インゲンマメの発育種子および莱におけるジベレリンの変化, 特に発育停止について

誌名	日本作物學會紀事
ISSN	00111848
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巻/号	46巻3号
掲載ページ	p. 371-378
発行年月	1977年9月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat

Japan. Jour. Crop Sci. 46(3) : 371-378 (1977)

Changes of Gibberellins during Seed and Pericarp Development in Common Bean with Special Reference to Abortion

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Received November 19, 1976

Introduction

In Mexico, common bean is an important main crop as well as maize and wheat, but abortion of seeds within developing pods has been known as one of the serious barriers for increasing bean yield. To explain the cause of this seed abortion researches from different fields such as the study of hormones may be necessary. In this report, changes of gibberellins (GAs) during seed and pericarp development was examined since GAs are known to play an important role in seed development.

Materials and Methods

Plant materials: Common bean, *Phaseolus vulgaris* c.v. Cacahuate was grown in pots by the usual procedures in a greenhouse in Chapingo. Pods were collected 5, 10, 15, 20, 25 and 40 days after anthesis and separated into pericarp and seed. After measuring length and weight, they were subjected to extractions. Aborted pods (those which had stopped their growth) were collected between 20 to 35 days after anthesis.

Extraction and fractionation: The materials were homogenized with 80% acetone. The homogenate stood for one night before filtering through cheese cloth and filter paper. The acetone was removed under reduced pressure and the aqueous solution was adjusted to pH 2.2 with phosphoric acid. The aqueous fraction was then extracted 3 times with equal volumes of ethyl acetate. The combined ethyl acetate fraction was further extracted with phosphate buffer at pH 7.0. The buffer was then adjusted to pH 2.2 with phosphoric acid and extracted 5 times with equal volumes of ethyl acetate. The second combined ethyl acetate fraction was dehydrated overnight with anhydrous sodium sulfate and concentrated under reduced pressure.

Thin-layer chromatography and bioassay: Each concentrated extract was dissolved in a small volume of acetone and applied as a 0.4 cm band on 20×20 cm, 0.6 mm thick silica-gel (Kieselgel GF_{254} nach stahl, Typ 60-MERCK) thin-layer plates. The plates were developed in ethyl acetate: chloroform: acetic acid (9:6:1, v/v/v) to a distance of 10 cm. After drying, chromatograms were divided into 10 equal zones (the first zone was re-divided into 2 equal zones), and the silica gel in each zone was placed into a 10 ml beaker. About 3 ml of 50% acetone was added to each beaker. The eluate was transferred to a 5 ml beaker and evaporated to dry. The residue was redissolved in 100 μl of 50% acetone and 5 μl was used for the bioassay by the micro-drop application method, 1 μl each to 5

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plants. The dwarf rice Tan-ginbozu was used for the bioassay. The length of the second leaf sheath was measured 3 days after the application. Test plants were grown at 32°C under continuous light of about 5000 lux. The amount of GAs (the sum of different Rf activities) was expressed as GA_8 equivalent in μg per 100 g fresh weight and in mμg per seed or pericarp.

GA activities on the histograms were separated into two groups for convenience according to their Rf values; from Rf 0.0 to 0.4 was called to fraction I and from Rf 0.4 to 1.0 was called to fraction II.

Rechromatography: Using one extract from seeds (25 days after anthesis) and one from pericarp (20 days after anthesis), rechromatography was made with isopropanol: 28% ammonia: water (10:1:1, v/v/v) as the developing solvent. Fraction I (Rf 0.0–0.4) and fraction II (Rf 0.4–1.0) were separately subjected to thin-layer chromatography using the residue of microdrop bioassay described above. After developing, those are subjected to the same bioassay.

