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INFLUENCE OF PHOSPHORUS APPLICATION TO FLOODWATER ON OXYGEN CONCENTRATIONS AND SURVIVAL OF RICE DURING COMPLETE SUBMERGENCE

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SUMMARY

Low concentrations of phosphorous were added to the floodwater of submerged rice in an attempt to regulate algal growth, so as to increase floodwater O_2 concentration and plant survival during submergence for up to 12 days. Increasing the phosphorus concentration of the floodwater by 1 ppm increased algal growth by at least 4-fold based on chlorophyll concentrations, and increased floodwater O_2 concentrations to over twice air saturation. However, additions of phosphorus reduced rice plant survival during submergence by up to 35%. The adverse effects of a high phosphorus concentration in the floodwater were mainly attributed to competition between the algae and the submerged rice for CO_2 and, perhaps, light. The importance of photosynthesis during the submergence of rice was supported by the results of experiments in which floodwater CO_2 concentration was manipulated by altering pH. The survival of an intolerant rice cultivar during submergence increased from 0 to 17 and 62% at pH 8, 7 and 5 respectively, while floodwater CO_2 concentrations at these pHs would have increased from 0.02 to 0.3 and 1.0 mol m^{-3} respectively. The results were used to question the importance of floodwater O_2 concentrations above anoxia for submergence tolerance of rice.

INTRODUCTION

During the monsoon season in South and Southeast Asia, flash-flooding may result in partial or complete submergence of rice crops, and subsequently either complete or partial loss of grain yields may occur. A total of 20 million hectares of rice-growing area is adversely affected by flash-flooding (IRRI, 1975), with about half of this occurring in eastern India (Reddy and Sharma, 1992).

The frequency of complete or partial submergence is not quantified for any location, but it will depend on several factors including the maximum water depth, duration of flooding, the rate of water increase and decrease, and the plant height and elongation during flooding. Recent data produced by Ram *et al.* (1999) in eastern India suggest that partial and complete submergence occur at approximately equal frequencies. In this study complete submergence was evaluated since (i) it results in a consistent exposure of all tissues to the same

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treatment, and (ii) there is considerable published data on exposure of rice and other plants to complete submergence (see Discussion). This is in contrast to the more complex treatment of partial submergence where some tissues are submerged and some are not, and this proportion usually changes during the course of flooding treatments.

Adverse effects of complete submergence on plant growth and survival are complex (Setter *et al.*, 1995a), and submerged plants may also be adversely affected after submergence when post-hypoxic injury can occur (Crawford, 1992). Gas diffusion is limited during submergence and is about 10 000-fold slower in water than in air (Armstrong, 1979). Therefore any gases important in plant metabolism may affect growth or survival during submergence. Reduced O_2 supply is one of the major factors which may limit growth and survival during waterlogging of plants (Armstrong, 1979; Jackson and Drew, 1984) and submergence of rice (Waters *et al.*, 1989; Setter *et al.*, 1989a).

In earlier studies of rice, treatments resulting in reductions in O_2 concentrations of shoots during submergence had effects which were rapid and severe. For example, lowering the O_2 concentration in the shoot medium, darkness, or reducing the CO_2 supply, resulted in reductions in root O_2 supply and reduction or cessation of root growth within minutes (Waters *et al.*, 1989). Furthermore, when a submergence-intolerant rice cultivar, IR8, was exposed to anoxia for only 16 h there was no recovery, while complete recovery occurred for the submergence-tolerant cultivar FR13A (Crawford, 1989).

Information is limited on the importance of different O_2 concentrations above anoxia on the growth and survival of rice during submergence. However, the range of O_2 concentrations of floodwater which occurs during submergence of rice in farmers' fields in India varies from nil to supersaturated (0.6 mol m^{-3} O_2; compare 0.24 mol m^{-3} O_2 in aerated water at 30 °C (Setter *et al.*, 1995b; Ram *et al.*, unpublished)). The generation of supersaturated O_2 by algae, particularly in eutrophic lakes (Wetzel, 1983) and estuaries (Lukatelich and McComb, 1986), is well known. In a recent study with submerged rice, algal growth was probably responsible for floodwater supersaturation with O_2 at high levels of phosphorus supply. Such pronounced effects of algal growth on floodwater oxygenation suggested that controlling algal growth by the addition of phosphorus could be a way of improving the O_2 supply to rice plants and hence increasing survival during submergence (Setter *et al.*, 1995b).

