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Circadian Gibberellins Production in Sorghum

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Abstract

The possibility of circadian production of plant hormone gibberellin (GA) was examined in phytochrome B mutant (*phyB-1*) and wild-type sorghum. GA_{12}, GA_{20} and GA_1 levels were found to cycle circadianly in both *phyB-1* and wild-type. The periods (33 h) of GA_{20} and GA_1 cycling in constant light were longer than normal photoperiods in both genotypes and typical average free running periods in plants of 22 to 28 h. The biological clock was thus shown to function properly in *phyB-1*. However, circadian regulation of GAs productions were not clear as compared to circadian ethylene regulation reported by Lee (1996). Although, in sorghum, EOD FR treatment hasten floral initiation, the differences in GA concentrations between treatments and untreated control were generally less dramatic than expected. Thus, it can be concluded that FR does not act primarily by changing absolute levels of GAs but rather by increasing flowering responsiveness to GAs.

Key words : *Circadian rhythm, Gibberellin*

INTRODUCTION

Sorghum *phyB-1* genoptype (phytochrome B mutant) is characterized as a tall, elongated mutant similar to other phytochrome B mutants including *hy3* (long *hypocotyl*) of *Arabidopsis*, the *lh* (*long hypocotyl*) mutant of cucumber, the *lv* mutant of pea, and the *ein* (*elongated internode*) mutant of *Brassica rapa*[1,2,3]. The elongated hypocotyls in these mutants arise primarily from increased cell elongation[4]. To explain this increased cell elongation of these mutant, comparison of absolute gibberellin (GA) levels and resposiveness to GAs between mutant and wild-type were extensively studied. The differences in the phyB mutants are emphasized by the fact that some mutants do not exhibit elevated GA levels, but they do exhibit increased sensitivity to applied GAs (*lv* of pea, *phyB of Arabidopsis*). In contrast, the *ein* mutant of *Brassica* and ma_3^R mutant of sorghum have increased levels of GAs. Another type of phytochrome B mutant, the *lh* mutant of cucumber exhibits increased GA levels under light condition which promote cell division and lower GA levels in light conditions which achive hypocotyl growth via cell elongation[5].

Furthermore, another progress has been made in the field of phytochrome control of GA metabolism. Foster and Morgan[6] and Lee et al.[7] reported the diurnal regulation of GA concentrations in sorghum. Under 12 h photoperiods, rhythmic patterns of GA^{12} and GA^{53} levels in ma_3^R (*phyB-1*) and non-ma_3^R (wild-type) genotypes were similar, peaking at mid-day with minima at

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night. However, GA_{20} and GA1 exhibited a different pattern between ma_3^R and non-ma_3^R genotypes ; levels peaked near dawn in ma_3^R and at mid-day in the non-ma_3^R genotype[6,7]. This result reveals that relative GA concentration differences between ma_3^R and non-ma_3^R genotypes depend on the time of day ; the maximum difference occurs around dawn. The differences in GA metabolism between genotypes is not the absolute content, but a shift in the rhythmic biosynthesis of GA_{20} and GA_1. The timing of the rhythmic pulse of GA_1 is altered or possibly uncoupled in 58M, and this change is correlated with the absence of a functional phytochrome B, altered photoperiodic sensitivity, early flowering, inhibition of tillering and promotion of shoot growth. Recently, Lee[8] discovered a second hormone rhythm in sorghum when he measured the production of ethylene by *phyB-1* and wild-type. There is a strong over production of ethylene by phyB mutant. Most of the ethylene is synthesized in the shoots. Since the rhythm in ethylene release occurs in plants grown on 12 h L/12 h D photoperiods and persists when plants are moved into continuous light or dark, it is clearly a clock-driven, circadian rhythm. Additionally, another circadian expression of the mRNA's from two genes involved in photosynthesis was found. These mRNA's were expressed circadianly in phytochrome mutant and wild-type plant of sorghum. Thus, the functioning and timing of these particular biological clock seem not to be changed by the absence of the phytochrome B from *phyB-1* plant.

Many physiological processes including flowering in plants and reproductive behavior in animals are regulated by an endogenous, still mysterious circadian clock. The expression of many higher plant genes has been shown to be regulated by the circadian clock, including RNA binding proteins[9,10], catalase, nitrate reductase[11], and genes that play a role in photosynthesis, such as the small subunit of ribulose bisphosphate carboxylase (rbcS)[12] and chlorophyll a/b binding protein (CAB)[13,14]. Although, diurnal variation of gibberellins in sorghum have been well established, the possibility of circadian regulation of GAs was not tested. The purpose of this research was to determine the role of phytochrome B on the GA production, and to determine if biosynthesis is circadionally rhythmic.