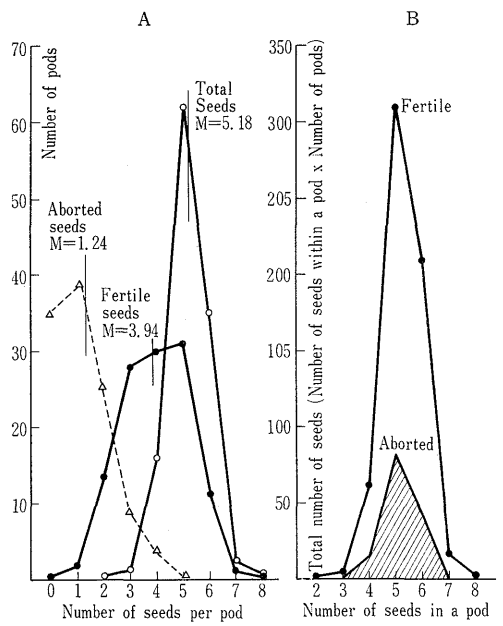

Fig. 1. A: Distribution of total, fertile and aborted seeds.
B: Distribution of fertile and aborted seeds.

Results

Distribution of the number of seeds observed in 115 pods is shown in Fig. 1A. Among all the seeds, about 22% were aborted. According to Fig. 1A, the average number of seeds per a pod was 5.18; however, that of fertile seeds was only 3.94; thus, an average of 1.24 seeds per pod were aborted. This relation is shown in Fig. 1B. The portion shown in black colour is aborted

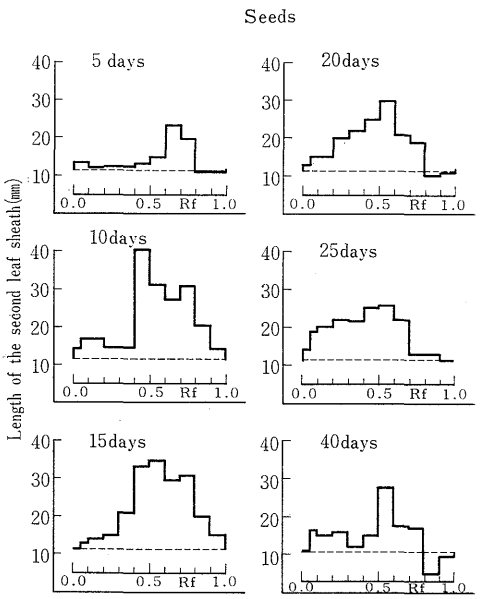

Fig. 2. Histograms showing GA activities in seeds harvested in different days after anthesis. Dwarf rice seedlings (Tan-ginbozu) were used as test plants. Materials used were as follows; 5 days (250 seeds, 0.1 g F.W.), 10 days (250 seeds, 0.2 g F.W.), 15 days (150 seeds, 5.36 g F.W.), 20 days (100 seeds, 25.0 g F.W.), 25 days (100 seeds, 47.8 g F.W.) and 40 days (100 seeds, 89.0 g F.W.). Solvent system used was ethyl acetate: chloroform: acetic acid (9:6:1, v/v/v).

