

## MITOTIC ACTIVITY IN RICE SEEDLINGS GERMINATING UNDER OXYGEN DEFICIENCY

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### SUMMARY

Rice seedlings germinating in oxygen-deficient environments do not manifest visual evidence of seminal or adventitious root growth or normal leaf growth, the coleoptile being the only seedling structure to emerge under these environmental conditions. Microscopic evidence, however, shows that mitotic activity occurs in the different tissues comprising the shoots of the seedlings germinating under oxygen deficiency. Thus evidence is presented here which shows that mitotic activity in rice seedlings germinating in oxygen-deficient environments is not confined solely to the emerging coleoptiles of the germinating seedlings.

### INTRODUCTION

Taylor (1942*a, b*) and Vlamis & Davis (1943) have shown that rice seeds are capable of germinating under low oxygen tensions and in oxygen-free environments but did not investigate mitotic activity in the rice seedlings germinated under these environmental conditions. Investigations concerning the occurrence of mitosis in rice seedlings germinating in low and normal oxygen environments as well as under anaerobiosis appear to have been concerned primarily with coleoptilar growth (Wada, 1961; Opik, 1973). Evidence is presented in this investigation which shows that mitotic activity occurs in the different tissues comprising the shoots of rice seedlings germinating in oxygen-deficient environments.

### MATERIALS AND METHODS

Unhulled, unwashed, and unsterilized rice seeds (*Oryza sativa*, varieties Calrose or Hokkaido-Carny) were germinated in hydrogen (H<sub>2</sub>) and nitrogen (N<sub>2</sub>) environments in the dark at 27 °C as described previously (Kordan, 1976*a*). Quiescent embryos as well as excised shoots (5-18 mm in length) of 6- to 7-day-old germinating seedlings were fixed, sectioned, and stained with safranin and fast-green FCF as in the previous investigation (Kordan, 1976*a*).

### RESULTS

More than 90% of the seedlings in the H<sub>2</sub> as well as in the N<sub>2</sub> environments manifested shoot emergence only, there being no visual evidence of seminal or adventitious root growth or normal leaf growth, and no visual evidence of fungal

growth on the shoots of the seedlings or on any of the grains of the germinated or ungerminated seeds. This pattern of rice seedling germination was the same as that observed previously in stagnant water (Kordan, 1972, 1974, 1975) and in anaerobic environments (Opik, 1973; Kordan, 1976*a*).

Microscopic examination of shoots 5–18 mm long of the germinating seedlings revealed mitotic figures at various phases of activity in the different tissues comprising the shoots. They were evident in the foliar leaves, in the shoot apex, in the coleoptile, and in the meristematic zone beneath the coleoptilar node where adventitious root initiation normally occurs. No mitotic figures were evident in the cells comprising the quiescent ungerminated embryos, the nuclei being in the interphasic condition.

#### DISCUSSION

The manifestation of cell extension before cell division in cereals has led to the suggestion that the greater part of the observed early growth should be attributed to the former process (Ballard, 1964). Amoore (1961*a, b*) found that low oxygen tensions prevented interphase nuclei of excised plant root apices from entering into new mitoses. Since the cells of quiescent ungerminated embryos are in the interphasic condition in the rice seeds, the possibility could be suggested on the basis of the evidence of Amoore (1961*a, b*) and Ballard (1964) that the emergence of the coleoptiles of rice seedlings germinating under low oxygen tensions and under complete anaerobiosis may be solely a result of cell extension. Opik (1973), however, has shown that cell division as well as cell enlargement occur in rice coleoptiles emerging from rice seeds germinated under complete anaerobiosis. The evidence presented here shows that mitotic activity also occurs in tissues other than the coleoptiles of rice seedlings germinating in oxygen-deficient environments, even though there are no visible manifestations of the growth of seedling organs other than the coleoptiles.

Adventitious root primordia initiation has been observed in rice seedlings germinated under low oxygen tensions and in oxygen-deficient environments (Kordan, 1976*a*). Since adventitious root differentiation in germinating *Gramineae* seedlings occurs in a meristematic zone beneath the coleoptilar node (Avery, 1930; Hayward, 1938; Yung, 1938), the point was emphasized that new mitoses must have been associated with the initiation of adventitious root primordia in the rice seedlings germinated under low oxygen tensions and in oxygen-deficient environments (Kordan, 1976*a*). The findings presented here demonstrate the occurrence of mitotic activity during adventitious root primordia initiation in rice seedlings germinating under anaerobiosis.

