

COLEORHIZAL RESPONSES TO CERTAIN PLANT GROWTH REGULATORS IN CULTURES OF EXCISED BARLEY EMBRYOS

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ABSTRACT.—Excised embryos of barley in early stages of development (400-500 μ) cultured on synthetic media showed "precocious germination" when cultured on media containing either kinetin or gibberellic acid. In addition to coleoptile elongation, which is a normal germination response in vivo and in vitro, such embryos also developed elongate coleorhizas. The latter response is characteristic of germination in vivo, but not usually of germination in vitro. Coleorhizal elongation was most markedly stimulated by gibberellic acid.

There are two evanescent organs in embryos of grasses which play an important role in germination. The coleoptile, a sheath enclosing the leaf primordia, has received a great deal of attention, not only for the key part it plays in the early life of the seedling but also because of the significant research in auxinology which employs this structure as a reaction system. The coleorhiza, or root sheath, breaks open the covering coats of the caryopsis. In barley and oats, for example, which have tough husks in addition to the seed coats, the coleorhiza forces an opening through layers nearly a millimeter thick. Parenthetically, the coleorhiza is a delicate organ and the forces it em-

plays in its action have never been properly studied.

In studies of excised oat embryos it was observed (Norstog, 1955) that embryos grown in liquid media produced elongated coleorhizas, whereas those grown on solid media did not. In a series of experiments it was shown that embryos grown on a solid substrate in an atmosphere of 2% O₂ produced similarly elongated coleorhizas. That there was, in addition, an involvement with auxin was demonstrated by experiments in which the addition of indole-3-acetic acid to liquid media resulted in elongations somewhat greater than those occurring in liquid media alone. Aside from those cultures grown in low O₂, the only agar cultures showing coleorhizal elongations were in two isolated instances on a medium containing 40 mg/1 adenine sulfate.

When excised embryos are cultured at earlier stages of their development they may, depending on cultural conditions, either continue embryonic development or they may grow as small seedlings. The latter development has been termed precocious germination. Recently, in agar

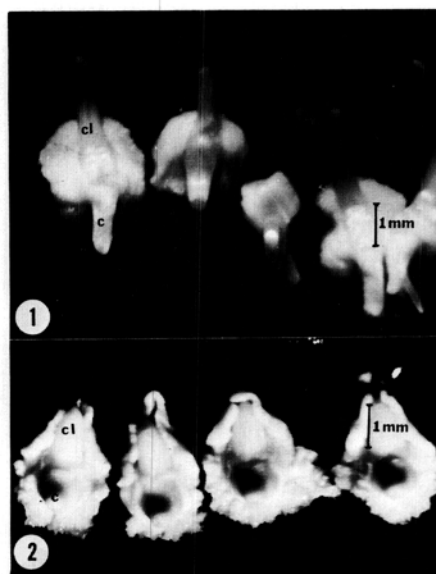


FIGURE 1.—Excised 500 μ barley embryos after 10 days' growth on a medium containing 0.1 mg/l GA_3 , at 25° C, in light. Cl-coleoptile, C-coleorhiza.

FIGURE 2.—Excised 500 μ barley embryo after 10 days' growth on control medium at 25° C, in light. Cl-coleoptile, C-coleorhiza.

cultures of barley embryos, coleorhizal elongations have been observed in some instances in association with precocious germination. The following brief report deals with those observations.

MATERIALS AND METHODS

Embryos of barley, *Hordeum vulgare*, var. "Moore", were excised aseptically from still-green caryopses and placed on a synthetic culture medium (Norstog, 1967) which in a ten-day period allowed 500 μ embryos to attain a state of development equal to or greater than that of normal full-term embryos (see Fig. 2). These cultures were used as controls against media containing 0.1 mg/l gibberellic acid (GA_3); 0.1 mg/l kinetin (6 furfurylaminopurine), (K); and 0.1 mg/l indole-3-acetic acid (IAA). The latter two substances were used

TABLE 1.—Responses of the barley coleorhiza to IAA, K and GA_3 .