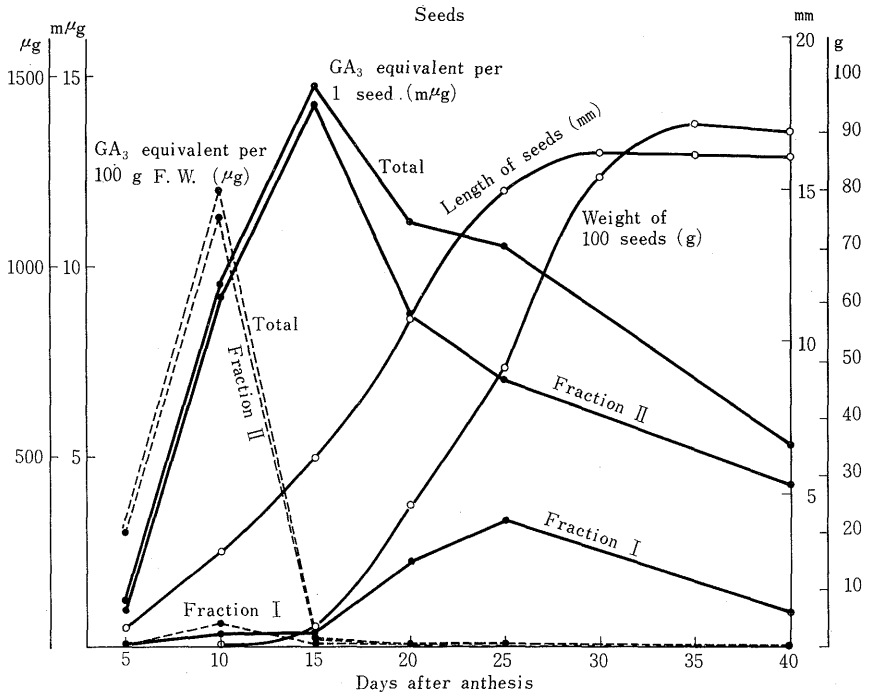

Fig. 3. Changes of GA activities, length and weight of seeds during seed development.

seeds. Thus, it is easy to understand that yield loss attributed to seed abortion within a developing pod is the serious problem.

In addition, abscission of flower buds or young pods is common but was not studied here. All these factors may contribute to the loss of yields. There may be exist many reasons to cause such phenomena, but here we consider only GAs.

Histograms indicating GA activities in seeds harvested in different days after anthesis are shown in Fig. 2. Fig. 3 shows changes in seeds of fresh weight, length and content of endogenous GAs, both per 100 g fresh weight and per seed. The seed length increased gradually and reached a maximum about 30 days after anthesis, on the other hand, the fresh weight increased very slowly until 15 days after anthesis, then increased rapidly and reached a maximum at 35 days after the anthesis.

Changes of GA content in seeds are somewhat different between fresh weight basis and seed basis. When calculated on a fresh-weight basis, the GA content increased rapidly toward the maximum at 10 days after anthesis and then decreased rapidly. On the other hand, GA content per seed reached a maximum at 15 days after anthesis and decreased gradually.

Histograms of GA activities in the extracts from the pericarps are shown in Fig. 4 and changes of fresh weight, length and content of endogenous GAs are shown in Fig. 5. In pericarps, the tendency of changes in GA content within tissues is almost the same as with seeds, but the maximum of GA concentration per 100 g fresh weight was seen at 5 days after anthesis; the first sampling date. This may be due to the

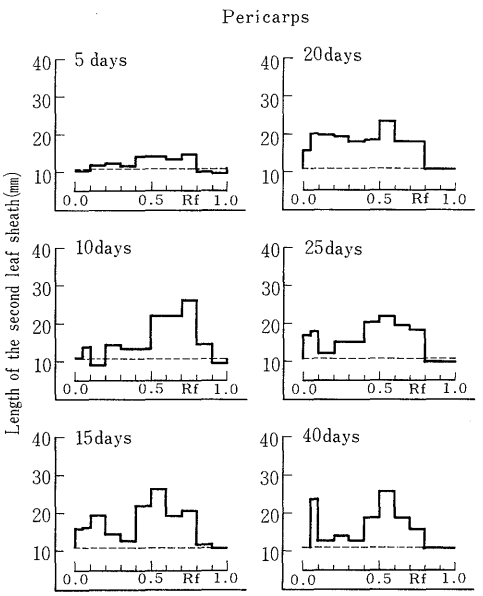

Fig. 4. Histograms showing GA activities in pericarps harvested in different days after anthesis. Dwarf rice seedlings (Tanginbozu) were used as test plants. Materials used were as follows; 5 days (50 pericarps, 4.5 g F.W.), 10 days (50 pericarps, 97.3 g F.W.), 15 days (40 pericarps, 186.4 g F.W.), 20 days (30 pericarps, 183.5 g F.W.), 25 days (30 pericarps, 167.4 g F.W.). and 40 days (30 pericarps, 47.7 g F.W.). Solvent system used was as same in Fig. 2.

fact that the length of pericarps at this stage is only 3.5 cm and the weight of 50 pericarps is only 4.5 gram so that the concentration is high but the content per pericarp is small. On the other hand, the absolute content of GA per pericarp reached a maximum at 20 days after anthesis; this coincided with the date when fresh weight of a pericarp also reached a maximum.

