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Communication

Growth and Mitochondrial Respiration of Mungbeans (*Phaseolus aureus* Roxb.) Germinated at Low Pressure[1]

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ABSTRACT

Mungbean (*Phaseolus aureus* Roxb.) seedlings were grown hypobarically to assess the effects of low pressure (21–24 kilopascals) on growth and mitochondrial respiration. Control seedlings grown at ambient pressure (101 kilopascals) were provided amounts of O_2 equivalent to those provided experimental seedlings at reduced pressure to factor out responses to O_2 concentration and to total pressure. Respiration was assayed using washed mitochondria, and was found to respond only to O_2 concentration. Regardless of total pressure, seedlings grown at 2 millimoles O_2 per liter had higher state 3 respiration rates and decreased percentages of alternative respiration compared to ambient (8.4 millimoles O_2 per liter) controls. In contrast, seedling growth responded to total pressure but not to O_2 concentration. Seedlings were significantly larger when grown under low pressure. While low O_2 (2 millimoles O_2 per liter) diminished growth at ambient pressure, growth at low pressure in the same oxygen concentration was enhanced. Respiratory development and growth of mungbean seedlings under low pressure is unimpaired whether oxygen or air is used as the chamber gas, and further, low pressure can improve growth under conditions of poor aeration.

Very few attempts to grow plants in reduced pressures have been made (1, 3, 7, 20), and yet this is an important consideration for space biologists in view of the engineering advantages associated with a reduced pressure differential between the growth chamber and space. It has been suggested that the poor growth and mitochondrial aberrations previously observed in plants grown in space (9, 17) might relate to aeration problems associated with microgravity, and that a low pressure growth area for plants might improve gas exchange between plant and atmosphere (13). The theoretical and experimental studies of gas exchange in plants by Gale (5, 6) provided evidence that the increase in diffusion coefficient with decreasing pressure compensates for the decreased partial pressure of carbon dioxide at low absolute pressures. Thus, low pressure growth of plants might serve to counteract effects of microgravity on gas exchange by increasing the diffusivity of metabolic gases and contaminants.

Janert (7) successfully grew oat seedlings and plants at 169 mm Hg in air and found no ill effects of low pressure on plant growth and development. Costes and Vartapetian (3) germinated rice *in vacuo* for 6 d and isolated functionally active mitochondria from the coleoptiles in a demonstration that early growth in this species can occur in the absence of oxygen. However, a study by Andre and Richaud (1) using rye grass and barley in a sealed low pressure chamber suggested that during germination and early growth, seedling respiration might be limited by low oxygen partial pressure even though respiration and photosynthesis proceeded at normal rates once the plants became autotrophic.

In the present study, seedling growth and mitochondrial respiration were determined following a growth period in a flow through low pressure chamber using either oxygen or air as the flow through gas. The goal was to determine whether low pressure had any effect on growth and respiration of mungbean seedlings, and further, whether oxygen availability was a limiting factor at these low pressures.

MATERIALS AND METHODS

Low Pressure and Ambient Pressure Germination Chambers. A bench level hypo/hyperbaric chamber (40 × 15 cm i.d.; Bethlehem Corporation) was used in the flow through low pressure system diagrammed in Figure 1. A direct-drive rotary vane vacuum pump (Edwards model EDM 2) provided continuous evacuation of the chamber. Either air or 100% O_2 was humidified by bubbling through glass-distilled, deionized water and continuously vented into the chamber at a rate measured by a rotameter and adjusted by means of a metering valve. A vacuum regulator (Matheson Co., model 3491) positioned between the vacuum pump and the chamber admitted and controlled a second continuous flow of ambient air to the pump as required to maintain the vented chamber at the desired pressure. Chamber pressure was monitored using a precision standard Hg barometer (Wallace & Tiernan, model FA-135) and was controlled to within ±5 mm-Hg (0.67 kPa) of the desired pressure throughout the course of each 3 d experiment. Since the vacuum regulator controlled pressure differentially, most of this variation reflected changes in the ambient barometric pressure during the course of the experiment.

A Plexiglas chamber with dimensions and port geometry comparable to the low pressure chamber served as an ambient pressure control. An air purge was forced through the chamber by a pump (GE 1/20 hp), and was humidified and regulated as in the low pressure set up.

