Journal of Experimental Botany, Vol. 33, No. 136, pp. 1030–1044, October 1982

The Effect of Oxygen and Turbulence on Elongation of Coleoptiles of Submergence-Tolerant and -Intolerant Rice Cultivars

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Received 16 November 1981

ABSTRACT

Coleoptiles of rice (*Oryza sativa* L.) elongated more rapidly in stagnant solution than in water-saturated air: elongation rates in aerated solution were intermediate. Elongation was stimulated between 170% to 390% in stagnant solution in 17 cultivars screened. On this basis, a relatively submergence-intolerant cultivar and two submergence-tolerant cultivars were studied further.

When seedlings were grown in a range of oxygen concentrations imposed by bubbling solutions rapidly with gas, germination and early coleoptile growth (up to 5·0 mm) were inhibited at low oxygen concentrations, particularly in the submergence-intolerant cultivar. Weight per unit length declined with time in this cultivar, even in mild oxygen deficits. However, later stages of coleoptile elongation were unaffected by low oxygen supply: elongation proceeded at control rates even in anoxia. It is concluded that processes unique to germination and early stages of coleoptile elongation (possibly cell division) have greater oxygen requirements than cell extension.

Rates of elongation in stagnant solution were much faster than those in any other treatment, including bubbled solutions at similar oxygen concentrations (0·125 mmol O_2 l^{-1}). Therefore, elongation appears to be stimulated by accumulation of endogenous compounds which are dispersed in bubbled solutions and in air. The principal volatile compound was almost certainly ethylene, since Ag^+ (an antagonist of ethylene) reduced the rate of elongation in stagnant solution by 53%.

Differences in turgor pressure were only partly responsible for rapid coleoptile elongation observed in stagnant solution: there must have also been differences in cell wall properties between coleoptiles grown in stagnant and bubbled solution. The rapid elongation in stagnant solution hastens coleoptile emergence from the solution, thus establishing an adequate oxygen supply for the seedling. Leaf and root growth then proceeds.

INTRODUCTION

Rice coleoptiles grow rapidly under submerged conditions and rice is one of the few higher plant species to elongate in the complete absence of O_2. Some situations in which tolerance of germination and/or seedling growth of rice to O_2 deficits is particularly important are (i) the cold areas of Northern Asia (e.g. Japan and Korea) where the seedling is submerged to buffer it from low air temperatures (Takahashi, 1978), (ii) the rice growing areas of Australia and the United States where seed is often sown directly into paddys and (iii) the river deltas of South-East Asia where deep water rices are cultivated. In this case stem elongation must keep pace with rising floodwaters.

Takahashi (1978) has demonstrated that lowland Japonica varieties from Northern Japan

form longer coleoptiles under water than those of Indica varieties which are not traditionally flooded at germination. Both germination and coleoptile growth, especially of Japonicas, are highly tolerant to O_2 concentrations lower than those in equilibrium with air ('hypoxia') and seedlings even develop in the absence of O_2 ('anoxia') (Taylor, 1942; Avadhani, Greenway, Lefroy, and Prior, 1978). A number of adaptive features may be responsible for the tolerance of germinating rice to submergence.

1. The 'Schnorkel Effect'

Conduction of O_2 through the coleoptile to the root and leaf meristems is thought to occur once the elongating coleoptile reaches the atmosphere (Kordan, 1974). The cylindrical cavity inside the coleoptile is an ideal pathway for O_2 diffusion as it is usually filled with gas when coleoptiles are grown in stagnant solution.

Coleoptile elongation is more rapid in stagnant solution than in air (Avadhani *et al.*, 1978), thereby reducing the time taken for the tip to intercept the atmosphere and the 'Schnorkel Effect' to operate. Rapid elongation is an adaptive feature which arises through:

(i) accumulation of volatile compounds such as CO_2 and ethylene which have growth-promoting effects (Ku, Suge, Rappaport, and Pratt, 1970). Konings and Jackson (1979) suggest that the stimulatory effect of ethylene on elongation of rice roots is due to low rates of synthesis and accumulation of endogenous ethylene. Ethylene accumulation would be most pronounced in stagnant solutions

and/or (ii) a strong inhibition of root and shoot growth, causing all substrates to be directed to the coleoptile.

2. Anaerobic metabolism

Available evidence indicates that alcoholic fermentation is the principal energy-generating pathway in rice coleoptiles subjected to anoxia (Taylor, 1942; Avadhani *et al.*, 1978; Bertani, Brambilla, and Menegus, 1980). These data suggest that the rate of glycolysis may increase in anoxia, a phenomenon referred to as the 'Pasteur Effect'. Metabolic adaptation of this type does not seem to occur in wheat (Taylor, 1942) or buckwheat (Effer and Ranson, 1967), both of which are less tolerant to waterlogging than rice. However, even if the rate of glycolysis increased 3-fold in anoxia there would be a 5–6 fold decrease in ATP regeneration. This in turn is likely to inhibit biosynthetic processes essential to growth.

3. Mitochondrial function after growth in anoxia

Vartepetian, Andreeva, and Kozlova (1976) claim that ultrastructure and function of rice mitochondria are more dependent on an adequate supply of organic substrate than O_2 supply. Similarly, Opik (1973) showed that mitochondria of coleoptiles grown in anoxia have virtually normal ultrastructure although the capacity for O_2 uptake and cytochrome oxidase activity are depressed. The maintenance of mitochondrial structure in anoxia is consistent with the rapid recovery in cytochrome oxidase activity (Opik, 1973) and respiration rate (our observations) after transfer from anoxia to air.

