

Regulation of Growth in *Avena* (Oat) Stem Segments by Gibberellic Acid and Abscisic Acid

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Abstract

The purpose of this study was to analyze the nature of the interaction between gibberellic acid (GA_3) and abscisic acid (ABA) in the regulation of growth in excised *Avena* (oat) stem segments. Growth, compared to sucrose controls, was inhibited by ABA in the range of 10^{-4} to 10^{-6} M. GA_3 -promoted growth was also inhibited by ABA in the same concentration range. A Lineweaver–Burk analysis of the interaction between GA_3 and ABA indicated that ABA acts in a non-competitive fashion with GA_3 . This same result was obtained previously with GA_3 -indoleacetic acid (IAA) and GA_3 -kinetin interactions with *Avena* stem sections. Our results indicate that ABA can inhibit GA_3 -promoted growth within physiological concentrations, and that it is probably acting at a different physiological site from that for GA_3 .

Introduction

Our previous studies on interaction between IAA and GA_3 (Kaufman *et al.* 1969) and kinetin and GA_3 (Jones and Kaufman 1971a, b) indicated that neither IAA nor kinetin alone promote growth of *Avena* stem segments. When either of these hormones is combined with GA_3 at concentrations between 10^{-4} and 10^{-7} M, the GA_3 -promoted growth is significantly suppressed. Lineweaver–Burk analyses indicated that both IAA and kinetin inhibited GA_3 -promoted growth non-competitively.

Other work on GA_3 -abscisic acid (ABA) interactions indicates that ABA and GA_3 may act synergistically to promote growth of rice mesocotyls (Takahashi 1972, 1973) and *Avena* coleoptiles (Thomas *et al.* 1965). However, the data of Dey and Sircar (1968) suggest that ABA may not interact with gibberellin in affecting the length of wheat coleoptile sections. On the other hand, ABA suppresses IAA-promoted rapid growth response in *Avena* coleoptile segments (Rehm and Cline 1973). The present study was undertaken to determine the nature of the interaction

between GA_3 and ABA in excised *Avena* stem segments. We report here on kinetics of the growth response, dose-response relationship, and a Lineweaver–Burk analysis of the kind of interaction taking place between ABA and GA_3 .

Materials and Methods

Source of seed: ‘Victory’ oat seed (*Avena sativa* L. cv. Victory) was obtained from the Swedish Seed Association, Svalöf, Sweden.

Culture of plants: Oat plants were grown in flats of soil maintained in a Sherer–Gillet growth chamber programmed for 18 h light, 6 h dark, 22,000 lux. light intensity, and 15.5°C during the dark phase, 21.5°C during the light phase. The plants were illuminated with General Electric cool-white fluorescent lamps (Type F72T12-CW-1500) plus four 100-watt incandescent lamps. When plants were 40 days old, stem segments were excised from the bases of next-to-last internodes (p-1 internode). The p-1 internodes were 10 to 15 mm in length at this time.

Isolation and culture of stem segments: The stem segments were excised from p-1 internodes with a 1-cm razor blade cutting device. Such segments included the basal node, enclosing sheath-base portion, and the basally located intercalary meristem of p-1 internode. All segments were collected in a dish of distilled water until cutting was completed. They were then surface-sterilized in 10% ‘Clorox’ (0.5% sodium hypochlorite) for 2 min, then thoroughly washed with 8 to 10 changes of sterile distilled water. This treatment effectively negates microbial contamination in the incubation medium. The washed segments were then supported in raised perforated plexiglas disks on filter paper in 5-cm Petri dishes, with bases of the segments (node portions) immersed in 2 ml of treatment solution. These Petri dishes were then placed in a large glass culture

chamber, containing water to saturate the atmosphere, and covered with "Saran-wrap" to maintain a high relative humidity in the chamber. In each experiment, 25 stem segments were used in each Petri dish for a given treatment. The segments were incubated in the dark at 23°C.

Growth measurements: Net growth of the segments was measured periodically with a millimeter ruler to the nearest 0.5 mm in a dark room provided with a green "safe-light" (a fluorescent lamp covered with one sheet each of green and amber "Cellon" Cellulose acetate film obtained from Cadillac Plastic Company, Detroit, Michigan).

