

RESPONSES OF THE OAT COLEORHIZA TO VARIOUS TREATMENTS IN CULTURE¹

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The coleorhiza or root sheath of the grasses has received little attention from plant physiologists and experimental morphologists. Nevertheless it is of importance in the germination of the oat seed and is interesting in its responses to certain factors in its immediate environment.

In a number of cultures of excised oat embryos, extreme coleorhizal elongations were observed. Such elongations occurred regularly in liquid media. However, the coleorhizae of oat embryos cultured on the surface of nutrient agar elongated only slightly. Instead they produced numerous epidermal hairs which appeared to be identical to root hairs. These phenomena have been reported by other investigators. Nishimura (1922) and Howarth (1927) consider that such coleorhizal hairs serve to supply water to the embryo during germination. Dietrich (1924) discovered that greatly lengthened coleorhizae were developed in subsurface cultures of excised oat embryos. LaRue (1936) observed that immature, excised embryos of certain grasses formed coleorhizal hairs *in vitro*.

Normally the coleorhiza is the first part of the oat embryo to break through the seed coat during germination. At this time it further elongates and forces itself through the husk or lemma surrounding the seed. When this is accomplished coleorhizal elongation ceases. Hairs then are often formed on the parts of the coleorhiza exposed to air. The following account deals with oat coleorhizae subjected to various treatments.

MATERIAL AND METHODS

Seeds of oats, *Avena sativa* L. variety *Andrew*, were obtained from the North Dakota Agricultural Experiment Station. Some of these were planted to provide embryos of various stages of development. Other seeds were cultured in sterile water or on moist filter paper. A simple apparatus was used in the latter cultures to obtain various dilutions of air with nitrogen gas. Excised embryos, of different ages, were cultured in White's solution (White 1943). All seeds were sterilized in a 10 percent solution of *Clorox* for ten minutes prior to culture.

RESULTS

Coleorhizal growth in liquid culture. Coleorhizal elongations were noted in excised embryos cultured for a week in White's solution but not in those cultured in sterile water alone. Coleorhizal elongations also occurred when intact seeds were placed either in White's solution or in sterile distilled water. Because of the ease with which they could be cultured in sterile water, whole seeds rather than excised embryos were used in the experiment reported in table 1. This experiment was performed in order to discover whether or not coleorhizal elongations were due solely to the presence of water. The effect of indole-3-acetic acid on coleorhizal elongation was also tested.

The effect of various factors on the growth of oat coleorhizae. As a result of experimentation, the data of which appear in table 1, some factors affecting coleorhizal behavior have been determined. Elongation of the coleorhiza was found to be inversely proportional to the amount of air present in the immediate environment, the greatest length being attained in liquid media. Hair formation was pro-

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portional to the amount of air present and was stimulated by the application of IAA. Lower concentrations of IAA (0.1 to 1.0 mg/lit.) in liquid cultures stimulated coleorhizal elongation, but higher concentrations (5.0 to 10.0 mg/lit.) of this substance proved inhibiting. The greatest length, 10 mm., was obtained in cultures with water and 0.1 mg/lit. IAA. Mitotic figures were not observed in aceto-carminic smears of the coleorhizae of cultured embryos. Coleorhizal growth in these instances, whether characterized by elongation or hair formation, occurred only by the enlargement of existing cells.

TABLE 1
The effect of various factors on the growth of oat coleorhizae.

Treatment	No. of embryos	Mean coleorhizal length			Length increase	Hairs ⁴
		Initial ³	Final	$\frac{\sigma}{\sqrt{n}}$		
20% O ₂ + 80% N ₂	20	0.8 mm.	2.6 mm.	±0.07	225%	moderate
20% O ₂ + 80% N ₂ + IAA ¹	21	0.8	2.5	±0.10	212%	considerable
10% O ₂ + 90% N ₂	20	0.8	3.2	±0.11	300%	slight
4% O ₂ + 96% N ₂	20	0.8	3.7	±0.07	362%	none
2% O ₂ + 98% N ₂	20	0.8	5.0	±0.13	525%	none
H ₂ O	47	0.8	6.1	±0.16	662%	none
H ₂ O + 0.1 IAA ²	22	0.8	7.1	±0.29	787%	none
H ₂ O + 1.0 IAA	27	0.8	7.1	±0.28	787%	none
H ₂ O + 5.0 IAA	32	0.8	5.7	±0.26	612%	none
H ₂ O + 10.0 IAA	33	0.8	5.4	±0.22	575%	none

¹Presoaked 24 hours in water and 10 mg/1. IAA.

