Kijne 2006; Rengasamy 2006). This statistic comprises up to 20% of the irrigated land worldwide, which is accountable to one-third of world food production (Munns & Tester 2008).

Rice, a crop favoured for irrigated cultivation, has a higher probability than most other crops of being exposed to the twin problems of salinity and waterlogging (Konukcu et al. 2006). In fields irrigated with poor-quality water, when exposed to transient waterlogging, salts dissolved in the water gradually increase in the soil, resulting in a saline, waterlogged environment. In spite of the fact that only 20% of arable land is under irrigated cultivation, and that rice is secondary to wheat as the major grain consumed around the world, rice is still largely cultivated via irrigation in over 100 countries around the world. This is due to a lack of adequate water supply throughout the year, since most of the areas cultivating rice are dependent on seasonal rainfall (Yeo et al. 1990).

Despite being widely known as an irrigated crop, rice can be grouped based on its four main habitat conditions (Hector 1936; Khush 1997):

1. **Upland, highland, mountain or dryland rice.** Grown in elevated areas in rainfed and naturally well-drained soils, but still under humid conditions. 16 million hectares of rice-growing areas fall under this category.

2. **Rainfed lowland rice**. This rice has diverse growing conditions, influenced by the amount and duration of rainfall, depth of standing water, soil type and frequency of flooding. These divisions lead to further subdivisions: rainfed shallow favourable, rainfed shallow drought-prone, rainfed shallow submergence-prone and rainfed medium deep. Due to the depth of varieties of rainfed lowland rice, it is not surprising that about one fourth of the world's rice cultivation area is of this ecological habitat.
3. **Irrigated or flood-water rice.** Comprising 55% of the world's rice area planted and the most productive rice growing regions of the world, this includes the most popular, economically significant and improved bred-rice varieties growing in these regions.
4. **Deep-water or flood-prone rice**. These varieties are grown in low-lying regions with intermittent and seasonal flooding, where the depth of standing water may vary from 50cm to 3m. These constitute 9 million hectares of the world's rice-growing areas.

Since the field has to be maintained with varying degrees of water, depending on the growth stage of the plant (IRRI 2009), rice plants might therefore have adapted to waterlogging with mechanisms and traits for tolerance that are of inducible nature.

The main problem of soil waterlogging for plant growth is the development of hypoxic to anoxic conditions due to a reduction in the diffusion rate of gases into the soil (Colmer 2003 [a & b]). The traits in plants that enable efficient internal gas-exchange processes for the roots are therefore of great significance. The main aeration traits that are required to enable efficient internal transport of gases between the shoots and the roots, and with the immediate surrounding environment of the roots are as follows:

1. Aerenchyma, for efficient longitudinal transfer of gases, especially oxygen and carbon dioxide, between the shoot-root system;
2. A barrier to radial oxygen loss for reducing lateral loss of oxygen from the roots to its surrounding hypoxic environment.

Rice is tolerant to waterlogging due to a combination of anatomical, physiological and morphological adaptations, which include: increased aerenchymal space (Yamasaki 1952; Armstrong 1979; Kawase 1981; Armstrong & Beckett 1987); thickening of the inherent apoplastic barrier (Justin & Armstrong 1987; Insalud et al. 2006); induction of a barrier to radial oxygen loss (Armstrong 1971; Colmer 2006; Colmer et al. 1998); increased thickness of the adventitious roots; decreased number and length of lateral roots (Armstrong 1979; Armstrong et al. 1990; Sorrell et al. 2000; Colmer 2003) and decreased shoot-root growth rate for decreasing the distance for longitudinal transport of gases (John et al. 1977; Drew & Lauchli 1985; Drew et al. 1988; Barrett-Lennard 2003). The presence of these traits in combination, and the sensitivity of the plant to salt (Yeo et al. 1985; Yeo et al. 1990; Lutts et al. 1996; Folkard et al. 2000; Anil et al. 2005), contributes immense opportunities to use rice as a model species for developing deeper insights into the relationships between waterlogging and salinity. In addition, the possible influences of these mechanisms in conferring tolerance to waterlogging and efficient gas transport on ion and water transport in rice roots is also an area requiring further study.