Sources of hormones: Gibberellic acid (GA_3) was obtained as a gift from Imperial Chemical Industries, Ltd., England. Abscisic acid (ABA) was kindly provided by F. Hoffman-La-Roche and Co., Ltd., Basel, Switzerland.

Results

Dose-response relationship: ABA- GA_3 interaction

Avena stem segments were cultured in varying concentrations of ABA (10^{-4} to 10^{-9} M) in the presence and absence of $3 \mu M$ GA_3 (concentration of GA_3 which just gives a maximal growth response). $0.1 M$ sucrose was also included in the incubation medium. The results in Figure 1

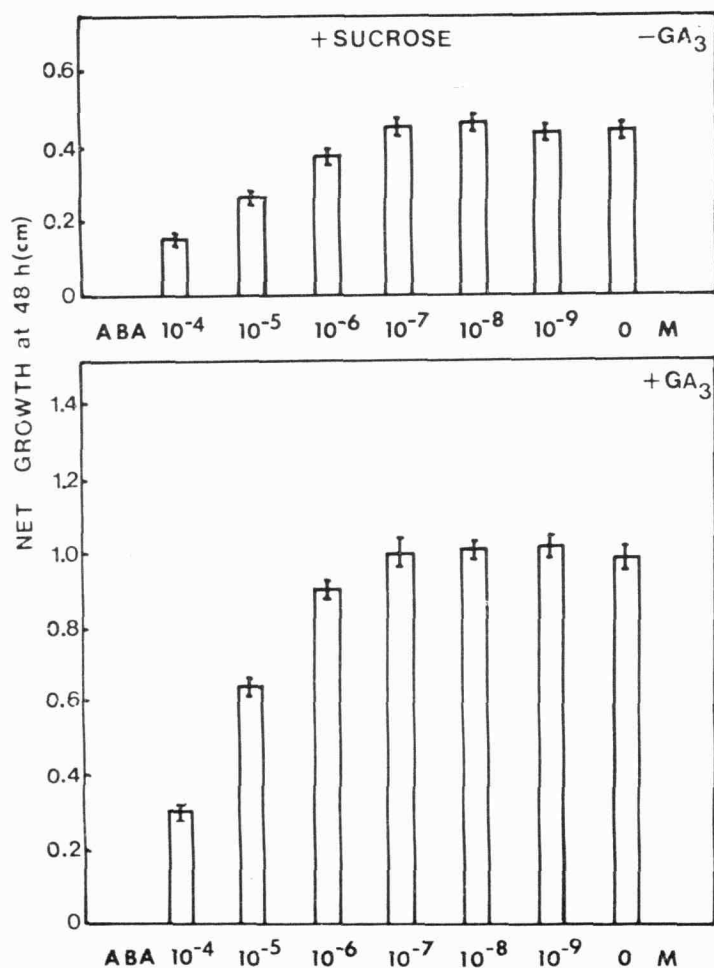


Figure 1. Net growth of *Avena* stem segments treated with varying concentrations of ABA in the presence and absence of GA_3 . Segments were incubated in the dark at 23°C in $0.1 M$ sucrose. GA_3 was used at $3 \mu M$; ABA concentration varied between 10^{-4} and 10^{-9} M. Standard errors of the mean are indicated by vertical line over each bar.

show the net growth response after 48 h of incubation. In the absence of GA_3 , the growth of the sucrose control is inhibited by 10^{-4} to 10^{-6} M ABA, with 60% inhibition at 10^{-4} M ABA and 12% inhibition at 10^{-6} M ABA. In the presence of GA_3 , the inhibition of GA_3 -promoted growth occurs between 10^{-4} and 10^{-6} M ABA. At 10^{-4} M ABA the amount of inhibition of GA_3 -promoted growth is 70%; at 10^{-6} M ABA, it is 10%. These results indicate that ABA inhibits both sucrose and sucrose + GA_3 growth at comparable ABA concentrations, and the ABA can inhibit growth in *Avena* stem segments at physiologically significant concentrations.

Comparable results are obtained with ABA used at 10^{-4} to 10^{-9} M in the presence and absence of GA_3 ($3 \mu M$) when sucrose is excluded from the medium.