MATERIALS AND METHODS

Two Sorghum maturity genotypes : *phyB-1* ($ma_3^R ma_3^R$; phytochrome B mutant) and wild-type (Ma_3Ma_3) were used in this study. Characteristics of these two genotypes are described in a previous reports[1,6,7,8,15]. Seeds were germinated and grown in pots (19 cm in diameter, 14 cm in width) filled with a soil mix which was developed to minimize chlorosis problems in pot-grown sorghum. Seedlings were watered with 170 ml 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 300 mol m^{-2} s^{-1} (400−800 nm) measured at the pot surface by a LI-COR Portable Spectroradiometer (LI-1800). Plants were grown for 13 days in a growth chamber under a 12 h photoperiod with 32℃ days and 22℃ night. Begining on day 14 with light on, the plants were subjected to a 48 h period of continuous light, and day time temperatures. Beginging at 0800 on day 13 (light on), and every 3 h for 72 h, plant were harvested for GA analysis.

For the phytochrome control of gibberellin metabolism experiments, plants were grown as described above under 12 h photoperiod in the growth chamber. Beginning upon the day of emergence, plants were treated at the end-of-day (EOD) with 5 min FR or 5 min FR followed by 5 min R. Red (R) light was obtained by covering fluorescent lamps with Roscolux #27 Medium Red theatrical gel, and far-red (FR) was created by covering

incadescent bulb with Roscolux #27 Medium Red plus Roscolux #83 Medium Blue theatrical gel. Fluence rates within the chamber averaged 2 $\mu molm^{-2}s^{-1}$ (600~700nm) and 4 $\mu molm^{-2}s^{-1}$ (600~700nm) for the R and FR source respectively. Control plants received no special light treatments. On day 14, plants were harvested for GA analysis at the light turn on time.

Plants were cut at the root-shoot junction, and at the top of the tallest leaf collar. Prior to extraction, the three oldest leaves were removed from the culm. The extraction of gibberellins followed the general procedure of Lee *et al*[15]. Following methanolic extraction, GA's were purified using a combination of preparatory chromatography, 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

There is clear evidence that GA_{12} levels were regulated by photoperiod in both *phyB-1* and wild-type (Fig. 1). In both genotypes, GA_{12} levels were high during the first two days, and low during the intervening dark period. This regulation may in fact be circadian, as GA_{12} levels decreased during the two subjective dark periods and increased during the intervening subjective day during the 48 h continuous light period. This pattern was expressed most strongly in *phyB-1*. There were neither obvious diurnal nor circadian patterns of GA_{53} and GA_{19} levels in either genotype (Fig. 1). GA_{53} levels increased during the first light period, and decreased during the subsequent dark period, however, this pattern did not extend into the second day. There was no evidence for circadian regulation of synthesis of these two GA's. GA_{20} levels followed a clear diurnal pattern in wild-type, with the highest peaks observed during the afternoon. GA_{20} levels slightly increased late in the 48h continuous light treatment. This occurred during a subjective light off period. In the first 24 h, GA_{20} levels in *phyB-1* were highest at the beginning of each photoperiod, declined during the first day, and increased during the night (Fig. 1). The day time pattern was not repeated during the second day. GA_{20} levels in *phyB-1* peaked late in the continuous light period. In both genotypes, GA_1 levels followed the patterns of GA_{20} levels. In wild-type, GA_1 levels peaked at 1700 h on each of the first two days. GA_1 levels in *phyB-1* also peaked twice during this period, but at 0800 h, out of phase with the peaks in wild-type. GA_1 levels either decreased (*phyB-1*) or remained low (wild-type) as the 48 h continuous light period progressed. The lengths of the observed periods in GA_{20} and GA_1, 33 h in both genotypes, are longer than the typical average free running periods in plants of 22 to 28 h[16]. Nevertheless, since these two genotypes demonstrate circadian rhythmicity of GA_{12}, GA_{20} and GA_1, it may be concluded that these genotypes possess a functioning biological clock.

