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Physiological Adaptation of Crop Plants to Flooding Stress

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ABSTRACT

When crop plants are subjected to soil waterlogging, or an anaerobic condition, their root and shoot systems respond differently. A variety of morphological and anatomical alterations develop in the root system. Reduction of the root respiration rate has been reported in both flooding-tolerant and intolerant species. Besides alcoholic fermentation, several diverse fermentative bypasses take place, which ameliorate the poisoning through excessive accumulation of specific metabolic intermediates. Root systems starved of oxygen are also poor providers of mineral nutrients for both themselves and the shoot systems. Stomatal closure and non-stomatal metabolic alterations are responsible for the reduction of leaf CO₂ incorporation. Plant hormones are much involved in regulation of these physiological adaptations.

Key Words: anoxia, hypoxia, flooding stress, physiological stress, stress

I. Introduction

In tropical and subtropical regions, severe crop losses are caused by prolonged seasonal rainfall. Excess water produces anoxic soil conditions within a few hours (Gambrell and Patrick, 1978). Plant roots, consequently, suffer hypoxia or anoxia. In flood-tolerant plants, the formation of aerenchyma and adventitious roots in the vicinity of cotyledonary nodes is an indicator of the presence of adaptive mechanisms (Kawase, 1981). The interaction of auxin and ethylene is important for the induction of adventitious root formation (McNamara and Mitchell, 1989). The root respiratory capacity of flooded plants is routinely taken as an indicator of the viability of the root system, as well as the integrity of the respiratory apparatus (Kennedy *et al.*, 1992). With the exhaustion of the terminal electron acceptor of aerobic respiration, the electron-transport system and the tricarboxylic acid cycle are blocked. ATP production must, then, depend upon fermentative metabolism; thus, the activity of alcohol dehydrogenase (ADH) is augmented and ethanol accumulation is enhanced (Davies, 1980). Anoxic fermentation in roots occurs through glycolysis, and the fermentation rate in roots is dependent on the available supply of soluble sugars. Vartapetian (1991) reported that exogenous glucose delayed the onset of ultrastructural disintegration in excised pea root cells. Zhang and Greenway (1994) found that feeding exogenous sugar to

beetroots prevented depletion of sugar supplies in the shoots and enhanced ethanolic fermentation. Wample and Davis (1983) confirmed that the accumulation of starch in leaves of flooded sunflower plants is a reflection of reduced phloem transport. The results of those studies generally implied that flooding reduces phloem transport and causes further accumulation of photosynthate in leaves and depletion of carbohydrates in roots.

Photosynthetic capacity has also been shown to be significantly inhibited in flooding-intolerant plants. Regehr *et al.* (1975) and Phung and Knipling (1976) suggested that the stomatal aperture governs CER (CO₂ exchange rate) in flooded plants. It was reported that a decrease of stomatal conductance coincided with a reduction of CER, and lower *C_i* (leaf internal CO₂ concentration) was observed in flooded rabbiteye blueberry (*Vaccinium ashei* Reade) (Davies and Flore, 1986a, 1986b). A decrease of CER, independent of stomatal conductance, in sunflower (*Helianthus annuus*) was, however, reported by Guy and Wample (1984). *C_i* has also been found to be similar for leaves of flooded and control pecan plants (*Carya illinoensis*) (Smith and Ager, 1988) and sweet orange grafted trees (*Citrus sinensis* Osbeck) (Vu and Yelenosky, 1991). It is conceivable that non-stomatal factors are responsible for the reduction of CER. Reduction of RuBP regeneration (Bradford, 1983) or feedback inhibition resulting from starch accumulation (Wample and Davis, 1983; Vu and

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Yelenosky, 1991; Topa and Cheeseman, 1992) was suggested to be involved in non-stomatal factors.