Opik (1973) prevented the accumulation of carbon dioxide (CO<sub>2</sub>) in the anaerobic jars containing H<sub>2</sub> by the use of 10% KOH. In the anaerobic environments used by Kordan (1976*a*) as well as here, CO<sub>2</sub> was allowed to accumulate in the anaerobic jars containing H<sub>2</sub>, whereas no CO<sub>2</sub> or other metabolic emanations were able to accumulate in the N<sub>2</sub> environments since the vessels were continually being flushed by a continuous flow of N<sub>2</sub>. This shows that the germinating rice seedlings were able to manifest mitotic activity under anaerobiosis irrespective of the presence or absence of CO<sub>2</sub> in the environments.

Fungal growth on the grains and/or shoots usually becomes evident when rice seeds are germinated under non-sterile conditions in stagnant water or in normal air environments (Kordan, 1972, 1976*a*, *b*). The absence of visual evidence of fungal growth on the grains or shoots of rice seeds germinated in non-sterile anaerobic environments in a previous investigation (Kordan, 1976*a*) as well as here indicates that such fungal growth requires the presence of oxygen. Since most fungi are obligate aerobes (Brock, 1970), this apparent absence of fungal growth on the grains and shoots of the rice seeds seems to be an additional indicator, along with the reduced methylene blue, of the anaerobicity of the non-sterile H<sub>2</sub> and N<sub>2</sub> environments.

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## REFERENCES

- AMOORE, J. E. (1961*a*). Arrest of mitosis in roots by oxygen lack or cyanide. *Proc. R. Soc. B* **154**, 95-108.
- AMOORE, J. E. (1961*b*). Dependence of mitosis and respiration in roots upon oxygen tension. *Proc. R. Soc. B* **154**, 109-129.
- AVERY, G. S. JR. (1930). Comparative anatomy and morphology of embryos and seedlings of maize, oats, and wheat. *Bot. Gaz.* **89**, 1-39.
- BALLARD, L. A. T. (1964). Germination. In *Grasses and Grasslands* (ed. C. Barnard), pp. 73-88. London: Macmillan.
- BROCK, T. D. (1970). *Biology of Microorganisms*. New Jersey: Prentice-Hall.
- HAYWARD, H. E. (1938). *The Structure of Economic Plants*. New York: Macmillan.
- KORDAN, H. A. (1972). Rice seedlings germinated in water with normal and impeded environmental gas exchange. *J. appl. Ecol.* **9**, 527-533.
- KORDAN, H. A. (1974). Patterns of shoot and root growth in rice seedlings germinating under water. *J. appl. Ecol.* **11**, 685-690.
- KORDAN, H. A. (1975). Relationship between oxygen availability and transverse and vertical shoot geotropisms during germination of submerged rice seedlings. *Ann. Bot.* **39**, 249-256.
- KORDAN, H. A. (1976*a*). Adventitious root initiation and growth in relation to oxygen supply in germinating rice seedlings. *New Phytol.* **76**, 81-86.
- KORDAN, H. A. (1976*b*). Anaerobiosis-induced etiolation of light-germinated rice seedlings. *Ann. Bot.* (in Press).
- OPIK, H. (1973). Effect of anaerobiosis on respiratory rate, cytochrome oxidase activity and mitochondrial structures in coleoptiles of rice (*Oryza sativa* L.). *J. Cell Sci.* **12**, 725-739.
- TAYLOR, D. L. (1942*a*). Effects of oxygen on respiration, fermentation, and growth in wheat and rice. *Science, N.Y.* **95**, 129-130.
- TAYLOR, D. L. (1942*b*). Influence of oxygen tension on respiration, fermentation, and growth in wheat and rice. *Am. J. Bot.* **29**, 721-738.
- VLAMIS, J. & DAVIS, A. R. (1943). Germination, growth, and respiration of rice and barley seedlings at low oxygen pressures. *Pl. Physiol., Lancaster* **18**, 685-692.
- WADA, S. (1961). Growth patterns of rice coleoptiles grown on water and under water. *Scient. Rep. Tohoku Univ. Ser. IV (Biol.)*, **27**, 199-207.
- YUNG, CHI-TUNG. (1938). Developmental anatomy of the seedling of the rice plant. *Bot. Gaz.* **99**, 786-802.

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