However, circadian regulation of GAs productions were not clear as compared to circadian ethylene and CAB mRNA regulation reported by Lee[8] and Childs et al[17], respectively. When he monitored ethylene production rates of whole seedlings growing in test tubes under the 12h photoperiod every 3 hours, the genotypes containing *phyB-1* (58M) and wild-type (100M) exhibited rhythmic variations in ethylene production rates. The wild-type showed very small amplitudes in ethylene production rhythms with a peak mid-day to the late in light period. In contrast, there was strong over production of ethylene in the phytochrome B mutant(*phyB-1*; 58M). Ethylene production was sharply increased with lights-on at 8000 and peaked around 1400 (6 h into the 12 h light period). The higher production of ethylene at the middle of day decreased dramatically before lights-off and reached a minima at the middle of the dark period. Ethylene production patterns under a 16 h photoperiod were similar to those under 12 h. Ethylene production in the phytochrome B mutant (*phyB-1*)

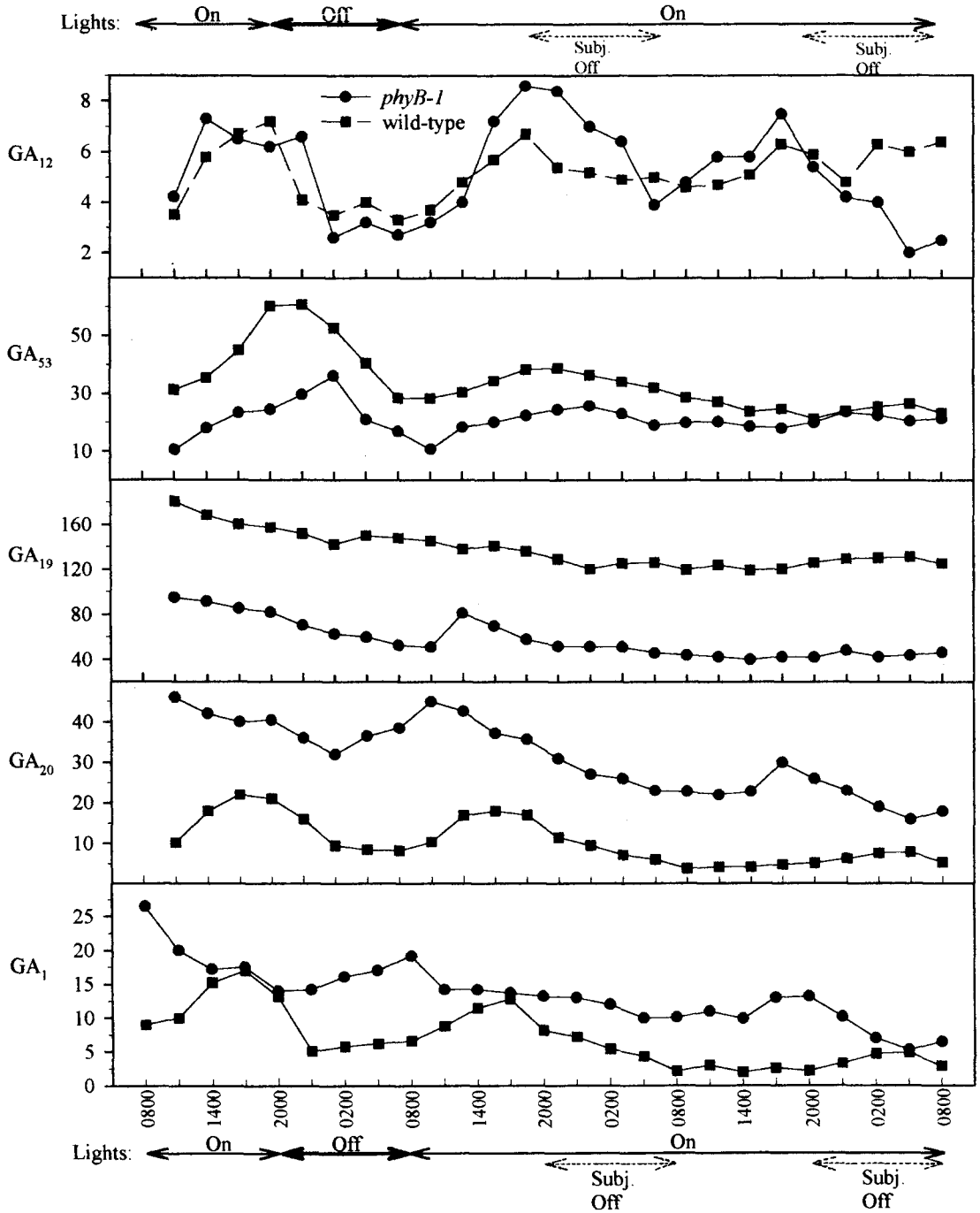

Fig. 1. Circadian GA rhythms in phyB-1 and wild-type sorghum seedlings. Plants were grown under 12h photoperiod for 14 days, after which daytime conditions were imposed for 28 h. Samples were harvested at 3 h intervals begining on day 13 at light on.

