

Plant Anaerobic Stress

I. Metabolic Adaptation to Oxygen Deficiency

Martin M. Sachs¹ • Boris B. Vartapetian^{2*}

¹ USDA/ARS/MWA-Soybean/Maize Germplasm, Pathology & Genetics Research Unit Urbana, IL, USA

² Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, 35, Botanicheskaya, Moscow, Russia

Corresponding author: * borisvartapet@ippras.ru

ABSTRACT

The establishment and further development of a novel branch of science in the field of ecological physiology, biochemistry and molecular biology, dedicated to plant life under hypoxic and anoxic stresses, is considered in the present review focusing upon strategies of adaptation and injury exhibited by plants under hypoxia and anoxia. Taking into account that the results of investigations over the last few decades confirmed the conclusions about the existence of two general strategies for plant adaptation to hypoxic and anoxic stresses: (a) true tolerance realized at the molecular level due to cardinal rearrangement of cellular metabolism under conditions of oxygen deficiency or complete absence of oxygen, and (b) apparent tolerance that is brought about by avoidance of anaerobiosis thanks to long-distance oxygen translocation, the accumulated experimental information is considered and discussed mainly with respect to basic strategies. The physiological role of anaerobic proteins synthesized under conditions of hypoxia and anoxia as well as advances in molecular biology and molecular genetics in the study of regulation of synthesis of these proteins at the level of transcription, translation and post-translation are discussed. The key significance of energy (glycolysis and fermentation) and related processes of carbohydrate (mobilization and utilization of reserved carbohydrate) metabolism in plant metabolic adaptation to oxygen deficiency is stressed. Special attention is given to an earlier period of active investigations in this field that played a substantial role in the establishment and international recognition of this new biological discipline. The strategy of avoidance of anaerobiosis by long-distance oxygen translocation and some other aspects of plant life under hypoxia and anoxia will be considered in the following publication (Vartapetian, Sachs, Fagerstedt 2008).

Keywords: anaerobic proteins, energy and carbohydrate metabolism, hypoxia and anoxia, molecular and genetics aspects, strategies of adaptation

Abbreviations: ABA, abscisic acid; ADH, alcohol dehydrogenase; AEC, adenylate energy charge; ENO, enolase; GA, gibberellin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LDH, lactate dehydrogenase; NMR, nuclear magnetic resonance; PDC, pyruvate decarboxylase; PHI, phosphohexose isomerase

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INTRODUCTION

The phenomenon of anaerobic stress experienced by intact plants or their parts under oxygen deficiency (hypoxia) or

its complete absence in the environment (anoxia) is widespread on our planet; sometimes, it results in the damage and even mass death of crops and wild flora, thus substantially impairing the economy of various countries and the

environment. Most often, hypoxia and anoxia damage plant roots and seeds on transiently or constantly excessively wet and flooded soils, which occupy vast areas in various countries (Maltby 1991). Low solubility and low diffusion rate of molecular oxygen in water (Armstrong 1979) reduce sharply its availability for plants. Anaerobic conditions could arise also under other environmental conditions, on compact soils, for example (Smucker and Allmaras 1993), or in autumn and winter, when plants and soil surfaces are covered with the ice crust impermeable to air, as frequently happens in the zones of north and moderate climate (Andrews 1997). In connection with global climatic changes, new vast areas could be flooded due to thawing in the permafrost zone and polar ice, resulting in the increase in the world's ocean levels. Oxygen deficiency could also be anthropogenic in its origin, due to irrigation of agricultural areas (for example, Ram *et al.* 2004), under conditions of space flights, and also during long-term bulk storage of fruits, grains, or vegetables (Knee 1991).

On excessively wet and flooded soils, plants suffer not only from oxygen deficiency but also from the accumulation of toxic organic and inorganic compounds in these soils (Ponnamperuma 1984; Gambrell *et al.* 1991).

Being aerobic organisms, plants respond sharply to oxygen deficiency. Crops, usually grown on dry aerobic soils, are most sensitive: when they are under conditions of oxygen deficiency, they are damaged and perish or adapt to these extreme conditions. Rice, one of the most important crops on the Earth, represents exclusion: it is mainly cultivated on flooded anaerobic soils (Swaminathan 1993). Nevertheless, even rice often suffers from oxygen deficiency, especially when its seedlings are completely submerged, as happens in East and South-East Asia (Jackson and Ram 2003).

Strangely enough, in spite of the obvious importance of the problem of plant anaerobic stress, little attention was paid to its study unlike other extreme ecological factors (high and low temperature, soil salinity, drought and biotic stresses). Considerable changes took place only during the last few decades, when physiological, biochemical, molecular-biological and molecular-genetic aspects of plant hypoxia and anoxia became a topic of increased interest for numerous researchers in various countries and their studies resulted in substantial progress. Rapid development of this branch of science as a new scientific discipline in the field of ecological physiology, biochemistry started in the 1970s, when the problem became a topic for discussion at international scientific meetings and in numerous special publications. It should be mentioned that, as early as in 1980, the phenomenon of anaerobic stress was recognized by the international scientific society as an essential problem important for applied agronomy, forestry, and environment protection.

It became evident that the efforts of scientists from various countries should be united and coordinated for more detailed and extensive studying of the problem. To this end, the International Society for Plant Anaerobiosis (ISPA) was founded. One of the principal topics discussed at the international symposium in the United Kingdom in 1992, which was organized by ISPA under the aegis of NATO in connection with the global climate warming (NATO *Advanced Research Workshop*, 1992), was directly related to the after-effects of hypoxia and anoxia for plants. Two ISPA symposia (USSR in 1985 and Iceland in 1992), financially supported by UNESCO, were destined wholly to plant anaerobic stress, similarly as two special international ISPA conferences were held in 2001 in the Netherlands under the aegis of the Green Cross, and in 2004 in Australia. Sixteen international meetings held in various countries and twelve comprehensive monographs and collected publications on plant life under anaerobic stress were the result of scientific and organizing activity of ISPA members (Vartapetian and Crawford 2007).

BACKGROUND INFORMATION

This section of the review mainly outlines the results of early studies, when active investigations were started, which led to the establishment of a new scientific discipline, dedicated to plant hypoxic and anoxic stresses. Before the discussion in the subsequent sections of the review the further development of investigations in the field of plant anaerobic stress and the current state of the problem, the authors would like to briefly summarize the basic advances of these early studies. Such an approach to the problem is significant for two reasons. First, a reminder of the sources of this new field of science will introduce the reader of this review to sense the logic of its further development. Second, in some cases, the results of these early studies gave an impetus and served as a cause for following active research and advances in the field of plant physiology, biochemistry, and molecular biology of plant anaerobic stress, which will be considered in subsequent sections of this review.

Crawford's metabolic theory

In this connection, it should be primarily noted that, in this early period, active experimental investigations were performed, which led to two alternative concepts put forward simultaneously by Crawford (McManmon and Crawford 1971; Crawford 1977, 1978) and by Vartapetian (Vartapetian *et al.* 1970, 1978; Vartapetian 1978). The metabolic theory of Crawford was the most accepted at that time. It was based on the investigation of alcoholic fermentation and the induction of alcohol dehydrogenase (ADH) synthesis in the roots and seeds of plants growing on flooded and dry soils. According to this theory, the flooding tolerance of hydrophytes is determined by biochemical and molecular properties of the cells of their roots and seeds, namely, these cells do not induce the activity of ADH and, thus, alcoholic fermentation and accumulation of toxic ethanol are substantially inhibited in them under the condition of oxygen deficiency. In contrast, mesophytes inhabiting dry soils activate sharply ADH and enhance alcoholic fermentation under anaerobic conditions, with subsequent ethanol accumulation and cell poisoning. In that time, as it was mentioned, metabolic theory was widely accepted and confirmed or supported by a number of researchers (Marshall *et al.* 1973; Francis *et al.* 1974; Larcher 1980; Moore 1982). It seemed likely that this theory was in good agreement with the results of earlier experiments of other researchers (Soldatenkov and Chirkova 1963), who also demonstrated high tolerance of root cells to anoxia in plants inhabiting flooded anaerobic soils (*Scirpus sylvaticus*, *Glyceria aquatica*) that tolerated 6-7 day-long anoxia, whereas the roots of plants growing on aerated soils (potato *Solanum tuberosum*, kidney bean *Phaseolus vulgaris*) manifested a much higher sensitivity to oxygen deficiency and perished after 24 h of anaerobiosis.

Two principal strategies of plant adaptation to anaerobic stress and a key role of energy metabolism in plant tolerance to oxygen deficiency

Strategy of avoidance of anaerobiosis

The results of Vartapetian's studies (Vartapetian *et al.* 1970, 1978) contrasted with the statements of metabolic theory. In fact, an opposite paradoxical phenomenon was demonstrated: the roots of plants inhabiting flooded anaerobic soils did not exhibit hypertolerance to anaerobiosis but, in contrast, were hypersensitive to oxygen deficiency. They were even more sensitive than the roots of plants inhabiting dry aerobic soils. These results led to the conclusion that the capability of tolerant plants to grow on flooded anaerobic soils depended not on root cell metabolism as was stated by Crawford's theory, but on the capability of wetland plants in contrast to plants inhabiting dry soils, to avoid anaerobiosis

in roots due to long-distance oxygen transport from aerated shoots, i.e., we deal here not with true but with **apparent** tolerance (Vartapetian 1978).

This conclusion was supported by physiological and biochemical studies (Webb and Armstrong 1983; Ap Rees and Wilson 1984) and also by long-distance oxygen transport measurements by a polarographic technique and other methods (electron microscopy, chemiluminescence analysis, and mathematical modeling) performed by Armstrong (Armstrong 1964, 1969, 1978, 1979) and Vartapetian (Vartapetian 1964; Vartapetian *et al.* 1970, 1978) considered in the second part of this review (Vartapetian, Sachs, Fagerstedt 2008). These studies showed in particular that the aerenchymatous roots of hydrophytes (rice and some wild species) obtain sufficient oxygen from above-ground organs and even release it partially into the rhizosphere. In contrast, in the mesophyte studied (cotton, *Gossypium hirsutum L.*), the proportion of oxygen transported from shoots to roots of 22-26 day old plants comprised only about 7% of roots' oxygen requirement in aerobic environment (Nuritdinov and Vartapetian 1981). Therefore, they perished on flooded soils when they could not develop secondary adventitious roots with a well-developed aerenchyma.

As it will be obvious also from the second part (Vartapetian, Sachs, Fagerstedt 2008) of this review, the following comprehensive studies of aerenchyma formation and oxygen translocation in wetland and non-wetland plants confirmed and substantially developed the above-mentioned observations.

Strategy of metabolic adaptation to anaerobiosis and key role of energy and related processes of carbohydrate metabolism

During this early period, notable progress was achieved also in the studies of metabolic adaptation to anaerobic stress, i.e. true tolerance to anaerobiosis, which is realized under the condition of deficiency or complete absence of oxygen. For this aim the technology of feeding of detached organs of rice seedlings with an exogenous glucose (Vartapetian *et al.* 1976) was used to stimulate glycolysis and fermentation under anoxic conditions. This permitted to demonstrate the key role of energy metabolism in the development of plant-cell tolerance to anoxia because such activation of glycolysis and alcoholic fermentation substantially improved cell tolerance even to strict anoxia. Just in these studies, it was demonstrated that extraordinary tolerance to the absence of oxygen of rice coleoptiles, which maintained an intact cell ultrastructure, including potentially physiologically active mitochondria (Costes and Vartapetian 1978), during many days of anaerobic growth, was lost when coleoptiles were detached from the seeds and exposed to anaerobiosis (Vartapetian *et al.* 1976). However their tolerance to anoxia could be restored when substrate transport from the seed was imitated by feeding of detached coleoptiles with exogenous substrate by incubation in 0.5% solution of glucose to maintain and stimulate glycolysis and alcoholic fermentation (Vartapetian *et al.* 1976).

It became evident that a phenomenal property of rice seeds to germinate under strict anoxia is explained by their capability to mobilize and transport seed storage compounds (starch) even under a complete absence of oxygen in the environment, thus supporting active glycolysis and alcoholic fermentation in the coleoptile cells. In this case, rice seeds, unlike roots, manifested **true** but not apparent tolerance to anoxia.

