Effect of Gibberellin Biosynthesis Inhibitor Ancymidol on Growth, Floral Initiation and Endogenous GA levels in *Sorghum bicolor*

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수수의 생육과 개화 및 내생 GA 함량에 미치는 지베렐린 생합성억제제 Ancymidol의 영향

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ABSTRACT

This study was conducted to correlate changes in plant growth and flowering behavior with in-hibition of gibberellin synthesis following application of GA biosynthesis inhibitor. Two sorghum genotypes, wild-type and *phyB-1*(phytochrome B mutant) which grow fast and flowers early relative to the wild-type, were used. Both growth and floral initiation of these two genotypes were greatly affected by ancymidol concentration increased. However, these growth inhibition and delayed flowering are almost completely overcome by simultaneous applications of 31.6ppm GA_3. The ability of GA_3 to reverse the effect of the inhibition on both growth and floral initiation in sorghum sug-gests a role for native GAs in sorghum flowering. This result was contrast to the fact that in some long day plants GA biosynthesis inhibitors will inhibit shoot elongation but not floral initia-tion. In sorghum, inhibition of vegetative growth by GA biosynthesis inhibitor is accompanied by a delay in flowering. Ten ppm of ancymidol treatments drastically reduced all early-13-hydroxyla-tion pathway GAs(GA_{12}, GA_{53}, GA_{19}, GA_{20}, GA_1, GA_3, and GA_8) levels.

Key words : GA biosynthesis inhibitor, Ancymidol, Floral initiation

INTRODUCTION

Exogenous GAs have been shown to promote the switch from vegetative growth to flowering in a variety of plants species. Applications of GA_3 and other GAs substitute for the requirement for long day(LD) or low temperature vernalization in many species[8]. In addition, recent work on flowering mutants in *Arabidopsis* has clearly supported the involvement of GAs in mechanisms of flowering control. The *ga1-3* mutant which is

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defective in *ent*-kaurene synthesis never flower in short day(SD) condition without treatment with exogenous GA[13].

Exposure of the SD plant *Pharbitis nil* to a single inductive night promoted flowering which was modified by application of GAs, depending on the GA-type, dosage, and timing[4]. Exceeding an optimum dosage changed the response from promotion to inhibition of flowering. Ten to 50-fold less GA was needed to promote flowering than stem elongation. In the LD plant *Lolium temulentum*, GA-like substances doubled 24h after an inductive short night[8]. A putative polyhydroxylated GA, with retention time between GA_3 and GA_{32}, increased $3 \sim$ to $5 \sim$ fold on LD one after the short night and then decreased. GA_{32}, GA_5, 2,2-dimethyl GA_4, GA_3, and GA_7 all induced flowering under noninductive SD. The most effective GAs for stem elongation were least effective for floral induction[9]. A role for GAs in flowering of sorghum is suggested by the fact that both GA_3 and end of day far-red promote floral initiation[12]. In the field under unfavorable photoperiods GA_3 treated plants initiate a floral meristem.

With the introduction of CCC for wheat in the mid-sixties and ethephon for barley in the mid-seventies, the use of growth regulators has become common practice in intensive cereal production. GA biosynthesis inhibitors are often used for prevention of lodging in small grain cereals, growth inhibition to prevent lodging and to improve harvesting abillity and yield in rape and growth inhibition of cool and warm season turf grasses. Ancymidol has also been shown to be effective in reducing stem elongation in numerous crops. Ancymidol(N-containing heterocyclics compounds) inhibit oxidation steps from *ent*-kaurene to *ent*-kaurenoic acid catalyzed by kaurenoic acid hydroxylase[10]. These reactions are catalyzed by microsomal(membrane-bound), cyto-chrome P-450-dependent monooxygenases[3]. However, the practical use of these inhibitors was somewhat restricted by the fact that they also affect other plant growth and development processes including delayed flowering.

