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# Timing of Floral Differentiation in Four Avocado (*Persea Americana* Mill.) Cultivars on the Main Island of Okinawa Prefecture, Japan

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## Abstract

We conducted a morphological investigation of flower bud differentiation in four cultivars of avocado (*Persea americana* Mill.): 'Pinkerton' (Guatemalan hybrid), 'Simmons' (West Indian), 'Hass' (predominantly Guatemalan × Mexican), and 'Monroe' (Guatemalan × West Indian). The avocado cultivars were planted on the main island of Okinawa Prefecture, which is the only subtropical area of Japan. Buds were collected on 15 November, and 1 and 19 December, 2011, and on 4 and 17 January 2012. The buds were sectioned longitudinally at a thickness of 15 µm through paraffin sectioning, double-stained with Safranin O and Fast green, and observed under an optical microscope to morphologically determine the flower bud differentiation stage. Flower bud differentiation occurred earliest in 'Pinkerton' in mid-December, followed by 'Simmons' in early January and 'Hass' in mid-January. Flower bud differentiation was not observed in 'Monroe' during the study period.

**Keywords:** Dome-shaped shoot apex; Flowering period.

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## 1. Introduction

Avocados have high nutritional value [1-4] and are widely consumed in Japan. A total of 76,694 tons of avocados were imported into Japan in 2021, mainly from Mexico (64,472 tons, 84%; [5]). Takenaga, *et al.* [6], compared Japanese and foreign avocados and reported that there was no significant difference in liquid and fatty acid composition. Therefore, it is desirable to increase domestic avocado production in Japan. Under recent global warming, avocado cultivation has become more feasible throughout Japan. Avocado cultivars are broadly classified as Mexican, Guatemalan, or West Indian according to their original distribution ranges [7]. Among these groups, Mexican avocado cultivars exhibit the best cold tolerance and are therefore cultivated in temperate regions [8]. On the other hand, 'Hass', which is mainly Guatemalan and has some Mexican genes, is vulnerable to low temperatures [9]. Thus, avocado production in Japan is focused mainly on Mexican cultivars and their hybrids. In 2019, the avocado cultivation area of Japan reached 23 ha, with 12.8 tons of fruit produced mainly in Wakayama, Ehime and Kagoshima prefectures [10].

The main obstacles to domestic avocado production are its low fruit setting rate compared to the number of flowers, and the high rate of fruit drop. Inoue and Takahashi [11], reported a maximum avocado fruit-bearing rate of 0.038% in Japan. Therefore, there is an urgent need to develop cultivation techniques to improve the avocado fruit bearing rate. Clarifying the flower bud differentiation period of each cultivation area is crucial for achieving fruit setting stability, and for establishing cultivation techniques such as appropriate tree nutrition and moisture and temperature control. The transition period from vegetative to reproductive growth has been reported for the 'Hass' [12], and a morphological study examined flower bud differentiation stages of avocado in the temperate climate of Shizuoka Prefecture, Japan [13]. However, flower bud differentiation morphology has not yet been investigated in subtropical Japan.

In this study, we conducted a morphological investigation of flower bud differentiation stages among four avocado cultivars of Mexican, Guatemalan, and West Indian origin on main island of Okinawa Prefecture, which is the only subtropical region of Japan.

## 2. Material and Method

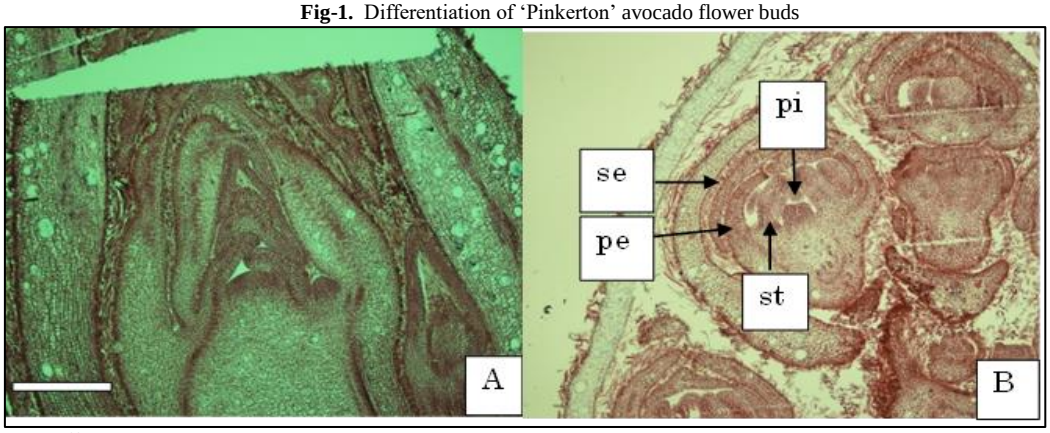
We studied the flower bud morphology of four-year-old avocado plants of the following cultivars: 'Pinkerton' (Guatemalan hybrid: 'Rincom'×'Hass'),'Simmons' (West Indian), 'Hass' (predominantly Guatemalan x Mexican)