In this study, we have used rice cultivars which vary in tolerance to submergence in order to investigate the effects of O_2 deficits and turbulence of the solution on coleoptile elongation. Elongation has been studied in steady state conditions and after perturbations in O_2 supply. Turgor pressure has been measured in coleoptiles showing different elongation rates and at different stages of development. The role of turgor pressure in cell extension is discussed.

MATERIALS AND METHODS

Seventeen cultivars of rice (*Oryza sativa* L.) were used in the screening experiment: three were selected for more detailed studies (Calrose, KR 90, and KR 56). Seeds were de-hulled in all but the screening

experiment. All seed was less than one-year-old and stored at 5 °C with a desiccant. However, there was some variation in coleoptile elongation rates between experiments, probably due to the different age of seed used. This was particularly obvious in seedlings grown in stagnant solution.

De-hulled seeds were sterilized in 0·1% (w/v) acidified $HgCl_2$ for 3 min and sown into 250 ml of 0·5 mM $CaSO_4$ plus 0·8 mM KH_2PO_4 (adjusted to pH 5·5 with KOH) at the rate of 30 seeds per jar. All solutions and glassware were sterilized. Stagnant solutions (5·0 cm deep) were left undisturbed whereas in bubbled solutions water-saturated gas of known O_2 concentration was introduced through sintered bubblers at high flow rates (1·0 dm^3 min^{-1}). Air and 'Industrial Dry' nitrogen gas were mixed to obtain the desired O_2 concentrations. In anoxic treatments, N_2 gas was bubbled through alkaline pyrogallol to remove traces of O_2. O_2 concentrations were measured with a Beckman Fieldlab Oxygen Analyser. Germination and growth proceeded in the dark with all jars immersed in a water bath at 28 °C.

Coleoptile elongation was measured with a ruler. Ethanol-insoluble dry weights were measured after extracting tissue for 10 min in refluxing 80% ethanol, rinsing three times with cold ethanol and drying at 70 °C for 24 h.

Osmotic pressure (π) was measured in the sap of coleoptiles which had been frozen in liquid air and stored in sealed vessels at −30 °C. π was measured in Wescor psychrometers (Model C-52) using 0·04–0·4 mol kg^{-1} NaCl solutions as standards.

A 10 μl sample of sap was boiled for 10 min in 80% ethanol and then extracts were dried at 70 °C for K^+ and sugar assays. K^+ was measured by flame photometry. It was found that sucrose was completely inverted during extraction of the sap which would have resulted in spuriously high values of π. Therefore the following correction was made: coleoptiles not used for psychrometry were harvested and killed immediately in boiling 80% ethanol to de-activate invertase (cf. freezing first). Extracts were dried at 70 °C and sucrose, glucose and fructose estimated by the method of Bergmeyer (1965). The contribution of sugars to π_{sap} and π_{sap} measured by psychrometry were then reduced by the factor [mM sucrose inverted/400]MPa.

RESULTS

Screening for tolerance to submergence

The submergence tolerance of cultivars was evaluated by measuring coleoptile length of seedlings in stagnant solution as a percentage of coleoptile length in water-saturated air.

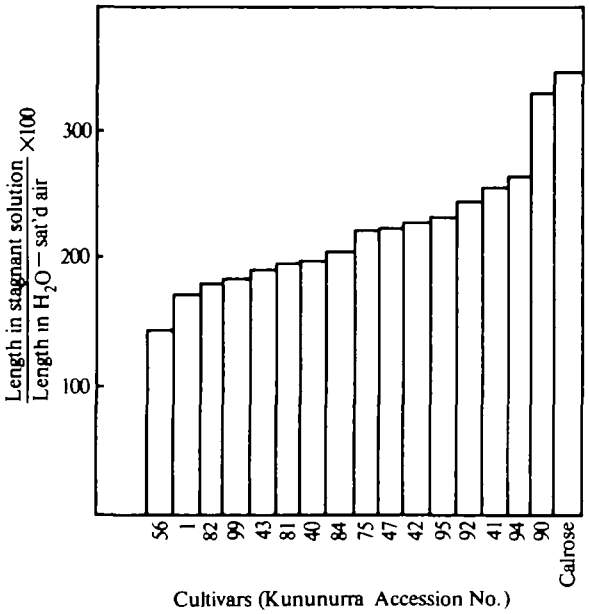

FIG. 1. Tolerance of 17 rice cultivars to submerged conditions. Tolerance is indicated by the ratio of coleoptile length in stagnant solution to that in air. Coleoptile length was measured 4 d after imbibition. (Based on 3 replicates; 10 seedlings per replicate.)

Measurement was made after 4 d. Stagnant solution simulated direct sowing into flooded paddys causing seedlings to be subjected to both low O_2 concentrations and accumulation of endogenous compounds. A water-saturated atmosphere simulated well-aerated soil.

The stimulation in coleoptile length after 4 d in stagnant solutions ranged from 40% to 230% (Fig. 1). Calrose and KR 90 (Inga), short and long-grained cultivars respectively, were chosen for tolerance to submergence. KR 56 (IR 22), a long-grained cultivar, was least tolerant to submergence even though coleoptiles were slightly longer in stagnant solution than in air. KR 56 is referred to as 'submergence-intolerant'. The large difference in response of these cultivars to submergence was reproducible in all subsequent experiments.

Elongation rates of coleoptiles in air compared to stagnant and aerated solutions

Elongation rates were measured at 5-h intervals on coleoptiles growing in water-saturated air, stagnant solution and aerated solution (Table 1). The O_2 concentration in stagnant solution declined over 24–48 h to approximately 0·125 mmol O_2 l^{-1} in the bulk stagnant solution: it was almost certainly lower near the surface of the coleoptile. Aerated solution contained 0·25 mmol O_2 l^{-1}.

