DORMIN AND CYTOKININ: GROWTH REGULATION OF LEMNA

J. VAN OVERBEEK and M. IONA R. MASON

Institute of Life Science, Texas A & M University, College Station

SUMMARY

Growth curves of *Lemna minor* are presented in which the fresh weight of the sterile cultures was plotted against time, and as a function of additions to the medium of 1 and 2 ppm of the dormin abscisic acid (ABA) and 0.1 ppm of the cytokinin benzyladenine (BA). The growth regulators were presented alone or in a variety of combinations and time sequences. Growth was halted or greatly inhibited by ABA and resumed upon addition of BA, and vice versa. *Lemna* plant growth thus can be made to stop or go at will, simply by varying the dormin and cytokinin concentrations.

1. INTRODUCTION

Recently we have presented evidence that abscisic acid (ABA) inhibits the synthesis of nucleic acids and that this is the reason for the growth inhibition caused by this new plant hormone. We also demonstrated that benzyladenine (BA) has just the opposite effect. It promotes nucleic acid synthesis which is followed by promotion of growth (VAN OVERBEEK *c.s.* 1967; VAN OVERBEEK 1968). In these experiments we used sterile cultures of *Lemna*, because preliminary screening trials had demonstrated that this floating water weed is so sensitive to ABA that a concentration of the order of 10^{-9} M is detectable. In these earlier papers we concentrated our attention on the mode of action of the growth regulating substances. In this paper we are presenting a summary of our findings on the effect of ABA and BA, alone or in combination, on the growth of *Lemna* cultures.

2. METHOD

We found *Lemna minor* extremely suitable for quantitative work. It is small and can be inoculated from sterile cultures into sterile tubes as one would microorganisms. It grows fast. The 3-plantlet inoculant (each plantlet composed of 3 leaves), weighing 10 milligrams, grows into a 100 mg mass in a week, and into a 220 mg plant cover in two weeks. This is shown by curve AD in *fig. 1.*

Hoagland nutrient solution 1 (1950), without the added copper, was used as a growing medium. The cultures grew in tubes containing 25 ml of the nutrient solution (medium), and they were slanted (15° with the horizontal) with aluminium foil below the tubes, on racks under constant fluorescent light (2500 ft candles, 25 kilo lux; no incandescent light was added). The temperature of the walk-in growth chamber was 22 $\pm$ 1 °C. Growth regulators were added to the