As shown in Table I, comparisons were made between seedlings grown with equal amounts of oxygen at different pressures. In the first type of experiment (experiment A), pure oxygen was bled through the hypobaric chamber maintained at 159 mm Hg

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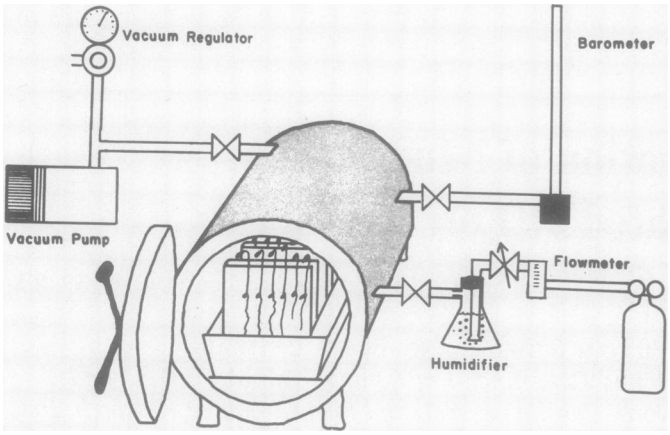

FIG. 1. A flow through hypobaric chamber system used for germinating mungbean seedlings.

Table I. *Experimental Design for Evaluating the Effect of Oxygen Concentration and Pressure on Seedling Growth and Respiration*

Gas	Pressure	Oxygen	
	kPa	*mol %*	*mmol/L*
Experiment A			
Air	101	21	8.4
Oxygen	21	100	8.4
Experiment B			
Air	101	21	8.4
Air	24	21	2.0
Experiment C			
Air	101	21	8.4
Air + N_2	101	5	2.0

(21.2 kPa), so that these seedlings and those in the control chamber (ambient pressure, air purge) received equal amounts of oxygen. In the second type of experiment (experiment B), air was bled through both chambers, and by maintaining the hypobaric chamber at 182 mm Hg (24.3 kPa) O_2 availability was equivalent to that in a 5% O_2 ambient pressure atmosphere. In the third type of experiment (experiment C), 5% O_2 (achieved by diluting air with N_2) or air flowed through the chambers at ambient pressure.

In the hypobaric experiments, flows entered the low pressure chamber at 0.2 L/min ± 10% (measured at ambient pressure). Volume flow rates equivalent to those inside the low pressure chamber at 159 or 182 mm Hg (0.95 or 0.84 L/min, respectively) were provided through the ambient pressure system. Allowing for the accuracy limits of our flowmeters and discrepancies arising from using oxygen in a flowmeter designed for air, we estimate that volume flow rates through the ambient and low pressure chambers were within 10% of being equal. For ambient pressure experiments (experiment C), air or air diluted with nitrogen to achieve 5% oxygen flowed through the chambers at 0.9 L/min.

Germination Sequence. Mungbean seeds (*Phaseolus aureus* Roxb. cv Berken, Tom Munger Seed Co., Enid, OK) were prepared for germination in 40 g (about 700 seeds) aliquots as follows. The seeds were soaked 16 h in 750 ml aerated deionized water. The imbibed seeds were sandwiched between germination papers (Anchor Paper Co., St. Paul, MN), and the packet folded three times and secured with a rubber band. Eight packets were placed on end in two shallow trays of water and arranged parallel with the long dimension of the chamber in pairs to facilitate gas flow. Water was supplied to the seedlings by the capillary action of the germination paper throughout the three day period. Light openings in the chambers were covered so that the seedlings were etiolated. Temperature was maintained at 20°C (±2°C). After the 3 d period, the low pressure chamber was slowly returned to ambient pressure (5–10 min recompression time) and the seedling growth was characterized by weighing and measuring seedlings from each packet. Statistical differences between the populations of seedlings were assessed using one-way ANOVA and Fisher's test of significance. Experiments were repeated at least three times with similar results.

Mitochondrial Preparation. Washed mitochondria were prepared following the methods of Siedow and Bickett (15) using 40 to 50 g of starting material. Respiration was assayed in 100 μl aliquots in 1.8 ml Reaction Buffer (0.3 M mannitol, 10 mM K_2HPO_4, 10 mM KCl, 5 mM $MgCl_2$, pH 7.2). Cyanide-resistant respiration was determined as a percentage of the state 3 rate (plus 150 μM ATP, 10 mM succinate, 150 μM ADP) which was inhibited by 1 mM SHAM in the presence of 0.25 mM KCN.