Rates of elongation were identical in all cultivars growing in air whereas in stagnant solution they were stimulated 170% and 390% in the submergence-intolerant and submergence-tolerant cultivars respectively. These high rates were unique to stagnant conditions. The response of the cultivars was consistent with the screening experiment, where differences in coleoptile length could have resulted from effects on both germination and coleoptile elongation.

TABLE 1. *Rates of coleoptile elongation for Calrose, KR 90 and KR 56 in water-saturated air, aerated solution and stagnant solution*

Based on 2 replicates; 30 seedlings per replicate. l.s.d. = 0·1 mm h^{-1}.

Cultivars	Water–saturated air (20·4% O_2)	Aerated solution (0·25 mmol O_2 l^{-1})	Stagnant solution (0·125 mmol O_2 l^{-1})[a]
Calrose	0·20	0·59	0·98
KR 90	0·19	0·57	0·88
KR 56	0·19	0·40	0·51

[a] As measured in the bulk solution.

Elongation rates in aerated solution were intermediate between those of coleoptiles grown in air and in stagnant solution: the response of the two cultivars was similar to that found in stagnant solution but was less marked. Thus, there appears to be an inverse relation between O_2 concentration and the rate of elongation. However, endogenous compounds would have accumulated more in stagnant solution than in bubbled solution or air, especially if they were volatile. Therefore, differences in elongation rates may have been due to accumulation of endogenous compounds such as ethylene, O_2 concentrations or a combination of these factors. These possibilities were investigated using two experimental approaches. Firstly, elongation rates were measured in the presence of Ag^+, an antagonist of ethylene (Beyer, 1976). Secondly, elongation was measured in bubbled solutions at O_2 concentrations ranging from 0·0 to 0·25 mmol O_2 l^{-1}. This allowed O_2 supply to be varied with a constant rate of dispersal of outwardly diffusing endogenous compounds.

(i) *The effect of Ag^+ on rate of elongation.* Table 2 shows elongation rates in the presence and absence of 1·0 μM Ag^+: this concentration was adequate to prevent aerenchyma

formation in maize roots when they were treated with ethylene or grown in stagnant solutions (Drew, Jackson, Giffard, and Campbell, 1981).

In stagnant solution (0·075–0·125 mmol O_2 l^{-1}), elongation rates were approximately halved when coleoptiles were grown in the presence of Ag^+, whereas dry weight was reduced by only 34%. There were no severe toxic effects of Ag^+ because the dry weight of coleoptiles was reduced by only 11% in bubbled solutions containing 0·0875 mmol O_2 l^{-1}. Furthermore, the time taken to germinate was not affected by 1·0 μM Ag^+. In bubbled solution (containing 0·0875 mmol O_2 l^{-1}), Ag^+ reduced the elongation rates of the coleoptiles by only 19% (Table 2), compared to the 53% reduction observed in stagnant solution. It is also of interest that the rate of elongation in the presence of Ag^+ was the same in both stagnant and bubbled solutions (Table 2). This suggests that some ethylene accumulated even in bubbled solutions, causing a higher rate of coleoptile elongation than in water-saturated air.

TABLE 2. *Elongation rates and dry weights of rice coleoptiles grown in stagnant and rapidly bubbled hypoxic solutions in the presence and absence of 1·0 μM silver nitrate ($AgNO_3$). Hypoxic solutions contained 0·0875 mmol O_2 l^{-1} and stagnant solutions contained 0·075 to 0·125 mmol O_2 l^{-1} in the bulk solution. Elongation rates were constant during the entire period of coleoptile growth*

Based on 3–4 replicates; 30 seedlings per replicate

	Stagnant Solution (0·075–0·125 mmol O_2 l^{-1})		Hypoxic Solution (0·0875 mmol O_2 l^{-1})	
	−Ag	+Ag	−Ag	+Ag
Elongation rate (mm h^{-1})	0·66 ± 0·03[b]	0·31 ± 0	0·37 ± 0·04	0·30 ± 0·01
% Inhibition		53%		19%
Dry weight (μg/coleoptile)[a]	366 ± 4	242 ± 6	435 ± 26	388 ± 15
% Inhibition		34%		11%

[a] Harvested at 68 h and 72 h respectively.
[b] s.e. of the mean.

(ii) *The effect of a range of O_2 concentrations imposed by bubbling.* In these experiments imbibition, germination and seedling growth all took place at the same O_2 concentrations. In contrast to ethylene build-up, which seemed to specifically stimulate coleoptile elongation, O_2 deficits probably affected both germination and seedling growth. The actual definition of germination under these conditions was difficult due to the failure of the coleorhiza to emerge at low O_2 concentrations. Germination is therefore defined as coleoptile emergence.

Figures 2 (i) and (ii) show the elongation of coleoptiles of Calrose, KR 90 and KR 56 over time. Germination was delayed at very low O_2 concentrations, particularly in KR 56. Furthermore, KR 56 showed a distinct lag phase during coleoptile development: coleoptiles remained about 1·0 mm long for up to 8 h in hypoxia and failed to extend beyond 1·0 mm in anoxia. A lag phase was also observed when Calrose and KR 90 were subjected to anoxia. The lag phase was investigated more thoroughly in a subsequent set of experiments.

Elongation rates were approximately equal in all cultivars at all O_2 concentrations except anoxia, this contrasted with the inhibition of germination and early coleoptile growth at low O_2 supply. However, rates were invariably much lower in hypoxia (Fig. 2) than in stagnant solution (Table 1): this could *only* have arisen through turbulence due to bubbling. The concentration of a range of compounds including ethylene and CO_2 would have been lower in bubbled solutions.