Treatment	Coleorhizal elongation*:	
	Dark	Light
Control	—	—
GA_3	+	++
K	+	+
IAA	—	—
K + IAA	+	+

* As compared to controls.

alone and in combination. Cultures were maintained at 25°C in light (20-100 FC) and in dark. The results are summarized in Table 1 and are represented photographically in Figs. 1 and 2. Coleorhizal elongations were observed in all treatments except in those which contained IAA alone. The most consistent results were obtained with GA_3 in light-grown embryos. Coleorhizal elongations occurring in K and K + IAA cultures were somewhat obscured because embryo germination was greatly promoted by these treatments, both in light and in dark, and as germination progressed growing roots ruptured the coleorhiza and its elongation ceased. This was also true of GA_3 cultures in dark, but not so much so in light. It has been observed repeatedly that light tends to inhibit GA_3 -induced precocious germination in our barley cultures. Thus dark-cultured embryos with GA_3 displayed ruptured coleorhizas, whereas those grown in light on GA_3 had intact and elongate coleorhizas.

Coleorhizal hairs resembling root hairs are a feature of coleorhizal development in some circumstances (Nishimura, 1922; Norstog, 1955). IAA, while not active in the induction of coleorhizal elongation in cultures of barley embryos on solid media, does stimulate coleorhizal hair production in such cultures. There is an interesting morphogenetic dichotomy apparent in this response, in that coleorhizal elongation is usually not accompanied by hair formation, and vice versa.

DISCUSSION

Precocious germination in barley embryo cultures, as in embryo cultures of other species (Rijven, 1952,

1956) represents a marked morphogenetic departure from an embryonic type of development. In the latter, development consists of rapid cell division and differentiation with little if any cell elongation taking place. Precocious germination, on the other hand, may not involve much cell division but is characterized by cellular elongation. Haber and Luippold (1960) have shown in their studies of gamma-irradiated wheat embryos that cell division is not necessarily a corollary of GA₃-induced cell elongation in germination. Differentiation may or may not occur, depending on the nature of the medium and conditions of culture. Although precocious germination may mimic normal germination in many aspects, it occurs without a dormant interval between embryogenesis and the seedling state. Moreover, it may occur before the embryos have reached a full-term state of development. Aside from these differences, there is no reason to believe that precocious germination, as a biochemically-mediated response, differs significantly from normal germination.

The relationship between gibberellin and germination in cereals has been explored by a number of investigators. Gibberellins are involved in the germination response in barley (see for example Paleg, 1960; Yomo and Inumina, 1966; MacLennan and Palmer, 1967; Naylor and Simpson, 1961).

Since in grass embryos germination involves coleorhizal elongation, a case may be made for involvement of gibberellin in the response of this organ in vivo as in vitro.

It is thought that growth in plants

is an expression of balanced actions of growth regulators such as cytokinins, gibberellins, and auxins (van Overbeek, 1966). The promotion of coleorhizal elongation in cultures on K and K + IAA media, as well as by GA₃, suggests that interactions between growth substances may affect coleorhizal development. However, biochemical evidence of such relationships has not been obtained as yet.

The observation (Norstog, 1955) that adenine sulfate stimulated coleorhizal elongation in cultured oat embryos is perhaps evidence of cytokinin activity. Doerschug and Miller, 1967, observe that adenine sulfate is a less effective substitute for kinetin in bud induction in *Lactuca* explants. One also may postulate a similarity of action by adenine and kinetin in coleorhizal elongation, with adenine giving a weaker response.

The role of the grass coleorhiza in germination is fundamental. Although it now appears clear that several growth regulators are involved in this action, the interaction of these substances with factors of the physical environment such as light and O₂ tension is not well understood, and it is apparent that further study of these effects is required.

ACKNOWLEDGMENTS

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