Waxapple (*Syzgium samarangense* Merr. Et. Perry) is a tropical fruit tree. Farmers in southern Taiwan (23°N) induce early flowering of waxapple by continuously flooding the orchard for 30 – 40 days during the summer, and no adverse physiological impact has been observed (Wang, 1983). Furthermore, two vegetative cucurbitaceae plants, luffa (*Luffa cylindrica* Roem. cv. Cylinder #2, a flood-tolerant species) and bitter melon (*Momordica charantia* L. cv. New Known You #3, a flood-intolerant species), were studied in our laboratory recently (Liao and Lin, 1994, 1995, 1996; Su and Lin, 1996; Su *et al.*, 1998). Our goal was to better understand the physiology of these plants while they are withstanding flooding stress.

II. Root Respiratory Capacity

Reduction of root respiration is one of the earliest responses of plants under anoxia, regardless of whether the plants are flooding-tolerant or intolerant (Carpenter and Mitchell, 1980; Hsu *et al.*, 1999b; Kuo and Chen, 1980; Lambers, 1976; Lin and Lin, 1992; Liao and Lin, 1995; Su and Lin, 1996; Marshall *et al.*, 1973; McNamara and Mitchell, 1991; Tripepi and Mitchell, 1984). In excised roots of *Oryza sativa* L. and *Cucurbita pepo* L., the integrity of mitochondria was found to be highly dependent on oxygen availability (Vartapetian *et al.*, 1978). Vartapetian (1991) also reported that upon exposure of detached pea roots to anoxia for more than 9 hours periods, there was an irreversible change in the membrane structure and loss of oxidative and phosphorylative activities of mitochondria. Furthermore, the K_m values of the alternative oxidase for oxygen were found to be higher than those of cytochrome oxidase (Lambers and Smakman, 1978). During prolonged flooding, the seminal roots of luffa were able to maintain 60% of the respiratory capacity of nonflooded controls (Su and Lin, 1996). Flooded luffa retained higher root respiratory capacity than did flood-intolerant bitter melon. The respiratory capacity of bitter melon fell to 28% that of the nonflooded bitter melon level after 5 days of flooding (Liao and Lin, 1995). This phenomenon may be valuable for survival and may help flooded roots scavenge any oxygen available around the roots. A high oxygen consumption rate in root tips is associated with respiration, which is required for related metabolic activities, such as generation of ATP. Under flooded conditions, plant roots are in a state of hypoxia, their metabolic activity is inhibited and ATP production decreases (Saglio *et al.*, 1980). The decreased ATP production restricts the supply of energy for root growth, thus reducing vegetative growth.

III. Anaerobic Metabolism of Roots

Plants also respond to anoxia by altering the pattern of

protein synthesis. The proteins which are synthesized as a specific response to anaerobiosis are called the anaerobic polypeptides (ANPs) (Sachs *et al.*, 1980). Several ANPs have been characterized by means of protein chemistry and the molecular approach. All the characterized polypeptides are glycolytic enzymes (Miernyk, 1990; Mujer *et al.*, 1993). Among the ANPs, ADH is predominant and has been extensively studied (Sachs *et al.*, 1980). Newly synthesized ADH isozymes emerge during flooding in many plants (Harberd and Edwards, 1983; Lin and Lin, 1992; Liao and Lin, 1995) and with different biochemical properties. The reaction catalyzed by the enzyme is, however, not the rate-limiting step in ethanolic fermentation, and the regulatory significance of the massive induction of ADH remains unclear (Bertani and Reggiana, 1991).