In view of the above findings, experiments were conducted to manipulate the O_2 concentrations of floodwater during submergence by the addition of different concentrations of phosphorus to the stagnant water. The lack of any beneficial effects due to the high O_2 supply found in these experiments and the importance of other factors contributing to growth and survival of rice during submergence are discussed.

MATERIALS AND METHODS

Submergence-tolerant FR13A and submergence-intolerant IR42 rice cultivars (Mazaredo and Vergara, 1982) were raised in pots containing 1.5 kg soil from the Central Rice Research Institute, Cuttack, India (lat 20°28′N, long 85°55′E). The soil type was an Inceptisol with pH 6.8, 0.69% organic carbon, 0.065% total nitrogen, a cation exchange capacity of 15.4 per 100 g soil and an electrical conductivity of 0.31 dS m^{-1} (Chakravorty *et al.*, 1987).

Plants were submerged 25 d after germination in cement tanks (2.1 × 1.1 × 1.1 m in depth) with a water depth of 0.8 m above the soil surface. Eighteen tanks were used with three replicates of six phosphorous (P) treatments. Each replicate contained 20 pots of FR13A and of IR42, and there were 10 plants per pot. Phosphorus was added at floodwater concentrations of 0.00, 0.00 plus 5 ppm $CuSO_4$ (added to reduce the algal growth), 0.25, 0.50, 0.75 and 1.00 ppm P in the form of single superphosphate and this was added to the floodwater on the first day of submergence (day 0). Plants were maintained completely submerged for 10 d and then suddenly desubmerged to a water depth of 5 cm by removing the pots from the tanks; recovery was for 14 d after desubmergence.

A second experiment was conducted with no added P and at pH 5.0, 6.9 or 8.0. The pH 5 treatment consisted of natural floodwater at pH 6.9 adjusted with H_2SO_4 and the pH 8 treatment was obtained by adding potassium hydroxide to the floodwater. Plants were submerged for 12 d in the tanks. The pH was measured and readjusted daily in each treatment, and there was less than 0.5 pH unit change per day.

Dissolved O_2 concentrations in floodwater were determined using a submersible polarographic O_2 electrode (Syland Model 610, Heppenheim, Germany) which was attached to a measuring scale held at specific depths in the floodwater. The O_2 measurements were made daily at 1100 hours at water depths of 40 and 80 cm (approximately the top and bottom of the submerged rice canopies). The data presented are the means of these measurements. In 22 of the 24 measurements of floodwater O_2 there were no significant differences for the two cultivars used and so the data were pooled. The CO_2 concentrations in floodwater were measured using a CO_2 polarographic electrode and calculated according to Setter *et al.* (1987; 1995b) using the Henderson–Hasselbalch equation:

$$\mathrm{pH} = \mathrm{pKa} + \log\,[\mathrm{HCO_3^-}]/[\mathrm{CO_2}]$$

and pKa is 6.36 at 30 °C (Umbreit, 1964; Ram *et al.*, 1999).

Light was measured using an underwater quantum light sensor (LI-COR, Model 185B, Massachusetts, USA), and measurements were made at 1100 hours at a water depth of 50 cm.

Algal growth was measured in terms of the total chlorophyll concentrations in floodwater. Samples of floodwater measuring 1 L were taken from each tank at 1100 hours 10 d after submergence. Samples from water depths of 40 and 80 cm were mixed and filtered through Whatman No. 42 filter paper and extracted in

25 mL 80% *V*/*V* acetone. The extract was assayed for total chlorophyll (mg L^{-1} floodwater) according to Arnon (1949).

Survival was measured by the ability of plants to regrow following desubmergence, and data are presented as a percentage of the number of seedlings submerged in each treatment.

