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LEAF SENESCENCE IN SUBMERGED RICE PLANTS

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SUMMARY

Leaf senescence in the rice (*Oryza sativa*) cultivars FR13A and IR42 under submergence was assessed in terms of changes in total chlorophyll, soluble amino acids and protein concentrations and peroxidase activity in crude extracts. The objective was to determine whether delay in leaf senescence was related to the submergence tolerance of a rice cultivar. Submergence induced senescence and its extent was notably different in the two cultivars. Results indicated a reduction in chlorophyll and protein concentrations in leaves during submergence but an increase in amino acid concentration and peroxidase activity. These relative changes were more pronounced in submergence intolerant IR42 than in tolerant FR13A even at four days after complete submergence. When plants were desubmerged and returned to standard irrigated conditions after 10 days of complete submergence the rate of recovery was different for the two cultivars. FR13A showed a greater protein and chlorophyll restoring ability compared with the intolerant IR42. The differences observed between the responses of FR13A and IR42 to submergence were likely to be due to differences in proteolysis. Senescence of leaves due to submergence was similar to the senescence of non-submerged excised leaves. Results demonstrate that, in the two cultivars studied, leaf senescence is an important biochemical mechanism in plants under submergence and its slower development in tolerant cultivars is, in part, responsible for submergence tolerance.

INTRODUCTION

Rice (*Oryza sativa*) is subjected to partial or complete submergence ranging from a few days to several weeks at different stages of growth. At the extremes, this will result in poor yields and large crop losses. 'Submergence tolerance', the ability of rice plants to survive during and after submergence in water, is a genetic character expressed in the complex environment of floodwater (Setter *et al.*, 1987; 1989). However, the extent of tolerance varies among different rice cultivars. Submergence tolerant cultivars include FR13A and Kurkurappan (Mazaredo and Vergara, 1982). IR36 has moderate submergence tolerance while IR42 is an intolerant cultivar (Mazaredo and Vergara, 1982). Submergence tolerance in rainfed lowland cultivars is desirable, as submergence escape mechanisms such as elongation of leaves and internodes can be disadvantageous in the rainfed lowland areas where this leads to excessive lodging when flood water recedes. This is supported by Jackson *et al.* (1987) who showed that lowland rice cultivars which

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survived submergence the longest elongated less under submergence than a less tolerant cultivar. In contrast, leaf and internode elongation is appropriate to the deepwater and floating rice areas (>100 cm water) where water remains at these depths for several months. Tolerance is relative and never absolute, and it is imparted through both structural and metabolic features.

The study of senescence is of significance in understanding the mechanisms by which a cell undergoes programmed cell death and in gaining insight into cell maintenance under non-senescing conditions (Smart, 1994). Under partial or complete submergence, the response of rice plants in relation to senescence is of practical significance in view of the possibility of rapid recovery during post-submergence periods. Considerable information on the relationship between flooding tolerance and gas transport and metabolic processes involving hormones (Armstrong *et al.*, 1994), and on the genetics of submergence tolerance (Setter *et al.*, 1997) is available. However, the relationship between leaf senescence and submergence tolerance is little understood.

Leaf senescence and its characterization and regulation have been studied extensively in rice, but under irrigated conditions (Kamachi *et al.*, 1992; Yeh and Kao, 1994). The course of senescence in leaves can be determined either in attached (normal senescence) or detached (enhanced senescence) leaves. Senescence occurs more rapidly when the leaves are detached from the plants (Thimann *et al.*, 1974) but it remains that the processes involved under these two conditions may be different. Cell maintenance and growth may cease if submergence is prolonged. It was hypothesized that delay or avoidance of senescence is related to the tolerance of a rice cultivar to submergence, that is, that the tolerant cultivar simply dies more slowly. In the present study the changes in leaf senescence in a submergence tolerant (FR13A) and an intolerant cultivar of rice (IR42), grown under submerged and non-submerged, irrigated conditions were investigated.