1. Ethanolic Fermentation

Under anoxia conditions, whereby oxidative phosphorylation of mitochondria is blocked, cells inevitably undergo anaerobic fermentation, thus replacing the Krebs cycle in fulfilling the cellular demand for ATP (Davies, 1980). During alcoholic fermentation, ADH is responsible for the recycling of NAD^+ needed for the glycolysis process to continue (Saglio *et al.*, 1980). The inducible formation of ADH that accompanies ethanol production has been studied in the course of characterizing flood-tolerant and intolerant plants (Crawford, 1967; McManmon and Crawford, 1971). High levels of ADH activity and ethanol production during anaerobiosis have been reported for flood-tolerant plants (Avadhani *et al.*, 1978; Chirkova, 1978; Smith and ap Rees, 1979; Tripepi and Mitchell, 1984). The activity of ADH was positively correlated with the magnitude of flood injury in different genotypes (Francis *et al.*, 1974; Liao and Lin, 1995; Lin and Lin, 1992), and species with higher ethanol production were less tolerant to flooding, compared to tolerant plant species (Barta, 1984; Crawford, 1967, 1978). It has been proposed that the accumulated ethanol may have a “self-poisoning role” in flood-intolerant plants. However, other studies have not supported this idea (Jackson *et al.*, 1982). A pH stat hypothesis was proposed by Davies (1980) to explain the short-term flood tolerance of plants in which ethanol rather than lactate is the less deteriorating end product of fermentation. An accumulation of lactate promotes acidification of the cytoplasm (Roberts *et al.*, 1984) of anoxia “sensitive” plants, such as maize, wheat and barley (Menegus *et al.*, 1989, 1991). The pH stat hypothesis is also supported by the observation of alkalization of the cytoplasm in “pH resistant” plants, such as rice and *Echinochloa* (Menegus *et al.*, 1989, 1991).

The level of ethanol in flooded sunflower and luffa roots does not continue to increase in proportion to the levels of PDC (pyruvate decarboxylase) and ADH activities but declines after an initial rise on day one (Jayasekera *et al.*, 1990; Su and Lin, 1996). This phenomenon may be due to diffu-

sion and/or venting of the produced ethanol to the surrounding medium. Even though many plants can accommodate ethanol concentrations much higher than those found endogenously (Jackson *et al.*, 1982), the ethanol tolerance of plant tissues is not infinite. Barclay and Crawford (1981) reported that an internal ethanol concentration of 60 mmol/L appeared to be the threshold value for survival of pea seedlings, and that anoxic death occurred when this concentration was exceeded. To conclude, for poisoning due to excessive accumulation of ethanol to be avoided, it may be important for flooded roots to be able to excrete the ethanol produced by aerobic metabolism.

Notably, the activity of PDC is usually many-fold lower than that of ADH, suggesting that the rate-limiting enzyme for ethanol synthesis in flooded roots is more likely to be PDC than ADH (Chang *et al.*, 1983; Su and Lin, 1996; Waters *et al.*, 1991). Why do flooded roots require such high ADH activity? Perata and Alpi (1991) showed that the toxic effects of exogenously added ethanol on carrot-cell cultures were due to the formation of acetaldehyde. Waters *et al.* (1991) suggested that a high ratio of ADH/PDC may be required to prevent accumulation of potentially toxic acetaldehyde. Kundu *et al.* (1993) reported that the correlation between the survival rate and aldehyde production of rice plants after submergence was strongly negative. In flooded luffa roots, the acetaldehyde concentration did not rise in proportion to the induction of PDC activity, and it increased only slightly after 5 days of flooding (Su and Lin, 1996). These reports suggest that avoidance of accumulation of toxic acetaldehyde is a determining factor for flooding tolerance.

2. Alternative Fermentative Pathways

According to the metabolic theory of R.M.M. Crawford (Crawford, 1967; McManmon and Crawford, 1971), flooding tolerance is achieved by minimization of ethanol production and is associated with re-routing from ethanol fermentation to malate production. The activities of PEPC (phosphoenolpyruvate carboxylase) and MDH (malate dehydrogenase) are required for synthesis of malate. In addition, NADP-ME (NADP-malic enzyme) must be absent or repressed for decarboxylation of malate to be avoided. However, malate produced in flooded luffa roots is easily decarboxylated, and adaptation of luffa seedlings to flooding appears to be incompatible with R.M.M. Crawford's theory (Su and Lin, 1996).