and ‘Monroe’ (Guatemalan x West Indian) as described previously, these lines[14]. Two trees of each cultivar were planted in Yaese-cho, Okinawa Prefecture. The trees were sampled on 15 November, and 1 and 19 December, 2011, and on 4 and 17 January 2012. We selected three ca. 15-cm non-fruit-bearing branches grown in the current year at a near-horizontal angle. Two buds from the tip of each branch were collected from two trees of each cultivar (i.e., 12 buds per cultivar). The buds were immediately preserved in formalin acetic acid alcohol solution (50% ethanol: formaldehyde solution : acetic acid=90 : 5 : 5). Then, the buds were sectioned longitudinally to a thickness of 15 µm through paraffin sectioning, and double-stained with Safranin O and Fast green. Under an optical microscope, we conducted morphological observations of flower bud differentiation, which is distinguished by the thickening of the growing point, completion of the flower bud primordium, and first appearance of the primary projections of the perianth [13]. We also observed the avocado flowering stages. Avocado flowering begins when the buds begin to open continuously; the full bloom stage occurs when ca. 80% of all flower buds have opened, and the end of the flowering period occurs when 50% of flowers have turned brown or dropped [15]. Japan Meteorological Agency data from the nearest station to the cultivation area (Nanjo City, Okinawa Prefecture) were used to obtain the seasonal maximum, minimum and average air temperatures during the experiment period.

## 3. Results and Discussion

‘Pinkerton’ avocado buds collected on 1 December 2011 had a flat growing point and no morphological flower bud differentiation was observed (Fig. 1A). Buds collected on 19 December exhibited the formation of sepal, petal, stamen, and pistil primordia; thus, flower bud differentiation was observed (Fig. 1B).

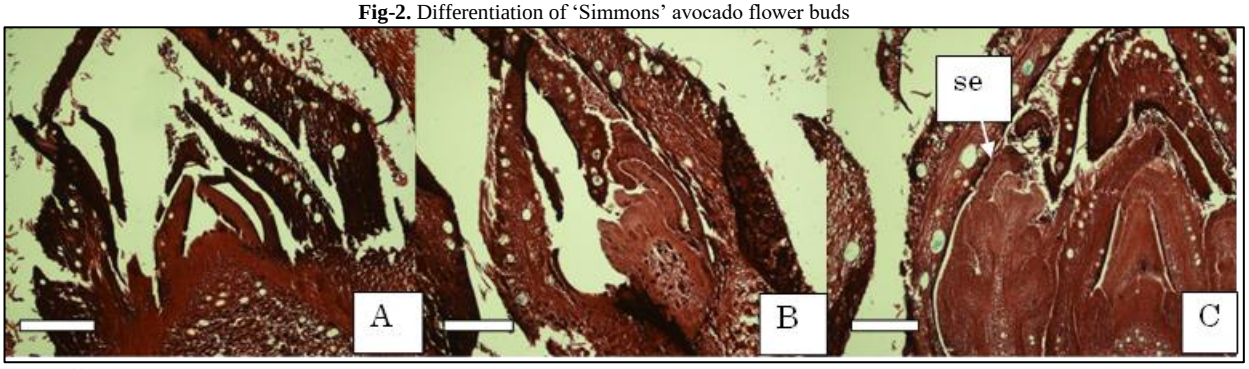
**Fig-1.** Differentiation of ‘Pinkerton’ avocado flower buds




A: Undifferentiated bud (1 December 2011)
B: Differentiated buds (19 December 2011)
pe : petal primordium pi : pistil primordium
se : sepal primordium st : stamen primordium
Scale bar length indicates 500 µm.

Morphological flower bud differentiation was not observed in ‘Simmons’ buds collected on 19 December, 2011 (Fig. 2A). Buds collected on 4 January 2012 were in the early stage of flower bud differentiation, as the shoot apex had thickened and begun to acquire a flattened or dome-like shape (Fig. 2B). Sepal primordia were observed in buds collected on 17 January 2012 (Fig. 2C).