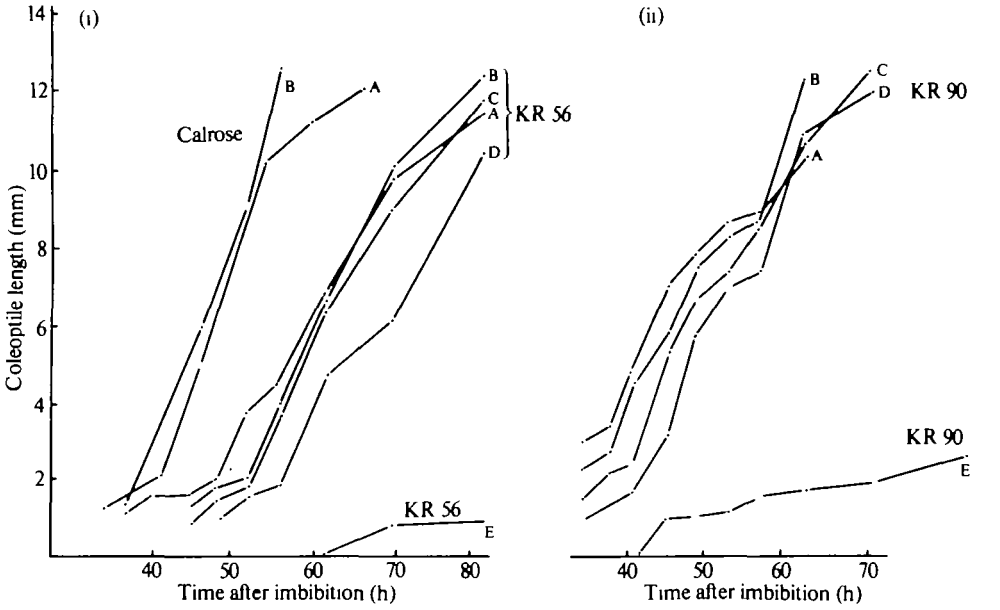

FIG. 2. Elongation of coleoptiles of (i) Calrose and KR 56 and (ii) KR 90 over time in a range of O_2 concentrations from aerobic to anoxic (0·25 (A), 0·125 (B), 0·08 (C), 0·03 (D) and 0·0 (E) mmol O_2 l^{-1}). (Based on 3 replicates; 30 seedlings per replicate.)

The effect of perturbations in oxygen supply on coleoptile elongation

The lag phase observed in steady state experiments indicates that coleoptile elongation may be most strongly inhibited by O_2 deficits during the first few millimetres of growth. Therefore, experiments were designed to test the effect of O_2 supply during coleoptile development.

The following treatments were imposed on Calrose and KR 56, the O_2 concentrations being in mmoles O_2 l^{-1}:

(i) Solutions were continuously bubbled with O_2 concentrations of zero, 0·0625 and 0·125 (Controls).

(ii) An initial treatment with an O_2 concentration of 0·0625 was followed by zero O_2 (transfer from hypoxia to anoxia).

(iii) An initial treatment with anoxia was followed by transfer to an O_2 concentration of 0·125 (transfer from anoxia to hypoxia).

Perturbations (ii) and (iii) were imposed either at germination (coleoptile emergence) or when the mean coleoptile length reached 5·0 mm (± 1·0 mm) after the lag phase.

(i) *Controls (continued exposure to known O_2 concentrations)—Figure 3 (i).* Coleoptiles grown continuously at 0·0625 and 0·125 mmol O_2 l^{-1} elongated linearly with time, Calrose elongation was slightly faster than KR 56. In anoxia, Calrose elongated slowly at first but the elongation rate increased 4·4 fold when the coleoptile reached 2·5 mm in length. In contrast, KR 56 elongated very slowly in continuous anoxia. This suggests that the most adverse effects of O_2 deficits were during or before the first 2–3 mm of coleoptile elongation.

(ii) *Transfer from hypoxia to anoxia—Figure 3 (ii).* Coleoptiles of both cultivars transferred from hypoxia to anoxia at the time of germination elongated at the same rate as controls exposed to anoxia since imbibition (Fig. 3 (i) cf. Fig. 3 (ii); curves A and B). That is, hypoxia prior to germination did not increase the rate of elongation in anoxia, even though germination occurred earlier than in the anoxic controls.

In contrast, sudden transfer from hypoxia to anoxia during the rapid phase of elongation