This chapter will focus on: (i) physiological and anatomical mechanisms responsible for waterlogging tolerance in plants (specifically rice), (ii) adaptations to salt stress, and (iii) plant (rice plant) responses to the interactive effects of waterlogging and salinity when in combination.

1.1 Waterlogging

1.1.1 Flooding versus Waterlogging

What is the difference between flooding and waterlogging?

Waterlogging is a condition in which the field is flooded with stagnant water; by contrast, flooding involves running water (lotic system). In flooded conditions, the water is flowing, submergent and stirring with the convective movement of solutes and gases, more so than normal conditions. However, with time, after the flood subsidises the field is slowly exposed to waterlogged/submergent conditions depending on the amount of water accumulated. In waterlogged conditions, the soil in the field slowly turns from hypoxic conditions to anoxic conditions (Colmer & Flowers 2008). This is due to the fact that oxygen is consumed and there is a reduction in the diffusion rate of oxygen, which is 10,000 times slower in water than in air (Grable 1966).

Irrigation

Any system that is used to deliver water to plant-growing regions is known as irrigation (Taiz & Zeiger 2006). Irrigation is a common system of agriculture in regions that experience limited water access, and although it is a beneficial system of agriculture, it has also been the causative agent for the unresolved problem of salinity.

The two criteria which are of importance in irrigation with respect to salinity and waterlogging are the quality of the irrigated water that influences salinity, and the amount of water that is used to irrigate a field. It is these criteria which determine the difference between waterlogging and flooding.

1.1.2 Effects of Flooding and Waterlogging in the soil

Physicochemical changes in the soil

When a field is waterlogged, a number of physicochemical changes occur in the soil, particularly a number of redox reactions mediated not only by the chemical changes and physical properties of the soil but also as a result of microbial activities and the type of plant cultivated. Consequently, the fertility index of a paddock is usually measured as a factor of electrical conductivity (EC) value after four weeks of flooding, which is believed to give a measure of the concentration of nutrients mobilized in the soil solution (Sahrawat & Narteh 2002) required for good productivity.

According to Chandler (1979), the main changes that occur under waterlogged conditions, especially in a paddy field, are:

1. Decreased oxygen supply, except for a thin layer on the soil surface;
2. Changes in the pH of the soil; an increase in pH for acidic soil and a decrease in pH for calcareous soil. On the whole the pH comes to near-neutral conditions (as shown in Fig. 1.1, a survey conducted on various soil types);
3. Iron compounds are converted from ferric source to ferrous source. For example, the conversion to ferrous sulphide by oxidizing hydrogen sulphide (Armstrong & Armstrong 2005), which results in a large amount of soluble form of iron being released into the soil solution (Begg et al. 1994);
4. An increase in the supply of nitrogen, phosphorus (Huguenin-Elie et al. 2003), silicon, and molybdenum.

Other changes include a decrease in zinc and copper sources (depending on the availability of organic substrates), an increased production of organic acids, and increased accumulation of carbon dioxide, ethylene and hydrogen sulphide (Ponnamperuma 1984).

