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# Gibberellins in Buds of *Euphoria longana* Lam. during different stages of growth

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**Abstract.** Buds of longan (*Euphoria longana* Lam.) trees, cv. Fen Kur were collected at intermittent flushing, bud dormancy, and floral initiation for analysis of gibberellin (GA) content. The highest concentrations of free $GA_1$-like and $GA_8$-like were found during intermittent flushing, diminishing thereafter. The concentrations of $GA_9$-like and $GA_{32}$-like increased immediately during bud dormancy and floral initiation, respectively. $GA_{32}$-like was likely an important factor in eliciting strong flowering response. Floral initiation was induced by exogenous injection of 2,2-dimethyl $GA_4$ and 2,2-dimethyl $GA_7$.

**Keywords:** Gibberellin; Growth stage; Longan.

## Introduction

It is clear that gibberellins (GAs) are implicated in several aspects of floral initiation in certain thermoperiodic and photoperiodic plants (Evans et al., 1990; Guttridge, 1963; Jones and MacMillan, 1984; Pharis et al., 1987;Tromp, 1982; Michniewicz and Lang, 1962). Different GAs have different affects on floral initiation, even when applied to the same species at the same stage of development. Tromp (1982) reported that while $GA_3$, $GA_7$, and $GA_{4+7}$ inhibited flowering in apple, $GA_4$ was ineffective. Michniewicz and Lang (1962) studied the affect of $GA_{1-9}$ on floral induction of five species and found that some GAs could induce flowering under noninductive conditions, whereas other GAs could not. Pharis et al. (1987) found that the florigenic properties of a GA increase as its degree of hydroxylation increases.

Recently, Nishijima et al. (1989, 1990, 1992) found that the GA sensitivity of the microdrop test was enhanced when the plants in the assay were pre-treated with uniconazole [S-3307; (±)-(E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazole-1-yl)-1-penten-3-ol], an inhibitor of GA biosynthesis. In this paper, I present data on some of the endogenous GAs of *Euphoria longana* Lam., determined during different growth stages of buds by the methods of Nishijima et al. The results indicate different specificities of GAs to intermittent flushing on the one hand and to bud dormancy and floral initiation on the other.

## Materials and Methods

### Plant Material

Eighty buds were detached each time from longan (*Euphoria longana* Lam.) trees, cv. Fen Kur (12 years old), in an orchard at Kaohsiung, Taiwan. Collections were made on August 16 (middle of intermittent flushing: After all the mature and maturing leaves are removed, the apical meristem is finally revealed as a small group of cells located between the youngest leaf primordia. The young bud flushes intermittently, and so it is called intermittent flushing), October 20 (end of intermittent flushing), November 30 (middle of bud dormancy: The onset of bud dormancy appears to be directly related to the cessation of extension growth), December 26 (end of bud dormancy), January 4 (just prior to floral initiation), and January 12 (floral initiation: The flattening and elongation of the apical meristem, and the concurrent appearance of flower primordia). Bud development was observed by a non-destructive replica technique for scanning electron microscopy (Hernández et al., 1991), allowing individual meristem surfaces to be imaged sequentially. Buds for gibberellin extraction were weighed, immediately frozen in liquid $N_2$, and freeze-dried.