RESULTS

The first type of experiment was designed to determine the effects of low pressure alone on seedling growth and respiration. Seedlings from a chamber at ambient pressure with air as the flow through gas were compared with those from the hypobaric chamber in which 100% O_2 had been the flow through gas. This treatment provided the seedlings with 8.4 mmol O_2/L, regardless of the total chamber pressure (Table I). The results are summarized in Table II. Respiration, assayed as state 3 rates of washed mitochondria, was unaffected by the low pressure treatment and percentage of cyanide-resistant respiration was not affected. Seedling growth was somewhat stimulated in the low pressure chamber, with significantly greater fresh weight and length.

When seedlings grown at low pressure with air as the flow through gas were compared with ambient pressure controls, a stimulation of state 3 respiration rate and a decrease in the percentage of alternative pathway were observed (Table III). Again, seedling growth was stimulated by the low pressure treatment with significantly greater fresh weight (at the 0.05 level) and lengths.

The changes in respiratory characteristics observed at low pressure with air as the flow through gas were quite similar to those occurring at ambient pressure when 5% O_2 was the flow through gas (Table IV). Five percent O_2 at ambient pressure is equivalent to 2.0 mmol O_2/L, the amount available when air

Table II. *Growth and Mitochondrial Respiration of Mungbean Seedlings following a 3 d Period at 21 kPa with 100% O_2 as the Flow through Gas*

Mean values (and their standard errors in parentheses) for one representative experiment. Underlined values are significantly larger between treatments at the 0.01 level. Stimulation by the low pressure treatment is expressed as a percentage of the ambient value (LP/A).

	Ambient	Low Pressure	LP/A
			%
Mitochondrial respiration			
State 3 rate (nmol O_2/mg·min)	127 (4)	133 (7)	105
Alternative pathway (% State 3)	24.5 (0.9)	24.1 (0.9)	98
Respiratory control	2.3	2.5	109
Seedling growth			
Fresh weight/seedling (mg)	252 (10)	282* (10)	112
Dry weight/seedling (mg)	40 (2)	42 (2)	105
Hypocotyl length (cm)	2.7 (0.1)	3.1 (0.1)	117
Root length (cm)	4.6 (0.1)	6.1 (0.2)	132
Total length (cm)	7.3 (0.2)	9.2 (0.2)	122

* Significantly larger at the 0.05 level.

Table III. *Growth and Mitochondrial Respiration of Mungbean Seedlings following a 3 d Period at 24 kPa with Air as the Flow through Gas*

Mean values (and their standard errors in parentheses) for one representative experiment. Underlined values are significantly larger at the 0.01 level. Stimulation by the low pressure treatment is expressed as a percentage of the ambient control (LP/A).

	Ambient	Low Pressure	LP/A
			%
Mitochondrial respiration			
State 3 rate (nmol O_2/mg·min)	187 (7)	260 (7)	140
Alternative pathway (% State 3)	28.3 (0.9)	18.3 (0.9)	65
Respiratory control	2.5	2.5	100
Seedling growth			
Fresh weight/seedling (mg)	250 (10)	300 (10)	120
Dry weight/seedling (mg)	43 (2)	47 (2)	111
Hypocotyl length (cm)	2.0 (0.1)	2.6 (0.1)	130
Root length (cm)	3.9 (0.2)	5.5 (0.1)	141
Total length (cm)	5.9 (0.2)	8.1 (0.2)	137

Table IV. *Growth and Mitochondrial Respiration of Mungbean Seedlings following a 3 d Period at Ambient Pressure (101 kPa) with Air or Air + Nitrogen to Achieve 5% Oxygen as the Flow through Gas*

Mean values (and their standard errors in parentheses) for one representative experiment. Underlined values are significantly larger at the 0.01 level. Low oxygen effects are expressed as a percentage of the ambient values (LO/A).