RESULTS

Floodwater characteristics

The application of increasing concentrations of P to floodwater with submerged rice plants tended to increase concentrations of dissolved O_2 at 4, 8 and 10 d after submergence (Table 1). By 10 d after submergence the O_2 concentration in floodwater was 2- to 3-fold greater than for floodwater with no added P or with $CuSO_4$ which was used to minimize algal growth. Between 0 and 10 d after submergence there was a large increase in floodwater pH from about 7 to 10 for all treatments with and without added P (Table 1). At 8 to 10 d after submergence the pH ranged about 1 pH unit between the different P treatments, and there was little difference in pH between treatments with or without added $CuSO_4$ but no added P (Table 1).

Algal growth was measured by increases in the chlorophyll concentrations of the floodwater and algal chlorophyll increased with increase in P concentrations (Table 2). The treatment of floodwater with $CuSO_4$ was successful in reducing

Table 1. Oxygen concentrations (mol m^{-3}) and pH in floodwater during submergence of rice at different floodwater phosphorus concentrations and with 5 ppm $CuSO_4$ to suppress algal growth. Measurements were made at 1100 hours at water depths of 40–80 cm. The O_2 concentration of air saturated water at 30 °C was 0.23 mol m^{-3} (7.5 ppm) and plants were submerged at approximately 0800 hours on day 0. Standard errors of the means are in parentheses.

Treatment (ppm phosphorus)	Days after submergence			
	0	4	8	10
			Oxygen concentration	
0.00	0.23 (0.05)	0.25 (0.01)	0.37 (0.01)	0.36 (0.02)
0.00 (plus $CuSO_4$)	0.20 (0.01)	0.28 (0.01)	0.27 (0.02)	0.20 (0.01)
0.25	0.26 (0.02)	0.29 (0.03)	0.38 (0.03)	0.37 (0.07)
0.50	0.27 (0.03)	0.29 (0.02)	0.41 (0.01)	0.43 (0.00)
0.75	0.27 (0.02)	0.31 (0.01)	0.47 (0.03)	0.46 (0.04)
1.00	0.27 (0.02)	0.31 (0.01)	0.57 (0.02)	0.50 (0.05)
			pH	
0.00	7.0 (0.3)	7.1 (0.3)	9.3 (0.2)	9.9 (0.1)
0.00 (plus $CuSO_4$)	—	—	9.2 (0.0)	9.4 (0.0)
0.25	—	—	10.3 (0.2)	10.3 (0.2)
0.50	—	—	10.3 (0.2)	10.3 (0.1)
0.75	—	—	10.4 (0.2)	10.4 (0.2)
1.00	—	—	10.8 (0.0)	10.6 (0.1)

Table 2. Algal chlorophyll (mg L^{-1}) in floodwater during submergence of rice at different floodwater phosphorus (P) concentrations. Chlorophyll was measured at 10 d after submergence. In one treatment without added phosphorus 5 ppm $CuSO_4$ was added to suppress algal growth. Standard errors of the means are in parentheses.

Treatment (ppm P)	Floodwater chlorophyll
0.00	0.83 (0.01)
0.00 (plus $CuSO_4$)	0.01 (0.02)
0.25	2.34 (0.04)
0.50	2.64 (0.05)
0.75	2.95 (0.03)
1.00	3.81 (0.07)

algal growth to about 1% of water without added P (Table 2). The regression coefficient (r^2) between algal chlorophyll and O_2 concentrations in floodwater across all treatments was 0.88 (calculated from data at 10 d, Table 1 and 2).

Irradiance in floodwater at the centre of the submerged plant canopies (water depth 50 cm) was approximately 80% of the incident irradiance above the floodwater at the commencement of submergence (0 d, Table 3). However, irradiance at this depth decreased to as low as 37% of incident irradiance with time of submergence. The irradiance in floodwater tended to be reduced more where algal growth was highest (compare Table 2 and 3); and at 10 d, irradiance at a water depth of 50 cm was negatively correlated to algal chlorophyll ($r = -0.76$ calculated from Table 2 and 3).