## Extraction and Analysis Procedures for Gibberellins

The equivalent of 3 g dry weight of longan buds was homogenized and extracted four times with 80% (v/v) methanol (500 ml). The methanol extract was concentrated in vacuum and the residue was diluted with $H_2O$ to 2 ml, mixed with 0.5 g Celite, dried in a gentle air stream, and loaded onto a $SiO_2$ partition column (prepared from 5 g of deactivated Woelm $SiO_2$ slurried in 95/5 v/v ethyl acetate/n-hexane). This was first eluted with 80 ml ethyl acetate/hexane (95/5, v/v) to remove most free GAs, and then with 100% methanol (150 ml) to remove highly water soluble GAs and GA conjugates. The ethyl acetate/hexane eluate was dried in vacuum, and the 100% methanol eluate was neutralized with 0.1 N $NH_4OH$ and then dried in vacuum. Both were adjusted to pH 8.0 with 0.1 N NaOH and then each was extracted three times: the fraction containing free GAs with hexane, and the fraction containing GA glucosyl conjugates with ethyl acetate. The hexane and ethyl acetate were discarded. The aqueous phase was diluted with $H_2O$ to 100 ml, adjusted to pH 3.0 with 3 N HCl, and extracted three times with ethyl acetate. The ethyl acetate was evaporated in vacuum to give an acidic ethyl acetate fraction. The acidic ethyl acetate was then chromatographed on a reversed-phase $C_{18}$ μBondapak (Waters Associates) HPLC column, eluting with a linear gradient of methanol (30% to 100%) in 1% aqueous acetic acid, run in 25 min; flow rate 2.0 ml/min. One-minute fractions were collected and dried in vacuum before a bioassay with 1/400 aliquots. Authentic $GA_1$ and $GA_8$ were purchased from J. MacMillan (Univ. of Bristol), and $GA_9$ and $GA_{32}$ were their retention times based on literature values (Pharis et al., 1987 and Koshioka et al., 1988, respectively). The dwarf rice (Oryza sativa L. cv. Tan-ginbozu and Waito C.) microdrop method developed by Nishijima et al. (1989, 1990, 1992) was used to assay each fraction. Tan-ginbozu is known to respond to many kinds of GA, while Waito-C responds only to a limited range of GA, such as C-3-hydroxy GAs (Murakami, 1970). The differences of specificity between the two dwarf rices were used to

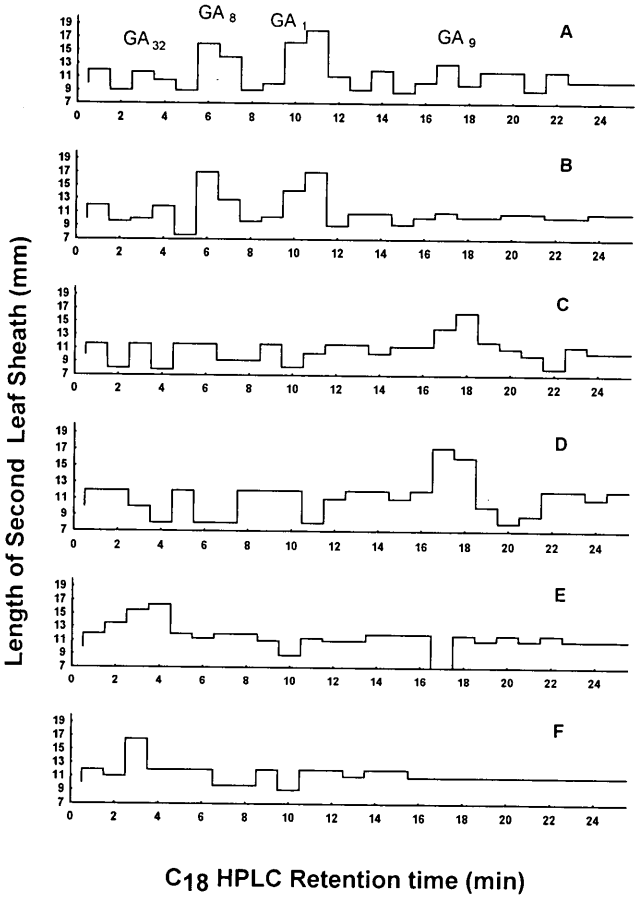


**Fig.1.** Bioassay responses of reversed-phase $C_{18}$ HPLC fractions from extracts of shoot apices harvested at different growth stages of buds Microdrop assay was used, at 1/400 dilution, with cv. Tan-ginbozu rice seedlings. All apex samples were approx. 1 mg dry weight. **A**) middle of intermittent flushing; **B**) end of intermittent flushing; **C**) middle of bud dormancy; **D**) end of bud dormancy; **E**) just prior to floral initiation; **F**) floral initiation.