Thus, the above-mentioned studies for the first time demonstrated the key role both of energy metabolism (glycolysis and fermentation) and adequate substrate provision by mobilization and utilization of reserved carbohydrates, to support and stimulate long-term energy metabolism and plant adaptation to anaerobic stress (Vartapetian *et al.* 1976, 1978). As will be discussed in the next section of this review, detailed subsequent studies of the mechanisms of mobilization of reserved carbohydrate of rice seed under

anoxia (Perata *et al.* 1997; Loreti *et al.* 2003a, 2003b), long-term tolerance to anoxia of wetland plant rhizomes (Brändle 1991; Aragaus and Brändle 2000) and rapid underwater growth of some hydrophytes (Summers *et al.* 2000; Sato *et al.* 2002; Voesenek *et al.* 2003; Harada *et al.* 2005) substantially confirmed the above-mentioned conclusions.

In general, the studies described above, on the one hand, demonstrated an incorrect statement of Crawford's metabolic theory about plant injury and adaptation under conditions of anaerobic stress. On the other hand, based upon (a) a paradoxical phenomenon of hypersensitivity but not hypertolerance to oxygen deficiency of the roots of food tolerant rice (Vartapetian *et al.* 1970; Vartapetian 1978), (b) a facilitated oxygen transport from shoots to roots of these plants (Armstrong 1969; Vartapetian *et al.* 1970; Armstrong 1978; Vartapetian *et al.* 1978), and (c) a key role of energy and related processes of carbohydrate metabolism in the hypertolerance of anaerobically grown rice coleoptiles (Vartapetian *et al.* 1976, 1978), the concept of two principal strategies of plant adaptation to anaerobic stress was put forward (Vartapetian 1978). These strategies are (1) metabolic adaptation to deficiency or complete absence of oxygen realized at the molecular level, i.e., **true** tolerance and (2) adaptation at the level of the whole plant due to long-distance oxygen transport from aerated parts to the organs located in the anaerobic environment (roots, rhizomes), i.e., avoidance of anaerobiosis, or **apparent** tolerance. In the case of true tolerance, the importance of seed stored carbohydrate (starch) mobilization and utilization was especially emphasized (Vartapetian *et al.* 1976). The products of starch hydrolysis are used in glycolysis and alcoholic fermentation, thus providing for long-term tolerance of germinating rice seeds to anaerobic stress. Nevertheless, in spite of the fact that Crawford's theory turned out to be inconsistent, it stimulated an active discussion and experimental verification of its basic statements. As a result, more complete information was obtained about plant adaptation and damage under oxygen deficiency. Hence, Crawford's metabolic theory was beneficial from this point of view.

As will be seen from further discussion, subsequent investigations of the physiological role of anaerobic proteins and the phenomenon of plant hypoxic acclimation to anoxia considerably supported the notions about a key role of energy and related processes of carbohydrate metabolism in plant tolerance to anaerobic stress.

In that early period the formation of specific anaerobic stress protein in plants was also demonstrated (Maslova *et al.* 1975; Sachs *et al.* 1980). It was shown by gel electrophoresis that rice coleoptiles actively growing under strict anoxia comprised seven specific proteins, which were absent in aerobically grown coleoptiles (Maslova *et al.* 1975). When aerobically grown rice coleoptiles were transferred to anoxic conditions, anaerobic proteins also appeared in them. At that time, the physiological role of these stress proteins was not clear, except for one of them, ADH, whose activity increased substantially during lasting anaerobic incubation of coleoptiles. Based on the above results the authors concluded that "anoxia causes qualitative and quantitative reconstruction of protein metabolism" (Maslova *et al.* 1975). In subsequent years, the synthesis of stress proteins in plants was virtually demonstrated under all ecological stresses (high and low temperature, salinity, water stress, biotic stress) (Sachs and Freeling 1978; Sachs *et al.* 1980; Altschuler and Mascarenhas 1982; Sachs and Ho 1986; Ho and Sachs 1989). Sachs *et al.* (1980) demonstrated the selective synthesis of 20 anaerobic stress proteins in oxygen-sensitive maize roots. Synthesis of anaerobic proteins was also detected in seedlings of *Echinochloa* species (Mujer *et al.* 1993), whose seeds, like those of rice, germinate under anoxia. In addition, experiments by Mocquot *et al.* (1981) showed that both proteins and DNA were synthesized in rice coleoptiles under anaerobic conditions.

As will be seen in the next section of this review, the

expression of genes encoding anaerobic proteins, the control of their synthesis at the transcription, translation, and posttranslational levels, as well as the functional role of anaerobic proteins in plant adaptation to oxygen deficiency were studied in detail in subsequent years in a number of research groups, especially in relation to the phenomenon of hypoxic acclimation. In the case when their identification was successful they turned out to be mainly the enzymes of glycolysis and fermentation as well as of related processes of carbohydrate metabolism and aerenchyma formation.

In this period, an important discovery with far-reaching consequences was made by Davies *et al.* (1974) in experiments with cell-free extracts from pea *Pisum sativum* var. 'Alaska' seeds. These authors followed the kinetics of lactic and alcoholic fermentation after plant transfer from an aerobic to an anaerobic environment, which permitted them to put forward a pH-stat theory (Davies 1980). As will be discussed in the second part of this review this direction was greatly activated by the use of nuclear magnetic resonance (NMR) (Roberts *et al.* 1982, 1985; Fox *et al.* 1995; Ratcliffe 1997; Felle 2005). It is believed that cytoplasmic acidification and the control of this process are the mechanisms responsible for plant injury and adaptation under anaerobic stress.

Just in this period, active studies were started in the Pradet laboratory on the energy status of plants under conditions of oxygen deficiency (Pradet and Bomsel 1978). They successfully applied a technique allowed to measure the cell energy charge as a criterion of cell energy status under hypoxia and anoxia. In particular, regular and sharp modulation of energy charge depending on cell supply with oxygen was demonstrated. The investigations performed later by Pradet and coworkers showed a high efficiency of this approach for studying the effects of hypoxia and anoxia on plants (Saglio *et al.* 1980, 1988, 1999). In particular, they showed that, when maize root tips were transferred from aerobic to anaerobic environment without feeding with exogenous glucose, AEC ($\text{ATP} + 0.5 \text{ATP} / (\text{ATP} + \text{ADP} + \text{AMP})$) dropped from 0.9 to 0.2 as soon as in 30 min, whereas when root tips were then fed with glucose under lasting anoxia, the value of AEC immediately increased to 0.6 at simultaneous acceleration of lactic and ethanolic fermentation.

Other research teams were also successfully engaged in the problem of anaerobic stress. Some of their results were presented in the First International Symposium on Plant Anaerobiosis, and later they were included as separate chapters in the book edited by Hook and Crawford (1978). Among them, important studies of Gambrell and Patric (1978) and Ponnampuruma (1984), who investigated in detail the properties of flooded anaerobic soils, chemical and microbiological conversions in them, and also the distribution in such soils of the zones differing in their capability of oxidation/reduction are worth mentioning.

Ethylene evolution and its physiological role in plant adaptation to hypoxia and anoxia were actively studied in this period by Jackson's group (Jackson and Gambrell 1975; Jackson *et al.* 1978) and Kawase (1981).

Thus, reports in the First International Symposium on plant anaerobiosis and the chapters in the aforementioned book edited by Hook and Crawford, on the one hand, reflected the state of our knowledge in the early period of the establishment of this new scientific discipline. On the other hand, the results of these early studies gave an impetus to a swift break-through in this science field resulted in the formation of the International Society for Plant Anaerobiosis (ISPA), whose members played an important role in the further development and international recognition of this new scientific discipline.

Further sections of this review deal with the results of more recent investigations and the current state in this field of science. They are based on the concept on two main strategies of plant adaptation to anaerobic stress (Vartapetian 1978) that is now widely accepted (Brändle 1991;

Waters *et al.* 1991; Vartapetian and Jackson 1997; Jackson and Armstrong 1999; Saglio *et al.* 1999): metabolic adaptation, i.e., true tolerance, which is determined by major rearrangements of mainly proteins, carbohydrates, and energy metabolism, and which plays a critical role in plant adaptation to hypoxia and anoxia, and the strategy of avoidance of anaerobiosis due to long-term oxygen transport, i.e., apparent tolerance. Correspondingly, in the first part of this review the metabolic adaptation to anaerobic stress is discussed while in the second part we are going to focus mainly on the avoidance strategy and some other aspects of plant hypoxia and anoxia.

MOLECULAR GENETICS ASPECTS OF PLANT ANAEROBIC STRESS

Molecular studies of the maize anaerobic response stemmed from the extensive analysis of the maize ADH system by Drew Schwartz and coworkers (reviewed in: Freeling and Birchler 1981; Freeling and Bennett 1985; Gerlach *et al.* 1986). Hageman and Flesher (1960) were the first to show that ADH activity increases in maize seedlings as a result of flooding. Freeling (1973) later reported that ADH activity increased at a zero order rate between 5 and 72 hours of anaerobic treatment, reflecting a simultaneous expression of two unlinked genes, *adh1* and *adh2*. By producing ADH null mutations, Schwartz (1969) showed that ADH activity is essential for the survival of maize seeds and seedlings during flooding. Normal seedlings survive ~3 days of anoxia (Schwartz 1969; Lemke-Keyes and Sachs 1989b), while ADH nulls only survive a few hours of flooding. ADH is the major terminal enzyme of fermentation in plants and is responsible for recycling NAD⁺ during anoxia.

Most animal cells are very sensitive to anoxia. One reason for this is that animals rely solely on lactic acid fermentation, since they lack the enzyme pyruvate decarboxylase (PDC), which catalyzes a necessary intermediate step in ethanol fermentation. A model proposed by Davies (1980) postulates that there is a tight regulation of pH stat to prevent cytoplasmic acidosis during anoxia in plants. Initially upon anaerobiosis, lactate dehydrogenase (LDH) is active causing a drop in cytoplasmic pH with the accumulation of lactic acid. However, as the pH drops LDH (which has a relatively high pH optimum) activity decreases while PDC (which has a relatively low pH optimum) activity increases (Laszlo and Lawrence 1983). Thus, it is proposed that after a short period of oxygen deprivation, ethanol fermentation would predominate. This hypothesis was supported by NMR studies (Roberts *et al.* 1984a, 1984b, 1985, 1992). Thus, as in animal cells, maize ADH null mutants, might rely only on LDH for recycling NAD⁺ and succumb to cytoplasmic acidosis. Alternatively, a plant that is ADH-null may accumulate toxic levels of acetaldehyde, the substrate for ADH produced by the enzymatic reaction catalyzed by PDC. Hence, the occasion of high sensitivity of maize ADH- null mutants to oxygen deficiency, demonstrated in experiments of Schwartz (1969) could be accumulation of toxic levels of acetaldehyde or cytoplasmic acidosis.

While some minimum level of ADH activity appears to be required for any flooding tolerance, natural variation observed in long-term seedling survival of anaerobic stress does not appear to be correlated with variation in the levels of ADH activity (Lemke-Keyes and Sachs 1989b). In their study, more than 400 ADH⁺ maize lines were examined, and variation in flooding tolerance found among these lines was from 2 to 6 days. Selected lines, showing variation in flooding tolerance, also varied in induced ADH activity levels over a 5 fold range, but there was no apparent relationship between these two traits (Lemke-Keyes and Sachs 1989b). A mutation (*atn1*-; Lemke-Keyes and Sachs 1989a), that allows ADH null seedlings to survive 24 hours of anoxia, may allow further understanding of the role of ADH in flooding tolerance.

The anaerobic response

The maize anaerobic response is analogous to the heat-shock response observed in many organisms, including plants. There is a repression of pre-existing protein synthesis and *de novo* synthesis of a new set of proteins (Sachs and Freeling 1978; Sachs *et al.* 1980; Ferl *et al.* 1979; 1980; Rowland and Strommer 1986; Bailey-Serres *et al.* 1988; Dennis *et al.* 1989; Russell and Sachs 1989; Webster *et al.* 1991; Russell and Sachs 1992; de Vetten and Ferl 1995; Manjunath *et al.* 1999; Subbaiah and Sachs 2001). Except for one possible overlap, anaerobiosis induces a different set of proteins in maize than does heat shock (Sachs *et al.* 1980; Kelley and Freeling 1982; Cooper and Ho 1983). Regulation of both transcription and translation appear to be involved in the anaerobic stress response (Sachs *et al.* 1980; Dennis *et al.* 1988b). This is most likely a plant's natural response to flooding.