Alternatively, Vanlerberghe *et al.* (1990) proposed that succinate, rather than malate, is the fermentation end product. They developed a model in which succinate is accumulated through a partial tricarboxylic acid pathway from fumarate via oxaloacetate and malate, thus regenerating NAD⁺ and producing a net gain in ATP per mole of succinate produced. The elevated NADP-ME activity may, however, indicate that malate is decarboxylated to pyruvate as soon as it is formed. Neither malate nor succinate is, therefore, the fermentative

end product in flooded luffa roots (Su and Lin, 1996).

Lactate dehydrogenase activity increases 20-fold during prolonged hypoxia, which may be an important factor contributing to long-term adaptation to hypoxia (Hoffman *et al.*, 1986). Moreover, lactic fermentation in luffa roots has been found to be important as an early mechanism of adaptation to flooding, and may contribute to resistance to long-term flooding (Su and Lin, 1996).

3. Regulation of Gene Expression

ANPs that are induced show increased levels of mRNA, and regulation of specific genes occurs at the level of RNA synthesis (Rowland and Strommer, 1986; Dennis *et al.*, 1988). At the same time, translation of mRNAs of aerobically expressed proteins is inhibited. The effects of anaerobiosis on gene expression have mainly been studied in maize. Transcriptional induction under anaerobic conditions has been investigated in glycolysis and ethanolic fermentation enzymes, *e.g.*, pyruvate decarboxylase (Kelley, 1989; Peschke and Sachs, 1993), alcohol dehydrogenase (Gerlach *et al.*, 1982; Rowland and Strommer, 1986; Andrews *et al.*, 1993, 1994), glucose phosphate isomerase (Kelley and Freeling, 1984a), aldolase (Kelley and Freeling, 1984b), and glyceraldehyde phosphate dehydrogenase (Martinez *et al.*, 1989; Russell and Sachs, 1989).

Walker *et al.* (1987) found that a region critical for anaerobically inducible expression of "the Anaerobic Responsive Element" (ARE) was located between -140 and -90 relative to the transcription start site of maize *Adh1*. Furthermore, several of the genes encoding ANPs (*Adh1*, *Adh2*, *Sh1*, *Ald*) in maize, pea, and Arabidopsis also share a consensus motif (ARE) in the promoter regions (Dennis *et al.*, 1988; Liewellyn *et al.*, 1987; Olive *et al.*, 1991; Walker *et al.*, 1987). The ARE was found to contain all the sequences necessary for hypoxic induction, and when the ARE linked to another gene, it could confer hypoxic responsiveness (Dennis *et al.*, 1993). Nuclear proteins have been identified that bind to the ARE of the maize *Adh1* promoter (Olive *et al.*, 1991) or to sequences outside the ARE (Paul and Ferl, 1991).

Hypoxic stress rapidly induces the expression of *Adh1* and *Adh2* genes in maize within 4 hours through hypoxic treatment of cell suspension cultures (Paul and Ferl, 1991). Likewise, Arabidopsis *Adh* genes can be induced in tissues within 4 to 8 hours (Chung and Ferl, 1999; Dolferus *et al.*, 1994). Furthermore, transcripts of *Adh1* are more effective at loading ribosomes under anoxia than are transcripts of genes that are only aerobically expressed, and elongation is greater on *Adh1* transcripts (Fennoy and Bailey-Serres, 1995).

IV. Hormonal Regulation of Developmental Responses

Plants exhibit a wide variety of morphological and ana-

tomical responses to submergence of roots or entire plants. Some appear to have adaptive significance. In a wide range of upland plants, the prominent responses are the formation of lysigenous aerenchyma as observed in *Zea mays* (Kuo, 1993), fast underwater elongation of shoots or leaves of aquatic or amphibious species, and stomatal closure and epinastic leaf curvature. Each of these reactions is mediated by plant hormones, with ethylene and abscisic acid playing prominent roles (Jackson *et al.*, 1993).