MATERIALS AND METHODS

Plant material

Healthy seeds (five per pot) of submergence tolerant (FR13A) and intolerant (IR42) cultivars of rice were sown in 2-L pots filled with alluvial soil (Typic Haplaquept) from the experimental farm of the Central Rice Research Institute, Cuttack (lat 20°25′N, long 85°55′E), India. All seedlings were maintained under irrigated conditions until 20 d after sowing by allowing a water level of 2 cm above the soil surface in the pots. Throughout the study period, one set of plants, maintained under irrigated, non-submerged conditions as mentioned above, served as controls. Another set of plants (20-day-old) in pots was maintained under continuous complete submergence for 10 d by placing them in concrete tanks (1 m × 1 m × 1 m) with an 80-cm water column above the surface of the soil in the pots. After 10 d of submergence, the plants were removed from the tanks and returned to standard irrigated conditions. The characteristics of the flood

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water in terms of temperature and of oxygen concentration were measured using a Clark electrode (Syland Model 610, Syland Scientific, Heppenheim, West Germany) at 1100 hours on every day of plant sampling according to Setter *et al.* (1987).

Normal leaf senescence

The course of normal leaf senescence was characterized in plants under irrigated (control) and submerged conditions, and after desubmergence. For chemical analysis, the penultimate leaf from the apex was sampled (Thimann *et al.*, 1974) from each plant in control and submerged treatments. When samples were harvested they were placed in moist polyethylene bags on ice and extracted within 3 h of sampling.

Harvesting and preparation of excised (detached) leaves for senescence studies

Shoots of plants from irrigated or submerged conditions were excised at the soil surface and stored on ice for 4 h until preparation of plant tissues for senescence studies. The fully expanded penultimate leaves were cut at the base from the shoots and then 10 leaves (approximately 1 g fresh weight) were placed in 60-mL screw-capped test tubes containing sterile distilled water (Thimann *et al.*, 1974). Excised leaves were kept in the dark at $30 \pm 1\,^{\circ}C$ and the sterile water was replaced every 24 h. After 72 h of incubation, the detached leaf samples were extracted and analysed for senescence parameters.

Extraction and analyses

Chlorophyll pigments from leaves (100 mg tissue) were extracted in chilled 80% acetone (Krishnan *et al.*, 1996). The chlorophyll concentration of leaves was determined in a UV/VIS Beckman (Model 24) spectrophotometer. For protein concentration, leaves were homogenized in trichloroacetic acid (TCA) (200 mg fresh tissue: 6 mL 10% w/v TCA). The precipitate was then washed with acetone and dissolved in 5 mL 1M NaOH. The NaOH-treated fraction (0.1 mL) was used for determining protein following Lowry's method (Lowry *et al.*, 1951). Soluble amino acids were extracted in 80% ethanol and estimated by the method of Rosen (1957).

For peroxidase activities, crude extracts were prepared by extracting the leaves (1 g tissue) in 5 mL chilled 0.1M phosphate buffer (pH 6.8) using a pre-chilled mortar and pestle. The homogenate was centrifuged at $2\,^{\circ}C$ for 15 minutes at 17 000 g. The clear supernatant was diluted 10-fold and taken as the enzyme source. The peroxidase (EC 1.11.1.7) activity was measured using pyrogallol (Yeh and Kao, 1994; Mahadevan and Sridhar, 1996). The assay mixture (5 mL) for the peroxidase activity contained 125 μmol phosphate buffer (pH 6.8), 50 μmol pyrogallol, 50 μmol H_2O_2; and the change in absorbancy at 460 nm was measured. Peroxidase activity was expressed in units (g^{-1} fresh weight) where one unit refers to a change of 0.001 of absorbancy following the addition of substrate.