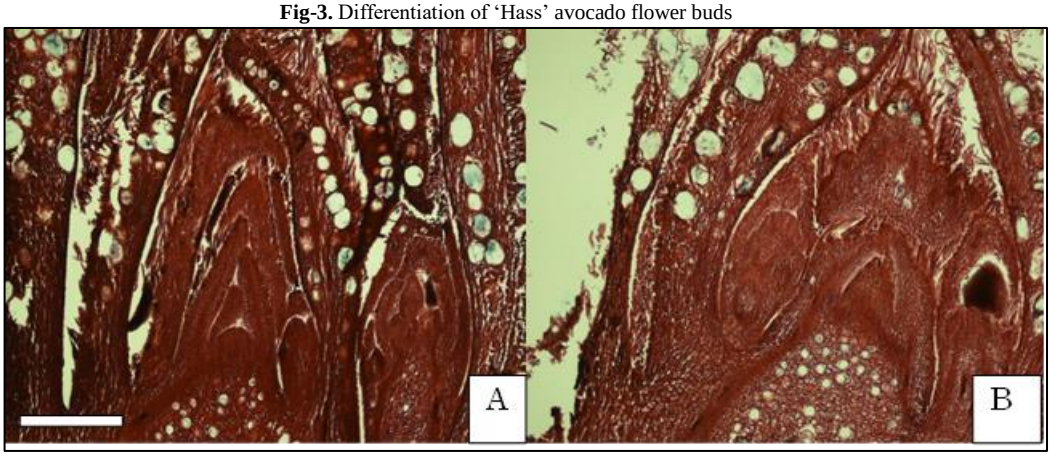
**Fig-2.** Differentiation of ‘Simmons’ avocado flower buds




A: Undifferentiated bud (19 December 2011)
B: Early bud differentiation (dome stage; 4 January 2012)
C: Differentiated bud (17 January 2012)
se: sepal primordium
Scale bar length indicates 500 µm.

‘Hass’ avocado buds collected on 4 January 2012 were in an undifferentiated state (Fig. 3A), whereas those collected on 17 January 2012 had begun to acquire a dome shape, indicating the early stage of flower bud differentiation (Fig. 3B).

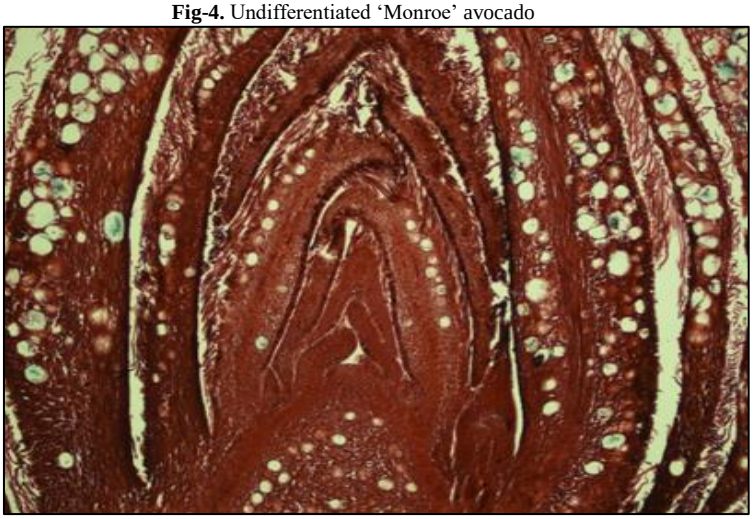
**Fig-3.** Differentiation of 'Hass' avocado flower buds




A: Undifferentiated bud (4 January 2012)
B: Differentiated bud (dome stage; 17 January 2012)
Scale bar length indicates 500 µm

'Monroe' buds collected on 17 January 2012 were undifferentiated (Fig. 4).

**Fig-4.** Undifferentiated 'Monroe' avocado



Scale bar length indicates 500 µm. Flower bud (17 January 2012).