The most conspicuous anatomical response of crop roots to soil waterlogging or anoxia is the development of an extensive aerenchyma system in their cortex (Konings and Lambers, 1991), greatly facilitating gas transport in waterlogged or anoxia root systems (Armstrong *et al.*, 1991). These plants include wheat, barley, maize, tomato, and various forage species. It has been clearly demonstrated that ethylene is the principal mediator promoting the development of aerenchyma in maize as well as other plants (Jackson, 1985, 1987, 1989, 1990a, 1990b). The formation of aerenchyma in rice roots has, however, been considered to be a result of genetic control (Jackson and Drew, 1984) since the aerenchyma always forms in rice roots, regardless of environmental conditions (Kuo, 1993).

A principal developmental characteristic of rice is an enhanced rate of upward extension, enabling coleoptiles and shoots to gain access to oxygen, carbon dioxide and light (Jackson and Pearce, 1991). This occurs under the influence of ethylene, together with an accumulation of carbon dioxide and limited supply, but not a total lack of, oxygen (Jackson *et al.*, 1993). Hormonal regulation is based on an increase of the concentration of ethylene, which interacts with gibberellins (Musgrave *et al.*, 1972; Kende, 1987) and auxins (Cookson and Osborne, 1978; Horton, 1987). Auxins and gibberellins are prerequisites for ethylene action and play triggering rather than regulatory functions. Gas diffusion into the plant is impeded, leading to low oxygen partial pressure. The low partial pressure stimulates biosynthesis of ethylene by increasing ACC (1-aminocyclopropane-1-carboxylic acid) synthase activity, thus further enriching the stem with ethylene (Pearce *et al.*, 1992).

However, the effects of flooding or submergence are not confined to the inundated parts of the plant. Stomatal closure and epinastic curvature take place in some roots and shoots; hence, the effects may involve adjustments to the translocation of hormones or their precursors between the above- and below-ground parts (Jackson *et al.*, 1993). ABA concentrations were found to increase in roots of pea plants during the 2nd, 3rd, and 4th days of flooding, causing stomata to partially close and enriching the leaves with the hormone (Zhang and Davies, 1987). Neuman and Smit (1991) found a doubling of the ABA concentration in the xylem sap of detopped *Phaseolus vulgaris* plants after flooding, thus allowing the plants to gain access to the sap which was expressed under pressure. Furthermore, epinastic curvatures can be in-

duced by small amounts of exogenous ethylene, and shoots of flooded plants contain increased amounts of endogenously produced gas (Jackson and Campbell, 1976). Bradford and Yang (1980) also discovered that the ethylene precursor ACC increased in xylem sap of detopped tomato plants after 12 hours of flooding. This suggests that ACC ascends from the roots to the shoots of intact plants.