Growth kinetics for ABA- GA_3 -treated stem segments

Figure 2 illustrates a typical set of time-course growth data for *Avena* stem segments incubated in $10 \mu M$ GA_3 + $0.1 M$ glucose (control) compared with the same treatment solution plus ABA at 10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7} M. Glucose

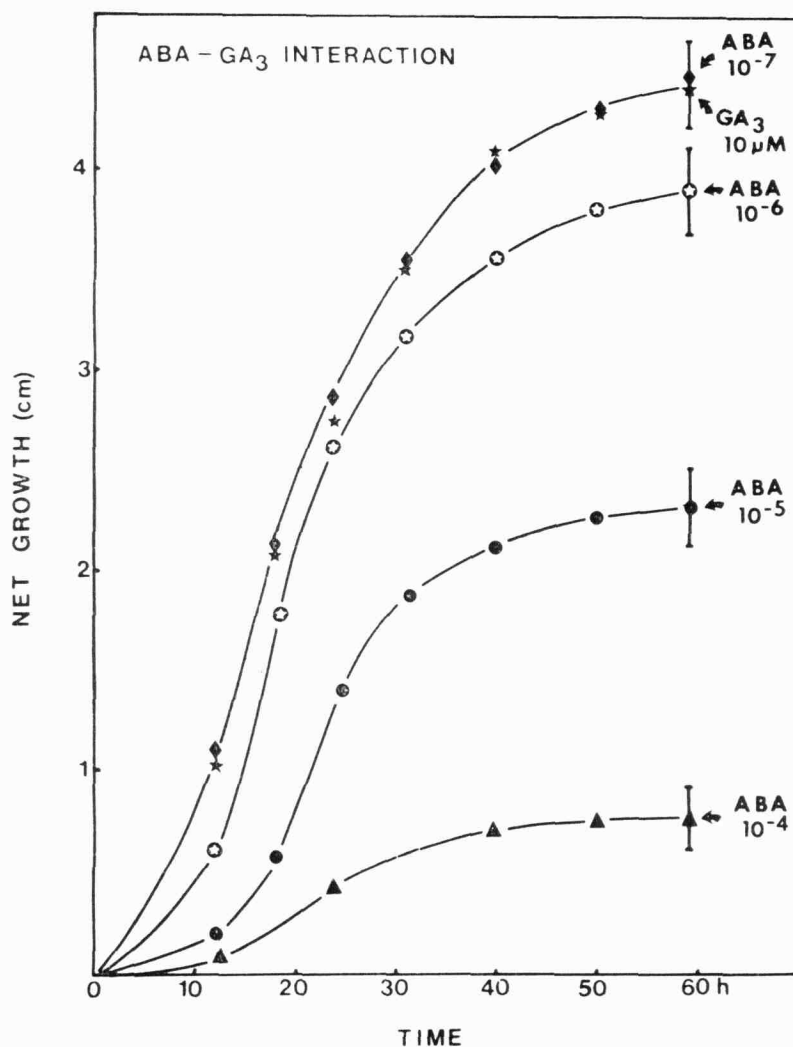


Figure 2. Time-course changes in net growth of *Avena* stem segments treated with GA_3 and GA_3 + ABA. Glucose at $0.1 M$ was included in all treatment solutions. GA_3 was used at $10 \mu M$ in the control and with ABA at 10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M. This is a typical set of results for the experiment, which was repeated four times.

gives as good a growth response as sucrose, and $10 \mu\text{M GA}_3$ is nearly equivalent to the level ($3 \mu\text{M}$) just giving maximal growth in our system.

The results indicate that the inhibition of GA_3 -promoted growth by ABA starts very early, within the first 10 h of incubation. This inhibition is seen with 10^{-4} , 10^{-5} , and 10^{-6} M ABA , but not with 10^{-7} M ABA which parallels the growth of the GA_3 control. ABA at the successively higher concentrations clearly reduces the growth rate during the log phase of GA_3 -promoted growth. The segments also reach the plateau phase earlier, as especially well-illustrated with 10^{-4} M ABA .