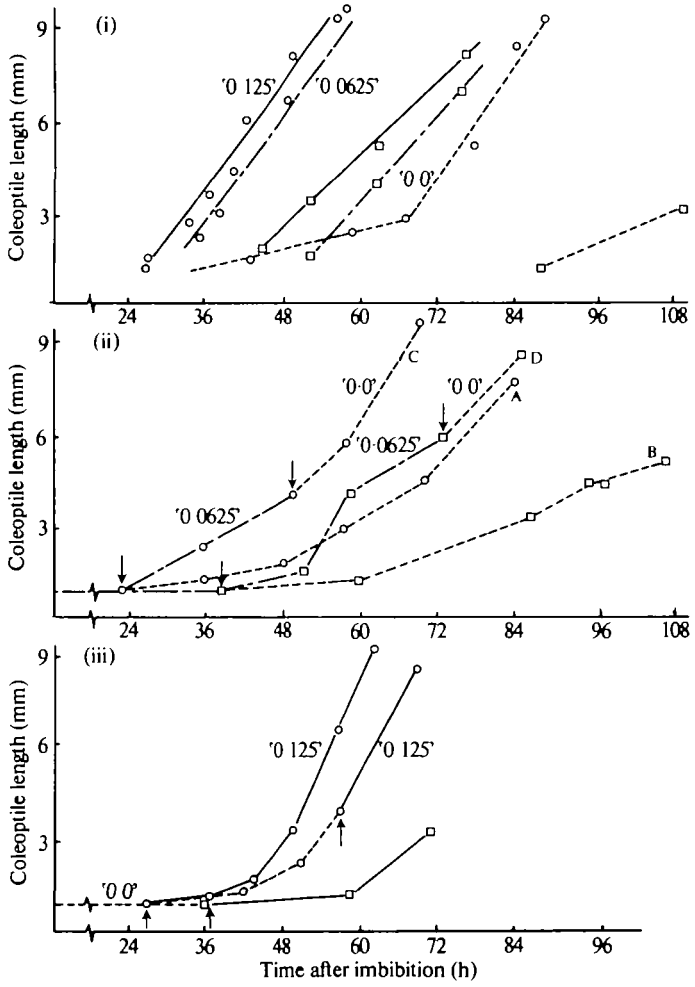

FIG. 3. Elongation of coleoptiles of Calrose and KR 56 in (i) 0·125, 0·0625 and 0·0 mmol O_2 l^{-1} continuously, (ii) 0·0625 followed by 0·0 mmol O_2 l^{-1} and (iii) 0·0 followed by 0·125 mmol O_2 l^{-1}. Switchovers were made either at germination or when the coleoptiles were approximately 5·0 mm long. These are denoted by arrows. (Based on 3–4 replicates; 30 seedlings per replicate). ○ Calrose, □ KR 56. —— denotes 0·125 mmol O_2 l^{-1}, – – – – – denotes 0·0625 mmol O_2 l^{-1} and – – – – – denotes 0·0 mmol O_2 l^{-1}. These O_2 concentrations are also indicated on the figures by '0·125', '0·0625' and '0·0'. The letters A–D in Fig. 3 (ii) refer to particular treatments discussed in the text.

did not reduce elongation rates (Fig. 3 (ii); curves C and D), even in KR 56 which grew very slowly in steady state anoxia. It would be interesting to know the elongation rate of KR 56 in steady state anoxia, had it extended beyond 3·0 mm. These results again support the view that the adverse effects of low O_2 supply were most pronounced during early stages of elongation.

(iii) *Transfer from anoxia to hypoxia—Figure 3 (iii).* Transfer of Calrose from anoxia to hypoxia at the time of germination resulted in an elongation rate of 0·09 mm h^{-1}, increasing to 0·40 mm h^{-1} by the time coleoptiles were 3·0 mm long (cf. 0·28 mm h^{-1} in hypoxic controls). This early acceleration in elongation was probably accompanied by O_2-dependent reactions which could not proceed during the anoxic period.

Calrose seedlings were also transferred from anoxia to hypoxia during the rapid phase of

elongation when most cell division presumably had already ceased. The increase in O_2 supply did not further increase elongation rates. These various approaches show conclusively that germination and the first stages of coleoptile growth are strongly inhibited by O_2 deficits but later phases of elongation are independent of O_2 supply.

The effect of hypoxia on coleoptile dry weight

After finding that elongation rates were similar in all cultivars in bubbled, hypoxic solution, the rate of increase in ethanol-insoluble dry weight was measured to see whether it too was independent of O_2 supply. Ethanol-insoluble dry weight consists mainly of polymers (proteins, cell walls, etc).

Table 3 shows that the weight per unit length increased with time in Calrose in all treatments except anoxia and decreased with time in KR 56, even in hypoxia. Furthermore, in the period over which these measurements were made, Calrose elongated to an average length of 12·7 mm and KR 56 to an average length of 7·8 mm. Had KR 56 elongated as much as Calrose, the effect would have probably been more marked.

TABLE 3. *Increase in length (Δ length), and ethanol-insoluble dry weight (Δ weight) of coleoptiles of Calrose and KR 56 when grown at 0·125, 0·078 and 0·0 mmol O_2 l^{-1}*

The change in ethanol-insoluble dry weight per unit length is also given. The mean length of coleoptiles at harvest 1 was 5·9 ± 1·0 mm and at harvest 2 was 10·8 ± 1·4 mm. Harvests were between 11 h and 21 h apart, depending on the elongation rate of the particular treatment. Based on 3–4 replicates; 30 seedlings per replicate.

Cultivar	O_2 Concentration (mmoles O_2 l^{-1})	Δ Length (mm)	Δ Weight (μg/coleoptile)	Weight/length at: Harvest 1 (μg mm^{-1})	Harvest 2
Calrose	0·125	7·9	102	8·7 ± 0·9[a]	11·3 ± 0·6
KR 56	0·125	3·8	26	9·0 ± 0·8	7·9 ± 0·5
Calrose	0·078	5·6	68	8·4 ± 0·6	9·9 ± 0·4
KR 56	0·078	3·1	7	7·9 ± 0·5	5·8 ± 0·5
Calrose	0·0	4·2	5	7·0 ± 1·0	5·0 ± 0·2

[a] s.e. of the mean.

The role of turgor pressure in coleoptile elongation

Cell extension is a function of turgor pressure (P) and cell wall properties (threshold turgor (P_{th}) and extensibility). In plant cells, these are related by the function:

$$\text{cell extension} = \text{extensibility} \times (P - P_{th}) \quad \text{(Hsaio, 1973)}$$

P can be estimated in plant cells by measuring osmotic pressure (π) at a known water potential (ψ) and using the formula:

$$P = \pi + \psi \quad (\psi \text{ negative}, \ \pi \text{ positive})$$

The P of submerged coleoptiles is approximately equal to π because the bathing solution has a ψ of only 0·006 MPa. It has been shown in the Appendix that this formula can be applied to both growing and non-growing tissues. In previous experiments, the free space of the coleoptiles was measured using ^{14}C-mannitol (Greenway, 1974) and found to be less than 10% of the total coleoptile volume.