V. Metabolic Imbalance between Shoots and Roots

Among the biochemical changes observed in plants under flooding, a high level of fermentative metabolism in roots has been shown to be important for plant survival because it supplies a high enough energy charge that can sustain metabolism in roots (Jackson and Drew, 1984; Mohanty *et al.*, 1993). Thus, maintaining adequate levels of fermentable sugars in flooded roots is undoubtedly important for long-term survival of plants during flooding. Several studies showed that the starch levels in intact roots of alfalfa and rice did not significantly vary under increased durations of flooding, suggesting that root starch is not readily mobilized and converted to fermentable substrates (Barta, 1988; Bertani *et al.*, 1981). However, Perata *et al.* (1992) reported that germinating rice seeds could degrade starch reserves under anoxia, whereas wheat seeds did not germinate and were unable to degrade the starch in the endosperm. These distinct behaviors are due to the successful induction of α -amylase under anoxia in rice seeds but not in wheat seeds. Starch reserves in roots are considered to be easily mobilized during flooding and can readily provide sugars for anaerobic metabolism in flooded roots (Perata *et al.*, 1992). The starch levels in roots of both flood-tolerant luffa and flood-intolerant bitter melon were found to decrease markedly during the early flooding stages (Su *et al.*, 1998). The amount in luffa started to increase after 6 days of flooding while the amount of bitter melon remained low throughout the entire flooding period. The variation of soluble sugar in roots of luffa and bitter melon during flooding period agreed strongly with studies on alfalfa by Barta (1988) and Castonguay *et al.* (1993) that the root starch can be mobilized and converted to soluble sugars at the early stage of flooding. Analyses of soluble sugars in roots revealed that the amounts of sucrose and hexoses (glucose and fructose) in flooded roots of both species increased 3.5 – 4.0 fold during the early stage of flooding, then gradually decreased and finally reached levels that were similar to those of the controls (Su *et al.*, 1998). It was found that the roots of flood-intolerant bitter melon eventually underwent necrosis, and that the plants died (Liao and Lin, 1995; Su *et al.*, 1998).

Continuous supply of fermentable sugars to roots is considered to be critical for long-term survival of peas, pumpkin, and several herbaceous plants under anoxia or flooding (Jackson and Drew, 1984; Saglio *et al.*, 1980; Webb and Armstrong, 1983). In theory, if the translocation path is

blocked, assimilates in leaves will not be able to reach the roots, thus resulting in a sugar deficiency in the roots. The sugar level in flooded roots of bitter melon was, however, found to be higher than that in the unflooded control (Su *et al.*, 1998). Root starch tends to play a role in increasing the level of sugar in the early flooding stage.

Accumulation of starch has been reported in leaves of various flooded plants, *e.g.*, *H. annuus* (Wample and Davis, 1983), *C. sinensis* grafted onto rough lemon and sour orange rootstocks (Vu and Yelenosky, 1991), *Pinus serotina* (Topa and Cheesmann, 1992), *M. charantia* (Liao and Lin, 1994), *L. cylindrica* (Su *et al.*, 1998) and *S. samarangense* (Hsu *et al.*, 1999). Under root hypoxia, starch accumulation in leaves has been attributed to a reduced rate of translocation of carbohydrates from leaves to roots (Barta, 1987) and to retarded growth and a lower level of metabolism in roots, which apparently causes the carbohydrate demand to decrease (Hsu *et al.*, 1999; Wample and Davis, 1983).

The total nitrogen content in plant tissue has been reported to decrease under flooding stress in various fruit species, such as citrus (Labanauskas *et al.*, 1972), apple (Olien, 1989), avocado (Slowick *et al.*, 1979), and blueberry (Herath and Eaton, 1968). In the wax-apple tree, the total nitrogen in the leaves was found to be significantly lower after 35 days of flooding treatment compared to the unflooded control while the total amount of carbohydrates increased, resulting in a significant increase in the C/N ratio (total carbohydrate/total nitrogen) (Hsu *et al.*, 1999). The carbohydrate-nitrogen ratio in fruit trees has often been related to bud formation, flowering and fruiting, but this relationship also varies with the species (Goldschmidt, 1982; Scholefield *et al.*, 1985; Smith *et al.*, 1986).

Under flooding stress, the composition and quantity of proteins and amino acids, and the activities of related enzymes are important. In particular, nitrate reductase and glutamine synthetase, the two key enzymes in nitrate reduction and ammonia assimilation influencing the total nitrogen balance, are affected by flooding (Buwalda *et al.*, 1988; Garcia-Novo and Crawford, 1973; Reggiani *et al.*, 1988). Nitrate reductase is the key enzyme in nitrate reduction. Garcia-Novo and Crawford (1973) and Lambers (1976) reported that the activity of nitrate reductase in roots of flood-tolerant plants increased rapidly during flooding, as did the amino acid synthesis capability. However, wax-apple trees tend to respond differently to flooding. The activity of nitrate reductase in roots of flooded tree was not found to increase with flooding, as it did in other plants; instead, it decreased significantly (Hsu *et al.*, 1999). This might be attributable to reduced nitrate uptake by the roots. The synthesis of nitrate reductase is substrate regulated (Beever and Hageman, 1969; Hewitt, 1975). The half-life of the newly synthesized enzyme is only a few hours (Taiz and Zeiger, 1991). Therefore, reduced uptake of nitrate by roots will reduce the level of synthesis of nitrate reductase.