In an early study (Lin and Key 1967), anaerobic treatment was shown to cause a near complete dissociation of polysomes and a rapid repression of protein synthesis in soybean roots. Anaerobic treatment of maize seedlings also disrupts polysomes (Bailey-Serres and Freeling 1990). This phenomenon was shown to result in a redirection of protein synthesis (Sachs *et al.* 1980; Russell and Sachs 1992) in maize, and soybean (Russell *et al.* 1990). As is the case in many heat-shock systems (c.f., Schlesinger *et al.* 1982) maize seedlings subjected to anoxia (e.g., an argon atmosphere) display an immediate repression of pre-existing (aerobic) protein synthesis along with the induction of a new set of proteins (Sachs *et al.* 1980). Post-translational regulation of initiation factors and ribosomal proteins by reversible phosphorylation appears to play a role (Webster *et al.* 1991; Perez-Mendez *et al.* 1993; Manjunath *et al.* 1999) in addition to the structural determinants in the untranslated regions of mRNA (Bailey-Serres and Dawe 1996). Other post-translational changes leading to apparent new isozymes of proteins have also been reported to occur under anoxia (Chang *et al.* 2000; Subbaiah and Sachs 2001).

In maize seedlings, the induction of anaerobic polypeptide synthesis occurs in two phases. In the first 5 hours of anaerobic treatment there is a transition period during which there is a rapid increase in the synthesis of a class of polypeptides with an approximate molecular weight of 33 kDa. These have been referred to as the transition polypeptides (TPs). After approximately 90 minutes of anoxia, the synthesis of an additional group of ~20 polypeptides is induced. This group of 20 anaerobic polypeptides (ANPs) represents greater than 70% of the total labeled amino acid incorporation after 5 hours of anaerobiosis. By this time, the synthesis of the TPs is at a minimal level; however, these polypeptides accumulate to a high level during early anaerobiosis and have been shown by pulse-chase experiments to be very stable. The synthesis of the ANPs continues at a constant rate for up to ~72 hours of anaerobic treatment, depending on which maize line is being examined. After 72 hours, protein synthesis decreases concurrently with the start of cell death (Sachs *et al.* 1980). In similar experiments, it was shown that the rate of amino acid uptake in the anoxic root was about half that of the aerobic control, while incorporation into polypeptides was reduced to 1% (Russell 1990). Similar effects of anoxia on amino acid uptake and incorporation were also observed in rice roots (Mocquot *et al.* 1981).

Hypoxia (low oxygen) has been found to cause induction of the ANPs in maize primary roots, but does not cause the complete repression of pre-existing protein synthesis. Significant levels of "aerobic" protein synthesis are still observed at O₂ concentrations as low as 0.2% (Kelley and Freeling 1982). In addition, a novel set of polypeptides, not normally observed under aerobic or anaerobic conditions, is synthesized under hypoxic conditions (Kelley and Freeling 1982). Paul and Ferl (1991a) tested the effects of different concentrations of O₂ (15, 10, 5 and 0%) on ADH1

and ADH2 transcript accumulation. They found that 15% O₂ will induce transcript levels by ~4-fold while lower concentrations have a more dramatic effect. The largest effect was seen in the 0% samples. Submergence of seedlings in a flooding solution, or treatment with 0.2% O₂ appears to have the same effect on ADH activity and transcript levels as true anoxia (e.g., an argon environment; Russell 1990).

Most of the anaerobic proteins (ANPs) identified were found to be enzymes of glycolysis or sugar-phosphate metabolism; such as aldolase (Kelley and Freeling 1984b; Kelley and Tolan 1986), pyruvate decarboxylase (Kelley 1989; Kelley *et al.* 1991), enolase (Lal *et al.* 1998), glucose-6-phosphate isomerase (Kelley and Freeling 1984a), glyceraldehyde-3-phosphate dehydrogenase (Russell and Sachs 1991), sucrose synthase (Springer *et al.* 1986), and alcohol dehydrogenase (Freeling 1973).

In light of the inability of maize seedlings to survive even 6 hours of flooding in the absence of an active *adh1* gene (Schwartz 1969) or *pdh3* gene (Cui *et al.* 2000), it appears that at least one function of the anaerobic response is to enable the plant to produce as much ATP as possible when there is an oxygen deficit, as would occur in waterlogged soils.

In the presence of air, roots, coleoptile, mesocotyl, endosperm, scutellum, and anther wall of maize all synthesize a tissue-specific spectrum of polypeptides. Most organs (e.g., the scutellum and endosperm of the developing kernel) actually synthesize many or all of the ANPs constitutively, along with many other proteins under aerobic conditions (Okimoto *et al.* 1980). Only the root tissue of mature plants and seedlings and other pre-emergent seedling organs (e.g., the coleoptile) show a dramatic increase in the level of synthesis of the ANPs by anaerobic induction. This has also been observed in maize tissue culture (J.C. Woodman, pers. comm.) and while most culture strains tested have high constitutive levels of the ANPs under aerobic conditions, one particular culture strain (P3377) actually displays anaerobic induction (Duncan *et al.* 1985; Paul and Ferl 1991a, 1991b). On the other hand, maize leaves that have emerged from the coleoptile do not incorporate labeled amino acids under anaerobic conditions, and do not survive even a brief exposure to anaerobiosis (Okimoto *et al.* 1980).

The maize primary root tip is also very sensitive to anoxic treatment, surviving only a few hours in the absence of oxygen. However, it has been shown (Saglio *et al.* 1988) that a hypoxic pretreatment allows longer-term survival of the root tip under anoxia. In contrast, the more proximal part of the primary root axis and the preemergent shoot (i.e., those with an intact coleoptile) can survive anoxia for a few days without a hypoxic pretreatment (Sachs *et al.* 1980).

The phenomenon of plant metabolic acclimation to anoxia was demonstrated by Andrews and Pomeroy (1981, 1983). They flooded winter wheat plants at low temperature (2°C) and demonstrated a considered improvement of their tolerance to anoxia when plant surface was covered with the ice crust impermeable for gases. It was also shown that plant preliminary flooding resulted in the substantial activation of the enzymes of anaerobic respiration, PDC and ADH, and also in the notable improvement of the cell energetic status and acceleration of fermentation. Andrews (1997) considers such pretreatment as hypoxic acclimation. Later, the occurrence of hypoxic acclimation was confirmed, and until now, this approach is widely used by many researchers in diverse physiological, biochemical, and molecular-biological studies.

This phenomenon was confirmed in experiments with various plant species. Thus, when maize root tips were incubated at a low partial oxygen pressure (2-4 kPa) at 20-25°C for 18 h, root resistance to subsequent anoxia was greatly increased (Saglio *et al.* 1988). Such roots could tolerate 22-h anoxia, whereas control roots of the same plants (without hypoxic treatment) perished in 7 h of anoxia. In

experiments of Johnson *et al.* (1989), maize roots retained the capacity to grow even after 90-h anoxia when they were preliminary exposed to hypoxic conditions, whereas they lost this capacity after 24-h anoxia without such a pre-treatment. Similar results were obtained by other researchers as well in experiments with maize and tomato roots (Hole *et al.* 1992; Andrews *et al.* 1993; Germain *et al.* 1997).

It is worth mentioning that maize roots pretreated with hypoxia had a higher rate of anaerobic respiration, an increased concentration of ATP in tissues, a higher level of energy metabolism, and a higher ATP/ADP ratio. Even in wheat seedling roots, which are extremely sensitive to oxygen deficiency, hypoxic pretreatment of detached root tips or the roots of intact seedlings activated substantially the enzymes of alcoholic fermentation, increased the rate of aerobic respiration, and improved root tolerance to anaerobic stress (Waters *et al.* 1991).

The shift in the pattern of protein synthesis due to anaerobiosis has been observed in many other plant species including rice (Maslova *et al.* 1975; Bertani *et al.* 1981; Pradet *et al.* 1985; Ricard and Pradet 1989), *Arabidopsis* (Dolferus *et al.* 1985), soybean (Russell *et al.* 1990), *Echinochloa* (Mujer *et al.* 1993), cotton (Millar and Dennis 1996), tomato (Germain *et al.* 1997) and *Potamogeton* (Dixon *et al.* 2006). In anaerobically treated barley aleurone and root cells, LDH activity increases (Hanson and Jacobsen 1984; Hoffman *et al.* 1986) as do ADH mRNA and activity levels (Hanson *et al.* 1984). In the barley system, the gibberellic acid (GA₃) induction of α-amylase synthesis and its mRNA accumulation is suppressed by anaerobiosis. The “anaerobic” pattern of protein synthesis is perturbed only slightly by GA₃ when aleurone cells incorporate labeled amino acids in a nitrogen atmosphere (Hanson and Jacobsen 1984). A maize genomic sequence with homology to a barley cDNA encoding LDH has also been described (Hondred and Hanson 1990; Good and Paetkau 1992).

Identification and characterization of the genes involved in the anaerobic response

Cloned anaerobic-specific cDNAs were identified by differential hybridization to recombinant bacterial colonies using radiolabeled cDNAs of mRNA from anaerobic and aerobic roots (Gerlach *et al.* 1982). The anaerobic-specific cDNA clones were grouped into families on the basis of cross hybridization to each other, and several of these families were analyzed by hybrid-selected translation and by RNA gel blot (Northern) hybridization. The ADH1 and ADH2 cloned cDNA families were subsequently identified (Gerlach *et al.* 1982; Dennis *et al.* 1985). These cDNA clone families and the genes encoding them were then analyzed extensively (Dennis *et al.* 1984, 1985; Sachs *et al.* 1986; Peschke and Sachs 1994).

The anaerobic-specific cDNA clones were used as probes to measure gene expression in maize seedling roots and shoots. In both tissues, the levels of mRNA hybridizable to these cDNAs increase during anaerobic treatment (Peschke and Sachs 1994; Russell and Sachs 1989). This has been quantified rigorously in the case of ADH1 and ADH2. The kinetics of mRNA increase is the same for *adh1* and *adh2*. In both cases, the mRNA level first appears to increase at 90 minutes of anaerobic treatment. The mRNA level continues to increase until it plateaus at ~50 fold above the aerobic level at 5 hours of anaerobiosis. This level is maintained until after 48 hours in the case of *adh1* but starts declining after 10 hours in the case of *adh2* (Dennis *et al.* 1985). This pattern of mRNA level increase and decrease is reflected in the previously described rates of *in vivo* anaerobic protein synthesis for ADH1 and ADH2 (Sachs *et al.* 1980). PDC1 and PDC2 also appear to have similar kinetics of mRNA level increase (Peschke and Sachs 1993), as does inducible cytosolic GAPDH (Russell and Sachs 1989, 1991, 1992; Manjunath and Sachs 1997),

PHI1 (Lal and Sachs 1995), and ENO1 (Lal *et al.* 1991, 1994, 1998). However, aldolase (Hake *et al.* 1985; Kelley and Tolan 1986; Dennis *et al.* 1988a), sucrose synthase (ANP87; Hake *et al.* 1985; Springer *et al.* 1986), PDC3 (Peschke and Sachs 1993; Cui *et al.* 2000) and two other anaerobically inducible mRNAs (Peschke and Sachs 1994) show different kinetics of induction. *In vitro* run-off transcription experiments show an increase in transcription rate of the *adh1* and *adh2* genes in root cells (Rowland and Strommer 1985, 1986; Dennis *et al.* 1988b) and P3377 tissue culture (Paul and Ferl 1991a) during anoxia, strongly indicating that the increase in the levels of anaerobic-specific mRNAs resulting from anaerobiosis is due to induced transcription of the anaerobic-specific genes. All of these anaerobically inducible genes have been mapped to their chromosomal locations by RFLP analysis (c.f., Peschke and Sachs 1994; Subbaiah and Sachs 2003). Many additional anaerobically induced genes are being identified by microarray analyses (Branco-Price *et al.* 2005; Gonzali *et al.* 2005).

A comparison of the regions of the *adh1* and *adh2* genes upstream from the site of transcription initiation reveals only a few islands of homology (Dennis *et al.* 1985). Promoter analyses of these two genes revealed a particular nucleotide sequence that has been called the “anaerobic response element” (ARE). Its core nucleotide sequence is GGTTT (Walker *et al.* 1987b). Upstream sequences homologous to the “ARE” detected by Walker *et al.* (1987a) are found in both the *adh1* and *adh2* genes of maize, an *adh1*-like gene from pea (Llewellyn *et al.* 1987), the *adh* gene of *Arabidopsis thaliana* (Chang and Meyerowitz 1986), and also in the anaerobically inducible genes encoding sucrose synthase and aldolase in maize (for a review, see Walker *et al.* 1987b). However, the region containing the “ARE” from the maize *adh2* gene was found to be incapable of driving anaerobic induction (Walker *et al.* 1987a), and a region that is homologous to the “ARE” has been found upstream from a maize gene encoding a constitutive GAPDH (*gpc1*), whose expression actually decreases during anaerobic treatment (Martinez *et al.* 1989; Russell and Sachs 1989). Additionally, several other genes, which are not at all related to the anaerobic response, have been noted to have similar “ARE” sequences in similar positions (Russell and Sachs 1991). Reports by Paul and Ferl (1991a) and Olive *et al.* (1991) indicate that the “ARE”, and protein factors binding to it, may be necessary but not sufficient for hypoxic activation of gene expression. It may be that the region called the “ARE” is actually a general enhancer element and not the anaerobic analog of the heat-shock response element (Pelham and Bienz 1982).