A closer examination of the time-course changes in the ABA inhibition of GA_3 -promoted growth (Table 1), expressed in per cent of the growth of the control, reveals that the per cent inhibition by ABA is very marked at first (see 12-h data), and then gradually decreases until the end of the incubation at 60 h. This is well exemplified by 10^{-5} M ABA , where the growth, compared with that of the control, is depressed 80% at 12 h, but only 48% at 60 h. The primary reason for these changes in the per cent inhibition with time is that the control segments are initially growing at much higher rates (e.g., at 12 h), whereas later (e.g., between 30 and 60 h), this differential in growth rates is not nearly so great (see Figure 2).

*Interaction between GA₃ and ABA:
Lineweaver–Burk analysis*

To determine the kind of interaction between GA_3 and ABA, a Lineweaver–Burk analysis was performed. GA_3 was varied in concentration from 0.6 to $5 \mu\text{M}$; ABA was used at both 10^{-4} and 10^{-5} M . 0.1 M glucose was included in the incubation medium. The experiment was repeated four times. A typical set of results, when ABA was used at 10^{-5} M , is shown in Figure 3. The curves conclusively show that we have a *non-competitive* type of interaction between ABA and GA_3 . The same result was obtained

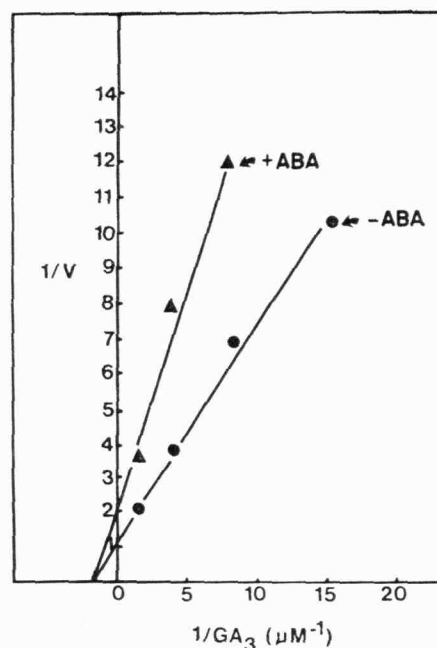


Figure 3. Lineweaver–Burk plot showing interaction between GA_3 and ABA. $V =$ growth (cm/24 h). 0.1 M glucose was included in the incubation medium, and ABA was used at 10^{-5} M . Experiment was run in the dark at 23°C . The experiment was repeated four times with essentially the same results.

with an incubation time of 24 h as with 48 h. It thus appears that GA_3 and ABA are not acting at the same physiological site. The interaction may represent secondary effects of the inhibitor (ABA). Indeed, ABA could be drawing off substrate, or inhibiting the production of reducing sugars (as seen in barley endosperm, where α -amylase synthesis is inhibited by ABA — Khan and Downing 1968), or lowering the levels of co-factors required for GA_3 -promoted growth, thus braking GA_3 -accelerated cell growth (here, cell lengthening). In similar Lineweaver–Burk analyses, we also found that IAA (Kaufman *et al.* 1969) and kinetin (Jones and Kaufman 1971a) act in a non-competitive fashion with GA_3 , paralleling the results obtained here with ABA and GA_3 .

Discussion

We have shown here that abscisic acid is a potent inhibitor of gibberellic acid-promoted growth in *Avena* stem segments at physiological concentrations tested (10^{-5} , 10^{-6} M). This compares favorably with results obtained in other systems where gibberellin-abscisic acid interactions have been studied (Addicott and Lyon 1969, Aspinall *et al.* 1967, Milborrow 1966). In most other systems, when kinetic analyses have been made, the interaction between ABA and gibberellin has been found to be of a non-competitive type (Addicott and Lyons 1969). The inhibition, in *Avena*, is also of a non-competitive nature, as seen in Figure 3, indicating that ABA must be acting at another physiological site from that of gibberellin. What that site is we do not know, but it is clear that the end-result is a braking action on gibberellin-promoted cell lengthening. ABA at 10^{-4} M , in the presence of $30 \mu\text{M GA}_3$, reduces the cell length of the cortical cells, at the level of the intercalary meristem, by 40%, compared with the control.