Plant characteristics

After 10 d submergence, plant survival was higher for FR13A than for IR42 at all floodwater P concentrations. The greatest difference was at 0.25 ppm P where

Table 3. Irradiance in floodwater (% of incident) during submergence of rice at different floodwater phosphorus (P) concentrations. Irradiance was measured at a depth of 50 cm at the centre of the submerged rice canopies and pooled for both cultivars; measurements at 0 d were immediately after submergence. The incident irradiance ranged from 1000 to 2800 $\mu E\ m^{-2}\ s^{-1}$ photosynthetically active radiation at the times of measurement. Standard errors of the means are in parentheses.

Treatment (ppm P)	Days after submergence 0	2	4	10
0.00	80 (2)	74 (0)	73 (5)	43 (3)
0.00 (plus $CuSO_4$)	82 (3)	80 (3)	82 (1)	72 (0)
0.25	—	82 (6)	70 (5)	53 (4)
0.50	—	80 (0)	66 (1)	48 (2)
0.75	—	78 (2)	62 (1)	37 (4)
1.00	—	75 (1)	59 (1)	37 (2)

100% of FR13A plants survived and no IR42 plants survived (Table 4). High concentrations of P in the floodwater resulted in a reduction in survival of up to 33% for both rice cultivars relative to floodwater with $CuSO_4$ but no added P during submergence (Table 4).

During the first 6 d of complete submergence, the continued growth of FR13A was demonstrated by significant increases in plant dry weight by up to 50% in all P treatments. In contrast, the dry weights of IR42 either remained the same or were reduced by up to 20% relative to the dry weights at 0 d (Table 5). By 10 d after submergence the dry weights of FR13A were still either greater or only slightly less than the weights at 0 d, while the dry weight of IR42 was reduced by more than 50% at the highest P concentration. During the 14-d recovery period following 10 d of submergence, only FR13A plants retained a dry weight; IR42 plants died and decayed in all treatments with added P (Table 5).

Leaf chlorophyll concentrations remained the same or increased in both cultivars under non-submerged conditions. However during submergence at 0.00 ppm P, chlorophyll concentrations decreased by 65% in IR42 but increased by over 30% in FR13A (both relative to 0 d, Table 6). Like dry weight, the increased P concentrations tended to reduce chlorophyll concentrations in both cultivars after 10 d of submergence, but in FR13A less than IR42 (Table 7). When plants were allowed to recover for 14 d, leaf chlorophyll concentrations tended to remain equal to concentrations at the end of the submergence period for treatments where plants survived (Table 6).

In another experiment where plants were submerged for 12 d with no added P there were consistent differences in the survival of submergence-tolerant (FR13A) and submergence-intolerant (IR42) cultivars (compare pH 6.9 in Table 7 with the 0.00 ppm P treatment in Table 4). When plants were submerged at pH 5, the

Table 4. Survival (%) of rice cultivars during submergence at different floodwater phosphorus (P) concentrations. Plants were submerged for 10 d, and survival was measured by the percentage of plants which were able to grow by 14 d after desubmergence. Standard errors of the means are in parentheses.

Treatment (ppm P)	Cultivar	Survival
0.00	IR42	17 (4)
	FR13A	100 (0)
0.00 (plus $CuSO_4$)	IR42	33 (4)
	FR13A	100 (0)
0.25	IR42	0 (0)
	FR13A	100 (0)
0.50	IR42	0 (0)
	FR13A	83 (2)
0.75	IR42	0 (0)
	FR13A	83 (1)
1.00	IR42	0 (0)
	FR13A	67 (4)

Table 5. Dry weight (g pot^{-1}) of rice cultivars during and after submergence at different floodwater phosphorus (P) concentrations. Plants were submerged for 10 d, and recovery was for 14 d in air following desubmergence. Standard errors of the means are in parentheses.