Genes involved in flooding tolerance

Initially, the only genes described in plants that are induced by oxygen deprivation (anoxia or hypoxia) encoded enzymes of glucose-phosphate metabolism (mostly glycolysis and fermentation), and thus apparently function to allow limited energy production in the face of limited oxygen supply (Sachs 1993, 1994; Subbaiah and Sachs 2003). However, some genes involved in responses to flooding-stress in plants have been discovered that appear to function outside the glycolytic pathway.

Anoxia-tolerance gene(s) identified from the analysis of natural variation in maize

Anaerobic stress can significantly reduce survival or growth of germinating maize seedlings in waterlogged soils. The majority of maize genotypes survive up to 3 days of anaerobic treatment at 27°C. On the other hand, mutants that are null for ADH activity only survive a few hours of anoxia. We screened several hundred inbred and exotic maize lines for their tolerance to anoxia. Nine exotic accessions showed greater tolerance to anaerobic stress, as they survived five to six days of anaerobic treatment at 27°C. In

addition, three inbred lines were found to be significantly less tolerant to anaerobic conditions, surviving only two days (Lemke-Keyes and Sachs 1989b). Results of crosses between the anaerobic tolerant accessions with one anoxia sensitive inbred line (Mo20W) and a "normal" inbred line (B73Ht) show that the anoxia tolerance trait(s) is dominant and shows very simple segregation. This indicated that only one or two genes are involved in each accession.

Submergence tolerance gene in rice

An indica rice cultivar (FR13A) has been found to be very tolerant of flooding and has been found to survive up to two weeks of complete submergence (Mazaredo and Vergara 1982; Mackill *et al.* 1993). A major quantitative trait locus responsible for this tolerance, designated *Submergence 1 (Sub1)*, was mapped near the centromere of chromosome 9 (Xu and Mackill 1996). This locus was isolated and found to include a cluster of three genes at the *Sub1* locus, encoding putative ethylene response factors. Two of these genes, *Sub1B* and *Sub1C*, are conserved and a single allele of each is present in the *Sub1* region of all rice accessions analyzed (including those not tolerant to complete submergence). In contrast, the presence of *Sub1A* is variable. An allele called *Sub1A-1* is found only in tolerant cultivars (e.g., FR13A) and another allele called *Sub1A-2* was found in non-tolerant cultivars (Xu *et al.* 2006). Further analyses of this gene show that the *Sub1* region haplotype determines ethylene- and gibberellic acid-mediated responses to submergence through differential expression of *Sub1A* and *Sub1C*. Submergence tolerance in Fr13A conferred by the *Sub1A-1* allele which dampens ethylene production and GA responsiveness, resulting in reduced growth during submergence that correlates with the capacity for recovery and continued growth when the flood water falls and the shoot is again exposed to air (Fukao *et al.* 2006). Over-expression of *Sub1A-1* in a submergence-intolerant japonica cultivar conferred enhanced tolerance to the plants (Xu *et al.* 2006).

Analysis of the signal transduction pathway leading to the anaerobic response

The genes encoding the ANPs (e.g., ADH) are rapidly turned on by oxygen deprivation (including mild hypoxia) and rapidly turned off by reoxygenation (Wignarajah and Greenway 1976; Paul and Ferl 1991). Such a response implicates a fast and precise O₂-sensing system operating in plant cells. However, very little is known about how plants sense the changes in O₂ availability and how this information is translated into an adaptive response. There is some evidence for the involvement of phytohormones such as ethylene (e.g., Jackson 1985; He *et al.* 1994) and ABA (e.g., Zhang and Davies 1987; Hwang and Van Toai 1991) as intercellular or long distance signals during anoxia (Drew 1990). However, the intracellular pathway of O₂ sensing is only beginning to be understood. The maize anoxic response is well characterized at the molecular level and hence provides a useful model to further our understanding of abiotic stress signaling in plants.

Involvement of calcium ions in the signal transduction pathway leading to the anaerobic response

Deprivation of O₂ leads to disturbances in the ionic balance of plant cells, reflecting energy depletion and membrane depolarization. These transient changes, particularly in Ca²⁺ and H⁺, are immediately recognized, amplified and finally translated into long-lasting biochemical and physiological responses by plant cells. Among the ionic changes induced by anoxia, alterations in cytosolic pH have been studied extensively. Preliminary evidence was presented for the triggering role of pH in metabolic adaptation (Roberts *et al.* 1984; Fox *et al.* 1995). Gene expression and phy-

siological changes in response to O₂ deprivation were shown to be preceded and signaled by an elevation of cytosolic Ca²⁺ in maize seedlings and cultured cells (Subbaiah *et al.* 1994a, 1994b). Studies have shown that changes in cytosolic Ca²⁺ are crucial for gene expression and physiological responses induced under O₂ deprivation in maize (Subbaiah *et al.* 1994a, 1994b).

The origin of the calcium signal was traced, as a part of an attempt to elucidate the nature and intracellular location of the oxygen sensor. Being the primary site of oxygen consumption, it was thought the mitochondrion might serve as a Ca²⁺ store in response to anoxia in maize cells. Confocal analysis using compartment-specific Ca²⁺ probes showed that the Ca²⁺ signal probably originates in mitochondria (Subbaiah *et al.* 1998). The release of Ca²⁺ from mitochondria during early anoxia is most likely not due to passive leakage of the ion, since it was not preceded by the depolarization of mitochondria. However, prolonged anoxia (longer than 30 min) leads to a loss of mitochondrial membrane potential and thus may be responsible for further Ca²⁺ release.

The elucidation of how O₂ deprivation initiates the Ca²⁺ release from mitochondria may indicate exactly where the changes in O₂ levels are sensed in the cell. Since oxygen is more diffusive than any potential signal molecule that has to traverse the cellular membranes, anoxia may be first sensed at the mitochondrial electron transport chain, where O₂ would no longer be available as an electron acceptor. However, in view of the sensitivity of gene expression changes even to mild alterations in the O₂ availability (Paul and Ferl 1991a), i.e., the genes are induced at much higher concentrations than the K_m (O₂) of cytochrome a₃, a low affinity system could be a more appropriate sensor (such as a component of the plasma membrane redox system). The Ca²⁺ released from mitochondria may communicate the metabolic changes occurring in the cytosol (and mitochondria) under O₂ deprivation to the nucleus. Consistent with this, large anoxia-induced changes in the nuclear localized Ca²⁺ levels were observed in maize cells (Subbaiah *et al.* 1998), which may be a prelude to the chromatin changes that occur in anoxic maize cells (e.g., Paul and Ferl 1991a, 1991b).

Work from several laboratories indicates that a similar pathway may operate in other plant species such as *Arabidopsis*, rice and wheat (Sedbrook *et al.* 1995; Chung and Ferl 1999; Baxter-Burrill *et al.* 2002; Klok *et al.* 2002; Bailey-Serres and Chang 2005). Oxygen deprivation is also accompanied by cytosolic acidification (Roberts *et al.* 1984a). The transient changes in Ca²⁺ and H⁺ that follow cell stimulation are immediately recognized even at sub-micromolar levels, amplified and finally translated into long-lasting biochemical and physiological responses by plant cells (e.g., Knight *et al.* 1996; Fasano *et al.* 2001; reviewed in Bush 1995; Sanders *et al.* 1999). Deprivation of O₂ leads to disturbances in ionic balance of plant cells, reflecting energy depletion and membrane depolarization (e.g., Roberts *et al.* 1984a; Buwalda *et al.* 1988). It was shown that gene expression and physiological changes in response to O₂ deprivation are preceded and signaled by an elevation of cytosolic Ca²⁺ in maize seedlings and cultured cells (Subbaiah *et al.* 1994a, 1994b). Using calcium channel antagonists and analyzing cytosolic free calcium ([Ca]_i) changes, it was demonstrated that, calcium acts as a transducer of low O₂ signals (Subbaiah *et al.* 1994a, 1994b).

FURTHER SUBSTANTIATION OF THE CONCEPT ON THE KEY ROLE OF ENERGY AND RELATED PROCESSES OF CARBOHYDRATE METABOLISM IN PLANT ADAPTATION TO ANAEROBIC STRESS

Further development of the investigations of tolerant and sensitive plant adaptation to anaerobic stress confirmed substantially the idea on the key role of energy and related processes of carbohydrate metabolism in plant adaptation to hypoxia and anoxia, which was first demonstrated in

electron-microscopic studies in experiments with glucose feeding of detached rice coleoptiles (Vartapetian *et al.* 1976, 1978) and was analysed later in detail (Vartapetian *et al.* 2003). The role of substrate starvation in plant cell damage and death under anoxia was emphasized especially: "rapid degradation of fine structure of the cells ... under conditions of anoxia is first of all a result of carbon starvation rather than asphyxia or poisoning by toxic products of anaerobic metabolism" (Vartapetian *et al.* 1978, p 77). The important capability of a tolerant plant (rice) to mobilize and utilize endogenous stored carbohydrates (starch) exerted considerable support to this notion (Vartapetian *et al.* 1974, 1976). Due to these processes, glycolysis and fermentation are provided with substrates for a long time and plant adaptive possibilities under hypoxia and anoxia are maintained. Great progress was outlined also in the investigation of induction of the synthesis of corresponding enzymes providing for the involvement of monomeric and polymeric carbohydrates in anaerobic energy metabolism. Below, these investigations are considered.

Short-term adaptation of plants sensitive to anaerobic stress

First, the results of important studies of Saglio *et al.* (1980) and Webb and Armstrong (1983) should be noted. Saglio and his coworkers (1980) were the first who confirmed in detail the above-mentioned role of carbohydrate and energy metabolism in cell adaptation to anoxia also in the case of sensitive plant (maize). They demonstrated that feeding of maize roots with glucose improved substantially both the energy status and the tolerance of their cells to anoxia, similarly as it was observed in experiments with different organs of flood tolerant rice seedling (Vartapetian *et al.* 1976, 1978).

In experiments with the roots of pea (*Pisum sativum*) and pumpkin (*Cucurbita maxima*), Webb and Armstrong (1983) showed that their feeding with glucose helped to maintain the post-anaerobic root capability for growth even after 24 hours of anaerobic incubation, whereas without feeding, post-anaerobic root growth was limited to approximately 6 and 12 hours, pea and pumpkin, correspondently.

Stimulation of energy metabolism and prolongation of plant organ tolerance under the conditions of hypoxia and anoxia by feeding with sugars were also demonstrated in numerous studies of other researchers in experiments with different kinds and organs of plants. (Johnson *et al.* 1989; Waters *et al.* 1991; Hole *et al.* 1992; Ricard *et al.* 1998; Tadege *et al.* 1998; Loreti *et al.* 2003a, 2003b; Ismond *et al.* 2003).

Perata and coworkers (1997), in agreement with the results of experiments of Vartapetian *et al.* (1976), demonstrated the role of α -amylase in mobilization and utilization of stored carbohydrates in long-term tolerance of anaerobically germinating rice seeds (Lorety *et al.* 2003a, 2003b). The key role of energy and related processes of carbohydrate metabolism in plant tolerance to oxygen deficiency was also shown in experiments with rhizomes of plants inhabiting flooded soils and exhibiting high resistance to anoxia (Brändle 1991; Arpagaus Brändle 2000) as well as some submerged wild plants (*Potamogeton pectinatus*, *P. distinctus*) (Summers *et al.* 2000; Sato *et al.* 2002; Harada *et al.* 2005) and seedlings of rice cultivars manifesting different tolerance to submersion (Jackson and Ram 2003). Below we consider the main results of these works.

