# Gibberellins in Immature Seeds of Winged Bean

## — In comparison with some other legumes —

By YUTAKA MURAKAMI,* MASAJI KOSHIOKA** and AWANG SOH BIN MAMAT***

*Department of Applied Physiology, National Institute of Agrobiological Resources (Yatebe, Ibaraki, 305 Japan)
**Department of Farm Chemicals, National Institute of Agro-Environmental Sciences (Yatebe, Ibaraki, 305 Japan)
***Malaysian Agricultural Research and Development Institute (Serdang, Selangor, Malaysia)

Young pods of winged bean (*Psophocarpus tetragonolobus*) have long been used as a vegetable in Southeast Asia. Legumes are one of the most intensively studied plants on endogenous GAs. But no work has been done on GAs of winged bean. Taxonomically the genus *Psophocarpus* belongs to the tribe Phaseoleae.[1] Hyacinth bean (*Dolichos lablab*), kidney bean (*Phaseolus vulgaris*) and yard-long bean (*Vigna sesquipedalis*), which are cultivated in Japan, also belong to the same tribe. Murakami[5,6] reported that endogenous GAs in immature seeds of the Gramineae and those of the Rosaceae can be related to the taxonomic scheme of each family. Thus it was of interest to study endogenous GAs of the immature seed of winged bean in comparison with those of other related legumes.

## Materials and methods

### 1) *Plant materials*

Young pods with immature seeds were taken from hyacinth bean (*Dolichos lablab*), kidney bean (*Phaseolus vulgaris*), winged bean (*Psophocarpus tetragonolobus*) and yard-long bean (*Vigna sesquipedalis*) of the tribe Phaseoleae, and broad bean (*Vicia faba*), pea (*Pisum sativum*) and sweet pea (*Lathyrus odoratus*) of the tribe Viciae. Winged bean was obtained from a local market in Malaysia and Okinawa Branch of the Tropical Agriculture Research Center. Other legumes were cultivated in the field of the National Institute of Agrobiological Resources at Tsukuba. Thirty grams in fresh weight of immature seeds of different growth stages were used for the extraction of endogenous GAs of each legume.

Abbreviations: GAs=Gibberellin As; GC−MS=Gas chromatography-mass spectrometry; GC−SIM=Gas chromatography-selected ion monitoring; TLC=Thin-layer chromatography.

### 2) *Extraction and fractionation*

Extraction, fractionation, thin-layer chromatography, and bioassay were similar to those reported in a previous paper.[3]

Sample material was homogenized in 70% aqueous acetone with a blender, kept for one day at room temperature, and filtered. After the acetone was evaporated, the aqueous solution was adjusted to pH 2.5 with phosphoric acid and partitioned three times against ethyl acetate. The combined ethyl acetate phase was extracted three times with 1 M phosphate buffer at pH 7. The buffer phase was adjusted to pH 2.5 with phosphoric acid and partitioned three times against ethyl acetate to give an acidic ethyl acetate fraction. After drying over anhydrous sodium sulfate, the acidic ethyl acetate fraction was evaporated to dryness.

### 3) *Thin-layer chromatography*

The evaporated extract was taken in a small volume of acetone and subjected to TLC. Thin-layers of Silica gel H were used with the solvent system, isopropyl ether/acetic acid (95:5). The TLC plate was divided into 10 equal zones between the starting line and the solvent front (the first zone was

further subdivided into two zones). The scraped Silica gel of each zone was eluted with 50% aqueous acetone, evaporated to dryness, and dissolved again in 0.1 m*l* of 50% aqueous acetone for bioassay.

*4) Bioassay*

Two dwarf rice varieties, Tan-ginbozu and Waito-C, were used to assay GAs. Tan-ginbozu is known to respond to many kinds of GAs, while Waito-C responds to limited kinds such as C-3-hydroxy GAs.[4] Such specificity between the two dwarfs was used to identify the GAs found in the extracts.