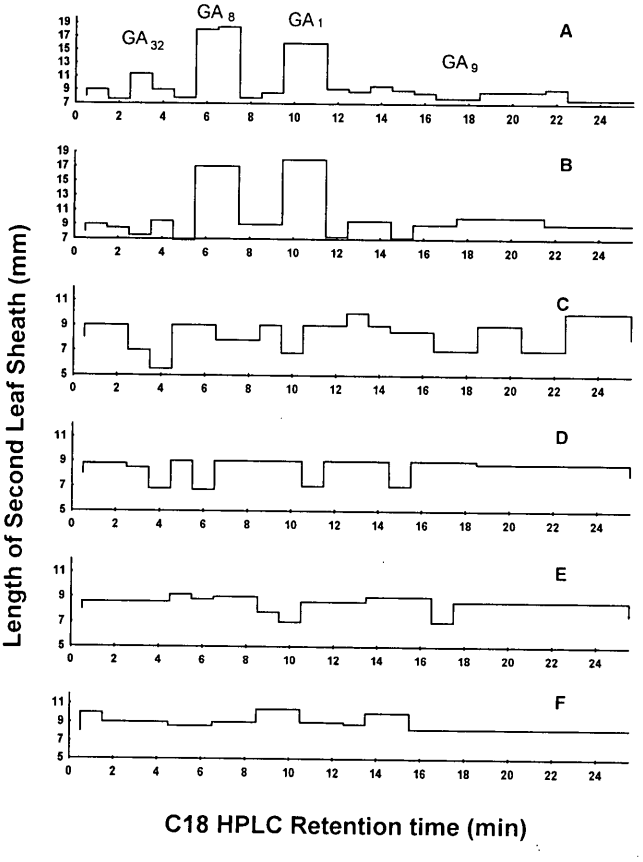


**Fig.2.** Bioassay responses of reversed-phase $C_{18}$ HPLC fractions from extracts of shoot apices harvested at different growth stages of buds. Microdrop assay was used at 1/400 dilution, with cv. Waito-C rice seedlings. All apex samples were approx. 1 mg dry weight. **A**) middle of intermittent flushing; **B**) end of intermittent flushing; **C**) middle of bud dormancy; **D**) end of bud dormancy; **E**) just prior to floral initiation; **F**) floral initiation.

**Table 1.** Influence of 2,2-dimethyl $GA_4$ and of 2,2-dimethyl $GA_7$ on flowering

| Time of Application | | [a]Flowering Response | | | | | |
|---|---|---|---|---|---|---|---|
| | | Apex length | | | Initiation | | |
| | | 2,2-dimethyl $GA_4$ | 2,2-dimethyl $GA_7$ | Control | 2,2-dimethyl $GA_4$ | 2,2-dimethyl $GA_7$ | Control |
| | | mm | | | % | | |
| Days before flower bud initiation | 21 | 0.5±0.1 | 0.5±0.1 | 0.5±0.1 | 0 | 0 | 0 |
| | 14 | 0.6±0.1 | 0.8±0.1 | 0.5±0.1 | 2±0.1 | 5±0.1 | 0 |
| | 7 | 0.8±0.2 | 1.2±0.1 | 0.6±0.1 | 6±1.0 | 13±1.0 | 0 |
| | 0 | 1.2±0.1 | 1.7±0.2 | 0.8±0.1 | 10±1.0 | 15±1.0 | 5±0.1 |
| Days after flower bud initiation | 7 | 1.8±0.2 | 2.8±0.2 | 1.2±0.2 | 18±1.5 | 28±2.0 | 17±1.0 |
| | 14 | 2.3±0.2 | 3.4±0.2 | 1.7±0.2 | 34±1.8 | 50±2.0 | 28±2.1 |

[a]The apex length and flower bud initiation were recorded 13 d after treatment.