Key role of mobilization and utilization of reserved carbohydrate in long-term plant adaptation to anaerobic stress

The publications of Perata's laboratory (Perata *et al.* 1997; Loreti *et al.* 2003a, 2003b) highlight the main results of investigations on the role of α -amylase in adaptation of anaerobically germinated rice seeds. Just this enzyme of the seed is responsible for anaerobic mobilization of stored

starch, which provides substrate not only for anaerobic glycolysis and fermentation but also for rice seedling active growth in the absence of oxygen. These studies showed that unlike rice seeds, the seeds of other cereals (wheat, barley, and oat) did not synthesize α -amylase under anoxia and, therefore, these seeds devoid of endogenously generated glucose could not germinate under anoxic conditions and perish. Their tolerance to anoxia could be improved to some degree only by feeding with exogenous sugars. In contrast to aerobically germinated seeds, under anoxic conditions a gene encoding specific α -amylase (Ramy3D) is expressed in rice seeds. The activity of this gene is controlled by the presence of sugar but not by a hormone (Loreti *et al.* 2003a). An excess of sugar suppresses the expression of this gene, whereas sugar shortage, in contrast, induces its expression. Enhanced sugar consumption in anaerobic fermentation results in a sugar shortage and thus activates the expression of the gene encoding Ramy3D (α -amylase) at early stages of anaerobic seed germination, thus providing seedlings with sugars. Simultaneously anoxia weakens the activity of the *Ramy1A* gene, which expresses predominantly under aerobic conditions. It was shown that the activity of the Ramy3D gene under conditions of anoxia was sufficient for the synthesis of corresponding α -amylase isoenzyme mobilizing stored carbohydrates of the rice seed.

The results of experiments by Brändle (1991) and Arpagaus and Brändle (2000) on the rhizomes of wild plants inhabiting flooded soils are also interesting. The rhizomes of *Acorus calamus* for example, containing a large store of starch, were capable of surviving during several winter months in the absence of oxygen in the state of quiescence. In spring, its shoots grew rapidly under water and contacted with the atmosphere. The duration of rhizome existence in the absence of oxygen depended on the amount of stored carbohydrates (Brändle 1991; Hanhijarvi and Fagerstedt 1995) and on the presence of active α -amylase (Arpagaus and Brändle 2000). Due to mobilization and utilization of stored carbohydrates during the entire winter period, the AEC in rhizomes was maintained at a rather high level (0.5-0.8).

The key role of anaerobic energy metabolism is manifested brightly during the rapid under-water growth of some wild submerged plants, for example, *Potamogeton* and *Rumex* species (Summers *et al.* 2000; Sato *et al.* 2002; Voesenek *et al.* 2003; Harada *et al.* 2005; Dixon *et al.* 2006). Experiments showed that in submerged plants rapid shoot growth under water was determined by a much higher rate of its elongation under anaerobic than aerobic conditions. Such a rapid growth under water was achieved due to the 3- to 6-fold increased rate of glycolysis (Pasteur's effect) and active mobilization of starch storage in the tuber.

Rice seedlings completely submerged in water are commonly observed under natural conditions in East and South-East Asia. The tolerant seedlings possess a specific system regulating energy and carbohydrate metabolism; therefore, they could survive under extreme conditions of oxygen starvation (Vriezen *et al.* 2003; Boamfa *et al.* 2003; Jackson and Ram 2003). However, under submerged conditions, plants suffer also from suppressed photosynthesis and thus from deficiency in the formation of a new substrate (Ellis and Setter 1999; Momer and Visser 2005). Seedlings of various rice cultivars differ substantially in their tolerance to total submersion. The investigation of carbohydrate metabolism and growth of rice cultivars contrasting in tolerance showed that flooding of the most tolerant cultivar ('FR13A') suppressed its growth substantially, which was accompanied by a more economic spending of carbohydrate stores. By analyzing the experimental material accumulated Jackson and Ram (2003) concluded that the basic cause for seedling damage and death of less tolerant rice cultivars under submersion is their energy shortage due to substrate starvation. Active leaf growth of non-tolerant cultivars result in exhaustion of substrates for glycoly-

sis because of more intensive spending of stored carbohydrates.

Induction of anaerobic synthesis of enzymes responsible for sugar involvement in glycolysis and fermentation

For active ethanolic and lactic fermentation under conditions of hypoxia and anoxia, along with polymeric carbohydrates (starch), sugars (glucose, fructose, and sucrose) should be involved in metabolism by corresponding enzymes, hexokinases and sucrose synthase. During hypoxic acclimation but not at anoxia, just these enzymes were activated providing for relatively high rates of glycolytic flow and ATP generation and thus cell survival under anoxia (Saglio *et al.* 1999). A critical role of sucrose synthase under anoxia was shown in experiments with the roots of maize double mutants deficient in sucrose synthase synthesis (Ricard *et al.* 1998). Even after hypoxic acclimation, such mutants could not metabolize sucrose and could not survive anoxia, whereas the roots fed with glucose were not damaged over the prolonged period and survived. The results of these experiments indicate that, under anoxia, sucrose could be involved in the glycolytic flow in roots only with the involvement of sucrose synthase but not invertase.

Under conditions of oxygen deficiency, the synthesis of enzymes of glycolysis, fermentation and sugar-phosphate metabolism is also induced (Kelley and Freeling 1984a, 1984b; Springer *et al.* 1986; Kelly 1989; Kelly *et al.* 1991; Russell and Sachs 1991; Lal *et al.* 1998; Saglio *et al.* 1999). Comparison of enzymes of fermentation (ADH, LDH, and PDC) showed that hypoxia and anoxia increased ADH activity sharply, although such a high activity is not required for cell survival under anaerobiosis.

Under hypoxia and anoxia, PDC activity is also markedly increased. Just this enzyme is believed to play a key role in regulation of ethanolic fermentation. It was shown that tobacco (Bucher *et al.* 1994; Tadege *et al.* 1998), rice (Quimio *et al.* 2000), and *Arabidopsis* (Ismond 2003) plants transformed with the PDC gene sharply accumulated acetaldehyde and ethanol. This was associated with the improvement of their tolerance to oxygen deficiency, at least in rice (Quimio *et al.* 2000) and *Arabidopsis* (Ismond *et al.* 2003).

Thus, accumulated experimental material indicates that carbohydrate and energy metabolism play key roles in plant tolerance to anaerobic stress. To synthesize a sufficient amount of ATP required for the maintenance of plant survival under these extreme conditions, the plant cell must be provided with both substrates and corresponding active enzyme systems capable of mobilization of polymeric and monomeric forms of carbohydrates for their subsequent usage in glycolysis and fermentation. Anaerobic energy metabolisms provide for not only long-term plant survival even under total absence of oxygen but also, in some cases, for active growth, as for example, during anaerobic germination of rice seeds and underwater growth of submerged wild plants, which was aforementioned.

CONCLUSION

In the present review we attempted, first of all, to follow the emergence and establishment of novel scientific branch in field of ecological physiology, biochemistry and molecular biology, dedicated to plant life under hypoxic and anoxic stresses that received international recognition as an independent original biological discipline.

An increased interest to the problem of low oxygen stress of plants during recent decades confirmed undoubtedly its importance from the point of view of fundamental science as well as of applied aspects of agronomy, forestry and environment protection. The coordination of investigations at the international level achieved mainly due to the efforts of ISPA members, who were the initiators of perio-

dic international meetings, cooperative studies, and also corresponding publications, favored this interest. All this resulted in the rapid development of investigation on plant hypoxia and anoxia and affected not only the volume of scientific production but also its quality.

In this first part of this review, in addition to background information about the establishment of a new branch of science, special attention was given to the further progress in investigation of plant metabolic adaptation to anaerobic stress, namely synthesis of anaerobic proteins and their physiological role, energy and related processes of carbohydrate metabolism that ensure plant life under condition of oxygen deficiency or complete absence of oxygen. The second general strategy of plant adaptation to anaerobic environment by long-distance oxygen translocation that is by avoidance of anaerobiosis will be discussed in the second part of this review (Vartapetian, Sachs, Fagerstedt 2008).

Analyzing investigations over the last four decades, the authors would like primarily to attract the readers' attention to the following points. As distinct from early period, when a novel scientific discipline only started to develop and two alternative theories of plant adaptation to anaerobic stress were actively discussed, namely, Crawford's metabolic theory (1977, 1978) and the concept of two general strategies of plant adaptation to anaerobiosis (Vartapetian 1978), further development of investigations showed the validity of just concept about two general strategies of adaptation, that is true tolerance which is realized at a molecular level due to fundamental rearrangements of protein, carbohydrate and energy metabolism under conditions of strict anoxia or hypoxia and apparent tolerance that is attained by avoidance of anaerobiosis by long-distance oxygen transport.

Certainly, keeping in mind a diversity of plants and environments they inhabit, one can admit that, in some cases, plant adaptation to anaerobic stress evidently occurs not in such a "pure" way as it was described above. Nevertheless, the analysis of experimental material accumulated up to now permits a statement that plant adaptation to the anaerobic stress is mainly realized within the framework of the two aforementioned strategies, true and apparent tolerance. It is especially brightly expressed in rice (*Oryza sativa*) plants: during germination of rice seeds in the absence of oxygen the mechanism of true tolerance functions, whereas in adult plants inhabiting flooded soils, the roots avoid anaerobiosis due to oxygen transport from shoot.

Understanding the response to anaerobic stress is also important for the future breeding of flood-tolerant or -resistant crop plants. In addition to molecular, physiological and biochemical approaches, genetic analysis has been essential in the establishment of causal relationships between the induction of the stress proteins and the establishment of resistance to the stress condition. In most stress responses, it is not difficult to detect the induction of new proteins during stress. However, the induction of new proteins does not necessarily establish a relationship with stress resistance. Novel protein synthesis may well be the consequence of damage caused by a stress condition. Thus, genetic mutants are necessary to test the physiological role of a stress protein. Perhaps the best example of this is the demonstration that ADH null mutants in maize fail to survive more than a few hours of anaerobic stress (Schwartz 1969) but grow well in non-flooding conditions. On the other hand, their ADH+ siblings will survive ~3 days of flooding (Schwartz 1969; Lemke-Keyes and Sachs 1989b).

With the advances in genetic transformation techniques, it is likely that genes encoding stress proteins, or other factors, from a tolerant plant will be introduced into plants that are normally sensitive to the stress condition. This approach is not only important for the molecular analysis of DNA sequences that are responsive to an anaerobic stimulus, but will also pave the way for constructing "tailor-made" flood resistant crop plants.