Table 1. Gibberellin concentrations of *phyB-1* and wild-type sorghum treated with end of day FR or FR+R. GA levels were measured by GC-MS-SIM.

Genotypes	Treatments	GA_{12}	GA_{53}	GA_{44}	GA_{19}	GA_{20}	GA_1	GA_3
		−ng/g D.W.−						
phyB-1	Control	16.5	34.1	24.3	108.7	26.5	15.4	3.8
	FR	17.8	36.8	23.1	120.1	28.4	16.2	3.6
	FR+R	18.2	39.4	25.8	111.6	25.6	14.8	2.9
Wild-type	Control	10.3	65.3	19.8	167.8	18.7	10.7	2.9
	FR	12.4	69.4	17.5	178.0	18.6	11.3	2.5
	FR+R	10.9	68.9	14.6	159.4	17.3	9.9	3.4

was 10-fold higher than the *PHYB*-containing 100M at peak ethylene production. Furthermore, when seedlings were moved into continuous light, the rhythmic production of ethylene persisted, indicating that this ethylene production is a clock-driven, circadian rhythm. During the first few hours of subjective day after lights-on, ethylene was produced as it was during a normal day and night cycle. Similarly, before the subjective night, ethylene production decreased as it did in a normal end of day. In addition, when seedlings were entrained and then moved to continuous dark, the rhythmic production of ethylene also persisted. Ethylene production was increased during the first subjective day after lights-off, and gradually decreased during the end of the subjective day.

Gibberellin contents of FR, FR+R and untreated control plants are shown in Table 1. The differences in GA concentrations between treatments were generally less dramatic than expected. And also, there was no discernable difference between the genotypes concerning the effect of R or FR treatments. Phytochrome control of gibberellin metabolism has shown before[18]. In those studies, R and FR were shown to control the responsiveness of plants to exogenous GA_{20}. It was concluded that phytochrome controlled the conversion of GA_{20} to GA_1. The approach taken in this experiment was different in that direct measurements were made of the GA content of end-of-day treated plants. EOD FR treatment of sorghum has previously been shown to hasten floral initiation and increase shoot elongation. The intent of this experiment was to show an increase in GA content in plants treated with FR and a reversal of the FR effect by R. However, the phytochrome control of GA metabolism experiments described here were rather disappointing in their lack of clarity. Also, growth of these plants was not significantly affected by either the FR or FR+R treatments. These results suggest that, although FR treatments promoted floral initiation in sorghum, FR does not act primarily by changing absolute levels of GAs but rather by increasing flowering responsiveness to GAs.

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초록 : 수수에서 식물호르몬 지베렐린의 Circadian 리듬

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수수에서 식물호르몬 에틸렌 생합성은 circadian 리듬 현상을 보인다고 이미 보고된바 있다. 따라서 본 실험은 또 다른 식물호르몬 지베렐린 생합성도 에틸렌과 같은 circadian 리듬 현상을 보이는지를 조사한 결과를 요약하면 다음과 같다. 일정기간 동안 12시간 주기의 밤과 낮 조건에서 생육시킨 식물체를 연속광 조건으로 옮긴후 매 3시간 마다 지베렐린의 함량변화를 조사하였다.

1. GA_{12}는 공시한 두 품종 모두에서 뚜렷한 circadian 리듬현상을 보였다.
2. GA_{53}과 GA_{19}는 일체의 리듬현상을 보이지 않았다.
3. GA_{20}과 GA_1은 circadian 리듬현상을 보였지만 연속광하에서 리듬의 정도는 12시간 주기의 정상적인 일장하에 비해 현저히 감소하였다.
4. 수수의 개화는 원적외선광의 조사에 의해 촉진됨에도 불구하고 이들 처리는 내생지베렐린의 함량을 변화시키지 않았다.