It is clear from the data in Figure 2 that ABA affects the lag, exponential, and stationary phases of growth in

Table 1. GA_3 –ABA interaction on the growth of *Avena intercalary meristem segments* incubated in the dark at 23°C . The incubation medium consisted of 0.1 M glucose, $10 \mu\text{M GA}_3$ (control) and varying concentrations of ABA. The growth is expressed in per cent of the control.

Time h	ABA, M		
	10^{-4}	10^{-5}	10^{-6}
12	7	20	57
18	10	28	85
24	16	52	95
31	16	53	90
40	17	51	86
50	17	52	89
60	17	52	88

gibberellin-treated *Avena* stem segments. If one uses cell elongation as the primary parameter of gibberellin action (see Kaufman 1965), ABA during lag phase seems to delay the response of these cells in, or limits the number of cells entering, active cell elongation. Further, during the exponential phase, ABA can limit the rate of cell elongation itself, as seen at 10^{-4} M ABA; this could occur either by reduced numbers of cells entering the elongation phase or by a reduced response of all cells which are elongating. At the stationary phase, those cells which do elongate, as a whole, cease elongating earlier in the presence of ABA. This could be a reflection of reduced numbers of meristematic cells entering the elongation phase, or be due to limiting factors which slow down the rate of cell elongation. Finally, the magnitude of these effects is well-correlated with ABA concentration.

How do these results with GA₃-ABA interaction compare with those obtained previously for GA₃-IAA and GA₃-kinetin interactions in *Avena*? In every case, the interaction has been found to be of a non-competitive type, based on Lineweaver-Burk analyses. Further, each of these hormones, IAA, kinetin, and ABA, represses GA₃-promoted growth in the same concentration range of 10^{-4} to 10^{-6} M (Table 2). No inhibition is seen with ABA at 10^{-7} and 10^{-8} M, and that for IAA and kinetin is small. At 10^{-4} to 10^{-5} M, however, ABA causes almost twice as much inhibition of GA₃-promoted growth as do either IAA or kinetin. All three hormones give about the same amount of inhibition at 10^{-6} M.

What this tells us is that each of these hormones could act to brake gibberellin-promoted cell lengthening in the elongating internode during intercalary growth. Based on experimental results with excised stem segments, we have previously postulated that IAA could play important roles in promoting lateral expansion of cells (perhaps through stimulation of ethylene synthesis) and determining planes of cell division in the intercalary meristem cells and their immediate derivatives (Kaufman *et al.* 1969). Kinetin appears to play a role in regulating asymmetric cell divisions in the intercalary meristem, since GA₃ abolishes all such

divisions, and kinetin restores the GA₃-blocked cell division activity. It also stimulates longitudinal divisions in intercalary meristem cells which bring about increase in internodal diameter (Jones and Kaufman 1971a).

What role could ABA play in regulating intercalary growth? It clearly represses shoot growth and induces bud dormancy under short days in many groups of plants (Addicott and Lyon 1969). One of its roles in regulating growth in grass shoots could be that of suppressing internodal extension, particularly in those short internodes which develop between seedling and inflorescence initiation. In *Avena*, we find 3 to 5 such "telescoped" short internodes; in rice, there are as many as 12 to 15 which are less than 0.5 cm long. Our hypothesis is that during development of the short internodes, the level of ABA is high compared to that of gibberellin; and later, when long internodes (up to 12 cm long) are forming, the ratio of gibberellin to ABA increases. To test this hypothesis, we must have definitive information on changes in levels of native gibberellins and ABA during shoot development in *Avena*. As of the present time, based on results obtained here, ABA would be a most likely candidate to participate with auxin and cytokinin in regulating GA₃-promoted cell lengthening during internodal extension.

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Table 2. Inhibition (%) of GA₃-promoted growth in *Avena* intercalary meristem segments. Sucrose or glucose was used as substrate. Segments were incubated in the dark at 23°C, for 48 h. GA₃ concentration was 0.1 M.

Hormone M	Hormone used		
	IAA	Kinetin	ABA
10^{-4}	40	35	83
10^{-5}	28	22	48
10^{-6}	19	16	12
10^{-7}	8	9	0
10^{-8}	1	5	0
10^{-9}	0	0	0

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