In LD plant, several GA biosynthesis inhibitors inhibited shoot growth but did not delayed floral initiation, and showed that shoot growth and floral initiation were clearly separated[11,14]. Sorghum showed more florally responsive to GAs and GA biosynthesis inhibitors than most LD plants which have been studied in that context. Furthermore, the results presented here indicated that in short day plant sorghum GA biosynthesis inhibitors applied at early stage of growth inhibited shoot growth and delayed floral initiation.

MATERIALS AND METHODS

Two Sorghum maturity genotypes : *phyB-1(ma₃^R^* *ma₃^R^* ; phytochrome B mutant) and wild-type(*ma₃* *ma₃*) were used in this study. Characteristics of these two genotypes are described in previous reports[6,7]. Seeds were germinated and grown in pots(19cm in diameter, 14cm in width) filled with a soil mix which was developed to minimize chlorosis problems in pot-grown sorghum. The mix consisted of 18.9L peat, 9.45L vermiculite, 9.45L perlite, 225g Osmotcote, 115g dolomite, 70g superphosphate, and 195g gypsum. Seedlings were watered with 170ml of full strength Hoagland solution every 4 days and with distilled water as needed. Plants were grown in growth chambers(EGC, Chagrin Falls, Ohio) which were equipped with a mixture of cool-white fluorescent and incandescent lights yielding a light intensity of 250 to 300mol m^{-2} s^{-1}(400-800nm) measured at the pot surface by a LI-COR Portable Spectroradiometer(LI-1800). Plants were grown in a growth chamber under a 11h photoperiod with 31℃ days and 21℃ night.

Ancymidol[α -cyclopropyl- α -(p-methoxyphenyl) -5-pyrimidine methyl alcohol] was dissolved in acetone and then were added to distilled water with stirring. All treatments(3.16, 10, 31.6, 100 and 316ppm) were applied as a soil drench in 50ml of each solution per pot at 5 days after seeding(DAS). Every 7 days another 50ml was applied until the experiments were completed. This volume, method and timing of application was chosen based on preliminary experiments. To test whether or not GA_3 could overcome growth inhibition and delayed floral initiation by GA inhibitor in sorghum, combination of ancymidol and GA_3 was applied as a soil drench in 50ml of each solution per pot. GA_3(70.3% purity, Eli Lilly) was dissolved in 95% ethanol and then diluted to each concentration(31 and 316ppm) with distilled water. To investigate the timing of growth retardant application influence on the date of floral initiation, 316ppm of ancymidol was applied to plants 7 days after seeding(DAS) to 14 DAS in 50mℓ of solution per pot. Three dasys after initial application, another 50mℓ of ancymidol solution was applied to each pot. Thus, each pot received two ancymidol treatments separated by 3 days(i.e. treatment 1 was a ancymidol application both 7 and 10 DAS, treatment 2 was an application on both 8 and 11 DAS, and so on).

Measurements of plant height(from soil to tip of the tallest leaf) and height to the tallest leaf collar were done at 14 and 21 days after seeding. At 2 ~ to 3 ~ day intervals, floral initiation was determined by splitting the shoot of harvested plants and examining the apical meristem under a dissecting microscope. Floral stage 2(visible flower primordia) was used as the criterion for floral initiation. After one plant in a population was recognized to have differentiated an inflorescence, several additional plants were examined to ensure that the response was typical.

For gibberellin anaysis, 14 day old plants treated with ancymidol were harvested at 2 : 00 ~ 3 : 00 PM. Plants were cut at the root-shoot junction, and at the top of the tallest leaf sheath. The resulting shoot samples were frozen in liquid N_2 within 10 min following the removal of the plant from the growth chamber. After lyophilization, the three oldest leaves were removed from the culm. The samples were stored at -20℃ until extracted for GAs. The extraction of gibberellins followed the general procedure of Lee et al.[6]. Deuterated internal standards(25ng each of $^2H_2$ GA_1, $^2H_2$ GA_8, $^2H_2$ GA_{12}, $^2H_2$ GA_{19}, $^2H_2$ GA_{20}, $^2H_2$ GA_{44}, and $^2H_2$ GA_{53} and 50ng of $^2H_2$ GA_3) were added to the extracts following methanolic extraction. Tritiated standards(1500Bq each of [1,2-^3H] GA_1 and [1,2-^3H] GA_4) were also added to monitor recovery throughout the purification procedure. GA′s were purified using a combination of preparatory chromatography(C_{18}, celite, SiO_2) solvent partitioning and HPLC. GA′s were quantified using GC-MS-SIM by calculating the area ratio of non-deuterated GA to deuterated [$^2H_2$] GA′s which had been added during extraction.

