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ROOT HAIR DEVELOPMENT IN *MARSILEA MUCRONATA* SPORELINGS

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ABSTRACT: The roots of sporelings of *Marsilea mucronata* do not generally form root hairs when grown in water, although they possess the genetic potential to do so. Rhizoid and root hair production is at least partially controlled by auxin (indole acetic acid). Two experiments were run to test the hypothesis that rapid leaching of auxin into the water prevents the accumulation of enough auxin at the outer cell walls of the root epidermal cells to initiate hair formation. Sporelings were grown suspended in 100% humid air to prevent leaching. Plants so grown produced typical root hairs. In addition, sporelings were grown in solutions of auxin at the following concentrations: 1×10^{-4} g/ml, 1×10^{-5} g/ml, 1×10^{-6} g/ml, and 1×10^{-7} g/ml. Plants grown in solutions containing auxin at concentrations of 1×10^{-5} g/ml produced numerous root hairs.

INTRODUCTION

Root hairs are elongated, tube-like extensions which extend outward radially from the external surface of the root. These cell extensions do not grow equally throughout, but only in a relatively small zone at the root tip. The cell wall found at the tip of the root hair consists primarily of loose fibrils. The cell protoplast exerts pressure on the tip, pushing the newly-formed wall over to the sides. Further development of the secondary wall along the sides prevents further growth in this area (Muehlethaler, 1961).

For some years, the formation of rhizoids on the megagametophytes of *Marsilea* spp. has been under investigation at Valparaiso University. These studies strongly indicate that the initiation of rhizoid formation is influenced by indole acetic acid (IAA) and that any outer cells under appropriate stimuli can form rhizoids (Bloom and Nichols, 1972). The rhizoids typically form first on the ventral surface as a gravitational response. The cells producing the rhizoids are actively-growing cells that seem to manufacture their own auxin. If these plants are grown on a vertical clinostat on agar plates, enough auxin appears to accumulate at the outer margins of all the peripheral cells to cause rhizoid formation over the entire surface of the megagametophyte (Bloom and Nichols, 1972).

Current theory suggests that auxin activates the hydrogen ion pump in the plasma membrane. The efflux of hydrogen ions acidifies the cell wall, weakening the bonds between the cellulose fibrils. Turgor pressure then causes cell expansion in the weakened area. Auxin has longer term effects as well; it affects gene transcription and the synthesis and secretion of new secondary cell wall material (Alberts, *et al.*, 1983).

The study of gametophyte development in *Marsilea* spp. also showed that the megagametophytes produce numerous rhizoids in water. In contrast, the young sporelings do not form root hairs in water, although they have the genetic potential to do so. One reason why the sporelings do not form root hairs in water might be that, since IAA is a small, readily-diffusible molecule, the concentration of auxin in the epidermal cells of the root remained too low to initiate root hair formation due to leaching. Two experiments were designed to test this hypothesis.

A common laboratory procedure is to grow radish seedlings on moist filter paper in petri dishes to demonstrate root hair formation in beginning biology classes. Root hairs readily form in the humid atmosphere above the filter paper. Perhaps, *Marsilea* sporelings could be induced to form root hairs using this same procedure.

Bloom and Nichols (1972) showed that by growing megagametophytes on agar containing low concentrations of IAA, rhizoids could be induced to form on their dorsal surfaces. If sporelings were grown in solutions containing low concentrations of IAA, thus countering the leaching effect, perhaps the roots could be induced to form root hairs.

MATERIALS AND METHODS

Sporocarps of *Marsilea mucronata* were scarified and placed in water to hydrate. Microspores and megaspores were released and underwent rapid development. Fertilization occurred in about eight hours, and within 72 hours, the first juvenile leaves and roots emerged. The sporelings were grown at room temperature (22° C) under constant illumination from a Westinghouse 40W cool white fluorescent tube and a Norelco 40W Growlume fluorescent tube.

When the sporelings were 96 hours old, a number of plants were transferred to a 125 ml jar in which a thin layer of plain agar had been poured and cooled. The sporelings remained attached to the agar surface when the jar was inverted in a shallow dish of water.

A 0.1 g/L solution of IAA in tap water was prepared to which seven drops per liter of Schultz-Instant Liquid Plant Food were added. Solutions having IAA concentrations of 1×10^{-4} g/ml, 1×10^{-5} g/ml, 1×10^{-6} g/ml, and 1×10^{-7} g/ml were prepared from this stock solution. Plants were grown in nutrient solution lacking IAA as a control. Sporelings were transferred to separate jars containing 100 ml of the various IAA solutions. After several days growth, the plants were examined for the presence of root hairs.

RESULTS

Sporelings grown in the humid air possessed numerous long, straight root hairs along the entire length of the root.

Of the plants grown in aqueous media containing IAA, only the plants grown at a concentration of 1×10^{-5} g/ml IAA possessed numerous, long root hairs. Plants grown at a concentration of 1×10^{-4} g/ml IAA had short root hairs. Most of the roots grown at other concentrations of IAA had many short protuberances, such as form at the beginning of root hair formation, as well as occasional root hairs, usually near the base of the root but not in the younger portions. These few root hairs may have formed during the short period of time when the older parts of the root were not fully immersed in the IAA solution. The controls grown in the nutrient solution without IAA were similar to the experimental plants grown at the lower concentrations of IAA.

CONCLUSIONS

These results suggest that leeching of IAA from the roots of sporelings grown in water is the cause of their failure to form root hairs. A number of angiosperms, grown from both seedlings and cuttings, were also studied. Some angiosperms (lima beans (*Phaseolus limensis*), barley (*Hordeum vulgare*), and popcorn (*Zea mays*)) responded as did the *Marsilea* sporelings, while others (tomato (*Lycopersicon esculentum*) and wandering-Jew (*Zebrina pendula*)) produced numerous root hairs in water.

A third way to explore the possibility that leaching causes the failure of root hair formation in *Marsilea* sporelings would be to measure the accumulation of IAA in a culture of sporelings. As yet, no method is generally available to measure such small amounts of IAA.

LITERATURE CITED

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