Treatment (ppm P)	Cultivar	Days after submergence			Recovery†
		0	6	10	
			Non-submerged		
0.00	IR42	1.9 (0.5)	—	3.6 (0.3)	—
	FR13A	1.6 (0.0)	—	3.5 (0.2)	—
			Submerged		
0.00	IR42	—	2.1 (0.2)	1.4 (0.3)	0.6 (0.2)
	FR13A	—	2.3 (0.1)	2.0 (0.4)	3.0 (0.1)
0.00 (plus $CuSO_4$)	IR42	—	1.7 (0.1)	1.6 (0.3)	1.2 (0.2)
	FR13A	—	2.4 (0.1)	2.0 (0.1)	2.3 (0.3)
0.25	IR42	—	1.6 (0.1)	1.4 (0.1)	‡
	FR13A	—	2.2 (0.1)	1.8 (0.1)	1.5 (0.1)
0.50	IR42	—	1.6 (0.1)	1.1 (0.1)	‡
	FR13A	—	2.1 (0.2)	1.2 (0.0)	1.7 (0.1)
0.75	IR42	—	1.4 (0.0)	1.1 (0.0)	‡
	FR13A	—	1.9 (0.0)	1.6 (0.1)	1.1 (0.1)
1.00	IR42	—	1.3 (0.0)	0.7 (0.0)	‡
	FR13A	—	1.9 (0.1)	1.4 (0.1)	1.0 (0.0)

†After 10 d of submergence; — not measured; ‡ sample decayed and unsuitable for analysis.

Table 6. Leaf chlorophyll concentrations (mg g^{-1} fresh weight) of rice cultivars during and after submergence at different floodwater phosphorus (P) concentrations. Plants were submerged for 10 d, and recovery was for 14 d in air following desubmergence. Standard errors of the means are in parentheses.

Treatment (ppm P)	Cultivar	Days after submergence			Recovery†
		0	6	10	
			Non-submerged		
0.00	IR42	2.56 (0.2)	2.90 (0.1)	2.73 (0.2)	—
	FR13A	1.75 (0.1)	2.85 (0.2)	2.86 (0.1)	—
			Submerged		
0.00	IR42	—	1.8 (0.2)	0.9 (0.1)	‡
	FR13A	—	2.3 (0.1)	1.6 (0.1)	1.8 (0.1)
0.00 (plus $CuSO_4$)	IR42	—	1.59 (0.1)	1.13 (0.3)	0.82 (0.0)
	FR13A	—	2.65 (0.1)	1.72 (0.0)	0.83 (0.1)
0.25	IR42	—	1.36 (0.1)	1.08 (0.0)	‡
	FR13A	—	2.34 (0.1)	1.56 (0.1)	1.43 (0.3)
0.50	IR42	—	1.32 (0.2)	1.17 (0.1)	‡
	FR13A	—	2.04 (0.1)	1.22 (0.0)	1.23 (0.1)
0.75	IR42	—	1.29 (0.2)	1.07 (0.0)	‡
	FR13A	—	1.82 (0.1)	1.07 (0.0)	1.03 (0.0)
1.00	IR42	—	0.99 (0.1)	0.81 (0.0)	‡
	FR13A	—	0.55 (0.1)	0.85 (0.0)	0.10 (0.0)

†After 10 d of submergence; — not measured; ‡ sample decayed and unsuitable for analysis.

Table 7. Survival (%) of rice during submergence at different floodwater pH. Plants were submerged for 10 d as per Table 5. The CO_2 concentration (mol m^{-3}) in floodwater was estimated from the CO_2/HCO_3^- equilibrium at the specified pH based on the measured CO_2 concentration in water (pH 6.4) of about 0.5 mol m^{-3} (pKa of CO_2/HCO_3^- = 6.4, calculated from Setter *et al.*, 1995b). Standard errors of the means are in parentheses.

Treatment pH	Floodwater CO_2 concentration	Cultivar	Survival
5.0	0.96	IR42	62 (4)
		FR13A	100 (0)
6.9	0.31	IR42	17 (4)
		FR13A	100 (0)
8.0	0.02	IR42	0 (0)
		FR13A	100 (0)

survival of intolerant IR42 increased more than 3-fold, but when plants were submerged at pH 8.0 the intolerant cultivar had no survival. Reducing pH from 8 to 5 resulted in a 50-fold increase in the dissolved CO_2 concentration in floodwater from about 0.02 to 1 mol m^{-3} (Table 7).