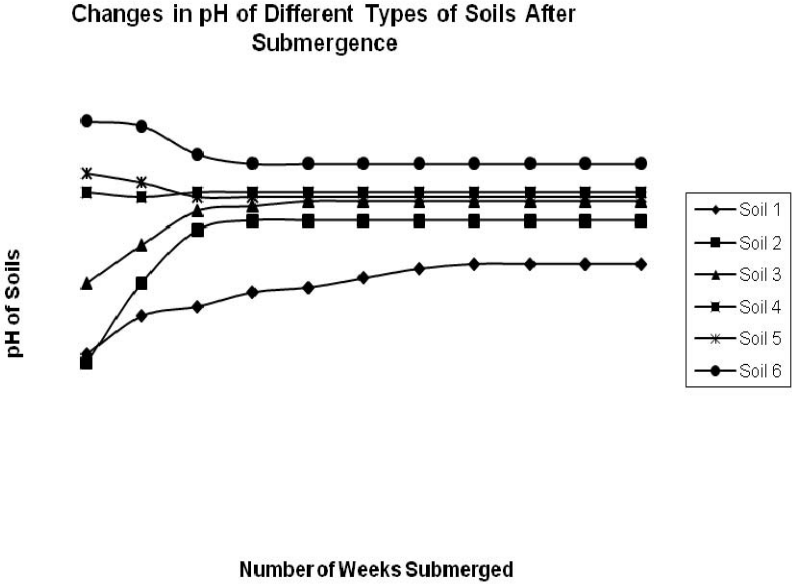

Fig. 1.1. Changes in pH of different types of soils after submergence

As a result of the various changes to the chemical nature of the soil, a number of related changes in the physical properties of the soil also occur. These include changes to the texture of the soil, such as the proportion of sand, silt, and clay, and the type of minerals which could be altered based on the chemical nature of the soil (Gambrell 1994). For example, clay soil will be hard, cracked and difficult for root penetration under drought conditions, whereas when the soil gets fully hydrated, it becomes loose and accessible to root penetration. Other changes in the soil structure include the conditions of aeration, since despite there being an increase in the porosity of the soil owing to penetration of the water molecules, there is a decrease in aeration, owing to the fact that the pores are occupied by water molecules. However, the impedance against penetration by roots decreases.

Biological changes in the soil

Biological changes will include slow transformation of the soil micro flora from obligate aerobes to anaerobic microbes. Metabolic activity of these microbes, in conjunction with that of the plant roots, will result in changes in the physical and chemical nature of the soil.

In a paddy field, the nitrification and denitrification processes of the soil are dependent on the reducing environment of the soil. In fact, a reduced soil environment results in a three-fold enhanced absorption of the total nitrogen absorbed by the rice plants as nitrates (Kirk 2001). Despite the fact that plants tolerant to waterlogging are adapted to grow in a reduced environment consisting of a number of reduced compounds such as hydrogen sulphide, ammonia and other volatile compounds (resulting in ebullition), in reality, the rhizosphere is not really a completely reduced environment. By re-oxidising reduced compounds like organic acids, hydrogen sulphide and ferrous iron to ferric iron in the rhizospheric region (Kirk 2004; Armstrong 1970), the radial oxygen lost from these tolerant plant roots into the reduced soil results in a partly oxidised zone.

It is worth noting, however, that the range of microbial products produced both under the oxidised and the reduced rhizospheric environment influences the growth and development of the plant (Lynch & Whipps 1991).

1. On the plant

Tolerance to waterlogging stress is genetically determined. Plants less tolerant to waterlogging usually fail as a result of hypoxic and anoxic stresses.

Morphological

Plants susceptible to waterlogging stop growing upon the onset of stress, and wilting and yellowing usually become apparent as a consequence. Depending on the level of tolerance, the morphological changes that are usually encountered have varying effects on the growth rate of the plant.

Contrary to waterlogging, flooding will result in wear and tear mechanical damage. As a result of the massive flow of water, effects such as the breaking of culms and the uprooting of plants due to erosion of the soil will occur.

Depending on the level of flooding, if submergence occurs after the flow of the flood subsides, no terrestrial or semi-hydrophytic plant, even a submergence-tolerant plant (other than those that grow underwater) will survive for more than a short period of time.

Biochemical and Physiological Changes

A number of metabolic changes occur as a result of waterlogging. These are dependent on various factors such as oxygen concentration, temperature and pressure affecting various physiological processes.

1. Oxygen Concentration

The main biochemical changes that occur in plants under waterlogged conditions are caused by oxygen starvation. This leads to ATP shortage, and occurs because there is a decline in efficiency of aerobic respiration and a tendency towards anaerobic respiration, especially if the plants are waterlogging intolerant.