VI. Leaf Gas Exchange

Leaf gas exchange parameters have been used to study the photosynthetic capacity of plants during flooding. Except in some flood-tolerant plants that have developed adaptive mechanisms so as to maintain better photosynthetic capacity (Sena Gomes and Kozlowski, 1980; Topa and Cheeseman, 1992), flooding causes a significant decrease in the capacity for leaf gas exchange in most flooding-intolerant plants. Examples are *Citrus sp.* (Phung and Knipling, 1976), *Triticum aestivum* (Trought and Drew, 1980), *Quercus macrocarpa* (Tage and Kozlowski, 1982), *Lycopersicon esculentum* (Bradford, 1983), *Pisum sativum* (Jackson and Kowalewska, 1983), *Actinidjo chinensis* (Save and Serrano, 1986), *Betula platyphylla* (Tsukahara and Kozlowski, 1986), *Vaccinium sp.* (Davies and Flore, 1986b), *Catya illinoensis* (Smith and Ager, 1988), and *M. charantia* (Liao and Lin, 1994). Studies showed that in pea plants that were subjected to soil flooding, the foliar ABA content increased, accompanied by closure of stomates (Jackson and Drew, 1984; Zhang and Davies, 1987). Similar results have been observed in tomato (Jackson, 1990a, 1990b) and apples (Jackson, 1991). Furthermore, a decrease of stomatal conductance were found to be correlated with an increase of leaf water potential in flooded bitter melon (Liao and Lin, 1994). Those observations indicate that marked stomatal closure results in an increase in leaf water potential. It can be hypothesized that stomatal closure slows the transpiration rate, thus preventing leaf dehydration (Bradford and Hsiao, 1982). In control and flooded plants, stomatal conductance were shown to be correlated with CER (CO₂ exchange rate) (Liao and Lin, 1998; Smith and Ager, 1988; Vu and Yelenosky, 1991). These results imply that stomatal aperture is a limiting factor for CER.

The internal CO₂ concentration (C_i) of leaves was found to increase linearly with the duration of flooding, despite a reduction of stomatal conductance (Liao and Lin, 1994). The ambient CO₂ concentration was estimated to average 350 μ bars, but less than 300 μ bars were detectable in the leaf intercellular space in leaves of bitter melon plants that had not been subjected to flooding. The C_i was found to be 380 μ bars in flooded bitter melon on the 6th day of flooding (Liao and Lin, 1994). These data suggest that factors such as (1) reduced photosynthetic capacity, (2) a respiratory CO₂ evolution rate exceeding the CO₂ fixation rate and (3) increased stomatal resistance may be involved in C_i elevation. It was found that respiration of leaves increased significantly during flooding (Liao and Lin, 1994). Flooding has been reported to cause stomatal closure directly, without affecting the photosynthetic capacity, in *V. ashei* (Davies and Flore, 1986a, 1986b), thus decreasing C_i . It has been suggested that the stomatal aperture regulates the decline of CER. However, in flooded bitter melon seedlings, C_i was observed to increase (Liao and Lin, 1994). Other plants, in which C_i was found to remain unchanged with flooding are *C. illinoensis* (Smith and

Ager, 1988), *C. sinensis* grafted onto *C. jamthiri* and *C. aurantium* rootstocks (Vu and Yelenosky, 1991), and bitter melon grafted onto luffa rootstocks (Liao and Lin, 1996). These observations suggest that stomatal aperture is not the only limiting factor for CER but is partly responsible for the decrease in the photosynthetic capacity of mesophyll tissue.