	Air	Low Oxygen	LO/A
			%
Mitochondrial respiration			
State 3 rate (nmol O_2/mg·min)	84 (2)	100 (2)	119
Alternative pathway (% State 3)	33.6 (2)	14.1 (2)	42
Respiratory control	2.2	2.2	100
Seedling growth			
Fresh weight/seedling (mg)	276* (9)	246 (9)	89
Dry weight/seedling (mg)	43 (2)	44 (2)	102
Hypocotyl length (cm)	3.4 (0.1)	2.2 (0.1)	65
Root length (cm)	6.1 (0.2)	4.6 (0.2)	75
Total length (cm)	9.5 (0.3)	6.8 (0.3)	72

* Significantly larger at the 0.05 level.

was flowed through the low pressure chamber in experiment B (Table I). Under these conditions, as compared with ambient control, the 5% O_2 seedlings again showed a decrease in cyanide-resistant respiration and an increase in state 3 respiration rate. Growth, however, was somewhat diminished by the 5% O_2, ambient pressure treatment. No difference was seen between dry weights of the two treatments, although the fresh weight of the low oxygen seedlings was somewhat less than control.

DISCUSSION

In the present work, both low pressure and ambient pressure control chambers were continuously vented to maintain constant atmospheric composition and provide a convective gas flow over the seedlings. In all cases comparing growth of seedlings at ambient and low pressure (one-fifth atmospheric), seedlings developed normally at low pressure and in fact were somewhat larger than ambient pressure controls. These results contrast with those reported by Andre and Richaud (1), who observed poor growth of wheat and rye grass seedlings early on in low pressure treatments (one-fourth atmospheric and below) and suggested that during germination, seedling respiration might have been depressed by lower oxygen availability. Subatmospheric oxygen levels have been reported to either inhibit or enhance radicle growth after emergence depending on species (16). Our experiments were designed to separate the effects of total pressure and oxygen availability on growth and respiration of mungbean seedlings.

The present finding that respiration responds to oxygen concentration and not to total pressure is in line with earlier reports. Bridgen and Staby (2) used low pressure and low oxygen storage to preserve tissue cultures and found that the rate and amount of growth decreased when the partial pressure of oxygen was below 50 mm Hg regardless of total pressure. Unger and Danielson (18) determined that 200 mm Hg was the optimum oxygen pressure for corn germination and growth with a linear decrease in radicle length as O_2 pressure (and total pressure) was dropped to 0 mm Hg.

In the present experiments, respiration responded to oxygen concentration in two ways: low oxygen (2 mmol O_2/L) evoked both a decrease in the proportion of alternative (cyanide-resistant) respiration and an increase in the total respiration rate. The relative proportion of the cyanide-resistant pathway to total respiration is of interest because this value has been observed to increase in seedlings subjected to environmental stress during germination (10, 11). The sensitivity of cyanide-resistant respiration to oxygen concentration was demonstrated by McCaig and Hill (12) who found less alternative pathway activity in mitochondria from wheat seedlings germinated at 21% O_2 than at 100% O_2. Similarly, present results show less alternative pathway activity at 5% oxygen (or equivalent at low pressure) than at 21% oxygen (or equivalent at low pressure). The higher state 3 rate observed in mitochondria from seedlings grown at the lower oxygen concentration (2 mmol O_2/L) may be a consequence of assaying the mitochondria under more highly oxygenated conditions than those in which they developed. Vartapetian *et al.* (19) have reported this phenomenon for mitochondria from rice coleoptiles grown anaerobically.

The mechanism for increased seedling growth at low pressure is unknown. External pressure effects on turgor might account for the 1 to 1.5% increase in water content ([fresh weight-dry weight]/fresh weight) of seedlings grown under low pressure. This very small increase in fresh weight attributable to turgor effects is in line with predictions based on other tissues (14). Another possible contributor to the improved growth is the enhanced dissipation of contaminants such as ethylene at low pressure which, along with the reduced concentration of oxygen, delays ripening and senescence during hypobaric storage of fruits and vegetables (8). The present results suggest that some aspect of gas exchange during germination and early growth is indeed improved at low pressure since at a given O_2 concentration, seedling growth at low pressure was consistently enhanced over corresponding ambient pressure controls, even though convective gas flows were equal.