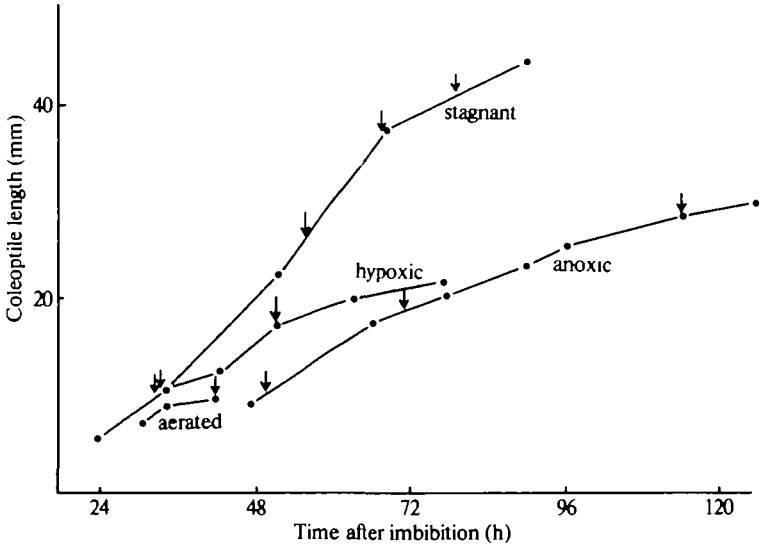

FIG. 4. Elongation of coleoptiles of Calrose over time in aerated (0·25 mmol O_2 l^{-1}), stagnant, hypoxic (0·125 mmol O_2 l^{-1}) and anoxic (0·0 mmol O_2 l^{-1}) solution. The sampling times for measurements on turgor pressure and potassium concentration shown in Table 4 are denoted by arrows. (Based on 3 replicates; 30 seedlings per replicate.)

TABLE 4 (i). *Coleoptile length, elongation rate and turgor pressure[a] of the expanding tissue or 'base' (0–5 mm) and expanded tissue or 'tip'[b] of Calrose coleoptiles in aerated, stagnant, hypoxic and anoxic solutions*

Figure 4 shows the elongation rates and sampling times for these treatments. Based on 3 replicates; 30–50 coleoptiles per replicate.

Treatment	Coleoptile length (mm)	Elongation rate (mm h^{-1})	π_{sap}[a] (MPa) 'base' (expanding)	'tip' (expanded)
Aerated	10	0·45	0·50 ± 0·14[c]	0·60 ± 0·10
Hypoxic	10	0·41	0·56 ± 0·10	0·69
Hypoxic	17	0·50	0·47 ± 0·05	0·42 ± 0·05
Stagnant	10	0·49	0·55 ± 0·02	0·57 ± 0·01
Stagnant	26	0·91	0·63 ± 0·03	0·57 ± 0·05
Stagnant	37	0·49	0·48 ± 0·03	0·48 ± 0·0
Stagnant	41	0·22	0·38 ± 0·05	0·44 ± 0·04
Anoxia	10	—	0·52 ± 0·0	0·61 ± 0·03
Anoxia	19	0·32	—	—
Anoxia	28	0·20	0·68 ± 0·03	0·42 ± 0·02

[a] *P* approximately equals π_{sap} (see Appendix).

[b] The tip was the 5–10 mm segment in 10 mm long coleoptiles and in all other cases it was taken as 10 mm to the tip of the coleoptile.

[c] s.e. of the mean.

The component of π due to K^+ and to sugars, and π_{sap} were measured at different O_2 concentrations and stages of elongation (Fig. 4 and Table 4) to determine whether: (i) *P* is as insensitive to low O_2 concentrations as elongation rates (after 5·0 mm of growth) in bubbled solutions. The effect of anoxia on *P* was of particular interest since reduced ATP yields could

TABLE 4 (ii). *Contribution of K^+ and sugars to π_{sap}*

These values represent the range in K^+ and sugar concentrations over all the sampling times given above. The contribution of K^+ includes the π of a divalent anion (e.g. malate) at half the K^+ concentration. 'Base' denotes expanding tissue and 'tip' denotes expanded tissue.

$\frac{\pi \text{ due to } K^+ + \text{sugars}}{\pi_{sap}}$ (%)		$\frac{\pi \text{ due to } K^+}{\pi_{sap}}$ (%)		$\frac{\pi \text{ due to sugars}}{\pi_{sap}}$ (%)	
Base	Tip	Base	Tip	Base	Tip
50–100%	70–100%	25–55%	45–60%	15–50%	10–50%

reduce solute transport, (ii) the rapid rates of elongation in coleoptiles in stagnant solution is reflected in high P or the result of changes in cell wall properties and (iii) decreased P was responsible for cessation of coleoptile growth.

Cell extension is confined to the basal part of the coleoptile according to our observations on cell size and the work of Wada (1961). Hence, π was measured in expanded (10 mm plus) and expanding (0–5 mm) sections of coleoptiles to give better estimates of P in these respective tissues. They are also referred to as 'tip' and 'base' respectively.

Table 4 (i) shows that P was remarkably constant in all treatments. For example, coleoptiles in anoxia had P values of greater than 0·5 MPa in the base. Coleoptiles in stagnant solution elongating at 0·91 mm h^{-1} did not have P values significantly greater than coleoptile tips which had ceased elongating. Thus cell wall properties must have been largely responsible for differences in elongation rates.

P declined from 0·63–0·38 MPa in the base of coleoptiles in stagnant solution as they elongated from 10–41 mm. Therefore a decline in P may have been partly responsible for cessation of elongation in stagnant solutions.

