Changes in Endogenous Gibberellin Level in Strawberry Plants Induced by Light Breaks¹

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Summary

Three-hour light break with a light intensity of 55 lux from 60-watt incandescent lamps increased the leaf growth of strawberry plants grown under natural short days of about 11 hours, and it was especially true when the light break was applied in the middle of the dark period.

The major gibberellin(GA)-like substance in the acidic ethyl acetate fraction extracted from strawberry leaves and crown was confirmed to be GA₁₉ or a GA-like substance strikingly similar to GA₁₉, by using thin-layer chromatography and dwarf rice bioassay.

The GA₁₉-like substance level in plants treated with light break became higher than that in plants grown under natural short days after 9 days of treatment. But differences in the GA₁₉-like substance level were not so clear among plants treated with light breaks at different times during the dark period. The possibility of GA turnover from the GA₁₉-like substance to other GAs induced by light break was discussed.

Introduction

The forcing system of strawberry plants in Japan consists of autumn-planting and winter-harvesting. Temperature required for growth is provided by covering plants with a plastic film. The day-length of autumn and winter, however, induces so called relative dormancy(8) i.e. retardation of leaf growth under enough higher temperature conditions, and providing smaller yield of fruit. Then, application of gibberellin(GA) (5) and/or long day (LD) treatment by lighting(9, 10, 20, 21, 22) have become popular techniques to prevent the relative dormancy.

Applications of GA and LD have similar effects in respect to increasing the vegetative growth(3, 7, 17, 19, 21). Hence it is very reasonable to consider that an increased endogenous GA level induced by LD might

stimulate the leaf growth, as presented by Porlingis and Boynton(16). Although they indicated that further work was necessary to determine any relationship between amounts of the GA-like substances and day-length, this relationship has not been confirmed yet (4, 12, 18). And endogenous GA-like substances in strawberry plants have also not been identified, though there are a few reports referring to them(1, 6, 13, 16). This work was carried out in order to learn the characteristics of the major endogenous GA-like substances present in strawberry plants and to clarify the relationship between the changes in the endogenous GA-like substance level and the growth of strawberry leaves regulated by day-length.

Materials and Methods

Plant materials

Strawberry plants (*Fragaria* × *ananassa* Duch. cv. Hohkohwase) were grown by the method of forcing system as described pre-

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Fig. 1. Schedule of 3-hour light breaks applied at various times during the dark period.

Light was given from 60-watt incandescent lamps at an intensity of 55 lx.

viously(20). Plants were transplanted to the field on October 3 and protected by plastic houses beginning from October 15. Three-hour light breaks as LD treatment, applied under natural short day conditions with about 11-hour day-length (Fig. 1), were started from October 20 and continued until the end of the experiment. A light intensity of about 55 lux at the top of the plants was provided from 60-watt incandescent lamps.

Extraction, fractionation and thin-layer chromatography (TLC)

Young leaves and upper 1 cm of crowns of strawberry plants were collected from the field and were used as materials for extraction of GA-like substances. The material was ground with 70% aqueous acetone 8 times its fresh weight. The mixture was left overnight at room temperature, and then filtered. After the filtrate was evaporated to remove acetone, the aqueous phase was adjusted to pH 2.5 with phosphoric acid and was extracted 3 times with ethyl acetate. The ethyl acetate fraction was extracted 3 times with 0.75 M phosphate buffer at pH 7.0. The buffer fraction was adjusted to pH 2.5 with phosphoric acid and extracted

3 times with ethyl acetate. The acidic ethyl acetate fraction was dried over anhydrous sodium sulphate and evaporated to dryness.

The residue was redissolved in a small volume of acetone and applied as a narrow band to the starting line of a 20×20 cm silica gel (G) thin-layer plates with a thickness of 0.5 mm. The chromatograms were developed using the following solvent systems: Isopropyl ether/acetic acid (95:5, v/v); Isopropanol/water (4:1, v/v); Isopropanol/ammonia (28%)/water (10:1:1, v/v); n-Butanol/1.5 N ammonia (3:1, v/v). The solvent was allowed to run 10 cm on the plate. After drying, the plates were divided into 10 equal zones from the starting line to the solvent front (the first zone was further subdivided into two zones). Each zone was scraped off and eluted 3 times with 2 ml of 50% acetone. The eluates were reduced to dryness, and redissolved again in 0.1 ml of 50% acetone for bioassay.

Bioassay

Bioassay was carried out by the method of microdrop application using dwarf rice cultivars, Tan-ginbozu and Waito-C as described by Murakami(14). The two dwarfs are known to have different sensibilities to different GAs. The former responds to various GAs, while the latter responds weakly to GAs having no OH group at the C-2 position of the gibbane ring(14).

Results

Growth response of leaves to 3-hour light break.