2. Temperature

Temperature adversely affects waterlogging. A reduction in temperature reduces the respiratory activity of the plants, leading to increased ROL with a concomitant increase in cell wall permeability and a decrease in metabolic efficiency, which ultimately affects the nutrient, water, ion transport and uptake (Armstrong 1971), and oxygen consumption (Colmer 2003 [b]). Conversely, an increase in temperature leads to a reduction in the solubility of gases like O_2 and CO_2, thus creating an anaerobic environment and thereby accelerating anaerobic respiration (Ram et al. 2002). However, temperature is of no significance for a screening experiment, especially in a semi-aquatic species like rice, since ranking based on LD_{50} among rice cultivars does not seem to vary under differing waterlogging treatments.

3. pH

Under waterlogged conditions and the associated development of low-oxygen conditions, there is a tendency for an increase in apoplastic pH to 6 and above. This in turn impairs many enzymes (e.g. invertase) and transporters which work under a pH of 5. There is also a decrease in the proton motive force, and thus an increase in demand for anoxic energy.

This is because the H^+-ATPases has a reduced activity or is impaired (Felle 2005), both at the cytoplasmic and vacuolar levels. At the vacuolar level, the activity of this enzyme is usually replaced by VPPases. The onset of acidosis is the first reaction to anoxia, and a sequence of reactions follow, which will lead either to tolerance or intolerance (Greenway and Gibbs 2003[a]).

4. Light

An increase in sunshine or light is beneficial, since light enhances the oxidation of phytotoxins like hydrogen sulphide which actually prevent the build-up of these compounds to levels toxic for plants (Vamos & Koves 1971). The oxidation is facilitated by the production of free radicals during photolysis of water, oxygen given off by organic acids and by active oxygen which is lost radially from the roots.

1.1.3 Waterlogging tolerance in Poaceae

Waterlogging tolerance mechanisms can be classified into three groups: (a) anoxia-avoiding strategy involving promotion of gas transport; (b) accommodation strategy involving metabolic adaptations; and (c) tolerance involving hormonal control. However, no single strategy is sufficient for coping with low-oxygen conditions.

1. Physiological

The main effect of waterlogging is due to the decline in oxygen concentration resulting in hypoxic to anoxic conditions. Plants tolerant to waterlogging usually develop mechanisms that enhance aeration. This results in morphological and anatomical adaptations, such as root pathways for efficient longitudinal transport of oxygen which in turn reduce the demand for oxygen per unit distance of root (Armstrong et al. 1994). An increase in aeration pathways results in increased downward transport of oxygen. The formation of aerenchyma can be lysigenic, by the breaking down of cells due to cell death, or schizogenic, by cell separation without cell death (Shiono et al. 2008). However, both patterns of aerenchyma are formed as a result of eliciting responses and reactions, as well as hormone syntheses due to hypoxic conditions. Under anoxic conditions there is no aerenchyma formation, since there is a reduction in ethylene biosynthesis. The precursors and enzymes involved in ethylene biosyntheses, such as ACC oxidase, require ATP, the production of which is reduced under anoxic conditions as the metabolic processes shift from aerobic to anaerobic respiration.

2. Morphological

Morphological traits related to waterlogging tolerance include:

1. Reduction in the shoot-root relative growth, resulting from reduction in cell elongation, which is in turn influenced by the hormonal fluctuations elicited by the hypoxic and anoxic conditions of waterlogging;
2. Formation of thicker adventitious roots or air roots (Armstrong 1979; Armstrong et al. 1982) that ultimately may or may not replace the seminal roots (Thomson et al. 1990);
3. Development of surface roots, which under inundated conditions results in development of a surface air-water interface on the surface of waterlogged soil (Alberda 1953);
4. Small number of laterals (Armstrong et al. 1983; Sorrell et al. 2000); and
5. Wilting of older leaves.

(b) Anatomical

The anatomical adaptations to waterlogging and submergence must take into account that there is no room for entry of oxygen from the rhizospheric environment (Justin & Armstrong 1987). Whilst aerenchyma formation and cuboidal packing of cells enhances the longitudinal transport of gases, radial diffusion is prevented by the aerenchyma inside the adventitious roots, and the hypoxic to anoxic conditions in the rhizospheric environment are of prime importance in signalling the development of these adaptations. However, more is known about the physiological nature of the “ROL Barrier” than about the anatomical and structural modifications that constitute the barrier. The impedance against the radial diffusion is highly species-dependent. Based on observations made in other plants like *Phragmites australis* and *Glyceria maxima* (Soukup et al. 2007), it is generally believed that an increase in the number of cell layers and thickening of the secondary cell walls in hypodermal regions or the exodermis of the roots forms the ROL barrier.