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REFERENCES

- Altschuler M, Mascarenhas JP (1982) Heat shock proteins and effects of heat shock in plants. *Plant Molecular Biology* **1**, 103-115
- Andrews CJ (1997) A comparison of glycolytic activity in wheat and two forage grasses in relation to their tolerance to ice encasement. *Annals of Botany* **79**, 87-92
- Andrews CJ, Pomeroy MK (1981) The effect of flooding pretreatment on cold hardiness and survival of winter cereals in ice encasement. *Canadian Journal of Plant Science* **61**, 507-513
- Andrews CJ, Pomeroy MK (1983) The influence of flooding pretreatment on metabolic changes in winter cereal seedlings during ice encasement. *Canadian Journal of Botany* **63**, 142-147
- Andrews D, Cobb BG, Johnson JR, Drew MC (1993) Hypoxic and anoxic induction of alcohol dehydrogenase in roots and shoots of seedlings of *Zea mays*: adh transcripts and enzyme activity. *Plant Physiology* **101**, 407-414
- Ap Rees T, Wilson PM (1984) Effect of reduced supply of oxygen on the metabolism of roots of *Glyceria maxima* and *Pisum sativum*. *Zeitschrift für Pflanzenphysiologie* **114**, 493-503
- Armstrong W (1964) Oxygen diffusion from the roots of some British bog plants. *Nature* **204**, 801
- Armstrong W (1969) Rhizosphere oxidation in rice: an analysis of intervarietal differences in oxygen flux from the roots. *Physiologia Plantarum* **22**, 296-303
- Armstrong W (1978) Root aeration in the wetland condition. In: Hook DD, Crawford RMM (Eds) *Plant Life in Anaerobic Environments*, Ann Arbor Science, Michigan, pp 269-297
- Armstrong W (1979) Aeration in higher plants. *Advances in Botanical Research* **7**, 225-332
- Arpagaus S, Brändle R (2000) The significance of α -amylase under anoxia stress in tolerant rhizomes (*Acorus calamus* L.) and nontolerant tubers (*Solanum tuberosum* L. var. Désirée). *Journal of Experimental Botany* **51**, 1475-1477
- Bailey-Serres J, Chang R (2005) Sensing and signalling in response to oxygen deprivation in plants and other organisms. *Annals of Botany* **96**, 507-518
- Bailey-Serres J, Dawe K (1996) Both 5' and 3' sequences of maize ADH1 mRNA are required for enhanced translation under low-oxygen conditions. *Plant Physiology* **112**, 685-695
- Bailey-Serres J, Freeling M (1990) Hypoxic stress-induced changes in ribosomes of maize seedling roots. *Plant Physiology* **94**, 1237-1243
- Bailey-Serres J, Kloeckener-Gruissem B, Freeling M (1988) Genetic and molecular approaches to the study of the anaerobic response and tissue specific gene expression in maize. *Plant Cell and Environment* **11**, 351-357
- Baxter-Burrill A, Yang Z, Springer PS, Bailey-Serres J (2002) Rop GAP4-dependent Rop GTPase rheostat control of *Arabidopsis* oxygen deprivation tolerance. *Science* **296**, 2026-2028
- Bertani A, Menegus F, Bollini R (1981) Some effects of anaerobiosis on protein metabolism in rice roots. *Zeitschrift für Pflanzenphysiologie* **103**, 37-43
- Boamfa EI, Ram PC, Jackson MB, Reus J, Harren FJM (2003) Dynamic aspects of alcohol fermentation of rice seedling in response to anaerobiosis and to complete submergence: relationship to submergence tolerance. *Annals of Botany* **91**, 279-290
- Branco-Price C, Kawaguchi R, Ferreira RB, Bailey-Serres J (2005) Genome-wide analysis of transcript abundance and translation in *Arabidopsis* seedlings subjected to oxygen deprivation. *Annals of Botany* **96**, 647-660
- Brändle R (1991) Flooding resistance of rhizomatous amphibious plants. In: Jackson MB, Davies DD, Lambers H (Eds) *Plant Life Under Oxygen Deprivation. Ecology, Physiology and Biochemistry*, SPB Academic, The Hague, pp 35-46
- Bucher M, Brändle R, Kuhlemeier C (1994) Ethanolic fermentation in transgenic tobacco expressing *Zymomonas mobilis* pyruvate decarboxylase. *The EMBO Journal* **13**, 2755-2763
- Bush DS (1995) Calcium regulation in plant cells and its role in signaling. *Annual Review of Plant Physiology and Plant Molecular Biology* **103**, 7-13
- Buwalda F, Thompson CJ, Steigner W, Barrett-Lennard EG, Gibbs J, Greenway H (1988) Hypoxia induces membrane depolarization and potassium loss from wheat roots but does not increase their permeability to sorbitol. *Journal of Experimental Botany* **39**, 1169-1183
- Chang WWP, Huang L, Shen M, Webster C, Burlingame AL, Roberts JKM (2000) Patterns of protein synthesis and tolerance of anoxia in root tips of maize seedlings acclimated to a low-oxygen environment, and identification of proteins by mass spectrometry. *Plant Physiology* **122**, 295-318
- Chang C, Meyerowitz EM (1986) Molecular cloning and DNA sequence of the *Arabidopsis thaliana* alcohol dehydrogenase gene. *Proceedings of the National Academy of Sciences USA* **83**, 1408-1412
- Chung H-J, Ferl RJ (1999) *Arabidopsis* alcohol dehydrogenase expression in both shoots and roots is conditioned by root growth environment. *Plant Physiology* **121**, 429-436
- Cooper P, Ho T-HD (1983) Heat shock proteins in maize. *Plant Physiology* **71**, 215-222
- Costes C, Vartapetian BB (1978) Plant grown in a vacuum: the ultrastructure and functions of mitochondria. *Plant Science Letters* **11**, 115-119
- Crawford RMM (1977) Tolerance of anoxia and ethanol metabolism in germinating seeds. *New Phytologist* **79**, 511-517
- Crawford RMM (1978) Metabolic adaptation to anoxia. In: Hook DD, Crawford RMM (Eds) *Plant Life in Anaerobic Environments*, Ann Arbor Science, Michigan, pp 119-136
- Cui XQ, Scheffler B, Simmons C, Meely RB, Schnable PS (2000) Mutations in the maize *pdcd3* gene cause reduced levels of tolerance to anaerobic stress, annual meeting of the American Society of Plant Physiologists 15-19 July, San Diego, CA
- Davies DD (1980) Anaerobic metabolism and the production of organic acids. In: Davies DD (Ed) *The Biochemistry of Plants*, Academic Press, New York **2**, pp 581-611
- Davies DD, Grego S, Kenworth P (1974) The control of the production of lactate and ethanol by higher plants. *Planta* **118**, 297-310
- de Vetten NC, Ferl RJ (1995) Characterization of a maize G-box binding factor that is induced by hypoxia. *The Plant Journal* **7**, 589-601
- Dennis ES, Gerlach WL, Pryor AJ, Bennetzen JL, Ingles A, Llewellyn D, Sachs MM, Ferl RJ, Peacock WJ (1984) Molecular analysis of the *Alcohol dehydrogenase (Adh1)* gene of maize. *Nucleic Acids Research* **12**, 3983-4000
- Dennis ES, Gerlach WL, Walker JC, Lavin M, Peacock WJ (1988a) Anaerobically regulated aldolase gene of maize: A chimeric Origin? *Journal of Molecular Biology* **202**, 759-767
- Dennis ES, Sachs MM, Gerlach WL, Beach L, Peacock WJ (1988b) The *Ds1* transposable element acts as an intron in the mutant allele *Adh1-Fm335* and is spliced from the message. *Nucleic Acids Research* **16**, 3815-3828
- Dennis ES, Sachs MM, Gerlach WL, Finnegan EJ, Peacock WJ (1985) Molecular analysis of the *Alcohol dehydrogenase 2 (Adh2)* gene of maize. *Nucleic Acids Research* **13**, 727-743
- Dennis ES, Walker JC, Llewellyn DJ, Ellis JG, Singh K, Tokuhisa JG, Wolstenholme DR, Peacock WJ (1989) The response to anaerobic stress: transcriptional regulation of genes for anaerobically induced proteins. In: Cherry JH (Ed) *Environmental Stress in Plants*, Springer, New York, pp 231-245
- Dixon MH, Hill SA, Jackson MB, Ratcliffe RG, Sweetlove LJ (2006) Physiological and metabolic adaptations of *Potamogeton pectinatus* L. Tubers support rapid elongation of stem tissue in the absence of oxygen. *Plant and Cell Physiology* **47**, 128-140
- Dolferus R, Marbaix G, Jacobs M (1985) Alcohol dehydrogenase in *Arabidopsis*: analysis of the induction phenomenon in plantlets and tissue cultures. *Molecular and General Genetics* **199**, 256-264
- Drew MC (1990) Sensing soil oxygen. *Plant Cell and Environment* **13**, 1013-1023
- Duncan DR, Willaims ME, Zehr BE, Widholm JM (1985) The production of callus capable of plant regeneration from immature embryos of numerous *Zea mays* genotypes. *Planta* **165**, 322-332
- Ellis MH, Setter TL (1999) Hypoxia induces anoxia tolerance to completely submerged seedlings. *Plant Physiology* **154**, 219-230
- Fasano JM, Swanson SJ, Blancaflor EB, Dowd PE, Kao TH, Gilroy S (2001) Changes in root cap pH are required for the gravity response of the *Arabidopsis* root. *Plant Cell* **13**, 907-921
- Felle H (2005) pH regulation in anoxia plants. *Annals of Botany* **96**, 519-532
- Ferl RJ, Brennan M, Schwartz D (1980) *In vitro* translation of maize ADH: evidence for the anaerobic induction of mRNA. *Biochemical Genetics* **18**, 681-691
- Ferl RJ, Dlouhy SR, Schwartz D (1979) Analysis of maize alcohol dehydrogenase by native-SDS two dimensional electrophoresis and autoradiography. *Molecular and General Genetics* **169**, 7-12
- Fox GG, McCallan NR, Ratcliffe RG (1995) Manipulating cytoplasmic pH under anoxia: a critical test of the role of pH in the switch from aerobic to anaerobic metabolism. *Planta* **195**, 324-330
- Francis CM, Devitt AC, Steele P (1974) Influence of flooding on the alcohol dehydrogenase activity of roots of *Trifolium subterraneum*. *Australian Journal of Plant Physiology* **93**, 1094-1101
- Freeling M (1973) Simultaneous induction by anaerobiosis or 2,4-D of multiple enzymes specified by two unlinked genes: differential *Adh1-Adh2* expression in maize. *Molecular General Genetics* **127**, 215-227
- Freeling M, Bennett DC (1985) Maize *Adh1*. *Annual Review Genetics* **19**, 297-323
- Freeling M, Birchler JA (1981) Mutants and variants of the *Alcohol dehydrogenase-1* gene in maize. In: Setlow JK, Hollaender A (Eds) *Genetic Engineering Principles and Methods*, Plenum Press **3**, pp 223-264
- Fukao T, Xu K, Ronald PC, Bailey-Serres J (2006) A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. *Plant Cell* **18**, 2021-2034
- Gambrell RP, de Laune RD, Patrick WH Jr. (1991) Redox processes in soils following oxygen depletion. In: Jackson MB, Davies DD, Lambers H (Eds) *Plant Life Under Oxygen Deprivation. Ecology, Physiology and Biochemistry*, SPB Academic, The Hague, pp 101-117