RESULTS AND DISCUSSION

To gain background information about which application methods were more reliable to reproduce the same results, a series of preliminary experiments were conducted. Observations of shoot growth, floral initiation dates and tiller growth were made. The results indicated that application of the inhibitors to roots as a soil drench produced more consistent results than application into the whorl or as a spray. This suggests that GA biosynthesis in the roots of sorghum is significant to growth of the whole plant or that the GA biosynthesis inhibitors were effectively absorbed through roots and transported to the site of action. In addition, the soil drench

method avoided localized leaf damage which occurred with the other two methods.

When ancymidol which inhibit *ent*-kaurene hydroxylase activity(between *ent*-kaurene and GA$_{12}$) was applied as a soil drench it inhibited shoot growth in both mutant(*phyB-1*) and wild-type. The height to the tallest leaf(TL) at 14 and 21 DAS was progressively reduced by increasing the concentrations of ancymidol(Table 1). The inhibition of the height of the tallest leaf sheath(TLS) also showed the same trend as TL. Inhibition of tallest leaf sheath length was more severe than that of height to tip of tallest leaf. Mutant(*phyB-1*) produces less tillers than wild-type[1]. Early step GA biosynthesis inhibitors ancymidol reduced height while promoting tillering in mutant(data not shown). GA biosynthesis inhibitors reduced shoot elongation and promoted tillering of sorghum. Generally, applied GA$_3$ reduced tillering of sorghum and other grasses during the period of application[1,2]. Increasing levels of ancymidol reduced plant height and culm height while promoting tillering of *phyB-1* causing them to be more like the wild-type. In addition to the effect on plant height and tillering, other aspects of development were markedly different in treated and untreated control plants. The leaves of inhibitor-treated plants were much darker green in color and wider than those of control plants.

Floral initiation of both genotypes was progressively delayed by increasing the concentrations of ancymidol(Table 2). 316ppm of ancymidol resulted in delay of floral initiation of phyB-1 by nearly 2 weeks and that of wild-type by more than one month. Because inhibitors working at the early steps in the GA pathway limit the substrate flow through the pathway, combinations of ancymidol and GA$_3$ were applied to test GA$_3$ alone overcome growth inhibition and delayed floral initiation. Both growth inhibition and delayed floral initiation were almost completely relieved by simultaneous application of 31.6ppm of GA$_3$. Further, growth was promoted and floral initiation was hastened by all treatments containing GA$_3$(Table 1 & 2). These results clearly demonstrated the role of GAs in floral initiation

Table 1. Plant growth of wild-type and *phyB-1* sorghum seedlings in response to exogenous ancymidol and combinations of GA$_3$ and ancymidol.