VII. Photosynthetic Capacity of Mesophyll Cells

The activation level of Rubisco in flooded bitter melon increased above the control value after 1 day of flooding and subsequently declined to a lower level (Liao and Lin, 1994). Changes in the level of activation of Rubisco reflect the level of carbamylation of Rubisco (Miziorko and Lorimer, 1983), which in turn is regulated by C_i and light intensity (Sage *et al.*, 1990). In general, the activation level of Rubisco generally declines as C_i increases (von Caemmerer and Edmondson, 1986; Sage *et al.*, 1988, 1990). The relationship between Rubisco activation and the internal CO_2 concentration of leaves has been extensively discussed by Liao and Lin (1994). The activation level of Rubisco was observed to be 92% at a C_i level of 240 μbars in control plants; this increased to 103% as C_i increased to 251 μbars and then declined as C_i further increased in flooded plants. When C_i was raised to 355 μbars , the activation level of Rubisco fell to 79% of the control level (Liao and Lin, 1994). The effect of elevated C_i on the activation level of Rubisco agrees well with the ambient CO_2 enrichment reported by von Caemmerer and Edmondson (1986) and Sage *et al.* (1990). Under elevated C_i , the pool size of RuBP was found to decline and the level of phosphoglycerate (PGA) to increase in three different plant systems (Badger *et al.*, 1984; von Caemmerer and Edmondson, 1986; Sage *et al.*, 1988). Sage (1990) suggested that excessive consumption of RuBP may switch off the active site of Rubisco, thus maintaining a balance between RuBP consumption and its regeneration, and regulating the activation of Rubisco. The bitter melon studies indicate that C_i elevation may affect Rubisco regulation in a similar manner (Liao and Lin, 1994). It has been established that the carbamylation/decarbamylation equilibrium of Rubisco involves reversible active enzyme- CO_2 - Mg^{2+} complex formation. Formation of this complex is pH dependent and has been observed in leaf homogenates (Lorimer *et al.*, 1976) and isolated chloroplasts (Bahr and Jensen, 1978). At a concentration of 0.2 M Mg^{2+} , optimal Rubisco activity was found to be between pH 8.5 to 10, whereas 90% of the enzyme activity was lost when the pH was lowered to 7.0 (Lorimer *et al.*, 1976). Andrews and Lorimer (1987) postulated that a high level of CO_2 favors overproduction of PGA and leads to acidification of the stromal phase, thus reducing the carbamylation level of Rubisco.

In bitter melon seedlings, Rubisco activity was found to average 59% of the control value on the 7th day after flooding (Liao and Lin, 1994). Inasmuch as Rubisco catalyzes the

initial reaction during the assimilation of atmospheric CO_2 (Andrews and Lorimer, 1987), the activation level of Rubisco should be positively correlated with the rate of photosynthesis. The leaf soluble protein of flooded bitter melon was found to decline slowly and to reach an average of 75% of the control level on the 7th day of flooding, but the Rubisco content was reduced significantly. Based on the positive correlation between Rubisco activity and Rubisco content ($R^2 = 0.89$) as well as on the decreased activation level of Rubisco, it can be inferred that a reduced quantity of Rubisco protein as well as reduced activity of existing enzymes may cause Rubisco activity to decline during flooding. Furthermore, it was reported that phloem transport of photosynthates was blocked (Saglio, 1985), and that the demand for sucrose loading was lowered. This may lead to an accumulation of starch in the chloroplasts (Wample and Davis, 1983; Liao and Lin, 1994). It can, thus, be suggested that feedback inhibition of starch accumulation may result in a reduction of CER in flooded plants. The many physiological responses observed indicate that both stomatal and metabolic factors are responsible for the reduction of CER during flooding stress.

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