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Statistical analysis

The experiment had a completely randomized design with a set of 50 pots for each replicate and each experiment described was performed at least three times. On each day of sampling, five pots with five rice seedlings were sacrificed for each treatment. For analysis of total chlorophyll concentration, total protein and amino acids, five separate samples (100 mg) of leaf tissue from each plant were used, and for peroxidase activity, replicated 1-g samples of tissue were used. Data were subjected to statistical analysis using IRRISTAT (Version 3/93, International Rice Research Institute, Manila, The Philippines).

RESULTS

The temperature of the flood water, measured at 1100 hours on every sampling day, increased from 25 to about 28 °C during 10 d of flooding (Table 1) At a depth of 80 cm the oxygen concentration of the flood water increased after 4 d from 0.24 to about 0.41 mol m^{-3}, and then declined to around 0.20 mol m^{-3} by 10 d of flooding. After 10 d of submergence, plants were returned to standard irrigated conditions. Survival, measured by the ability of plants to grow during desubmergence (that is, for submerged plants, after 7 d under standard irrigated conditions) and recorded as the percentage of submerged seedlings, was 100% for FR13A and 17% for IR42.

For non-submerged plants, the submergence intolerant cultivar IR42 had about 10% higher concentrations of chlorophyll than tolerant FR13A (Table 2). Leaf chlorophyll tended to increase with age between 25 and 45 d after seedling emergence under non-submerged conditions. Submergence for 10 d reduced leaf chlorophyll concentrations in both cultivars. The loss was more pronounced in IR42. During recovery from submergence, the chlorophyll concentration of FR13A either remained the same or increased while the chlorophyll concentration of IR42 decreased during both 7 and 14 d of recovery.

Detachment induced a rapid loss of chlorophyll pigments compared with normal conditions, but this was still faster in non-submerged or submerged IR42 compared with FR13A (Table 2). When exposed further to enhanced leaf

Table1. Oxygen concentration (mol m^{-3}) and water temperature (°C) of flood water in the experimental tanks determined at 1100 hours.

Water depth (cm)	Days of submergence					
	1		4		10	
	O_2	Temperature	O_2	Temperature	O_2	Temperature
1	0.27 (0.02)†	24.8 (0.5)	0.39 (0.03)	27.7 (0.5)	0.25 (0.02)	28.8 (0.5)
40	0.25 (0.01)	25.1 (0.3)	0.41 (0.02)	27.4 (0.2)	0.21 (0.01)	27.4 (0.3)
80	0.24 (0.02)	25.1 (0.2)	0.41 (0.01)	27.3 (0.2)	0.20 (0.02)	27.2 (0.3)

† Figures in parentheses are standard errors.

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Table 2. Chlorophyll concentration (mg g^{-1} tissue fresh weight)† of rice leaves in non-submerged, submerged or submerged and then desubmerged rice using either attached or detached leaves.

Growth treatment (plant age, d)	Rice cultivar			
	FR13A		IR42	
	Attached leaves	Detached leaves‡	Attached leaves	Detached leaves‡
Non-submerged				
(21 d)	1.90 (0.07)	1.42 (0.06)	2.03 (0.14)	1.35 (0.03)
(25 d)	1.96 (0.02)	1.50 (0.03)	2.23 (0.10)	1.46 (0.09)
(31 d)	2.07 (0.11)	1.79 (0.17)	2.46 (0.09)	1.55 (0.22)
(38 d)	2.12 (0.08)	1.61 (0.32)	2.70 (0.29)	1.59 (0.26)
(45 d)	2.59 (0.12)	1.99 (0.27)	2.91 (0.14)	1.76 (0.19)
Submerged				
4 DAS (25 d)	1.95 (0.06)	1.18 (0.09)	1.89 (0.04)	0.62 (0.18)
10 DAS (31 d)	1.71 (0.04)	0.96 (0.21)	1.43 (0.24)	0.34 (0.13)
Desubmerged				
10 DAS + 7 DAR (38 d)	1.86 (0.15)	1.05 (0.22)	1.13 (0.28)	NA
10 DAS + 14 DAR (45 d)	2.35 (0.05)	0.95 (0.24)	1.04 (0.12)	NA

† Each value is the mean of three observations from five replicate plants; ‡ analysed 72 h after dark incubation of detached leaves in test tubes of sterile distilled water; DAS = days after submergence; DAR = days after recovery from submergence and returned to standard irrigation conditions; NA = not analysed because samples were not suitable; figures in parentheses are standard errors.

senescing conditions by detachment and incubation in the dark for 72 h in sterile water, leaves of desubmerged IR42 plants became flaccid and were unsuitable for analysis of chlorophyll.