Suberin or lignin deposits are the main constituents of the cell walls which reduce the porosity of the cellulose matrix of the cell walls. So far suberin has been found to play a major role in acting as a barrier against radial oxygen loss (Simone et al. 2003; Enstone & Peterson 2005; Soukup et al. 2007). Apart from the chemical nature of the cell wall constituents, the microstructural arrangement of these constituents also plays an important role in the prevention of radial oxygen loss, especially in preventing the molecular diffusion of oxygen molecules. However, there is a major paucity of data regarding the permeability coefficient or the pore sizes of the cell walls of cell layers surrounding the aerenchyma. This poses a limitation upon the understanding of the mechanisms that control ROL, and prevents a quantitative comparison of ROL across species, different cultivars or various treatments (Colmer 2003 [b]).

Goller (1977) conducted a survey on the anatomical features of adventitious roots which involved 265 species of 127 grass genera. The roots were grouped into three types – ‘festucoid’ (hexagonal packing of cortical cells with stele diameters ranging from 200 µm to a maximum of 400 µm) [Pooideae], ‘panicoid’ (cuboidal packing of cortical cells with stele diameters ranging from 200 µm to greater than 400 µm) [Panicoideae, Chlorideae, Bambusoideae and Arundinoideae], and ‘intermediate’ (cuboidal packing of cortical cells with stele diameter ranging from 200 µm to a maximum of 400 µm) [Oryzoideae]. Of these, ‘intermediate’ root types are recognised as having the most enhanced longitudinal diffusion of gases because of the combination of increased porosity and decreased stele diameter. Aerenchymal patterns observed among a few genera of Poaeceae are tabulated based on the three groups (Fig. 1.2).

(c) Biochemical

A number of biochemical changes occur within the plant with the onset of decreased oxygen status. These are:

1. Control of energy metabolism under oxygen deprivation;
2. Making available of energy extensive resources;
3. Provision of essential gene products and the syntheses of macromolecules; and
4. Protection against post-anoxic injury (Armstrong et al. 1994).

The major shift occurs in the carbohydrate metabolism of the plants, which move towards lactate and ethanolic fermentation to provide the required ATP. When there is a signal that an energy crisis is about to occur with the development of initial hypoxic conditions or ‘hypoxic-pre-treatment’ in nutrient solutions, the breakdown of reserve carbohydrates is greatly reduced and there is a tendency towards accumulation of sugars (Ram et al. 2002). However, the energy thus provided can only maintain life; for sustained growth, the required ATP can only be provided by aerobic respiration (Armstrong et al. 1994).

As mentioned earlier, there is a tendency to increase the availability of reserve carbohydrates, with the most readily available resources being carbohydrates, while there are decreased amounts of proteins and amino acids, especially in the roots, and negligible amounts of lipids. Above all, the amount of carbohydrate reserves available is seasonal, meaning that it is higher in the vegetative phase and reduced during winter (Armstrong et al. 1994; Waters et al. 1989). Crucially, changes in amino acid-carbohydrate pattern can be used as a stress indicator (Koppitz 2004).

The synthesis of enzymes responsible for anaerobic respiration, proteins and hormones involved in aerenchyma formation, and both cell wall synthesizing and loosening enzymes increase in proportion under stressed conditions. Apart from the proteins and enzymes involved in the physiological changes that take place under anoxic conditions, synthesis occurs of stress response proteins, called anaerobic proteins (Greenway & Gibbs 2003 [b]; Dennis et al. 2000), and metabolites like proline and glycinebetaine. However, the role of these compounds in imparting biochemical tolerance to anaerobiosis is not clear.

