The Effect of Inflorescence Removal on the Formation and Flowering of Lateral Bud in 'Irwin' Mango (*Mangifera indica* L.)

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Key words: *Mangifera indica* L., inflorescence removal, flower initiation

Summary

In this study, the 'Irwin' (*Mangifera indica* L.) shoots were encouraged to flower out of season by removal of terminal inflorescences. Flower initiation date and readiness of the lateral bud to flower were studied through a collection of shoot samples which were carried out starting the day of inflorescence removal (February 11, 2013) and continuing every two days for 30 days. Microscopy of cross-sections of paraffin-embedded buds showed multi-lobed development of primary inflorescence occurring 4 days after inflorescence removal. These bud meristems, the precursors of the secondary inflorescence axes, bore flowers 12 days after inflorescence removal. Biochemical factors associated with inflorescence removal revealed that the total soluble sugar and C:N ratio increased during the process of inflorescence removal. These results suggest that increased total soluble sugar and C:N ratio may have a positive effect on flower formation in ‘Irwin’ mango during the initiation periods. Cross-sectioning and nutritional analysis confirmed that the changes within the bud were associated with flower initiation.

Introduction

Mango (*Mangifera indica* L.) is one of the most important tropical fruits of the world. In Taiwan 2011, the area of mango plantation was 16,695 hectares and yield production was 169,380 tons (COA, Taiwan, 2011). The main harvest season for mango in Taiwan is summer.

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Mango is a highly perishable fruit. However, the oversupply during June and July causes prices drop dramatically and making it a marginally profitable crop in Taiwan. The production of more profitable off-season mango may be possible by modifying the date of flowering which normally is in February and March.

Off-season fruit production depends on the ability to induce flowering out of season. Davenport (2009) reported that flower initiation can be stimulated by environmental factors, such as the change from dry to rainy season or a shift from cool to warm temperatures in the tropics. On the other hand, experiments have shown several methods that attempt to delay flowering and fruit development. One of which is tip pruning. Tip pruning is defined as pruning terminal stems anywhere from near the terminal apex to a point down the stem that is no larger than 1 cm in diameter (Davenport, 2006). Tip pruning causes a uniform flush of growth throughout the canopy, it removes growth and flower-inhibiting factors in stems derived from the previous flowering seasons and fruiting panicles (Davenport, 2000, 2006, 2009; Davenport and Nunez-Elisea, 1997). In order to delay flowering, other researchers have demonstrated that paradormancy exerted on axillary buds can be successfully removed by defoliation as soon as the new leaves emerge. Shoot tipping is, nonetheless, needed for the achievement of this intervention (Cautin and Razeto, 1999; Soler and Cuevas, 2008).

Removal of apical buds by pruning stimulates initiation of axillary shoots (Davenport *et al.*, 2006). Soler and Cuevas (2009) confirmed that leaf removal and tipping the new growth shoots delayed production of perfect flowers on cherimoya bud. This study theorized that by applying this methodology to inflorescence removal, the production of flowers on ‘Irwin’ mango buds could be delayed. The objectives of this study were 1) to document a reliable new technique for delay-season areas based on inflorescence removal to induce a secondary flower to produce out-of-season mango fruit; and 2) to confirm by a combination of microscopic observations of flower buds and by lab analyses of leaves response of flower buds by inflorescence removal.

Materials and Methods

Plant Materials

The experiments were carried out in February, 2013 using 25 year old 'Irwin' mango trees at the Grape Center, National Chung Hsing University, Wufong, Taichung, Taiwan (lat. 24° 4' 39.51N, long. 120° 43' 21.22E). All the mango trees were uniformly fertilized and received other recommended cultural practices such as shoot tipping, girdling, defoliation, irrigation and low temperature.

Flower initiation and development

To determine flower initiation date in mango, 190 shoots from the flower inflorescence of the 2013 season's growth were collected from 'Irwin' mango trees. Inflorescence removal was carried out on February 11th the day flower bud had just unfolded. This date is hereafter referred to as day 0. Successive bud samples were taken after 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30 days. These samples were used for light microscopy observations. For light microscopy, the mango buds detached every 2 days were fixed in FAA (formalin: acetic acid: 50% ethanol at 5:5:90 ratio), dehydrated through a graded ethanol series and embedded in Paraplast. The samples were cut at either a 10 µM thick sections using a Leica RM2125RT rotary microtome (Leica Microsystems), mounted on slides and stained with Gerlach solution (Tsai, 2000) before observation.