- Gambrell RP, Patrick WH** (1978) Chemical and microbiological properties of anaerobic soils and sediments. In: Hook DD, Crawford RMM (Eds) *Plant Life in Anaerobic Environments*, Ann Arbor Science, Michigan, pp 375-424
- Gerlach WL, Pryor AJ, Dennis ES, Ferl RJ, Sachs MM, Peacock WJ** (1982) cDNA cloning and induction of the *Alcohol dehydrogenase* gene (*Adh1*) of maize. *Proceedings of the National Academy of Sciences USA* **79**, 2981-2985
- Gerlach WL, Sachs MM, Llewellyn D, Finnegan EJ, Dennis ES** (1986) Maize *Alcohol dehydrogenase*: A molecular perspective. In: Blonstein AD, King PJ (Eds) *Plant Gene Research. A Genetic Approach to Plant Biochemistry*, Springer-Verlag Wein, NY **3**, pp 73-100
- Germain V, Ricard B, Raymond P, Saglio PH** (1997) The role of sugars, hexokinase, and sucrose synthase in the determination of hypoxically induced tolerance to anoxia in tomato roots. *Plant Physiology* **114**, 167-175
- Gonzali S, Loreti E, Novi G, Poggi A, Alpi A, Perata P** (2005) The use of microarrays to study the anaerobic response in *Arabidopsis*. *Annals of Botany* **96**, 661-668
- Good AG, Paetkau DH** (1992) Identification and characterization of a hypoxically induced maize lactate dehydrogenase gene. *Plant Molecular Biology* **19**, 693-697
- Hageman RH, Flesher D** (1960) The effect of anaerobic environment on the activity of alcoholdehydrogenase and other enzymes of corn seedlings. *Archives of Biochemistry and Biophysics* **87**, 203-209
- Hake S, Kelley PM, Taylor WC, Freeling M** (1985) Coordinate induction of alcohol dehydrogenase 1, aldolase, and other anaerobic RNAs in maize. *The Journal of Biological Chemistry* **260**, 5050-5054
- Hanhijarvi AM, Fagerstedt KV** (1995) Comparison of carbohydrate utilization and energy charge in the yellow flag iris (*Iris pseudocorus*) and garden iris (*Iris germanica*) under anoxia. *Physiologia Plantarum* **93**, 493-497
- Hanson AD, Jacobsen JV** (1984) Control of lactate dehydrogenase, lactate glycolysis, and α -amylase by O₂ deficit in barley aleurone layers. *Plant Physiology* **75**, 566-572
- Hanson AD, Jacobsen JV, Zwar JA** (1984) Regulated expression of three *Alcohol dehydrogenase* genes in barley aleurone layers. *Plant Physiology* **75**, 573-581
- Harada T, Satoh Sh, Yoshioka T, Ishizawa K** (2005) Expression of sucrose synthase genes involved in enhanced elongation of pondweed (*Potamogeton distinctus*) turions under anoxia. *Annals of Botany* **96**, 683-692
- He C-J, Drew MC, Morgan PW** (1994) Induction of enzymes associated with lysigenous aerenchyma formation in roots of *Zea mays* during hypoxia or nitrogen starvation. *Plant Physiology* **105**, 861-865
- Ho T-HD, Sachs MM** (1989) Stress-induced proteins: characterization and the regulation of their synthesis. In: Marcus A (Ed) *The Biochemistry of Plants*, Academic Press, New York **15**, pp 347-378
- Hoffman NE, Bent AF, Hanson AD** (1986) Induction of lactate dehydrogenase isozymes by oxygen deficit in barley root tissue. *Plant Physiology* **82**, 658-663
- Hole DJ, Cobb BG, Hole P, Drew MC** (1992) Enhancement of anaerobic respiration in root tips of *Zea mays* following low oxygen (hypoxic) acclimation. *Plant Physiology* **99**, 213-218
- Hondred D, Hanson AD** (1990) Hypoxically inducible barley lactate dehydrogenase: cDNA cloning and molecular analysis. *Proceedings of the National Academy of Sciences USA* **87**, 7300-7304
- Hook DD, Crawford RMM** (1978) *Plant Life in Anaerobic Environments*, Ann Arbor Science, Michigan, 564 pp
- Hwang S-Y, Van Toai TT** (1991) Abscisic acid induces anaerobiosis tolerance in corn. *Plant Physiology* **97**, 593-597
- Ismond KP, Dolferus R, Pauw MD, Dennis ES, Good AG** (2003) *Enhanced low oxygen survival in Arabidopsis* through increased metabolic flux in the fermentative pathway. *Plant Physiology* **132**, 1292-1302
- Jackson MB** (1985) Ethylene and responses of plants to soil waterlogging and submergence. *Annual Review of Plant Physiology* **36**, 145-174
- Jackson MB, Armstrong W** (1999) Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. *Plant Biology* **1**, 274-287
- Jackson MB, Gambrell DJ** (1975) Movement of ethylene from roots to shoots, a factor in the responses of tomato plants to waterlogged soil conditions. *New Phytologist* **74**, 397-406
- Jackson MB, Gales K, Gambrell DJ** (1978) Effect of waterlogged soil conditions on the production of ethylene and on water relationships in tomato plants. *Journal of Experimental Botany* **29**, 183-193
- Jackson MB, Ram PC** (2003) Physiological and molecular basis of susceptibility and tolerance of rice plants to complete submergence. *Annals of Botany* **91**, 227-241
- Johnson JR, Cobb BG, Drew MC** (1989) Hypoxic induction of anoxia tolerance in root tips of *Zea mays*. *Plant Physiology* **91**, 837-841
- Kawase M** (1981) Effect of ethylene on aerenchyma development. *American Journal of Botany* **68**, 651-658
- Kelley PM** (1989) Maize pyruvate decarboxylase mRNA is induced anaerobically. *Plant Molecular Biology* **13**, 213-222
- Kelley PM, Freeling M** (1982) A preliminary comparison of maize anaerobic and heat-shock proteins. In: Schlesinger MJ, Ashburner M, Tissières A (Eds) *Heat Shock from Bacteria to Man*, Cold Spring Harbor Laboratory Press, New York, pp 315-319
- Kelley PM, Freeling M** (1984a) Anaerobic expression of maize glucose phosphate isomerase I. *The Journal of Biological Chemistry* **259**, 673-677
- Kelley PM, Freeling M** (1984b) Anaerobic expression of maize fructose-1, 6-diphosphate aldolase. *The Journal of Biological Chemistry* **259**, 14180-14183
- Kelley PM, Tolan DR** (1986) The complete amino acid sequence for the anaerobically induced aldolase from maize derived from cDNA clones. *Plant Physiology* **82**, 1076-1080
- Kelley PM, Godfrey K, Lal SK, Alleman M** (1991) Characterization of the maize pyruvate decarboxylase gene. *Plant Molecular Biology* **17**, 1259-1261
- Klok EJ, Wilson IW, Chapman SC, Ewing RM, Somerville SC, Peacock WJ, Dolferus R, Dennis ES** (2002) Expression profile analysis of the low-oxygen response in *Arabidopsis* root cultures. *Plant Cell* **14**, 2481-2494
- Knee M** (1991) Fruit metabolism and practical problems of fruit storage under hypoxia and anoxia. In: Jackson MB, Davies DD, Lambers H (Eds) *Plant Life Under Oxygen Deprivation. Ecology, Physiology and Biochemistry*, SPB Academic, The Hague, pp 229-243
- Knight H, Trewavas AJ, Knight MR** (1996) Cold calcium signaling in *Arabidopsis* involved two cellular pools and a change in calcium signature after acclimation. *Plant Cell* **8**, 489-503
- Lal SK, Elthon TE, Kelley PM** (1994) Purification and differential expression of enolase from maize. *Physiologia Plantarum* **91**, 587-592
- Lal SK, Johnson S, Conway T, Kelley PM** (1991) Characterization of a maize cDNA that complements an enolase-deficient mutant of *Escherichia coli*. *Plant Molecular Biology* **16**, 787-795
- Lal SK, Lee C, Sachs MM** (1998) Differential regulation of enolase during anaerobiosis in maize. *Plant Physiology* **118**, 1285-1293
- Lal S, Sachs MM** (1995) Cloning and characterization of an anaerobically induced cDNA encoding glucose-6-phosphate isomerase from maize. *Plant Physiology* **108**, 1295-1296
- Larcher W** (1980) *Physiological Plant Ecology*, Springer-Verlag, Berlin, 450 pp
- Laszlo A, Lawrence P** (1983) Parallel induction and synthesis of PDC and ADH in anoxic maize roots. *Molecular and General Genetics* **192**, 110-117
- Lemke-Keys CA, Sachs MM** (1989a) Anaerobic tolerant null: A mutant that allows *Adh1* nulls to survive anaerobic treatment. *Journal of Heredity* **80**, 316-319
- Lemke-Keys CA, Sachs MM** (1989b) Genetic variation for seedling tolerance to anaerobic stress in maize germplasm. *Maydica* **34**, 329-337
- Lin CY, Key JL** (1967) Dissociation and reassembly of polyribosomes in relation to protein synthesis in the soybean root. *Journal of Molecular Biology* **26**, 237-247
- Llewellyn DJ, Finnegan EJ, Ellis JG, Dennis ES, Peacock WJ** (1987) Structure and expression of an *Alcohol dehydrogenase 1* gene from *Pisum sativum* (cv. 'Greenfeast'). *Journal of Molecular Biology* **195**, 115-123
- Loreti E, Alpi A, Perata P** (2003a) α -amylase expression under anoxia in rice seedlings: An update. *Plant Physiology* **50**, 737-742
- Loreti E, Yamaguchi J, Alpi A, Perata P** (2003b) Sugar modulation of α -amylase genes under anoxia. *Annals of Botany* **91**, 143-148
- Mackill DJ, Amante MM, Vergara BS, Sarkarung S** (1993) Improved seedling rice lines with tolerance to submergence of seedlings. *Crop Science* **33**, 749-753
- Maltby E** (1991) Wetlands – their status and role in the biosphere. In: Jackson MB, Davies DD, Lambers H (Eds) *Plant Life Under Oxygen Deprivation. Ecology, Physiology and Biochemistry*, SPB Academic, The Hague, pp 3-21
- Manjunath S, Sachs MM** (1997) Molecular characterization and promoter analysis of the maize cytosolic glyceraldehyde 3-phosphate dehydrogenase gene family and its expression during anoxia. *Plant Molecular Biology* **33**, 97-112
- Manjunath S, Williams AJ, Bailey-Serres J** (1999) Oxygen deprivation stimulates Ca²⁺-mediated phosphorylation of mRNA cap-binding protein eIF4E in maize roots. *The Plant Journal* **19**, 21-30
- Martinez P, Martin W, Cerff R** (1989) Structure, evolution and anaerobic expression of the nuclear gene for cytosolic glyceraldehyde-3-phosphate dehydrogenase from maize. *Journal of Molecular Biology* **208**, 551-565
- Maslova IP, Tchernyadeva IF, Vartapetian BB** (1975) Soluble proteins and alcoholdehydrogenase of rice seedlings in anoxia. Abstract. XII International Congress Leningrad Nauka **2**, 365
- Marshall DR, Broué P, Pryor AJ** (1973) Adaptive significance of alcohol dehydrogenase isozymes in maize. *Nature New Biology* **244**, 16-18
- Mazaredo AM, Vergara BS** (1982) Physiological differences in rice varieties tolerant of and susceptible to complete submergence. In: Proceedings of the 1981 international deepwater rice workshop. International Rice Research Institute, Manila, pp 327-341
- McMannon M, Crawford RMM** (1971) A Metabolic theory of flooding tolerance: The significance of enzyme distribution and behaviour. *New Phytologist* **70**, 299-306
- Millar AA, Dennis ES** (1996) Protein synthesis during oxygen deprivation in cotton. *Australian Journal of Plant Physiology* **23**, 341-348
- Mocquot B, Prat C, Mouches C, Pradet A** (1981) Effect of anoxia on energy charge and protein synthesis in rice embryo. *Plant Physiology* **68**, 636-640
- Mommer L, Visser EJW** (2005) Underwater photosynthesis in flooded terrestrial plants: a matter of leaf plasticity. *Annals of Botany* **96**, 581-589
- Moore P** (1982) Survival mechanism in wetland plants. *Nature* **299**, 581-582