The GA_3 equivalent per seed of fraction II reached a maximum at 15 days after the anthesis and then decreased (Fig. 3), whereas fraction I began to increase rapidly 15 days after anthesis and reached a maximum at 25 days. This tendency is also the same in pericarps (Fig. 5); fraction II reached a maximum at 15 days and then decreased, whereas fraction I reached its maximum at 20 days and decreased.

Fraction I and II from both seeds and pericarps were subjected to rechromatography and developed using a different solvent system. The results are presented in Fig. 6. Distribution of activities between seeds and pericarps is somewhat different, suggesting the existence of different kinds of GAs in the two different tissues. Harvesting the sufficient amounts of aborted pods in same age is difficult, thus aborted pods were collected during 20–35 days after anthesis and separated into pericarps and seeds. The average length of the pods collected was 12.4 cm and that of seeds ranged from 4.0 to 6.5 mm. According to the growth curve of pods (Fig. 5), a length of 12.5 cm was obtained at about 17 days after anthesis. Most pods used in this experiment might have stopped their growth around this time. Fig. 7 indicates that GA activities are greatly reduced in those aborted pericarps and seeds. In this case, comparison of GA concentration in fresh weight basis may be meaningless since aborted ones lose water greatly.

Discussion

Changes in the content of GAs in ripening seeds and pericarps have been surveyed in several plants; *Lupinus luteus* (OGAWA[8]), *Pharbitis nil* (MURAKAMI[6], OGAWA[9]). In most cases GA per seed increased remarkably at the early stage of development, when the seed and pericarp are small and GA content attained its maximum at an early age. This is also the case in common bean as shown in this study. This tendency is much increased when GA concentration was calculated per fresh weight base. HASHIMOTO

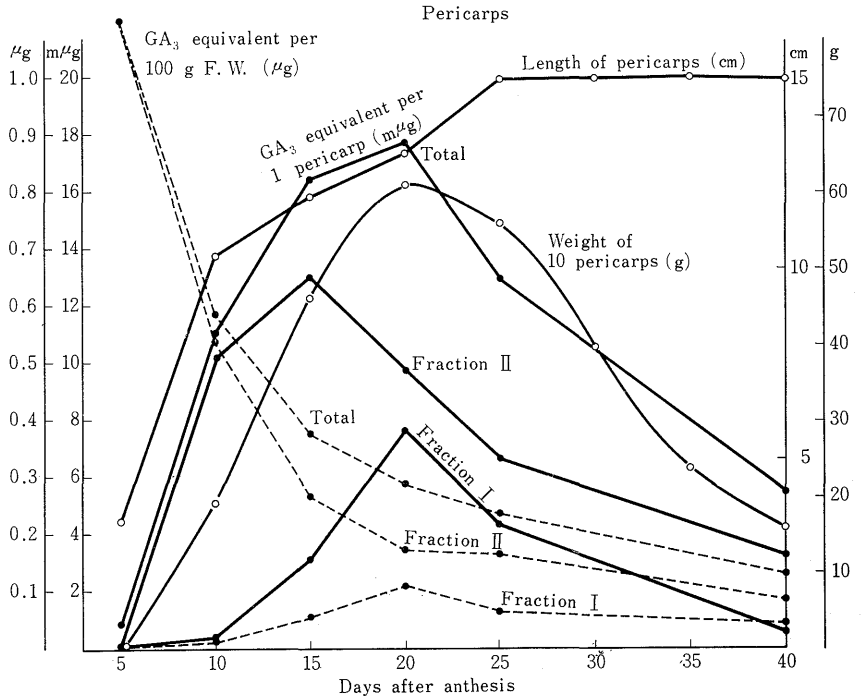

Fig. 5. Changes of GA activities, length and weight of pericarps during development.

and RAPPAPORT[4,5] separated GA-like substances of developing bean seeds into several different fractions. Within them, they concluded that the substances soluble in acidic ethyl acetate may be required for normal development of the bean seeds. They further observed that as the seed matures, neutral substances and one of the acidic butanol-soluble substances increase in concentration.