When the second leaf emerged from the first leaf, eluates were applied as a single 1 $\mu l$ droplet to the surface of each coleoptile with a micropipette. Test plants were grown at 32°C under continuous light of about 5000 lux. After 3 days the length of the second leaf sheath was measured with a ruler. The result

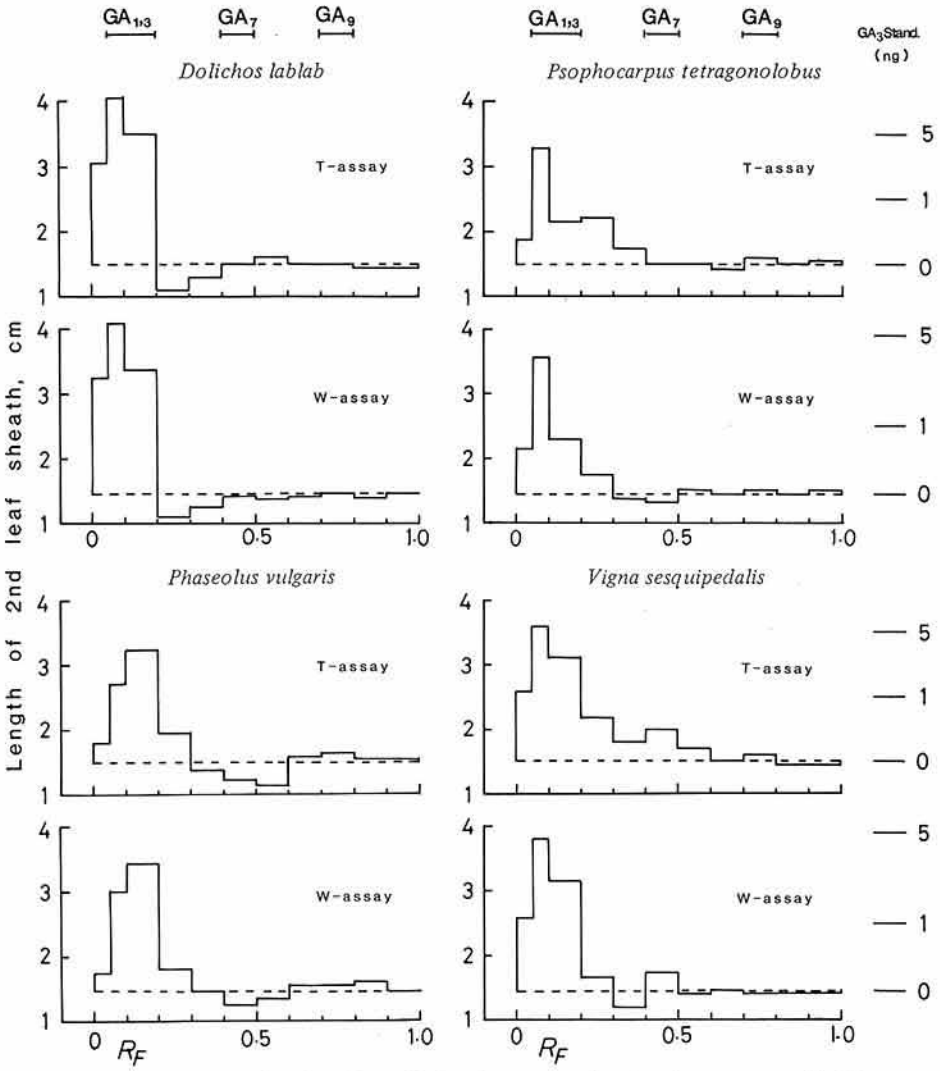


Fig. 1. Histograms showing GA activities of extracts taken from immature seeds of the tribe Phaseoleae

The acidic ethyl acetate fractions were separated with TLC using isopropyl ether/acetic acid (95:5) as the developing solvent. Eleven eluates were tested on Tan-ginbozu rice seedlings (T-assay) or Waito-C rice seedlings (W-assay). This legend also applies to Fig. 2.

was summarized as histograms, each indicating the average of 5 test plants.