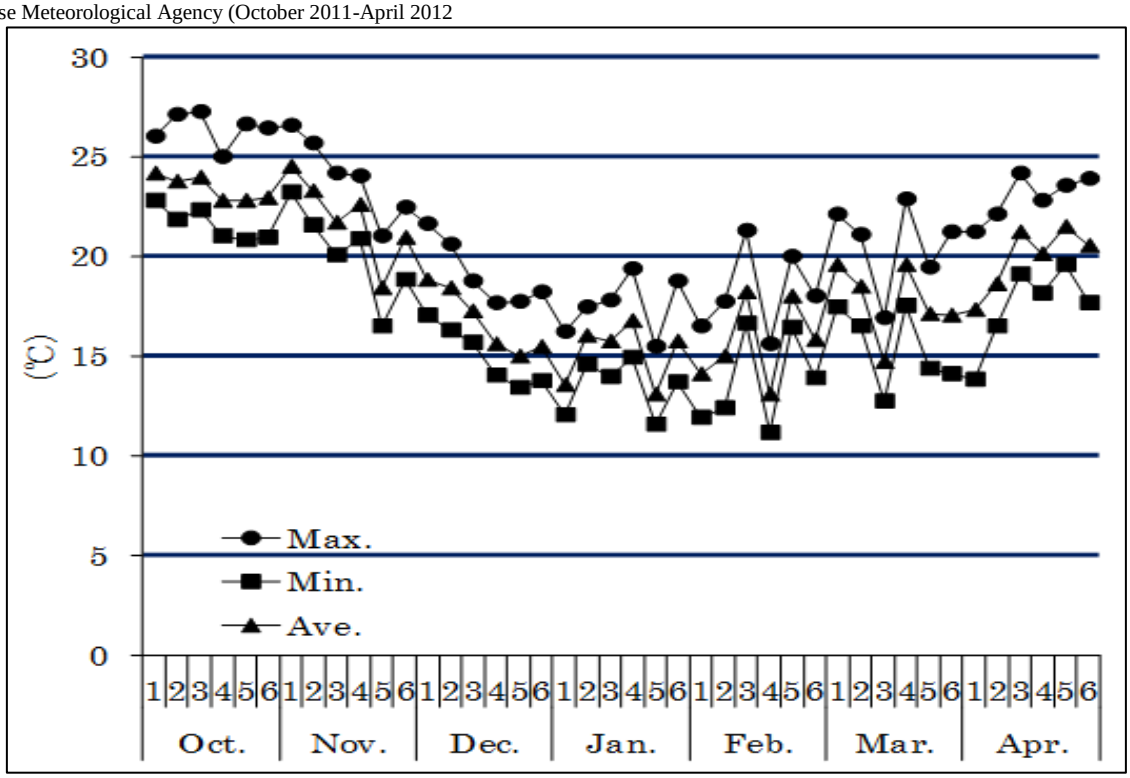
Among the four avocado cultivars, morphological flower bud differentiation was first observed in 'Pinkerton' in mid-December, followed by 'Simmons' in early January and 'Hass' in mid-January. Flower bud differentiation was not observed in 'Monroe' during the experiment period. Although the flower bud differentiation period differed among cultivars, no clear difference was observed. Inoue and Takahashi [13], investigated flower bud differentiation in the avocado cultivars 'Zutano' (predominantly Mexican x Guatemalan), 'Fuerte' (Mexican x Guatemalan) and 'Jalna' (Mexican) in Shizuoka Prefecture; all three cultivars exhibited flower bud differentiation in November. A simple comparison cannot be made between our studies because different cultivars were used; however, flower bud differentiation appears to occur slightly later in subtropical Okinawa Prefecture than in Shizuoka Prefecture.

Following a similar pattern, the flowering period began earliest in 'Pinkerton' (late January) and ended in early March (Table 1), after which 'Hass' began to flower, followed by 'Simmons' and 'Monroe'. The flowering period ended in early April for 'Simmons' and 'Hass', and mid-April for 'Monroe'. Thus, 'Pinkerton' required ca. 1 month between morphological flower bud differentiation and the flowering period, whereas 'Simmons' and 'Hass' required 1.5-2 months. We were unable to estimate this time lag for 'Monroe' because we were unable to determine the flower bud differentiation stage. Inoue and Takahashi [13], reported that after flower bud differentiation, avocado trees in Shizuoka Prefecture were affected by low winter temperatures that delayed the completion of flower organ development. Salazar-Garcia, *et al.* [15], also reported that avocado flowering was inhibited by low temperatures. In Okinawa Prefecture, 'Pinkerton' began to flower at the coldest time of year (minimum air temperature 13°C); floral development progressed as air temperature rose and the flowering season began in mid-February, when average air temperature reached 18°C (Fig. 5). The other three cultivars began to flower in March, when average air temperatures remained between 15 and 20°C. Thus, although 'Pinkerton' flowered earlier than the other three cultivars, flowering generally occurred within a shorter period of time after flower bud differentiation in Okinawa Prefecture than Shizuoka Prefecture.

**Table-1.** Flowering periods of four avocado cultivars in 2012

| Cultivar | Beginning of flowering period | Full bloom stage | End of flowering period |
|---|---|---|---|
| 'Pinkerton' | Late January | Mid-February | Early March |
| 'Simmons' | Mid-March | Late March | Early April |
| 'Hass' | Early March | Mid-March | Early April |
| 'Monroe' | Late March | Early April | Mid-April |

**Fig-5.** Seasonal changes in air temperature during the experimental period. Data were recorded at Nanjo City, Okinawa Prefecture by the Japanese Meteorological Agency (October 2011-April 2012




## 4. Conclusion

Our morphological investigation of four avocado cultivars on the main island Okinawa Prefecture, which has a subtropical climate, showed that flower bud differentiation occurred earliest in 'Pinkerton'(mid-December), followed by 'Simmons' (early January) and 'Hass' (mid-January). These thresholds occurred ca. 1-2 months later than those recorded in Shizuoka Prefecture, which has a temperate climate. The period between flower bud differentiation and flowering appears to be shorter in Okinawa Prefecture than in Shizuoka Prefecture.

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