As shown in this study, the substances soluble in acidic ethyl acetate initially increase and then decreased. The peak of activity in GA content was exist in early stage of pod growth. These results show that the acidic ethyl acetate soluble fraction of GA may play an important role in seed and pericarp development.

ALPI *et al*[1] analysed the growth regulator levels in embryos and suspensors of *Phaseolus coccineus* at two stages of development; heart-shaped embryo and cotyledonary embryo. They showed that at the former stage the total GA activity in the suspensor was about 30 times greater than in embryo and that a dramatic decrease of GA activity had occurred in suspensors of cotyledonary embryos when the level of total GAs in the embryo had increased to 10 times that at the former stage. CIONINI *et al*[2] cultured embryos of *Phaseolus coccineus* in different stage of development in vitro. They found that removal of the suspensor has no effect on the development of embryos which have reached a length of 5 mm. With younger embryos, removal of the suspensor reduces embryo development, the negative effect being the greater the younger the embryo. It was shown that GA_3 can replace the suspensor in heart-shaped and early cotyledonary embryos. Those results seem to support the view that the suspensor plays a

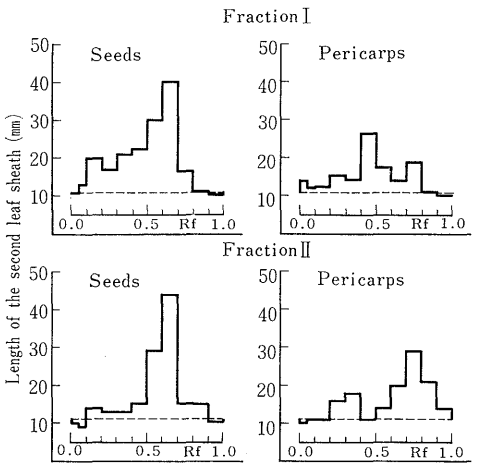

Fig. 6. Histograms showing GA activities in seeds and pericarps of different fractions. In seeds, sample shown in Fig. 2 (25 days after anthesis) was rechromatographed after subdivided into two groups; Rf 0.0–0.4 and 0.4–1.0). In pericarps, sample shown in Fig. 4 (20 days after anthesis) was rechromatographed after subdivided into two groups as well. Solvent system used was isopropanol: 28 % ammonia: water (10:1:1, v/v/v).

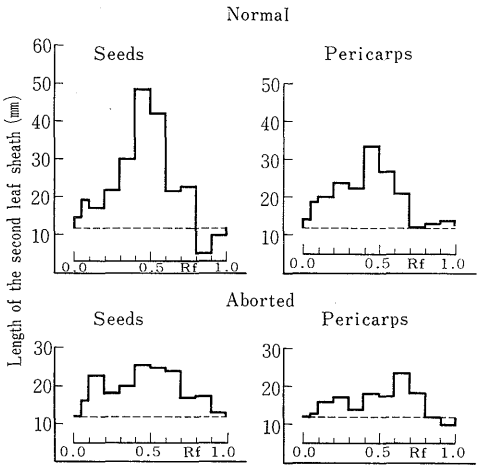

Fig. 7. Histograms showing GA activities in normal and aborted seeds and pericarps. Aborted pods were collected between 20 to 35 days after anthesis. For comparison, normal seeds were collected 30 days after anthesis and normal pericarps were harvested 35 days after anthesis. Materials used were as follows; normal seeds (100 seeds, 83.5 g F.W.), aborted seeds (100 seeds, 1.55 g F.W.), normal pericarps (30 pericarps, 67.9 g F.W.) and aborted pericarps (30 pericarps, 31.2 g F.W.).

role in embryogenesis by acting as a site of synthesis of GAs needed by the embryo.