FR13A showed significantly higher amino acid concentrations than IR42 under non-submerged, irrigated conditions. But submergence for 10 d resulted in an increase in amino acid concentration in both cultivars (Table 3). Amino acid concentrations were 1.2 to 3.0 times higher in IR42 than in FR13A at between 4 and 10 d submergence and they were seven times higher in the former than in the latter during recovery from submergence. Similarly, the detached leaves of both cultivars, either under non-submerged or submerged conditions, had higher amino acid concentrations than the attached leaves. The detached leaves of desubmerged IR42 in test tubes of sterile distilled water became flaccid after 72 h of incubation and were unsuitable for further analysis.

Protein concentrations in FR13A were slightly greater than in IR42 under non-submerged conditions. Submergence for 10 d reduced the protein concentration in FR13A (Table 4), but the reduction was much more pronounced in IR42. After 14 d of desubmergence (10 d of submergence plus 14 d of recovery), plants of FR13A showed an increase in protein concentration, but the protein concentration of IR42 declined markedly even after recovery from submergence. There were large decreases in the protein concentration of detached leaves of submerged IR42, but the changes were relatively small in the protein concentrations of

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Table 3. Free amino acid concentrations ($\mu g\ g^{-1}$ tissue fresh weight)† of rice leaves in non-submerged, submerged or submerged and then desubmerged rice using either attached or detached leaves.

Growth treatment (plant age, d)	Rice cultivar			
	FR13A		IR42	
	Attached leaves	Detached leaves‡	Attached leaves	Detached leaves‡
Non-submerged				
(21 d)	8.12 (0.02)	12.43 (0.05)	6.59 (0.06)	12.71 (0.10)
(25 d)	11.19 (0.09)	18.23 (0.11)	6.69 (0.08)	12.51 (0.26)
(31 d)	9.05 (0.21)	15.92 (0.26)	7.27 (0.50)	13.15 (0.14)
(38 d)	8.42 (0.28)	14.31 (0.21)	6.42 (0.28)	11.81 (0.11)
(45 d)	8.54 (0.28)	16.75 (0.17)	6.97 (0.12)	13.66 (0.33)
Submerged				
4 DAS (25 d)	8.19 (0.18)	16.46 (0.16)	10.20 (0.09)	21.52 (0.30)
10 DAS (31 d)	13.38 (0.18)	25.15 (0.31)	32.30 (0.31)	72.99 (0.31)
Desubmerged				
10 DAS + 7 DAR (38 d)	10.58 (0.36)	20.74 (0.19)	76.42 (0.14)	NA
10 DAS + 14 DAR (45 d)	9.13 (0.14)	18.44 (0.24)	86.67 (0.24)	NA

† Each value is the mean of three observations from five replicate plants; ‡ analysed 72 h after dark incubation of detached leaves in test tubes of sterile distilled water; DAS = days after submergence; DAR = days after recovery from submergence and returned to standard irrigation conditions; NA = not analysed because samples were not suitable; figures in parentheses are standard errors.

Table 4. Total protein concentrations ($mg\ g^{-1}$ tissue fresh weight)† of rice leaves in non-submerged, submerged or submerged and then desubmerged rice using either attached or detached leaves.