Table 4 (ii) shows that the sum of K^+ (+divalent anion) and sugars accounted for 50–100% of the π_{sap}. It has been assumed in calculation of π due to K^+ that the charge on K^+ is balanced by a divalent anion such as malate. The contribution of K^+ to π_{sap} was greater than that of sugars, especially in the tips where at least 45% of the π_{sap} was due to K^+. The contribution of K^+ to π_{sap} was smaller in growing regions where metabolites other than sugars must have substantially contributed to π_{sap}. The lowest contribution of sugars to π_{sap} (10–15% of π_{sap}) occurred in the later stages of elongation, possibly due to reduced sugar transport from the seed reserves. However, it has been shown that the supply of sugars does not limit growth or respiration at any O_2 concentration (unpublished data).

DISCUSSION

Tolerance of coleoptile growth to submergence in three rice cultivars tested was reflected in the following ways: (i) elongation was most rapid in stagnant solution, being 2-fold greater in the tolerant than in the intolerant cultivar. (ii) in anoxia, the elongation rate of the tolerant cultivar suddenly increased 4·4 fold when the coleoptiles were 2·5 mm long. This did not occur in the intolerant cultivar and (iii) the ratio of ethanol-insoluble dry weight to length in the tolerant cultivar increased in hypoxia but declined in the intolerant cultivar.

Elongation was much faster in stagnant solutions than in solutions flushed with gas of any O_2 concentration, including air. This implies that the rapid rates of elongation in stagnant solution were caused by accumulation of endogenous compounds around the coleoptile. The

fact that all cultivars elongated slowly and at the same rate in air saturated with water suggests that the growth-promoting compound is volatile and thus lost rapidly to the atmosphere. The most likely volatile compound involved is ethylene because Ag^+ an antagonist of ethylene strongly inhibited elongation in stagnant solution. There is substantial evidence in the literature to suggest that ethylene affects the growth of rice coleoptiles and roots. Rice coleoptiles synthesize ethylene in a range of O_2 concentrations and it markedly stimulates coleoptile elongation when supplied exogenously (Ku *et al.*, 1970). Similarly, Smith and Robertson (1971) found that the extension of rice roots was stimulated when they were exposed to less than 1·0 parts 10^{-6} ethylene. Konings and Jackson (1979) observed a 4·5 fold higher rate of ethylene synthesis in roots of white mustard than in rice: genetic variation in the rate of ethylene synthesis may also explain the differential response of rice cultivars to waterlogging.

CO_2 probably accumulated to higher concentrations in stagnant than in bubbled solutions. CO_2 would probably act as an ethylene antagonist, and is known to inhibit the export of seed reserves and coleoptile growth in oat seedlings (Mer, 1957). However, any effect of CO_2 was clearly insufficient to mask the stimulation in elongation thought to be due to ethylene. Removal of CO_2 from air did not affect coleoptile elongation in aerated solutions (unpublished data).

Observations (ii) and (iii) reflect variation in tolerance to low O_2 concentrations, as opposed to the stimulatory effects of endogenous compounds. Nevertheless, the tolerant cultivar is both most tolerant to low O_2 concentrations and most responsive to stagnant conditions. Tolerance to low O_2 concentrations is probably related to the efficiency of ATP regeneration rather than the supply of substrates for growth and respiration. Unpublished data show that the *in vivo* rate of ethanol synthesis in tolerant cultivars subjected to anoxia is at least 50% greater than in the intolerant cultivar (expressed on an ethanol-insoluble dry weight basis) supporting the view that metabolic efficiency varies between cultivars. Exogenous substrate did not influence the rate of ethanol synthesis in the intolerant cultivar.

Oxygen requirements during germination and coleoptile growth

Germination

The period from imbibition to germination was prolonged at low O_2 concentrations, especially in anoxia. However, the effect was most severe in the intolerant cultivar where germination at low O_2 concentrations was delayed at least 15 h. This cultivar germinated relatively slowly even in air. The rate of diffusion of O_2 to the embryo would have been similar in all three cultivars due to the similar seed sizes and morphology. Therefore, it is thought that slow germination at low O_2 levels is due to low rates of ATP regeneration resulting in inhibition of biosynthetic reactions. It appears that alcoholic fermentation does not provide sufficient ATP for rapid germination.

Early coleoptile growth

The first 2–5 mm of coleoptile growth was shown to be most intolerant to O_2 deficits, regardless of the O_2 supply prior to germination. Wada (1961) showed that cell division in the coleoptile ceases about 60 h after sowing. In his experiments, this occurred at 9·0 mm (35% of the final length) and 18 mm (25% of the final length) in air and stagnant solution, respectively. Considering the coleoptiles in our experiments reached half the final length of Wada's, cell division probably ceased when coleoptiles were considerably shorter than 9·0 mm and 18 mm. Light microscopy suggests that cell division is complete in 10 mm coleoptiles (unpublished data). Chu and Tang (1962) claimed that cell division is complete once the coleoptile is 0·87 mm long. These observations suggest that the period of greatest intolerance

to O_2 deficits in our experiments is concurrent with that of cell division and subsequent coleoptile elongation is due largely to cell extension.

Cell division requires DNA, RNA and protein synthesis, all relatively high-energy processes (Penning de Vries, Brunsting, and van Laar, 1974). In view of the low energy yield from anaerobic metabolism, it is possible that synthesis of these compounds may be inhibited in O_2 deficits. Amoore (1961) showed that anoxia severely inhibited mitosis in pea root tips, especially pre-prophase. Anoxia may have similar effects on mitosis in rice coleoptiles.

The possibility remains that there is an endogenous inhibitor of coleoptile elongation whose breakdown is O_2 dependent. If the concentration of this compound were higher in the intolerant than the tolerant cultivar, the effect of O_2 deficits during early stages of coleoptile elongation could be explained. However, de-hulling of seeds did not affect the response to O_2 deficits showing that no such inhibitor was located in the seed coats.