DISCUSSION

The oxygen supply of plants exposed to waterlogging or submergence is one of several factors affecting their growth and survival. Considerable work has been done on the effects of O_2 deficiency on plants (Greenway and Setter, 1996), but there is no published work where O_2 concentrations have been manipulated in the field during plant submergence. Generating a high O_2 supply by promoting algal growth in floodwater was proposed earlier as a means of increasing the growth and survival of rice during submergence, assuming that low O_2 supply was the main limiting factor affecting plant survival (Setter *et al.*, 1995b).

The results presented here confirm that high concentrations of P in the floodwater increase algal growth (Table 2), and this is consistent with numerous studies demonstrating the limitation of algal growth in aquatic ecosystems by P, N, or P plus N (Wetzel, 1983; Lukatelich and McComb, 1986). However, the result that high floodwater O_2 was associated with *reduced* survival during submergence was the opposite to what was expected. Such results reflect the complexity of environmental factors affecting survival during submergence.

The possibility that O_2 concentrations above anoxia in floodwater would have had little effect on the survival of rice during submergence would not be surprising since the k_m of cytochrome oxidase for O_2 is so low, i.e. only about 0.14 mmol m^{-3} (isolated soyabean mitochondria, Millar *et al.*, 1994), which is more than 10^3 lower than the O_2 concentration in air saturated water at 30 °C. However, O_2 deficiency of plant tissues could occur even in rapidly stirred, aerated solutions due to rapid rates of tissue respiration, the development of boundary layers in the microenvironment adjacent to submerged tissues, and the limited diffusion of O_2 in water and through tissues (Greenway and Setter, 1996). This is consistent with a k_m of growth for O_2 for rice roots in solution of about 1–2 mmol m^{-3} (calculated from Fig. 3 and 4 of Armstrong and Webb, 1985). It is possible that there may be little or no effect of O_2 concentration on the survival of submerged rice at a concentration greater than anoxia and less than air saturation (230 mmol m^{-3} at 30 °C; gas equilibrium of 21 kPa). This was suggested by a survival rate of 100% for rice seedlings in glasshouse experiments submerged for 2 d in continuous darkness in floodwater at 21, 10 or 5 kPa O_2, compared with 0% survival for plants submerged at 0 kPa O_2 (Ellis and Setter, 1999).

There are several possible explanations for the adverse effects found to be associated with high floodwater algal growth, high O_2 or high P concentrations during submergence of rice.

1. Increased algal growth may limit the light available for submerged rice plants to photosynthesize underwater. At low irradiance (approximately 300 μE m^{-2} s^{-1} photosynthetically active radiation), FR13A had more than 2-fold greater photosynthesis underwater than IR42 (Mazaredo and Vergara, 1982). The importance of photosynthesis during submergence is demonstrated by the severe reductions in survival during submergence in the dark (Palada and Vergara, 1972; Setter *et al.*, 1997) or in turbid water (Rama Krishnaya, field observations). For example, IR42 submerged for 10 d either with a natural diurnal irradiance or in complete darkness had 65 and 0% survival respectively (Setter *et al.*, 1997). There is no published information on the effects of low irradiance relative to complete darkness during submergence.

   It is unlikely that reduced irradiance in floodwater was the main explanation for reduced survival with high P concentrations since the differences in irradiance between treatments were often small, and sometimes treatments with high algal growth and high P concentrations had greater irradiance in floodwater than treatments with no added P. For example, at 10 d there was greater irradiance and lower survival of plants at 0.25 ppm P compared with 0.00 ppm P and no added $CuSO_4$ (Table 3 and 4).

   Measurements made here for irradiance were in the bulk floodwater solution. Hence, it was possible that greater algal growth occurred on the surfaces of submerged leaves at high P concentrations and this could have reduced irradiance more for plants than at low P concentration. Light reductions are well known as one of the adverse effects of high concentrations of nutrients in water resulting in epiphytic algal growth on submerged aquatic macrophytes like the marine seagrasses (Cambridge *et al.*, 1986). Epiphytic algae sometimes cause shading on leaves sufficient to reduce photosynthesis and growth of seagrasses (Sand-Jensen, 1977). In two locations in Western Australia which differed in eutrophication, the epiphyte loads were estimated to reduce leaf photosynthesis of submerged seagrasses by 15–63%; this was estimated using detached leaves at light saturation with 295 ppm CO_2 (equivalent to about 0.01 mol m^{-3} CO_2, Umbreit, 1964) in deoxygenated seawater (Silberstein *et al.*, 1986). In experiments presented here no measurements were made of the difference in epiphytes on rice submerged at low and high P concentrations and so reduced irradiance of leaves remains a possible contributing factor to the differences in survival. Whether epiphytic algal growth results in a reduced CO_2 supply for submerged rice grown in these experiments or for seagrasses in other published work is unknown.