Growth treatment (plant age, d)	Rice cultivar			
	FR13A		IR42	
	Attached leaves	Detached leaves‡	Attached leaves	Detached leaves‡
Non-submerged				
(21 d)	9.67 (0.53)	6.45 (0.73)	8.81 (0.62)	5.31 (0.81)
(25 d)	10.76 (0.81)	7.23 (0.66)	8.67 (0.49)	4.88 (0.66)
(31 d)	10.31 (0.79)	6.62 (0.43)	8.73 (0.73)	4.47 (0.54)
(38 d)	10.53 (0.71)	6.13 (0.52)	8.43 (0.41)	4.61 (0.49)
(45 d)	11.02 (0.71)	6.64 (0.71)	8.38 (0.51)	4.32 (0.59)
Submerged				
4 DAS (25 d)	10.87 (0.92)	7.24 (0.82)	6.31 (0.36)	1.97 (0.93)
10 DAS (31 d)	8.37 (0.86)	5.94 (0.36)	4.36 (0.58)	1.14 (1.10)
Desubmerged				
10 DAS + 7 DAR (38 d)	9.12 (0.89)	3.94 (0.68)	3.02 (0.62)	NA
10 DAS + 14 DAR (45 d)	10.36 (0.73)	3.78 (0.85)	2.53 (0.56)	NA

† Each value is the mean of three observations from five replicate plants; ‡ analysed 72 h after dark incubation of detached leaves in test tubes of sterile distilled water; DAS = days after submergence; DAR = days after recovery from submergence and returned to standard irrigation conditions; NA = not analysed because samples were not suitable; figures in parentheses are standard errors.

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Table 5. Peroxidase activity (units g^{-1} tissue fresh weight)† in crude extracts of rice leaves of plants exposed to submergence or enhanced senescence (dark conditions).

Growth treatment (plant age, d)	Rice cultivar			
	FR13A		IR42	
	Attached leaves	Detached leaves‡	Attached leaves	Detached leaves‡
Non-submerged				
(21 d)	506 (5)	1795 (20)	392 (10)	2834 (18)
(25 d)	637 (7)	2139 (31)	462 (15)	3096 (22)
(31 d)	668 (10)	2366 (16)	713 (18)	3295 (41)
(38 d)	680 (12)	2408 (51)	802 (9)	2934 (56)
(45 d)	639 (9)	2473 (32)	720 (6)	2876 (12)
Submerged				
4 DAS (25 d)	666 (4)	2525 (26)	781 (20)	NA
10 DAS (31 d)	810 (16)	2799 (35)	901 (16)	NA
Desubmerged				
10 DAS + 7 DAR (38 d)	705 (20)	2500 (46)	ND	NA
10 DAS + 14 DAR (45 d)	644 (15)	2498 (54)	ND	NA

† Each value is the mean of three observations from five replicate plants; ‡ analysed 72 h after dark incubation of detached leaves in test tubes of sterile distilled water; DAS = days after submergence; DAR = days after recovery from submergence and returned to standard irrigation conditions; NA = not analysed because samples were not suitable; ND = not detected; figures in parentheses are standard errors.

detached leaves of submerged FR13A. During desubmergence, decline in protein also occurred in detached leaves of FR13A.

Considerable differences in the peroxidase activities in crude extracts of both tolerant and intolerant cultivars were observed (Table 5). Submerged leaves of FR13A and IR42 showed up to two-fold higher peroxidase activity than non-submerged controls. During submergence the peroxidase activity in crude extracts was marginally higher in IR42 than in FR13A. After recovery from submergence, activity returned to the levels of non-submerged plants. Detached leaves had about three- to four-fold greater peroxidase activity than attached leaves and for FR13A the relative effects of submergence on detached leaves were similar to attached leaves.