The mechanisms to protect against post-anoxic injury include accumulation of antioxidants like ascorbic acid, glutathione or reduced phenolics, or induction of superoxide dismutase (SOD) or active-oxygen species removing enzymes like catalases and peroxidases (Monk et al. 1987).

The changes in the level of phytohormones under waterlogged or submerged conditions play a significant role in imparting tolerance to the plant. The phytohormone ethylene is a key player under inundation. It induces aerenchyma formation in most plants like *Rumex* sp*, Phragmites* sp*,* and *Oryza* sp (Evans 2004), but not *Juncus* sp (Visser et al. 2006). It is also involved in shoot or coleoptile elongation under submergence, and may be involved in inhibiting root extension in many plants. In *Rumex* sp, an increase in the accumulated ethylene concentration will influence a concomitant increase in gibberellin and a decrease in abscisic acid (ABA), and paves the way for cell elongation, with an accumulation of indole acetic acid (IAA) under submergence. The increased ethylene is also known to play a role in increasing apoplastic acidification, which in turn favours cell elongation, and upregulation of the expansin gene RPEXPA1,

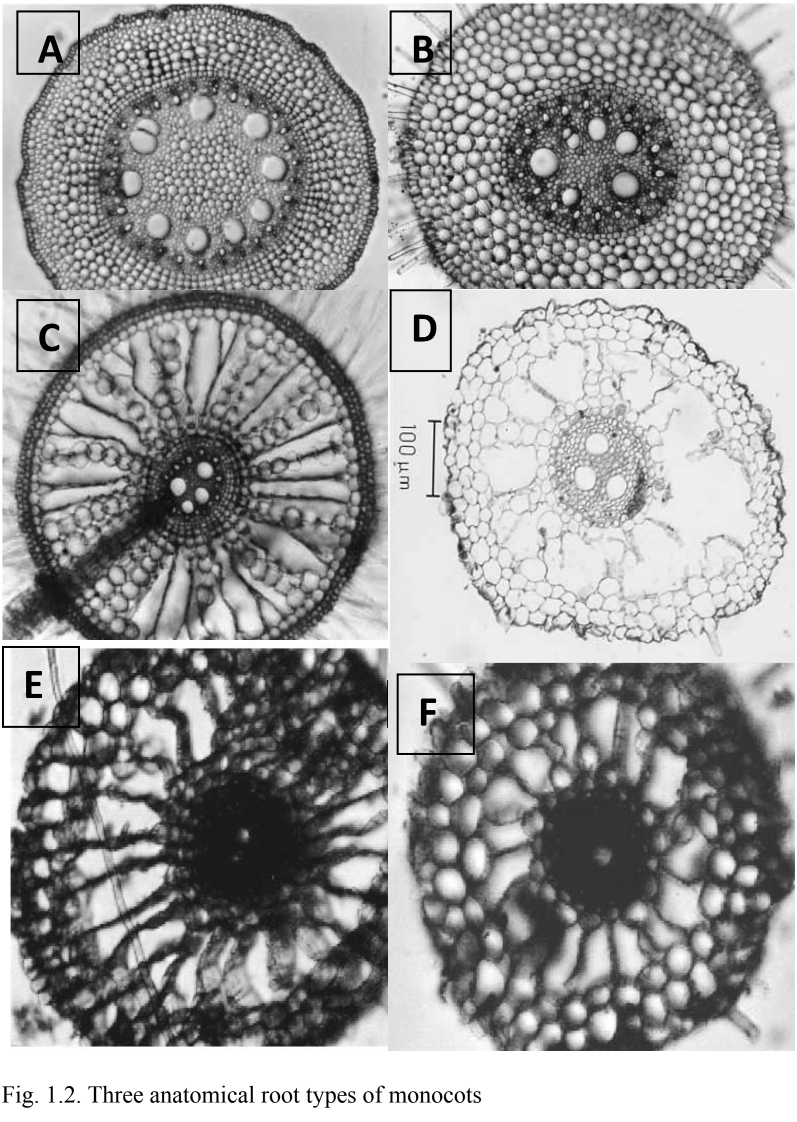

Fig. 1.2. Three anatomical root types of monocots

A. *Sorghum bicolor*; B. *Avena sativa* – Panicoid Type
D. *Zea mays*; C. *Oryza sativa* – Intermediate Type
E. *Phalaris arundinacea*; F. *Lolium perenne* – Festucoid Type

involved in cell wall loosening (Vreeburg et al. 2005). In many species, an increase in ABA also results in stomatal closure, especially under flooding (Armstrong et al. 1994).