Light break caused increased petiole elongation and increased leaf blade extension of newly formed leaves. Only leaf length was measured as an index showing the degree of leaf growth, since leaf length had been found to be highly correlated with petiole length or leaf area.

Plants treated with light break showed a slight growth increase after 9 days of treatment (Fig. 2). Differences in leaf growth due to the time of light break became clear after about 23 days of treatment. As previously reported(20), leaf growth was promoted most markedly when light break was

Fig. 2. Growth response of strawberry leaves to 3-hour light breaks applied at various times during the dark period. Length of the 3rd unfolded leaf (the 1st is basal) was averaged for 5 plants.

applied at the middle of the dark period. *Characteristics of endogenous GA-like substance in leaves and crown.*

Five plants grown under natural short days were used for one extraction. TLC was carried out using the solvent system, isopropyl ether/acetic acid (95 : 5, v/v). The results from the bioassays are illustrated in Fig. 3. In the Tan-ginbozu assay, one peak of GA activity was always detected at R_f 0.05~0.2, which corresponded to the known R_f value of GA_3 . But Waito-C dwarf did not respond to the extract of this zone.

Fig. 3. GA activity in the extracts from strawberry leaves and crown following TLC in the solvent system, isopropyl ether/acetic acid (95 : 5, v/v). Acidic ethyl acetate fractions extracted from 5 plants were chromatographed on silica gel (G). T, Tan-ginbozu assay. W, Waito-C assay. *Fresh weight of material.

Fig. 4. Behavior of GA active zone in the extracts from strawberry leaves and crown following T-LC in 4 different solvent systems. Acidic ethyl acetate fraction extracted from 25 plants was chromatographed in the solvent system, isopropyl ether/acetic acid (95 : 5, v/v). Then R_f 0.05~0.3 zone was eluted in 50% acetone, divided into 4 aliquots. Each aliquot was chromatographed in each solvent system and was bioassayed using rice seedlings.

Table 1. Comparison of behavior of GA active zone in extracts from strawberry leaves and crown and from rice and bamboo shoots following thin-layer chromatography in 4 different solvent systems.

Solvent system	R_f of GA_3	Plant material	Zone of growth promotion [*]
Isopropyl ether/acetic acid (95 : 5, v/v)	0.1	Strawberry	0—0.2
		Rice, Bamboo	0.1—0.2
Isopropanol/water (4 : 1, v/v)	0.75	Strawberry	0.7—0.9
		Rice, Bamboo	0.7—0.9
Isopropanol/ammonia(28%)/water (10 : 1 : 1, v/v)	0.65	Strawberry	0—0.1
		Rice, Bamboo	0.05—0.2
<i>n</i> -Butanol/1.5N ammonia (3 : 1, v/v)	0.4	Strawberry	0—0.1
		Rice, Bamboo	0.05—0.1

^{*} Bioassay was carried out using the Tan-ginbozu dwarf of rice. Data of rice and bamboo used are those reported by Murakami (14).

Fig. 5. Changes in GA level in strawberry plants induced by 3-hr light breaks. Acidic ethyl acetate fractions extracted from 5 plants were chromatographed on silica gel (G) in the solvent system, isopropyl ether/acetic acid (95 : 5, v/v). Bioassays were carried out by using rice seedlings. T, Tan-ginbozu assay. W, Waito-C assay. *Time of light break. **Fresh weight of material.

Next, the behavior of the active zone in the Tan-ginbozu assay in 4 chromatographic solvent systems was investigated. Twenty five plants treated with 3-hour light break (10 PM~1 AM) for 30 days were used as material. From the chromatogram developed in the solvent system, isopropyl ether/acetic acid (95 : 5, v/v), the zone at R_f 0.05~0.3 was eluted in 50% acetone and divided into 4 aliquots. Each aliquot was chromatographed in 4 different solvent systems. The behavior of the active zone in each system is given in Fig.4 and summarized in Table 1, in comparison to that of extracts from rice and

bamboo reported by Murakami(14). In the Tan-ginbozu assay, R_f value of the active zone did not correspond to the known R_f value of GA_3 in the solvent systems: isopropanol/28% ammonia/water (10 : 1 : 1, v/v) and *n*-butanol/1.5 N ammonia (3 : 1, v/v). But the active zone behaved almost similarly to the extracts from rice and bamboo shoots in each system.

Changes in endogenous GA level induced by light breaks.

Effect of light break on endogenous GA level was observed after 9 days of treatment. In the Tan-ginbozu assay, the extracts from

plants treated with light break showed higher GA activity at Rf 0.05~0.3 than those from plants grown under natural short days, but differences in the GA activity due to the time of light break were not clear (Fig. 5). In the Waito-C assay, weak activity was detected at the same Rf in some plots. After 16 days of treatment, however, no definite differences in GA activity due to the light break could be found in the Tan-ginbozu assay.

Discussion

Characteristics of endogenous GA-like substance in strawberry leaves and crown.