2. Algal growth during submergence could have resulted in competition for CO_2 with the submerged rice. The total inorganic carbon in these tanks was only about 1 mol m^{-3} (Setter *et al.*, 1995b) and, at a pH of 6.4, the CO_2 concentration would have been about 0.5 mol m^{-3} (pKa of CO_2/HCO_3^- is 6.39 at 30 °C, Umbreit, 1964). During submergence, treatments with no added P increased in pH by 2 units, whether there was low or high algal growth (compare pH and algal chlorophyll with and without $CuSO_4$, Table 1 and 2). Assuming that the pH increases measured in these experiments were a consequence of reductions in CO_2 concentration due to photosynthetic uptake, there would have been a 100-fold reduction in floodwater CO_2 concentration between 0 and 8 d after submergence (pH increase from 7 to 9, Table 1).

The increase in floodwater pH and the reduction in CO_2 concentration which would have occurred in these experiments would have a profound impact on the ability of rice to photosynthesize under water. For example, when pH increased from 6.5 to 7.9 the photosynthesis of submerged rice leaves immediately decreased more than 10-fold (cv. IR42, Setter *et al.*, 1989b). Since treatments here with and without $CuSO_4$ increased in pH to the same level, it suggests that plants in floodwater with $CuSO_4$ had greater photosynthesis than plants without $CuSO_4$ where algal growth was pronounced. The greater photosynthesis by submerged plants in floodwater with $CuSO_4$ could easily explain why the survival of IR42 was 2-fold greater in this treatment than in treatments without $CuSO_4$ (Table 4).

The adverse effects of low CO_2 supply were confirmed by effects of different floodwater pH levels on the survival of the submergence-intolerant cultivar IR42 (Table 7). When floodwater pH was reduced to pH 5 and the CO_2 concentration increased, the survival of rice plants increased significantly. However, when the floodwater pH was increased to pH 8 and the CO_2 concentration was reduced, plant survival was significantly reduced (Table 7). In the treatments with different floodwater pH (Table 7), one of the factors affecting pH was the tendency of the floodwater to readjust to the CO_2 partial pressure of the atmosphere (and hence change the floodwater pH). However, daily monitoring and readjustments of the floodwater pH ensured that the treatments and hence the $[HCO_3^-]/[CO_2]$ was maintained. In other experiments where the CO_2 concentration was manipulated similarly in equivalent volumes, it took at least two weeks for the floodwater to return to the equilibrium of the CO_2 partial pressure of the atmosphere. This lack of rapid equilibrium of large volumes of water is similar to the frequent measurements of CO_2 concentrations in the natural environment of rice fields that are often orders of magnitude different from equilibrium pressures (Ponnamperuma, 1984; Setter *et al.*, 1987; Ram *et al.*, 1999).

3. The negligible algal growth and minimal O_2 supersaturation of floodwater in the $CuSO_4$ treatment (Table 2 and 1 respectively) suggest that algae, and not the submerged rice, were largely responsible for floodwater O_2 supersaturation. This is consistent with floodwater O_2 supersaturation in trenches used for fish culture adjacent to this site where there was no submerged rice (Setter *et al.*, 1995b). Greater algal growth and associated supersaturated O_2 may have had adverse effects on submerged rice. Bean plants exposed to O_2 pressure in a gas phase above 70 kPa have an irreversible inhibition of leaf photosynthesis, while effects are reversible below this pressure (Bidwell, 1979, Fig. 15-6). The relevance of such effects in a gas phase to submergence are unknown. Rates of photosynthesis of submerged rice leaves declined at O_2 concentrations of 0.23–0.46 (water equilibrium of 21–42 kPa) relative to < 23 mol m^{-3}, and this was dependent on the CO_2 concentration of the floodwater. These results are consistent with an increasing rate of photorespiration at high O_2 concentration (Setter *et al.*, 1989b). There is no published information on the long-term effects of such high O_2 concentration on growth during submergence.