(d) Genetic

A number of genes involved in submergence and waterlogging tolerance have been isolated. Of these, the *Sub* gene cluster has been found to confer tolerance to submergence consistently. mRNA transcripts of these genes have been found to be dominantly isolated from many waterlogging- and submergent-tolerant species.

1.1.4 Waterlogging tolerance in rice:

Since 90 % of the world's rice cultivation is carried out in waterlogged fields during the growing season (Kirk 2001), the reputation of the crop as a semi-aquatic, waterlogging-tolerant species is indisputable. The plant adopts two main strategies – either to elongate and escape, or not to elongate and thus conserve resources (Ram et al. 2002). If the plant adopts the first strategy, the main features are then those that contribute to avoidance mechanisms of waterlogging; conversely, the latter results in developing mechanisms responsible for metabolic adaptations which result in imparting tolerance to the plant against waterlogging.

1. Morphological

Rice is the only crop that germinates under conditions of restricted or absent oxygen, and so acts as a model plant for true adaptation to anoxia (Vartapetian 1978). Adaptations include coleoptile elongation, development of thicker adventitious roots (also known as air roots) on exposure to low-oxygen conditions, increased number of adventitious roots, development of surface roots in well-oxygenated waterlogged conditions (Harrison & Ayyer 1913), decreased relative root-shoot length and increased leaf area. However, in rice plants grown under hypoxic conditions by continuous purging with nitrogen, it was found that there were no changes in hypoxic conditions but there was a reduction in the root dry weight, with shortening and increased branching of roots.

2. Anatomical

This relates to changes to the internal features and anatomy of the plant that are evident as morphological changes, contributing to the waterlogging tolerance mechanism of the crop are discussed in detail

Aerenchyma

Aerenchyma is a constitutive feature in rice, and has thus served as one of the two model plants (the other being corn) for studies on the formation of irregular lysigenic aerenchyma among the monocot, formed as a result of the breaking down of cortical cells (Esau 1965). In addition to being a constitutive feature, formation of aerenchyma has been observed to increase under hypoxic conditions (Armstrong 1971; Justin & Armstrong 1987; Kludze et al. 1994; Colmer 1998) although not under anoxic conditions (Evans 2004). However, some studies indicate that there is no significant increase in the proportion of aerenchyma with low-oxygen root-zone aeration treatment (John 1977; Jackson et al. 1985; Insalud et al. 2006). Thus an increase in the proportion of aerenchyma is dependent on the rice cultivar, and the developmental stage of the plant (Butterbach-Bahl et al. 2000; Aulakh et al. 2000). Despite this, the formation of air spaces is not restricted in rice to the roots alone. The cortical gas lacunae extends up from the root-shoot zone and into the shoots (leaves, leaf sheaths and tillers) to maintain efficient bi-directional longitudinal transport of gases (Aulakh et al. 2000) between the roots and the shoots, either from the atmosphere or from the oxygen generated via photosynthesis when the shoots are submerged, with some degree of resistance between the root-shoot, root-soil and shoot-atmosphere gas transfer (Butterbach-Bahl et al. 2000). The transaction of tillers at the root-shoot transition zone is a major obstacle for smooth internal aeration (Groot et al. 2005).

The formation of aerenchyma in rice roots when extensively studied by Kawai et al. (1998) resulted in the finding that aerenchyma formation usually begins at 10 mm behind the root apex, while the collapse or breaking down of cells begins from the fifth file layer from the endodermis, and extends radially. However, a strand of cortical cells or spike-like strands of the reminiscent cells connects the endodermis to the specialized hypodermal and epidermal layers and maintains the structural integrity of the plant (Clark & Harris 1981).