Acidic ethyl acetate fraction extracted from young leaves and crown of strawberry plants always showed one peak of GA activity at Rf 0.05~0.2 in the Tan-ginbozu assay, when TLC was carried out using the solvent system, isopropyl ether/acetic acid (95:5, v/v). And Rf value of this zone corresponded to that of GA₃. But the Waito-C dwarf did not respond to the extract of this zone. Since the Waito-C dwarf and the Tan-ginbozu dwarf respond to GA₃ almost in the same manner(14), the activity present in this zone may not be due to GA₃. This is supported by the result that the active zone differed in its behavior from GA₃, when TLC was carried out using the solvent systems, isopropanol/ammonia (28%)/water (10:1:1, v/v) and n-butanol/1.5N ammonia (3:1, v/v). The behavior of the active zone in each of 4 different solvent systems was almost similar to that of the active zone from rice and bamboo shoots. It is known that the GA activity in the extracts from rice and bamboo shoots is due to GA₁₉(11,14,15). From these results major GA-like substance present in strawberry leaves and crown is confirmed to be GA₁₉, known as bamboo GA, or a GA-like substance strikingly similar to GA₁₉. It is reported by Porlingis and Boynton (16), Leshem and Koller(13) and Avigdor-avidov *et al.*(1) that 2 or 3 regions of GA activity appeared in the chromatogram loaded with extracts or diffusates of strawberry leaves, buds or crowns. And Goodwin and Gordon(6) presented evidence that different

kinds of GA-like substances were present in the different parts of strawberry plants, but they did not identify the GA-like substances. Although no GA activities were found at Rfs other than that of GA₁₉ in this experiment, further work is necessary to identify other minor GA-like substances in strawberry leaves, buds and crowns, because it is possible that they play some role in leaf growth.

Changes in endogenous GA level induced by light breaks.

Strawberry leaf growth is regulated by day-length(20) as shown in this experiment. Differences in GA₁₉-like substance levels were observed after 9 days of treatment between plants treated with and without light break under natural short days, but they were not observed among plants treated with light breaks at different times during the dark period. This supports the hypothesis that increased endogenous GA level induced by long days may stimulate leaf growth. However the results mentioned above also suggest that there is no quantitative relationship between the GA₁₉-like substance level and the leaf growth when light breaks are applied at different times during the dark period. GA₁₉ is thought to be generally inactive GA. Even if quantitative changes in the GA₁₉-like substance level are found among the different photoperiodic conditions by using other minute methods, it is necessary for confirming the role of GA₁₉-like substance as the regulatory system in photoperiodicity to demonstrate that the GA₁₉-like substance extracted from strawberry plants shows activity in strawberry leaf growth. Zeevaart demonstrated that GA₂₀, which is also thought to be generally inactive, showed higher activity than GA₃ in spinach petiole elongation(25). So the possibility that the GA₁₉-like substance has some effect on the growth of the strawberry leaf cannot be denied. Although the GA-turnover reported by Zeevaart(24), Cleland and Zeevaart(2) and Van den Ende and Zeevaart(23) could not be confirmed in this experiment, the turnover from the GA₁₉-like substance to other active GAs also cannot be denied, be-

cause very weak but significant activity was detected in some plots in the Waito-C assay.

In the experiment reported here, we succeeded in making clear that LD treatment by light break induces the increase in the GA₁₉-like substance level but failed to clarify the direct inter-relationship between endogenous GAs level and leaf growth of strawberry plants varying with the time of light break.

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イチゴ内生ジベレリンの活性に及ぼす光中断の影響

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摘 要

長日によって促進されるイチゴの葉の生長反応にジベレリンが関与しているか知るため、白熱灯により暗期内の種々の時間帯に3時間の光中断を行ったときの内生ジベレリンの活性の変化を調査した。

葉の生長に光中断の影響が現われ始めたのは処理開始後9日目頃であった。その後、処理間に差が認められるようになり、葉の生長は暗期の中央の光中断区で最も促進された。

薄層クロマトグラフィとイネ苗テストを用いた結果、イチゴ茎葉から抽出した酸性酢酸エチル分画に含まれるジベレリン様物質のうち主要なものは、GA₁₉、もしくは

これに極めて類似した物質と考えられた。

光中断処理区における GA₁₉ 様物質の活性は、処理開始後9日目に調査したとき自然短日区より増加していたが処理区間差は明らかでなかった。したがって、イチゴの葉の生長反応を GA₁₉ 様物質の量的変化のみに基づくものと説明することは困難と考えられる。同じ処理開始後9日目には、一般的には活性が低いと考えられている GA₁₉ 様物質の一部が他の活性型のジベレリンに転換した可能性を、わずかではあるがうかがわせる結果が得られたことから、イチゴの葉の生長における活性型のジベレリンの同定、定量が必要と考えられた。