The biologically active zone in winged bean was further analyzed by GC-SIM.

*5) Gas chromatography-selected ion monitoring*

A Hewlett Packard 5890 GC and a 5970 B Series Mass Selective Detector, equipped with a splitless injector, were used with a cross-linked methyl silicone capillary column, 12 m long, 0.33 $\mu$m film thickness, and 0.2 mm internal diameter. The head pressure was 9 p.s.i.; E.M. volts, 1600; initial time, 2 min; initial temperature, 100°C; and final temperature, 240°C, with a program rate of 40°C/min. The interface temperature was 250°C. The sample was converted to trimethylsilyl ether of methyl ester (Me, TMsi) using ethereal diazomethane and *N,O*-bis(trimethylsilyl)-trifluoroacetoamide (Wako).

## Results and discussion

Histograms of GA activities of the acidic ethyl acetate fraction of extracts from immature seeds of *Dolichos lablab, Phaseolus vulgaris, Psophocarpus tetragonolobus,* and *Vigna sesquipedalis* are summarized in Fig. 1. Clear GA activities were detected around Rf 0.1 in the histograms of these species by both Tan-ginbozu and Waito-C rice seedlings. Tan-ginbozu seedlings respond to many kinds of GAs, while Waito-C seedlings respond to limited kinds such as C-3-hydroxy GAs.[4] On the basis of biological activity and Rf value, the activity near Rf 0.1 is probably due to $GA_3$-like compound. For the identification of this GA, the eluates from the zone of Rf 0.05–0.2 of *Psophocarpus tetragonolobus* were again chromatographed with a 10:1:1 mixture of isopropanol, 28% ammonia, and water and bioassayed. The biologically active eluate of Rf 0.4–0.6 was further analyzed by GC-SIM after conversion to the TMSi ether of the Me ester. The fragment ions at m/z 506 ($M^+$), 491 (M-15), 447 (M-59), 416 (M-90), 377 (M-129) and 313 (M-193), which are characteristic of $GA_1$-Me, TMSi, were selected for ion monitoring. As shown in Table 1, the fragment ion peaks appeared at the Rt of $GA_1$-Me, TMSi (10.06 min) on capillary GC with the same relative intensity of $GA_1$-Me, TMSi. Thus $GA_1$ was the major GA characterized from immature seeds of *Psophocarpus tetragonolobus.* Yokota et al.[9] reported the identification of $GA_1$ by GC-MS in immature fruits of *Dolichos lablab.* Hiraga et al.[2] examined in detail the GAs in developing seeds of *Phaseolus vulgaris.* They isolated $GA_1$, $GA_8$, and $GA_{38}$ and identified $GA_4$, $GA_5$, $GA_6$,and $GA_{37}$ by GC-MS. From the biologically active fraction on TLC of immature seeds extract of *Vigna sesquipedalis*, $GA_1$ has been identified by GC-SIM (Koshioka and Murakami, unpublished data). $GA_1$ seems to be widely distributed in immature seeds of the tribe Phaseoleae.

Histograms of Fig. 2 show GA activities of the acidic ethyl acetate fraction of extracts from immature seeds of *Lathyrus odoratus, Pisum sativum*, and *Vicia faba*. GA activities were detected when the eluate was assayed with Tan-ginbozu seedlings. But no clear GA activity was found when Waito-C seedlings were used as the test plant. This means that these legumes do not contain C-3-hydroxy GAs as the active GA in immature seeds. The results published so far indicate that immature seeds of *Pisum sativum* contain $GA_9$, $GA_{17}$, $GA_{19}$, $GA_{20}$, $GA_{29}$, $GA_{44}$, and $GA_{51}$.[8] Sponsel et al.[7] identified $GA_{17}$, $GA_{19}$, $GA_{20}$, $GA_{29}$, $GA_{44}$, and $GA_{53}$ in extracts from immature seeds of *Vicia faba.* Koshioka and Murakami identified $GA_9$ in the nonacidic ethyl acetate fraction of immature seeds of *Lathyrus odoratus* by using GC-SIM (unpublished data). All these GAs are not hydroxylated at C-3 and are inactive in the Waito-C seedling test. Thus *Pisum sativum, Vicia faba*, and