   It is possible that the high algal growth and supersaturated O_2 in floodwater may have been associated with much lower O_2 concentrations during the night and early morning, but this is unlikely in the experiments presented here. Early morning floodwater O_2 concentration was not measured, although earlier work demonstrated that during submergence of rice at this site when the development of supersaturated floodwater occurred, the floodwater remained saturated in O_2 during the nights and early mornings during the first couple of weeks after submergence (Setter *et al.*, 1995b, Fig. 3).
4. Phytoplankton and epiphytic algae may have produced toxic substances in treatments with high P concentrations. The production of animal phytotoxins are well known for dinoflagellates (Round, 1973) and for cyanobacteria species such as *Anabena* and *Microcystis* (Fay and Baalen, 1987). However, there is little or no published information on toxicity of such species to terrestrial or aquatic angiosperms. The algal species associated with supersaturation of floodwater O_2 were not identified here, though common cyanobacteria species found in this area include *Anabena*, *Aphanothece*, *Aulosira*, *Cylindrospermum*, *Gloeotrichia* and *Nostoc* (Singh and Bisoyi, 1989).
5. It is unlikely that high P concentrations in the floodwater adversely affected plant growth directly. In the experiments described here, seedlings were

grown at optimum soil P. Furthermore, P was applied to the floodwater immediately after complete submergence, thus minimizing the effect of P on the mineral nutrient status of plants. In earlier experiments, it was demonstrated that plants grown at high P concentrations tolerated submergence better and produced significantly higher grain yields relative to plants without added P (Reddy *et al.*, 1991), even though survival was reduced in these experiments.

In conclusion, the significant effects of different P concentrations on algal growth and environmental conditions in floodwater demonstrates the complexity of numerous factors that may affect the tolerance of rice to submergence. These experiments were conducted to evaluate a possible field solution for alleviating the adverse effects of complete submergence on rice during stagnant flooding, namely to increase the O_2 concentration of the floodwater using small additions of P. The manipulation of floodwater O_2 concentration was successful, but the beneficial effects of high O_2 concentration on survival during submergence were not demonstrated. The adverse effects of high P concentration shown here may have been due to the supersaturated O_2 that developed (up to equilibrium of 52 kPa, calculated from Table 1) or to other factors which were associated with promoting algal growth. Results presented here during submergence at different pH levels support the view that the adverse effects of high P concentration during submergence are the consequence of reduced plant photosynthesis underwater due to competition for CO_2 from algae. Other contributing factors may be a reduction in light due to greater phytoplankton in the floodwater and possibly epiphytic algal growth on the submerged leaves. Whether an adverse effect of supersaturated O_2 occurs during submergence remains unresolved although other studies suggest that this would be likely to reduce net photosynthesis of the submerged plants due to increases in photorespiration.

Future experiments to evaluate the impact of different O_2 concentrations during submergence of rice should use compressed gas to produce saturated and subsaturated floodwater O_2 treatments during submergence. However, unless the floodwater O_2 concentration is monitored at different times of the day and the water is continuously mixed, or unless plants are in continuous darkness, the photosynthetic O_2 evolution by submerged rice leaves during the day will affect treatments and hence make interpretation difficult. Such requirements imply that this work is better conducted in glasshouse or laboratory experiments (see Ellis and Setter, 1999 for further discussion). More worthwhile crop management experiments in the field aimed at increasing submergence tolerance during stagnant flooding may involve investigating ways of increasing floodwater CO_2 concentrations by supplying low concentrations of bicarbonate salts (Setter *et al.*, 1989b) or by maintaining moderately acidic floodwater pH. This could have major beneficial effects on submergence in regions like the Indo-Gangetic Plains of eastern India where there are large areas of alkali and sodic soils.

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