identify the GAs found in the extracts. The bioassay procedure used is similar to the microdrop method (Murakami, 1968) except for the treatment of the dwarf rice seed with uniconazole. Briefly, seeds of the dwarf rice cultivars (Oryza sativa L., cv. Tan-ginbozu and cv. Waito-C) are sterilized with Benlate (0.1% [w/v], Du Pont, Del., U.S.A.) and soaked in water containing 20 mg/l of uniconazole (Sumitomo Chemical Co., Japan) for 24 h in darkness at 30 °C. The seeds are then washed with water and germinated in water under the same conditions. When the coleoptiles are approx. 4 mm long, seven seedlings are planted in a vial (18 mm diameter ×58 mm high) containing 0.8 % (w/v) agar and incubated for 72 h at 30 °C under continuous irradiation (80 µmol $m^{-2}$ $s^{-1}$).

## GA Application

GA was applied by injection of 10 µl (50 µg) of an aqueous solution of 2,2-dimethyl $GA_4$ (Sigma) or 2,2-dimethyl $GA_7$ (Sigma) into the central part of the shoot apex. Control plants were treated with solvent alone.

## Results and Discussion

Gibberellin 1, 8, 9, and 32 were identified by comparing their retention times and bioassay results with those of authentic samples ($GA_1$ and $GA_8$) and literature values ($GA_9$ and $GA_{32}$) (Fig. 1). In the dormant buds, a peak of bioactivity near the retention time of $GA_9$ was present, but varied quantitatively depending on the stage. All GA-like substances present during vegetative budding peaked at or near the retention time of $GA_1$ and $GA_8$, on a dry-weight basis. It is noteworthy that $GA_1$ and $GA_8$ are C-13OH-GAs, suggesting that the early 13-hydroxylation GA-biosynthesis pathway operates in the buds during vegetative intermittent flushing. Assay with Tan-ginbozu seedlings indicated a different pathway for a large part of the biological activity of C-13H-$GA_9$-like substance in the dormant buds, but no clear GA-like activity was found when Waito-C seedlings were used as the test plant (Fig. 2). This means that dormant longan buds do not contain C-3-hydroxy GAs as the active GA.

There was a significant increase in putative polyhydroxylated $GA_{32}$-like substance in the shoot apex following floral initiation (Fig.1), but the bioactive free GA-like substances diminish. $GA_{32}$-like proved to be most like a promoter in eliciting floral initiation. $GA_9$ and $GA_{32}$ are not hydroxylated at C-3, and are inactive in the Waito-C seedling test. Luna et al. (1990) reported that the presence of endogenous $GA_1$/$GA_3$ does not increase immediately prior to bud break in peach flower bud. High $GA_1$/$GA_3$ concentrations are present, however, during those floral stages where the rates of growth and cellular differentiation of (mainly fertile) verticils can be influenced. Our results indicate that different GAs have different effects during different growth stages of longan buds. Our quantative analyses of GAs in the buds of *Euphoria longana* Lam. are preliminary. Determination of endogenous free and bound GA levels during vegetative bud → bud dormancy → bud break using more definitive methods (e.g. GC-SIM with stable-isotope-labeled internal standard) is needed.

Exogenous injection of 2,2-dimethyl $GA_4$ and 2,2-dimethyl $GA_7$ can induce flowering (Table 1) but has no

affect on the sex expression of flowers (data not presented). Application to the apex of even 50 μg per plant may not raise the apical levels beyond the physiological range. Exogenous application of GAs is most effective just before the end of bud dormancy.

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# 龍眼不同生長期之 gibberellin 活性

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龍眼（品種：粉殼）於間歇性抽梢期，休眠期及花芽分化期之各期分析芽體之 gibberellin 活性。間歇性抽梢期之 $GA_1$-like 及 $GA_8$-like 之活性甚強，隨後兩 GA-like 之活性消失。休眠期芽體之 $GA_9$-like 活性與花芽分化期之 $GA_{32}$-like 均有明顯的活性。$GA_{32}$-like 之活性增加似乎是引起花芽分化之重要因素。2,2-dimethyl $GA_4$ 與 2,2-dimethyl $GA_7$ 之外加處理顯著地促進龍眼之花芽發育。

**關鍵詞**：Gibberellin；生長期；龍眼。