**Table 1. GC-SIM relative intensity data from extract of immature seeds of *Psophocarpus tetragonolobus,* and authentic $GA_1$**

| GA | Retention time on GC-SIM | Peaks in SIM spectrum with relative abundance in parentheses (m/z value) | | | | Identity |
|---|---|---|---|---|---|---|
| Compound | 10.06 min | 100 (506) | 10 (491) | 10 (447) | 3 (416) | $GA_1$-Me, TMSi |
| | | 19 (377) | 12 (313) | | | |
| $GA_1$-Me, TMSi | 10.06 min | 100 (506) | 8 (491) | 11 (447) | 3 (416) | |
| | | 20 (377) | 13 (313) | | | |

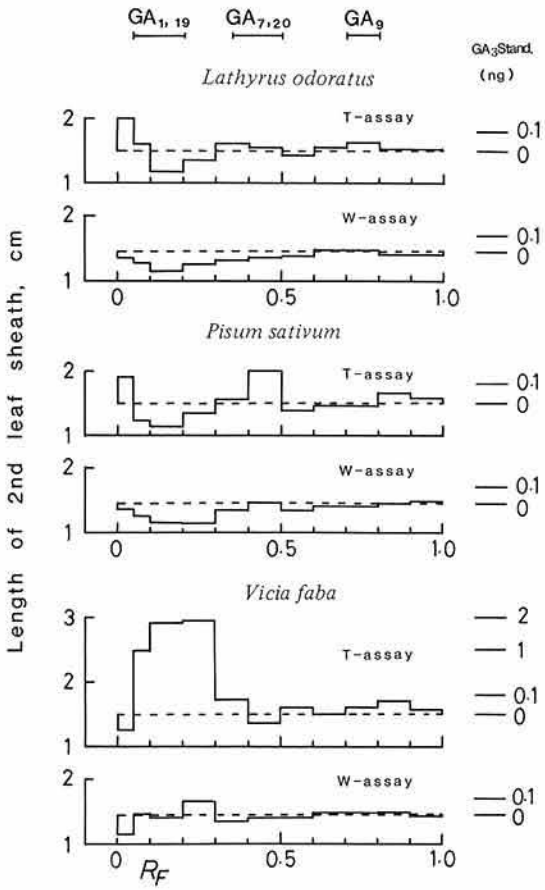


Fig. 2. Histograms showing GA activities of extracts taken from immature seeds of the tribe Viciae

*Lathyrus odoratus*, which are members of the tribe Viciae, do not contain $GA_1$ in immature seeds. *Psophocarpus tetragonolobus* is a species of the tribe Phaseoleae.[1] Both tribes belong to the same subfamily Lotoideae. Chemotaxonomically as well as taxonomically *Psophocarpus tetragonolobus* resembles other members of the Phaseoleae in the presence of $GA_1$ in developing seeds.

## Summary

Immature seeds of *Dolichos lablab*, *Phaseolus vulgaris*, *Psophocarpus tetragonolobus*, and *Vigna sesquipedalis* of the tribe Phaseoleae and those of *Lathyrus odoratus*, *Pisum sativum*, and *Vicia faba* of the tribe Viciae were analyzed for endogenous GAs. The acidic ethyl acetate fractions were subjected to TLC. Part of each eluate from TLC was tested in the rice seedling bioassay. The remainder of biologically active eluate of *Psophocarpus tetragonolobus* was analyzed by combined gas chromatography-selected ion monitoring. $GA_1$ was identified as a major endogenous GA of immature seeds of *Psophocarpus tetragonolobus*. Members of the Phaseoleae contained C-3-hydroxy GAs which are active in both Tan-ginbozu and Waito-C assay. But GAs in immature seeds of the Viciae were found not to be hydroxylated at C-3 using rice seedling bioassay.

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