Treatment[1]	TL(cm)				TLS(cm)			
	14DAS		21DAS		14DAS		21DAS	
	phyB-1	WT	*phyB-1*	WT	*phyB-1*	WT	*phyB-1*	WT
Control	44.3	33.3	70.3	58.3	13.8	7.4	20.6	12.6
Ancy 3.16	42.7	27.8	59.4	46.7	10.7	6.2	15.4	13.0
Ancy 10	35.2	21.1	49.1	38.5	8.6	4.8	11.6	11.9
Ancy 31.6	23.2	17.6	29.6	25.4	4.9	3.4	6.0	9.3
Ancy 100	17.3	13.5	17.3	14.3	2.8	2.3	3.3	5.6
Ancy 316	13.7	11.9	12.8	10.5	2.3	2.0	2.4	4.3
GA 31	49.4	38.9	78.3	64.9	14.4	9.9	22.8	13.3
GA 31+Ancy 3.16	48.6	38.5	76.7	66.1	13.2	8.5	22.3	12.7
GA 31+Ancy 31	42.7	36.2	75.9	62.3	12.7	7.6	22.5	12.8
GA 31+Ancy 316	42.6	37.4	74.2	63.6	12.3	7.8	20.8	13.1
GA 316	59.2	44.2	85.7	75.4	15.4	10.8	23.6	14.7
GA 316+Ancy 3.16	56.7	45.6	84.6	77.6	15.7	11.3	24.2	13.9
GA 316+Ancy 31	53.7	43.8	85.9	72.3	13.8	11.4	22.1	14.3
GA 316+Ancy 316	52.1	44.9	86.4	71.4	12.9	11.6	23.4	14.5

[1] Treatment concentrations are given in ppm

Table 2. Effect of ancymidol on floral initiation of wild-type and *phyB-1* sorghum

Treatments[1]	Days to floral initiation	
	phyB-1	Wild type
Control	20	28
Ancy 3.16	21	29
Ancy 10	22	33
Ancy 31.6	23	40
Ancy 100	23	45
Ancy 316	33	>60
GA 31	18	26
GA 31 + Ancy 3.16	18	26
GA 31 + Ancy 31	19	27
GA 31 + Ancy 316	21	33
GA 316	18	27
GA 316 + Ancy 3.16	18	26
GA 316 + Ancy 31	19	26
GA 316 + Ancy 316	20	30

[1] Treatment concentrations are given in ppm

Table 3. Effect of ancymidol application on different plant growth stage on floral initiation of *phyB-1* and wild-type sorghum. 316ppm of ancymidol was applied at different days after seeding(DAS).

Application Date	Days to floral initiation	
	phyB-1	Wild type
Control	19	26
5 & 12 DAS	30	>60
	26	45
7 & 10 DAS	23	38
8 & 11 DAS	23	32
9 & 12 DAS	21	33
10 & 13 DAS	20	29
	20	27
11 & 14 DAS	21	28
12 & 16 DAS	20	27

in short day plant sorghum.

The effect of ancymidol application on different days(plant growth stage) on the date of floral initiation was shown in Table 3. Floral initiation was significantly inhibited when ancymidol was applied at early in plant growth. Although applications of ancymidol 9 or 10 days after seeding were slightly delayed floral initiation of

sorghum, but this was not significant. This difference in floral initiation by the application of inhibitor on different growth stage may be explained by different levels of endogenous GAs which are already accumulated in plants before inhibitor application. In other words, GAs levels in the later application may be above a threshold need to permit floral initiation in sorghum. This result was similar to that obtained with CGA 163 935 and BX-112[5]. With increasing CGA and BX concentration, GA_1 concentrations declined linearly. GA_1 levels in 58M(*phyB-1*) averaged $2 \sim 3$ fold higher than those in 90M(wild-type), and floral initiation of *phyB-1* was not delayed. The results presented here indicated that in short day plant sorghum ancymidol applied at early stage of growth inhibited shoot growth and delayed floral initiation. However, ancymidol applied at later stage of growth did not delayed flowering.

Although activity at the enzyme level is an important criterion in determining the mode of action of GA biosynthesis inhibitor, its effect on the concentration of endogenous GAs indicates more directly their function in plants. To demonstrate the relationship between endogenous GA content and growth and flowering in the SD plant sorghum, ancymidol was applied and the content of native GAs was measured. Ancymidol lowered the concentrations of all the early 13-hydroxylation pathway GAs, including GA_{12}, GA_{53} and GA_{44}, as well as lowering concentrations of the GAs later in the pathway, GA_{19}, GA_{20} and GA_1(Table 4). Concentration of 10ppm ancymidol, lowed GA_1 levels of mutant(*phyB-1*) to around 20% control levels. This reduction of GA_1 was closely related to growth retardation by ancymidol(Table 1). In addition, 10ppm of ancymidol attained significant reduction of all 13-hydroxylation pathway GAs in both genotypes.