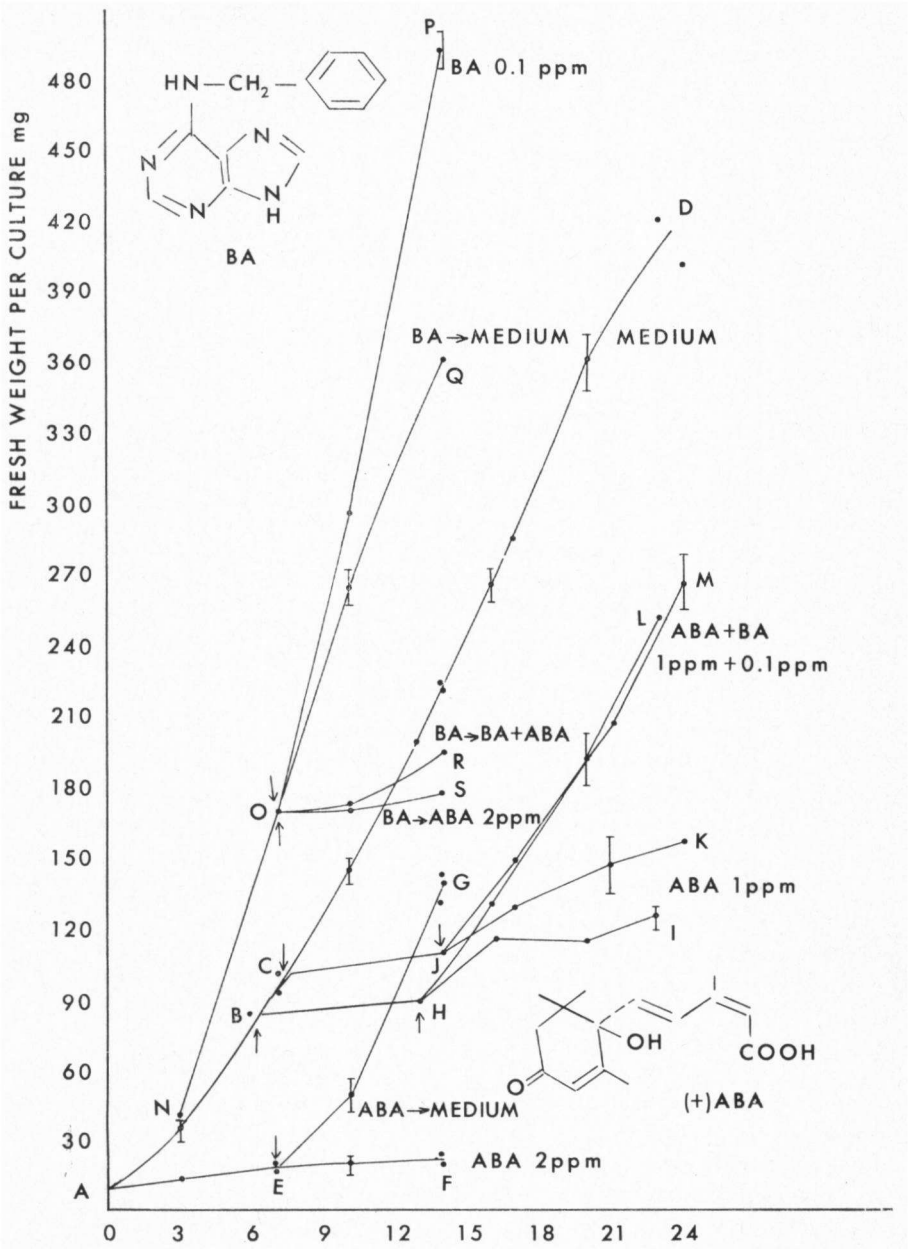

Fig. 1. Growth of *Lemna minor* cultures as a function of time (days) and treatment with benzyladenine (BA) and abscisic acid (ABA).

sterile medium by introducing 0.050 ml (for ABA) or 0.0050 ml (for BA) of a stock solution prepared in acetone.

Growth was determined by fresh weight, as this reflected the effect of the hormones better and lent itself to quantitative work better than air-dry weight or surface area determinations. Adhering liquid was blown off the plantlets with an air jet and the plantlets from each culture tube were placed in a plastic weighing bottle. Tests were replicated fivefold, so that every point on the graphs represents the average of not less than 5 cultures. Standard errors were calculated with each test. These were small as indicated by the small vertical lines. In order to avoid cluttering up the graphs unduly, only a few representative standard errors were drawn in. Reproducibility was so high that curves obtained as much as 5 months apart could be superimposed. The curve AD was drawn on points obtained from 4 different tests.

Synthetic (±) abscisic acid (ABA) came from Cornforth's laboratory (Shell Research, Sittingbourne, Kent), and synthetic benzyladenine (BA) came from Shell's Modesto, California laboratories.

In this paper BA is referred to under the general physiologically descriptive term cytokinin, proposed by Skoog for adenine materials that stimulate cells into activity. Similarly ABA is referred to under the general physiologically descriptive term dormin, proposed by Wareing for the hormone that changes summer buds into dormant winter buds.

3. RESULTS

Our results dealing with the effect of dormin (ABA) and cytokinin (BA) are summarized in *fig. 1*. AD represents growth in the Hoagland medium alone. It is a sigmoidal curve. When 2 ppm ABA was added to the tubes from the start of the test the curve AF was obtained. When after 7 days of inhibited growth induced by dormin, the cultures were transferred to medium without the inhibitor hormone, the curve EG was obtained. Not only is it evident that a normal growth curve is assumed without an apparent lag, but growth rates from cultures transferred from dormin were invariably greater than cultures transferred from plain Hoagland medium. This, we think, may be due to the building up of precursor (such as nucleotides) while growth (nucleic acid synthesis) is blocked by the inhibitor hormone.

When dormin is injected in the course of normal growth, such as at points B and C, growth is slowed at once and the curves BI and CK result. When cytokinin is injected into these dormin containing cultures, growth is rapidly resumed (curves HL and JM). The inhibitory effect of dormin is thus overcome by cytokinin. Promotive hormones of other classes, such as auxin and gibberellin, were ineffective on *Lemna*.

Not only does cytokinin overcome dormin-inhibited cultures, but cytokinin (optimum concentration 0.1 ppm) also promotes growth of normal *Lemna*, as shown by curve ANP. However, if dormin is injected, growth slows at once (OR). Growth is nearly completely halted when the cytokinin containing cul-

tures are transferred to tubes containing 2 ppm of the dormin without cytokinin (OS). When the cytokinin containing cultures were transferred to Hoagland medium alone, growth rate was reduced to about normal (OQ).

4. DISCUSSION

The results of our experiments, as presented in *fig. 1*, leave little doubt that growth of our *Lemna* system can be completely regulated by two hormones. The promotive hormone cytokinin accelerates growth. The inhibitory hormone dormin decelerates growth. The effects of either hormone are entirely reversible, at least when measured as fresh weight of the cultures. Because of the wide occurrence of cytokinins and of (+) abscisic acid in plants, we conclude that in nature, growth is regulated in a fashion similar to that demonstrated in our tubes: by a balance of promotive hormone and inhibitory hormone.

The faster recovery of the growth rate of cultures transferred from ABA compared to growth rate of cultures transferred from an untreated growing culture (curve EG) may be one of the factors contributing to the spectacular bud burst one witnesses every spring. During dormancy synthesis of nucleic acids, but not that of the nucleotides may be blocked. With dormin removed, growth can proceed at a rapid rate feeding, as it were, on this pool of accumulated nucleotides.

DEDICATION

This is a condensed version of a manuscript written and presented to Hans Söding on the occasion of the 70th birthday of this pioneer in plant hormone research. The experimental part was carried out at the laboratory of the Shell Development Company at Modesto, California. Present address of coauthor, M. Iona R. Mason, is Department of Pomology, University of California, Davis.

REFERENCES

OVERBEEK, J. VAN, J. E. LOEFFLER & M. IONA R. MASON (1967): Dormin (Abscisin II), inhibitor of plant DNA synthesis? *Science* **156**: 1497–99.

— & — (1968): Mode of action of abscisic acid. In press, *Proc. 6th International Conf. Plant Growth Hormones*. Ottowa. The Runge Press.