Later coleoptile growth

Ranson and Parija (1955) found that rice coleoptiles elongated most rapidly at intermediate O_2 concentrations in a gaseous environment. However, the response of these cultivars in bubbled solution was not tested. The data presented here showed that elongation rate was relatively insensitive to O_2 supply after the coleoptile reached 5·0 mm, even in the submergence-intolerant cultivar. The insensitivity is probably associated with a shift from cell division to cell elongation: there would be a consequential shift from protein synthesis to cell wall synthesis. The energy required for synthesis of cell walls is about one-third of that for protein synthesis on a dry weight basis (Penning de Vries *et al.*, 1974). This assumes that glucose and amino acids are supplied from the seed reserves. Hence, a shift from protein to cell wall synthesis would reduce the energy demand and delay cessation of growth.

Furthermore, energy for turgor generation appears to be available at all times, even in anoxia when the ethanol-insoluble dry weight per unit length was falling. Energy charge[1] is 0·1 to 0·2 units lower in coleoptiles adapted to anoxia than in air (Pradet and Prat, 1976). Atkinson (1968) suggested that energy charge controls the regeneration and expenditure of ATP such that a reduction in energy charge would stimulate pathways producing ATP and inhibit pathways utilizing ATP. The response of biosynthetic and amphibolic sequences to fluctuations in energy charge would be influenced by the concentration of feedback modifiers. Therefore, we propose that this 0·1 to 0·2 unit reduction in energy charge has inhibited a range of ATP-utilizing processes in rice coleoptiles, but to differing extents. Atkinson's theory for control of regulatory enzymes has been applied to growth of coleoptiles at low O_2 concentrations: it is proposed that cell division is very sensitive to lowered energy charge, polymer synthesis less so and solute transport relatively insensitive to low energy charge. Such a response would favour coleoptile elongation in submerged hypoxic conditions, often resulting in an improved O_2 supply and seedling survival.

Zarra and Masuda (1979) concluded that coleoptiles finally cease to elongate under stagnant conditions due to reduced turgor pressure. As the elongation rate declined from 0·60 mm h^{-1} to 0·11 mm h^{-1}, turgor pressure fell from 0·60 MPa to 0·0 MPa whereas we observed a decline in turgor from 0·63 MPa to 0·38 MPa accompanied by a fall in elongation rates from 0·91 mm h^{-1} to 0·22 mm h^{-1}. The response of coleoptile elongation in our experiments to relatively small changes in turgor indicates that either threshold turgor is high

[1] $\text{Energy charge} = \dfrac{[\text{ATP}] + 0{\cdot}5\,[\text{ADP}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}]}$.

(and extensibility is also high to permit rapid elongation) or threshold turgor is low and a decline in extensibility accounts for the fall in elongation rate.

Although further experiments are required to separate these possibilities, some evidence exists to support the case for both high threshold turgor and extensibility. Zarra and Masuda (1979) showed that the minimum stress-relaxation time (T_0) of coleoptiles in stagnant solution was constant throughout the coleoptile development whereas that of coleoptiles in bubbled solution or air rose. This suggests that the cell walls of coleoptiles in stagnant solution remain 'loosened' throughout development whereas those of other treatments became more rigid.

Thus, the rapid elongation rates under stagnant conditions may be due to both high extensibility and high threshold turgor, suggesting that cell wall properties are important determinants of cell extension under stagnant conditions. The accumulation of endogenous compounds may influence cell wall properties of the growing zone under stagnant conditions. For example, Grierson (1981) has shown that ethylene induces both softening of tomato fruit cell walls and synthesis of polygalacturonase, which is associated with softening during ripening. Similar studies on cell wall properties in rice coleoptiles would require clear separation of growing and non-growing tissue.

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APPENDIX

Measurement of Turgor Pressure in Rapidly Growing Rice Coleoptiles

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The use of a single value of ψ for the tissue implies that the tissue is very near hydraulic equilibrium. In rapidly growing tissue, however, the flux of water into expanding cells comes from the xylem along a pathway of finite hydraulic resistance and thus ψ will vary along this pathway. The following argument (Wolfe, pers. comm.) indicates that this variation is probably very small compared with typical turgor pressures.

Melkonian, Wolfe, and Steponkus, (1982) observe that in a simple hydraulic model of a leaf in which the resistance, R, of the pathway from each cell to the large xylem vessels is the same, a step change in ψ in the tissue causes an exponentially decreasing flow in the xylem with a time constant τ which is given by:

$$\tau = \frac{R\,V_c}{\varepsilon} \tag{1}$$

where V_c is the average cell volume. Further they observe that this model, however simple, provides a good fit to the measured hydraulic response of a wheat leaf. Since this notional resistance is by definition the ratio of the pressure drop across the xylem–protoplasm pathway (ΔP) to the flow along that pathway, we may write for a swelling cell:

$$\Delta P = R\frac{\mathrm{d}V_c}{\mathrm{d}t}$$

and using (1)

$$\Delta P = \varepsilon\tau \frac{1}{V_c} \frac{dV_c}{dt} \tag{2}$$

Melkonian *et al.* (1982) obtain $\tau \sim 20$ s and $\varepsilon \sim 20$ MPa for well-watered wheat leaves. We expect that for the small, easily extended, rapidly growing regions of rice coleoptile, the values would be lower than these. The maximum expansion rate we have observed in whole rice coleoptiles is 2×10^{-5} s^{-1}. Though some cells may be expanding faster than this average rate, we do not believe that $[(1/V_c)/(dV_c/dt)]$ is anywhere greater than 10^{-4} s^{-1}, the value reported for rapidly growing maize root by Erickson and Sax (1956). Thus equation (2) gives a crude upper estimate for ΔP of 40 kPa, much less than the turgor pressures considered here.