Nevertheless applied GA biosynthesis inhibitor ancymidol delayed floral initiation and exogenous

Table 4. Effect of ancymidol on endogenous GAs levels in wind-type and *phyB-1* sorghum seedlings. GA levels were measured by GC-MS-SIM. Data are the means of three replicate samples.

Treatments	Genotypes	GA_{12}	GA_{53}	GA_{19}	GA_{20}	GA_1	GA_3	GA_8
					ng/g D.W.			
Control	WT	12.6	60.0	124.2	19.8	10.1	2.1	3.2
	phyB-1	17.8	33.2	95.4	24.0	12.3	3.2	3.9
Ancy 3.16ppm	WT	6.1	20.0	45.3	4.7	3.6	tr	0.6
	phyB-1	3.8	10.4	23.4	5.6	4.7	tr	0.6
Ancy 10ppm	WT	3.4	10.6	16.5	3.4	1.2	tr	0.7
	phyB-1	2.8	8.2	18.3	4.3	2.4	tr	0.3
Ancy 31.6ppm	WT	1.1	2.3	8.7	2.3	0.9	tr	0.4
	phyB-1	1.5	4.3	10.2	3.8	1.4	tr	0.2
Ancy 100ppm	WT	0.8	0.3	3.4	0.9	0.7	tr	0.1
	phyB-1	1.4	0.5	4.2	1.2	0.4	tr	tr
Ancy 316ppm	WT	0.8	tr	1.1	1.0	0.3	tr	tr
	phyB-1	0.8	tr	0.3	0.4	0.4	tr	tr

[1] tr ; peaks too small for accurate intergration.

GA_3 overcome delayed flowering, interpretation of effects of ancymidol on flowering is difficult. There are complex structure-function relationships which often reveal differential efficacy on floral initiation/development versus shoot elongation. GAs which preferentially promote flowering are judged to be florigenic GAs while those which preferentially promote vegetative growth are judged to be growth promoters. When the LD plant *L. temulentum* was given one LD which caused floral induction, there was an increase in putative polyhydroxylated GA-like substances with HPLC retention times between those of GA_8 (three hydroxyls) and GA_{32}(four hydroxyls). The level of this fraction increased 3- to 5-fold on the day after the long day and then subsided. Effect of application of exogenous GAs on flowering not only depends on GA type but dosage and timing. Doses which promoted flowering when applied 11 to 17h before a single inductive dark period, inhibited flowering if applied 24h later. For several GAs, once the optimum amount of a GA was given to be promotive, higher amounts inhibited flowering.

In some long day plants GA biosynthesis inhibitors will inhibit shoot elongation but not floral initiation[14]. The ability of GA_3 to reverse the effect of the inhibition on both growth and floral initiation in sorghum suggests a role for native GAs in sorghum flowering. In sorghum, inhibition of vegetative growth by GA biosynthesis inhibitor is accompanied by a delay in flowering.

摘　　要

단일식물인 수수의 개화에 지베렐린이 관여하는지를 조사하기 위하여 지베렐린 생합성 억제제인 ancymidol을 처리한후 내생 지베렐린의 함량과 개화 및 생육에 미치는 영향을 조사한 결과는 다음과 같다.
1. 지베렐린 생합성 억제제 ancymidol은 공시한 두 품종 모두의 생육을 억제함과 동시에 개화를 지연시켰다.
2. GA_3를 Ancymidol과 동시에 처리할 경우 생육억제와 개화지연이 모두 회복되어 지베렐린이 수수의 개화에 관여함을 보였다.
3. Ancymidol 10ppm은 수수의 모든 지베렐린 (GA_{12}, GA_{53}, GA_{44}, GA_{19}, GA_{20}, GA_1) 합성을 현저히 억제하였다

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