The necessity of providing a gas flow sufficient to keep pace with the metabolism of germinating mungbean seeds in the Space Shuttle Plant Growth Unit (PGU), where quantities of gas carried on for an experiment are limited, has been demonstrated (4). Both carbon dioxide and ethylene can build up to undesirable levels in the PGU unless flow rates at least five times those currently used in flight are maintained. The present data suggest two advantages which would accrue by using low pressure as a solution to this problem. First, a given volume of air could be circulated through the growth chamber at one-fifth atmospheric pressure, providing five times the purge per volume carried on. Second, the low pressure treatment itself apparently improves gas exchange since at a given oxygen concentration, seedling growth at low pressure was consistently enhanced compared with ambient pressure controls. Low pressure technology should be

considered as a viable option in space biology applications, where availability of gas is a limiting factor, and a low pressure growth area would afford many engineering advantages.

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LITERATURE CITED

1. ANDRE M, C RICHAUD 1986 Can plants grow in quasi-vacuum? *In* RD MacElroy, NV Martello, DT Smernoff, eds, CELSS '85 Workshop (NASA Pub. TM 88215), pp 395–404
2. BRIDGEN MP, GL STABY 1981 Low pressure and low oxygen storage of *Nicotiana tabacum* and *Chrysanthemum* X *morifolium* tissue cultures. Plant Sci Lett 22: 177–186
3. COSTES C, BB VARTAPETIAN 1978 Plant growth in a vacuum: the ultrastructure and functions of mitochondria. Plant Sci Lett 11: 115–119
4. CUELLAR MD, CA MITCHELL 1986 Effects of static vs. flowing atmosphere on plant growth in the space shuttle Plant Growth Unit. Proceedings of the American Society for Gravitational and Space Biology, Charlottesville, VA, p 16
5. GALE J 1972 Availability of carbon dioxide for photosynthesis at high altitudes: theoretical considerations. Ecology 53: 494–497
6. GALE J 1973 Experimental evidence for the effect of barometric pressure on photosynthesis. *In* Plant Response to Climatic Factors. Proceedings Uppsala Symposium (UNESCO) 1970. (Ecology and Conservation, 5), pp 289–294
7. JANERT H 1922 Beitrag zur Beurteilung der klimatischen Wachstumfaktoren Kohlensaure, Sauerstoff and Luftdruck. Bot Archiv 1: 155–176, 201–210
8. KADER AA 1986 Biochemical and physiological basis for effects of controlled and modified atmospheres on fruits and vegetables. Food Technol 40: 99–104
9. KRIKORIAN AD, SA O'CONNOR 1984 Karyological observations. Ann Bot 54: Suppl 3, 49–63
10. LEOPOLD AC, ME MUSGRAVE 1979 Respiratory changes with chilling injury of soybeans. Plant Physiol 64: 702–705
11. LEOPOLD AC, ME MUSGRAVE 1980 Respiratory pathways in aged soybean seeds. Physiol Plant 49: 49–54
12. MCCAIG TN, RD HILL 1977 Cyanide-insensitive respiration in wheat: cultivar differences and effects of temperature, carbon dioxide, and oxygen. Can J Bot 55: 549–555
13. MUSGRAVE ME, HW SCHELD, BR STRAIN 1986 Physiological response of crop plants to non-earthnormal metabolic gas ratios and pressures. Proceedings of the American Society for Gravitational and Space Biology, Charlottesville, VA p 16
14. NOBEL PS 1983 Biophysical Plant Physiology and Ecology. WH Freeman and Co, New York
15. SIEDOW JN, DM BICKETT 1983 Binding of butyl gallate to isolated mungbean mitochondria. Relation to inhibition of the alternative pathway. Plant Physiol 72: 339–344
16. SIEGEL SM, LA ROSEN 1962 Effects of reduced oxygen tension on germination and seedling growth. Physiol Plant 15: 437–444
17. SLOCUM RD, JJ GAYNOR, AW GALSTON 1984 Cytological and ultrastructural studies on root tissues. Ann Bot 54: Suppl 3, 65–76
18. UNGER PW, RE DANIELSON 1965 Influence of oxygen and carbon dioxide on germination and seedling development of corn (*Zea mays* L.). Agron J 57: 56–58
19. VARTAPETIAN BB, IN ANDREEVA, N NUTRIDINOV 1978 Plant cells under oxygen stress. *In* DD Hook, RMM Crawford, eds, Plant Life in Anaerobic Environments. Ann Arbor Science Publishers, pp 13–88
20. WALKINSHAW C 1971 Lunar horticulture. HortScience 6: 518