DISCUSSION

In rainfed areas, submergence tolerance in lowland rice cultivars is beneficial; flash flooding in these areas leads to increases in water level and to complete submergence of plants for up to about 14 d. On complete submergence of rice seedlings, survival decreases with increased duration and depth of submergence (Palada and Vergara, 1972). If submergence lasts longer than 1–2 weeks, death of plants occurs. Mazaredo and Vergara (1982) reported that dry weight production of both submergence tolerant and intolerant cultivars ceased after 6 d of complete

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submergence. Upon desubmergence, tolerant cultivars suffered less plant mortality (15%) and recovered more rapidly. Survival was only 50% of the initial number of plants for the intolerant cultivars (Mazaredo and Vergara, 1982). Physiological changes which occur with up to 10 d of complete submergence of rice cultivars could determine the survival of plants.

Setter *et al.* (1989) reported alterations in the rates of photosynthesis and photorespiration of submergence tolerant (Kurkaruppan) and intolerant (IR42) rice cultivars under submerged conditions. Complete submergence of lowland rice cultivars reduces the rate of growth and also the concentrations of carbohydrates in shoot tissues. Under partial submergence, the magnitude of these changes depends on the proportion of the leaves below the water (Setter *et al.*, 1987).

Jackson *et al.* (1987) showed that the ethylene concentration in the leaves of submerged plants of the tolerant FR13A and Kurkaruppan, and the intolerant IR42 rice cultivars increased 1–7 times relative to non-submerged plants. The effects of ethylene on plants include chlorosis (Thimann, 1987). Jackson *et al.* (1987) showed that the loss of chlorophyll from fully expanded leaves after 3 d of submergence was the conspicuous feature of submergence intolerant IR42. Degradation of chlorophyll was much less marked in the two tolerant cultivars FR13A and Kurkaruppan.

Many traits such as seed or embryo dry weight, seed:shoot carbohydrate contents, shoot height, solute leakage, underwater respiration and photosynthesis, anoxia tolerance and high rates of alcoholic fermentation are all indicated as mechanisms explaining submergence tolerance of rice and have been evaluated by different researchers (Setter *et al.*, 1997). The physiological explanation of senescence in relation to submergence tolerance of rice is, however, lacking.

We postulated that one of the survival strategies of submerged plants is to delay leaf senescence. In the present study, we observed substantial differences in the chlorophyll concentrations of submerged and irrigated plants and between the two cultivars, notably after 10 d of complete submergence. Only in FR13A after recovery from submergence did chlorophyll return to levels comparable with those of non-submerged plants. It was postulated that there would be differences in the breakdown of chlorophyll between submergence tolerant and intolerant cultivars and this was supported by the results of the present study which showed that loss of chlorophyll was greater in the submergence intolerant cultivar IR42 than in the tolerant cultivar FR13A. However, the loss of chlorophyll is not the initial stage in the biochemical changes normally taking place during leaf senescence (Thimann, 1987).

Proteolysis is considered to be the foremost step, especially in leaf senescence (Thimann, 1987). During the course of senescence, proteinases increase and amino acids are produced. In detached senescing leaves of oats (*Avena sativa*), accumulation of free amino acids can be detected within 6 h, and a 65% loss of proteins occurs by 72 h (Thimann *et al.*, 1974). Our results with rice indicated clearly that submergence or enhanced senescing conditions, imposed by detaching leaves in the dark, caused substantial increases in leaf amino acid concentrations

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while the total protein concentration decreased. Changes in the concentrations of free amino acids and total protein mirrored the differences in the response of cultivars to submergence and subsequent survival after desubmergence. In IR42 senescence appeared to have progressed beyond a capacity for recovery due to proteolysis and a 50% loss of protein after 10 d of submergence. FR13A showed a remarkable ability to recover protein concentrations within a week after desubmergence while in IR42 protein concentrations were still only about one-third of those in non-submerged plants (Table 4). Continued loss of total protein concentration in IR42 plants after 14 d of desubmergence is consistent with their intolerance of submergence.