²The figures given for IAA are milligrams per liter.

³Initial lengths based on measurements of section embryos.

⁴Based on the amount of coleorhizal surface covered by hairs.

TABLE 2
The effect of adenine sulfate on the growth of coleorhizae of 0.8 mm. oat embryos.

Medium	No. of embryos	Mean coleorhizal length		Increase in length
		Initial	Final	
White's agar.....	46	0.24 mm.	0.74 mm.*	208%
White's agar + adenine sulfate.....	28	0.24	1.58	558%
White's agar + adenine sulfate + coconut milk..	21	0.24	1.96	771%

*Coleorhizal hairs were produced by some of these embryos.

In order to discover whether elongation would occur in the absence of oxygen, oat seeds also were placed on moist filter paper in several bottles. The oxygen then was removed from the air in the bottles by means of pyrogallol. Coleorhizal elongations were not observed in these cultures and germination was suppressed.

Cultures of detached coleorhizae. Coleorhizae were also dissected from mature embryos and placed in culture on White's agar. In these cultures hairs were formed as in the preceding cultures, but coleorhizal elongation did not occur. Other detached coleorhizae did not grow at all when cultured in White's liquid. It was found that not all coleorhizal cells were capable of forming hairs. The coleorhiza is a hollow organ made up of several layers of parenchymatous cells.

A number of coleorhizae were removed from embryos, slit open and flattened out on nutrient agar. Those which were placed so that the epidermal cells were exposed to the air formed hairs. Others, placed so that the epidermis lay in contact with the agar and the cells of the inner surface were exposed to the air, formed no hairs. Thus it appears that only the epidermal cells are able to produce coleorhizal hairs.

The growth of coleorhizae of smaller embryos. Many small oat embryos were excised from unripened seeds and placed in culture. The largest of these were less than half the size of full term embryos. Embryos in the size range of 1.0 to 1.5 mm. exhibited elongated coleorhizae in liquid media and produced coleorhizal hairs when grown on nutrient agar. Smaller embryos did not develop lengthened coleorhizae in liquid media. Only one instance of coleorhizal elongation was observed in the 125 0.8 mm. embryos grown in White's solution. Yet hairs were usually produced even by 0.8 embryos when they were cultured on nutrient agar. Combinations of certain growth substances were added to White's medium. Yeast extract, IAA and coconut milk had little effect on coleorhizal development. However, in two separate sets of cultures on media containing adenine sulfate, definite coleorhizal elongations and hair suppression were observed. The first of these two sets of cultures was made on White's agar to which 40 mg/lit. of adenine had been added. The second set contained coconut milk in addition to 40 mg/lit. adenine sulfate. Coconut milk was found to promote the overall growth of excised oat embryos but did not of itself stimulate coleorhizal elongation, since marked elongations were not observed in cultures made on coconut milk media without adenine sulfate.

The effect of adenine sulfate on the growth of coleorhizae of 0.8 mm. oat embryos. Not all cultures of 0.8 mm. embryos exhibited coleorhizal elongations when treated with adenine. Larger embryos did not respond to this substance at all. The reason for these failures is not clear at present.

DISCUSSION

The behavior of the oat coleorhiza during germination seems to depend on differences in oxygen tension inside and outside of the seed and its coverings. Low concentrations of oxygen (down to at least 2%) stimulate coleorhizal elongations, while higher concentrations promote the formation of epidermal hairs. Neither elongation nor the formation of the "root hairs" occurred, however, at extremely low concentrations of oxygen. The hairs of the coleorhiza are a regular feature of the surface cells of this structure. That they are not simply adventitious outgrowths is shown by the failure of sub-surface cells to produce them. Indole-3-acetic acid appears not to initiate but rather to determine the extent of such responses as hair formation and coleorhizal elongation.

Skoog and Tsui (1951) report that adenine sulfate may be used to induce differentiation of buds in cultures of tobacco callus on nutrient agar. They also obtained such differentiation without adenine in callus masses submersed in liquid media. Thus adenine sulfate used with surface cultures seems to duplicate the effects obtained with subsurface cultures. Adenine sulfate also seems to substitute for liquid media in embryo cultures at least to the extent that it may induce coleorhizal elongation.

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