- Mujer CV, Rumpho VE, Lin JJ, Kennedy RA (1993) Constitutive and inducible aerobic and anaerobic stress proteins in *Echinochloa* complex and rice. *Plant Physiology* **101**, 217-226
- Nurritdinov N, Vartapetian BB (1981) A quantitative assay of O₂ transport in cotton plants at different temperatures. *Physiologia Vegetal* **19**, 211-217
- Okimoto R, Sachs MM, Porter EK, Freeling M (1980) Patterns of polypeptide synthesis in various maize organs under anaerobiosis. *Planta* **150**, 89-94
- Olive MR, Peacock WJ, Dennis ES (1991) The anaerobic responsive element contains 2 GC-rich sequences essential for binding a nuclear-protein and hypoxic activation of the maize *Adh1* promoter. *Nuclear Acids Research* **19**, 7053-7060
- Paul A-L, Ferl RJ (1991a) *Adh1* and *Adh2* regulation. *Maydica* **36**, 129-134
- Paul A-L, Ferl RJ (1991b) *In vivo* footprinting reveals unique *cis*-elements and different modes of hypoxic induction in maize *Adh1* and *Adh2*. *Plant Cell* **3**, 159-168
- Pelham HRB, Bienz M (1982) A synthetic heat-shock promoter element confers heat-inducibility on the herpes simplex virus thymidine kinase gene. *The EMBO Journal* **1**, 1473-1477
- Perata P, Guglielminetti L, Alpi A (1997) Mobilization of endosperm reserves in cereal seeds under anoxia. *Annals of Botany* **79**, 49-56
- Perez-Mendez A, Aguilar R, Briones E, de Sanchez JE (1993) Characterization of ribosomal protein phosphorylation in maize axes during germination. *Plant Science* **94**, 71-79
- Peschke VM, Sachs MM (1993) Multiple maize pyruvate decarboxylase genes are induced by hypoxia. *Molecular and General Genetics* **240**, 206-212
- Peschke VM, Sachs MM (1994) Characterization and expression of anaerobically induced maize transcripts. *Plant Physiology* **104**, 387-394
- Ponnamperuma FN (1984) Effect of flooding on soils. In: Kozlowski TT (Ed) *Flooding and Plant Growth*, Academic Press, Orlando, FL, pp 9-45
- Pradet A, Bomsel JL (1978) Energy metabolism in plants under hypoxia and anoxia. In: Hook DD, Crawford RMM (Eds) *Plant Life in Anaerobic Environments*, Ann Arbor Science, Michigan, pp 89-118
- Pradet A, Mocquot B, Raymond P, Morisset C, Aspart L, Delseny M (1985) Energy metabolism and synthesis of nucleic acids and proteins under anoxic stress. In: Key JL, Kosuge T (Eds) In: *Cellular and Molecular Biology of Plant Stress. UCLA Symposia on Molecular and Cellular Biology, New Series* **22**, Alan R. Liss, NY, pp 227-245
- Quimio CA, Torrizo LB, Setter TL, Ellis M, Grover A, Abrigo EM, Oliva NP, Ella ES, Carpena AL, Ito O, Peacock WJ, Dennis E, Datta SK (2000) Enhancement of submergence tolerance in transgenic rice overproducing pyruvate decarboxylase. *The Journal of Plant Physiology* **156**, 516-521
- Ram PC, Singh PN, Singh BN, Singh BB, Setter TL (2004) Waterlogging induced anaerobiosis: a hidden constraint to wheat productivity in sodic soils of India. 8th Conf. ISPA, Perth, West Australia, p 48 (Abstract)
- Ratcliffe RG (1997) *In Vivo* NMR studies of the metabolic responses of plant tissues to anoxia. *Annals of Botany* **79**, 39-48
- Ricard B, Pradet A (1989) Anaerobic protein synthesis in different organs of germinating rice seeds. *Plant Physiology and Biochemistry* **27**, 761-768
- Ricard B, Van Toai T, Chourey P, Saglio PH (1998) Evidence for the critical role of sucrose synthase for anoxic tolerance of maize roots using a double mutant. *Plant Physiology* **116**, 1323-1331
- Roberts JKM, Andrade FH, Anderson IC (1985) Further evidence that cytoplasmic acidosis is a determinant of flooding intolerance in plants. *Plant Physiology* **77**, 492-494
- Roberts JKM, Callis J, Jardetzky O, Walbot V, Freeling M (1984a) Cytoplasmic acidosis as a determinant of flooding intolerance in plants. *Proceedings of the National Academy of Sciences USA* **81**, 6029-6033
- Roberts JKM, Callis J, Wemmer D, Walbot V, Jardetzky O (1984b) Mechanism of cytoplasmic pH regulation in hypoxic maize root tips and its role in survival under hypoxia. *Proceedings of the National Academy of Sciences USA* **81**, 3379-3383
- Roberts JKM, Hooks MA, Miaullis AP, Edwards S, Webster C (1992) Contribution of malate and amino acid metabolism to cytoplasmic pH regulation in hypoxic maize root tips studied using nuclear magnetic resonance spectroscopy. *Plant Physiology* **98**, 480-487
- Roberts JKM, Wemmer D, Ray PM, Jardetzky O (1982) Regulation of cytoplasmic and vacuolar pH in maize root tips under different experimental conditions. *Plant Physiology* **69**, 1344-1347
- Rowland LJ, Strommer JN (1985) Insertion of an unstable element in an intervening sequence of maize *Adh1* affects transcription but not processing. *Proceedings of the National Academy of Sciences USA* **82**, 2875-2879
- Rowland LJ, Strommer JN (1986) Anaerobic treatment of maize roots affects transcription of *Adh1* and transcript stability. *Molecular and Cellular Biology* **6**, 3368-3372
- Russell DA (1990) The genetics and expression of cytosolic glyceraldehyde-3-phosphate dehydrogenase. PhD Dissertation, Washington University, 140 pp
- Russell DA, Sachs MM (1989) Differential expression and sequence analysis of the maize glyceraldehyde-3-phosphate dehydrogenase gene family. *Plant Cell* **1**, 793-803
- Russell DA, Sachs MM (1991) The maize cytosolic glyceraldehyde-3-phosphate dehydrogenase gene family: Organ-specific expression and genetic analysis. *Molecular and General Genetics* **229**, 219-228
- Russell DA, Sachs MM (1992) Protein synthesis in maize during anaerobic and heat stress. *Plant Physiology* **99**, 615-620
- Russell DA, Wong DM-L, Sachs MM (1990) The anaerobic response of soybean. *Plant Physiology* **92**, 401-407
- Sachs MM (1993) Molecular genetic basis of metabolic adaptation to anoxia in maize and its possible utility for improving tolerance of crops to soil waterlogging. In: Jackson MB, Black CR (Eds) NATO ASI Series, **16 Interacting Stresses on Plants in a Changing Environment**. Springer-Verlag, Berlin, pp 375-393
- Sachs MM (1994) Gene expression in maize during anoxia. In: Basra AS (Ed) *Stress Induced Gene Expression in Plants*, Harwood Academic Publishers, Switzerland, pp 87-102
- Sachs MM, Dennis ES, Gerlach WL, Peacock WJ (1986) Two alleles of maize *Alcohol dehydrogenase 1* have 3' structural and poly(A) addition polymorphisms. *Genetics* **113**, 449-467
- Sachs MM, Freeling M (1978) Selective synthesis of alcohol dehydrogenase during anaerobic treatment of maize. *Molecular and General Genetics* **161**, 111-115
- Sachs MM, Freeling M, Okimoto R (1980) The anaerobic proteins in maize. *Cell* **20**, 761-767
- Sachs MM, Ho T-HD (1986) Alteration of gene expression during environmental stresses in plants. *Annual Review of Plant Physiology* **37**, 363-376
- Saglio PH, Drew MC, Pradet A (1988) Metabolic acclimation to anoxia induced by low (2-4 kPa) partial pressure oxygen pretreatment (hypoxia) in root tips of *Zea mays*. *Plant Physiology* **86**, 61-66
- Saglio PH, Germain V, Ricard B (1999) The Response of plants to oxygen deprivation: Role of enzyme induction in the improvement of tolerance to anoxia. In: Lerner HR (Ed) *Plant Responses to Environmental Stresses: From Phytohormones to Genome Reorganization. Books in Soils, Plants and the Environment*, Marcel Dekker, New York, **72**, pp 373-393
- Saglio PH, Raymond P, Pradet A (1980) Metabolic activity and energy charge of excised maize root tips under anoxia. *Plant Physiology* **66**, 1053-1057
- Sanders D, Brownlee C, Harper JF (1999) Communicating with Calcium. *Plant Cell* **11**, 691-706
- Sato T, Harada T, Ichizawa K (2002) Stimulation of glycolysis in anaerobic elongation of pondweed (*Potamogeton distinctus*) turions. *Journal of Experimental Botany* **53**, 1847-1856
- Schlesinger M, Ashburner M, Tissières A (1982) *Heat Shock From Bacteria to Man*, Cold Spring Harbor Laboratory Press, New York, 440 pp
- Schwartz D (1969) An example of gene fixation resulting from selective advantage in suboptimal conditions. *American Naturalist* **103**, 479-481
- Sedbrook JC, Kronebush PJ, Borisy CG, Trewavas AJ, Masson PH (1995) Transgenic *ARQUORIN* reveals organ-specific cytosolic Ca²⁺ responses to anoxia in *Arabidopsis thaliana* seedlings. *Plant Physiology* **11**, 243-257
- Setter TL, Laureles EV (1996) The beneficial effect of reduced elongation growth on submergence tolerance of rice. *Journal of Experimental Botany* **47**, 1551-1559
- Smucker AL, Allmaras RR (1993) Whole plant responses to soil compaction. In: Buxton D R, Shibles R, Forsberg RA, Blad BI, Asay KH, Paulsen GM, Wilson RF (Eds) *International Crop Science I*, Crop Science Society of America, Madison, Wisconsin, pp 727-731
- Soldatenkov SV, Chirkova TV (1963) The role of leaves in root respiration under anaerobic conditions. *Soviet Plant Physiology* **10**, 452-458 (in Russian)
- Springer B, Werr W, Starlinger P, Bennett DC, Zokolica M, Freeling M (1986) The *shrunken* gene on chromosome 9 of *Zea mays* L. is expressed in various plant tissues and encodes an anaerobic protein. *Molecular and General Genetics* **205**, 461-468
- Subbaiah CC, Bush DS, Sachs MM (1994a) Elevation of cytosolic calcium precedes anoxic gene expression in maize suspension cultured cells. *Plant Cell* **6**, 1747-1762
- Subbaiah CC, Bush DS, Sachs MM (1998) Mitochondrial contribution to the anoxic Ca²⁺ signal in maize suspension-cultured cells. *Plant Physiology* **118**, 759-771
- Subbaiah CC, Sachs MM (2001) Altered patterns of sucrose synthase phosphorylation and localization precede callose induction and root tip death in anoxic maize seedlings. *Plant Physiology* **125**, 585-594
- Subbaiah CC, Sachs MM (2003) Molecular and cellular adaptations of maize to flooding stress. *Annals of Botany Special Issue* **91**, 119-128
- Subbaiah CC, Zhang J, Sachs MM (1994b) Involvement of intracellular calcium in anaerobic gene expression and survival of maize seedlings. *Plant Physiology* **105**, 369-376
- Summers YE, Ratcliffe RG, Jackson MB (2000) Anoxia tolerance in the aquatic monocot *Potamogeton pectinatus*: absence of oxygen stimulates elongation in association with unusually Pasteur effect. *Journal of Experimental Botany* **51**, 1413-1422
- Swaminathan MS (1993) From nature to crop production. In: Buxton DR, Shibles R, Forsberg RA, Blad BL, Asay KH, Paulsen GM, Wilson RF (Eds) *International Crop Science I*, Crop Science Society of America, Madison, Wisconsin, pp 385-394
- Tadege M, Brändle R, Kuhlemeier C (1998) Anoxia tolerance in tobacco roots: Effect of overexpression of pyruvate decarboxylase. *The Plant Journal* **14**, 327-335
- Vartapetian BB (1964) Polarographic method for study of oxygen transport in plants. *Soviet Plant Physiology* **11**, 774-781 (in Russian)

- Vartapetian BB** (1978) Introduction: life without oxygen. In: Hook DD, Crawford RMM (Eds) *Plant Life in Anaerobic Environments*, Ann Arbor Science, Michigan, pp 1-12
- Vartapetian BB, Crawford RMM** (2007) The International Society for Plant Anaerobiosis: history, functions and activity. *Plant Stress* **1**, 1-3
- Vartapetian BB, Jackson MB** (1997) Plant adaptation to anaerobic stress. *Annals of Botany* **79**, 3-20
- Vartapetian BB, Sachs MM, Fagerstedt KV** (2008) Plant anaerobic stress. II. Strategy of avoidance of anaerobiosis and other aspects of plant life under hypoxia and anoxia. *Plant Stress* (accepted)
- Vartapetian BB, Andreeva IN, Maslova IP, Davtian NG** (1970) The oxygen and ultrastructure of root cells. *Agrochimica* **15**, 1-19
- Vartapetian BB, Andreeva IN, Kursanov AL** (1974) Appearance of unusual mitochondria in rice coleoptiles at conditions of secondary anoxia. *Nature* **248**, 258-259 (see also Erratum **250**, 84)
- Vartapetian BB, Andreeva IN, Kozlova GI** (1976) The resistance to anoxia and the mitochondria fine structure of rice seedlings. *Protoplasma* **88**, 215-224
- Vartapetian BB, Andreeva IN, Nuritdinov N** (1978) Plant cell under oxygen stress. In: Hook DD, Crawford RMM (Eds) *Plant Life in Anaerobic Environments*, Ann Arbor Science, Michigan, pp 13-88
- Vartapetian BB, Andreeva IN, Generozova IP, Polyakova LI, Maslova IP, Dolgikh Stepanova YI** (2003) Functional electron microscopy in studies of plant response and adaptation to anaerobic stress. *Annals of Botany* **91**, 155-172
- Voeselek LACJ, Benschop JJ, Bou J, Cox MCH, Groeneveld HW, Milleenaar FF, Vreburg RAM, Peeters AJM** (2003) Interactions between plant hormones regulate submergence-induced shoot elongation in the flooding-tolerant dicot *Rumex palustris*. *Annals of Botany* **91**, 205-211
- Vriezen WH, Zhou Z, van der Straeten D** (2003) Regulation of submergence-induced enhanced shoot elongation in *Oryza sativa* L. *Annals of Botany* **91**, 263-270
- Walker JC, Howard EA, Dennis ES, Peacock WJ** (1987a) DNA sequences required for anaerobic expression of the maize *Alcohol dehydrogenase-1* gene. *Proceedings of the National Academy of Sciences USA* **84**, 6624-6628
- Walker JC, Llewellyn DJ, Mitchell LE, Dennis ES** (1987b) Anaerobically regulated gene expression; molecular adaptations of plants for survival under flooded conditions. In: Mifflin BJ, Mifflin HF (Eds) In: *Oxford Surveys of Plant Molecular and Cell Biology* **4**, Oxford University Press, UK, pp 71-93
- Waters I, Kuiper PJC, Watkin E, Greenway H, Colmer TD** (1991) Effects of anoxia on wheat seedlings: I. Interaction between anoxia and other environmental factors. *Journal of Experimental Botany* **42**, 1427-1435
- Webb T, Armstrong W** (1983) The effects of anoxia and carbohydrates on the growth and viability of rice, pea and pumpkin roots. *Journal of Experimental Botany* **34**, 579-603
- Webster C, Gaut RL, Browning KS, Ravel JM, Roberts JKM** (1991) Hypoxia enhances phosphorylation of eukaryotic initiation factor 4A in maize root tips. *The Journal of Biological Chemistry* **266**, 23341-23346
- Wignarajah K, Greenway H** (1976) Effect of anaerobiosis on activities of alcohol dehydrogenase and pyruvate decarboxylase in roots of *Zea mays*. *New Phytologist* **77**, 575-584
- Xu K, Mackill DJ** (1996) A major locus for submergence tolerance mapped on rice chromosome 9. *Molecular Breeding* **2**, 219-224
- Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey-Serres J, Ronald PC, Mackill DJ** (2006) *Sub1A* is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature* **442**, 705-708
- Zhang J, Davies WJ** (1987) ABA in roots and leaves of flooded pea plants. *Journal of Experimental Botany* **38**, 649-659