Peroxidase activity in crude extracts of submerged leaves was higher than in non-submerged leaves for both the cultivars. During submergence IR42 showed a greater increase in peroxidase activity than FR13A. However, after desubmergence and returning to non-submerged conditions, there was no detectable level of activity in IR42. Possibly, the loss of peroxidase activity resulted from increased proteolysis which was evident from the protein loss which was more than 50% during submergence. Peroxidase activities were measured using crude extracts of plant tissues in the present study. Hence there could have been activators or inhibitors which affected samples in different treatments. This would have to be evaluated in subsequent experiments, for example, by mixing extracts or measuring recovery using commercial enzymes.

After recovery from submergence, the leaves of FR13A showed changes in chlorophyll, amino acid and total protein concentrations as well as in peroxidase activity in crude extracts. These were comparable with levels in the non-submerged plants. IR42 did not recover from submergence stress within 14 d after desubmergence.

In the present study, detached (excised) leaves were held under dark conditions to evaluate further the course of senescence according to Thimann *et al.* (1974). There are similarities and differences between the senescence of attached and detached leaves, and senescence due to light and darkness (Thimann *et al.*, 1974; Thimann, 1987). In the present study the results showed that the general characters of the senescence process remain similar in attached and detached (darkened) leaves of both submergence tolerant and intolerant cultivars for up to 10 d of complete submergence. However, the rate of this process was enhanced in detached leaves of both cultivars. Biochemical analysis using detached leaves of rice cultivars could lead to the development of a simple screening method for plant breeders selecting cultivars tolerant to submergence. In this context, identification of suitable biochemical marker(s) may also become important.

Many changes such as an increase in amino acid concentration and protease activity, a decrease in chlorophyll (Vaux, 1993) and an increase in peroxidase activity (Kar and Mishra, 1976; Yeh and Kao, 1994) are indices of senescence. The nature of induction of senescence (both in normal and enhanced senescing conditions) has a considerable effect on plant metabolism. Thimann *et al.* (1974) reported that there was a linear increase in amino acid concentrations in detached

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senescing leaves of oats while there was a decline in attached leaves due to the mobilization to other plant parts. Time courses of solutes in detached leaves were not measured in the present study but results indicated that the changes in chlorophyll concentration, free amino acids, total protein and peroxidase activity were similar for the detached senescing leaves over 72 h and for those of submerged plants, but with discernible differences between tolerant and intolerant cultivars.

The differences observed between the responses of FR13A and IR42 to submergence were possibly due to the differences in proteolysis; IR42 showed a five-fold increase in the concentration of free amino acids and a 50% reduction in the total protein concentration, relative to the control after 10 d of submergence. However, these changes were less marked in FR13A (Tables 3 and 4). High rates of protein hydrolysis may result in increased amino acid concentrations which (1) can contribute as substrates to respiration or alcoholic fermentation, (2) can leak out of the tissue, or (3) can contribute to a pool of peptides not assayed by the methods used in this study. All of these possibilities suggest the need for further experiments to investigate how the traits identified for submergence tolerance to date are related to the rate of proteolysis in submerged rice plants. Results from a comparative study using only two cultivars suggested that it might be worth obtaining more data on the role of senescence in submergence tolerance of rice cultivars, using a wide range of cultivars.

During submergence, the environmental conditions impose several stresses on the physiological activities of leaves. Presumably, metabolism may also be affected due to senescence. Predominantly anaerobic flooded soils can act as a source of ethylene. Ethylene plays a considerable role in regulating senescence in leaves (Thimann, 1978; John *et al.*, 1995), elongation of rice plants (Jackson *et al.*, 1987), and chlorosis (Thimann, 1987). Ethylene is found at high partial pressures (0.07–2.5 Pa) in ricefield flood water (Setter *et al.*, 1988). Jackson *et al.* (1987) reported that there were increases in the concentration of ethylene (0.69 ± 0.10 Pa) extracted from rice leaves during complete submergence. These observations and the results of this study indicated that leaf senescence is likely to be an important biochemical mechanism in plants during submergence, and that submergence tolerance of rice cultivars could be related to reduced senescence metabolism.

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