Recently, TAKAHASHI *et al*[10] analyzed the kinds of GAs in developing bean seeds c.v. Kentucky wonder. According to them, GAs in seeds at 10 days after anthesis were found in the following order of amount; GA_8, GA_1, GA_{29}, GA_5, GA_{17}, $GA_6=GA_{20}=GA_{38}$; however, at 18 days after anthesis this order was changed as follows; GA_1, GA_8, GA_{38}, GA_{37}, GA_{29}, GA_4, $GA_5=GA_6=GA_{20}=GA_{14}$. GAs were found throughout the developmental stages of the bean seeds in the free form (GA_1, GA_4, GA_5, GA_6, GA_8, GA_{17}, GA_{20}, GA_{29}, GA_{38} and GA_{44}), as glucosyl esters (GA_1, GA_4, GA_{37} and GA_{38}) and as the glucoside of GA_8. So many kinds of GAs were found to exist in developing seeds and their amounts are different depending on the stage of development. Thus, it is very difficult to estimate the kinds of GAs from an experiment using only thin layer chromatography and bioassy. However, tendency of change in total amounts may be estimated effectively, since the bioassay system used in present experiment has known recently as the most sensitive one to all kinds of GAs (CROZIER *et al*[3]).

There is no detailed informations on the kinds of GAs in bean pericarps, but results shown in Fig. 6 suggest that the kinds are somewhat different from seeds. MURAKAMI[7] reported similar examples in pea seeds and pericarps.

In aborted seeds and pericarps, GA activity was found to decrease as shown in Fig. 7. At the present it is not known whether this

decrease of GA activity is the result or cause of abortion, but all results presented in this study suggest that endogenous GAs may play an important role in seed and pericarp development in common beans.

Summary

Changes of GAs during seed and pericarp development in common bean were examined in relation to abortion. When calculated on a fresh-weight basis, the GA content increased repidly toward the maximum at 10 and 5 days after anthesis and then decreased rapidly in seeds and pericarps, respectively. Whereas, absolute GA content per seed reached a maximum at 15 days after anthesis and decreased gradually. In pericarps, the absolute content of GA per pericarp reached a maximum at 20 days after anthesis and decreased gradually. Distribution of GA activities between seeds and pericarps is somewhat different, suggesting the existence of different kinds of GAs in the two different tissues. GA activities are greatly reduced in aborted pericarps and seeds.

We would like thank Drs. J. KOHASHI S. and A. LARQUE S. for their kind help during the course of this experiment. We also would like thank Dr. M. ENGLEMAN for reading and improving the manuscript. The senior author also thanks the Japan International Cooperation Agency (JICA) for giving him a oppotunity to join the cooperative program between Japan and Mexico.

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〔和 文 献 要〕

インゲンマメの発育種子および莢におけるジベレリンの変化，特に発育停止について

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メキシコではインゲンマメはトウモロコシ，コムギと共に三大主食の一つであるが，発育中の種子の発育停止は，収量増加にとって重大な障害となっている．品種カカワテを用いて，これら発育停止との関連において発育種子および莢におけるジベレリン（酸性酢酸エチル分画）の変化をしらべた．

発育停止種子は全種子の約22%におよぶ．発育種子におけるGA含量は生体重ベースの相対濃度では開花10日後に，また種子1個当りの絶対含量では開日15日目に最高に達し成熟に伴って漸減した．

また，莢においては生体重ベースの相対濃度では開花5日目に最高を示し急減するが，莢当りの絶対含量では開花15～20日目に最高に達し成熟に伴って漸減した．

再クロマトグラフイの結果から判断すると，種子と莢のGAの種類には共通するものも勿論存在するであろうが，異なるものも含まれる様に思われる．

発育停止した種子や莢では，正常に発育しているものに比較してGA活性が著しく低下していることが認められた．これらの結果より，GAはインゲンマメの種子や莢の正常な発育に重要な役割をはたしているものと思われ，収量増加の障害となっている発育停止の原因追究のためにこの分野の研究が重要であることを示唆した．