Bud readiness to complete flower development after de-inflorescence

The experiments were carried out to confirm the capacity of the young buds to produce flowers and used a completely randomized block design (CRD). The treatment was inflorescence removal. In the experiment, inflorescence removal was made on February 10th 2013 in 190 shoots that were blossoming (Fig. 1 inflorescences removal). Bud sprouting and flowering percentages were recorded for 30 days after inflorescence removal and compared with control shoots. Leaf samples were also collected every 2 days after treatment until re-flowering for the determination of total soluble sugar, starch, total nitrogen and mineral elements. Meteorological data were recorded every day after treatment until re-flowering. The minimum temperature was below 16°C for the months of November to February during the experiment (Fig. 2).

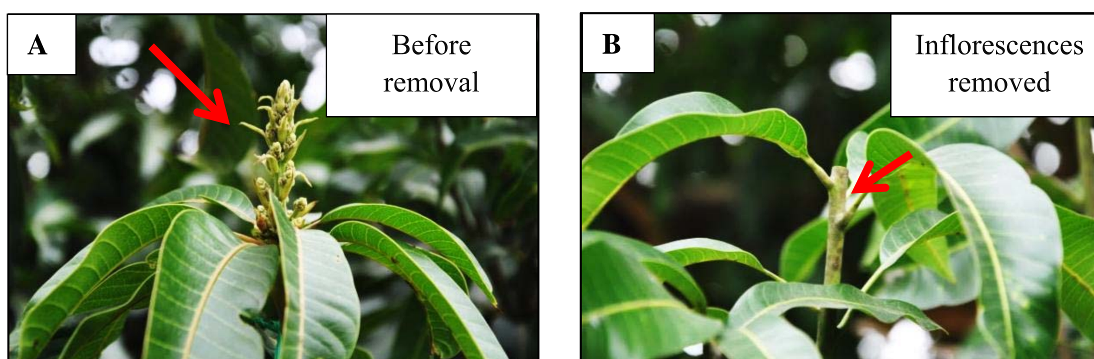


Fig. 1. The inflorescences of 'Irwin' mango. (A) Before removal. (B) Inflorescences removal.

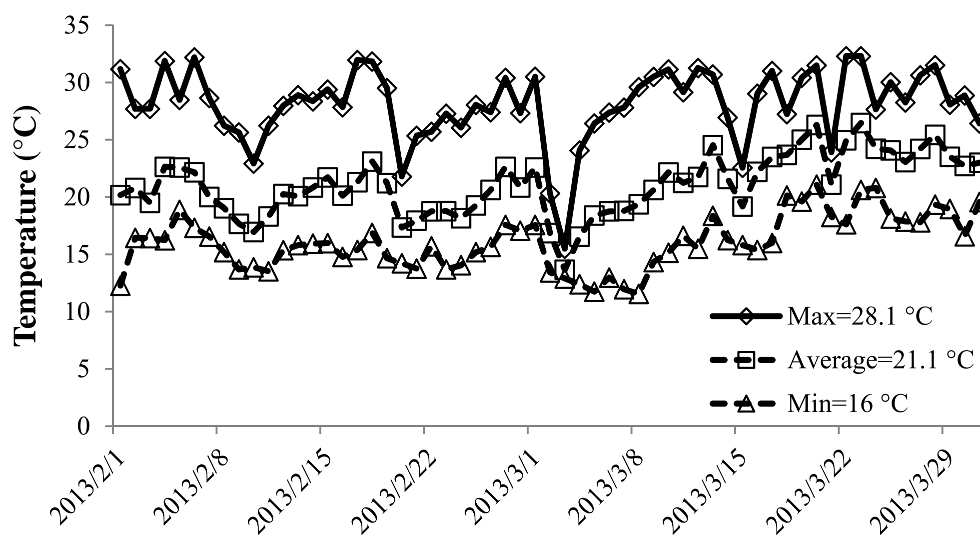


Fig. 2. Mean maximum, average and minimum temperature during the 2012-2013 winter season at the experimental orchard in Taichung, Taiwan.

Analysis of total soluble sugar and starch

The 'Irwin' mango leaves from 1 cm deep from removed inflorescences were collected and washed under tap water, rinsed with 1% HCl, and then thoroughly rinsed three times in distilled water. Samples were dried to constant weight in a hot air oven at 100 °C for 1 hour before the temperature was reduced to 70 °C for drying. The dried leaves were milled to a fine powder with an electric blender and subsequently stored until analysis. For the analysis, 10 mL distilled water was added to 0.1 g dried leaf samples in plastic test tubes. Samples were set in 30 °C water bath and shaken for 3 hours. After samples dissolved, they were centrifuged at 4,000 rpm for 10 min. The supernatants were removed and filtered through micro membrane cloth (Manufacturer) so that the supernatant could be used for total soluble sugar analyses while the residues were used in starch analysis.

Determination of total soluble sugar

A 2 mL aliquot of a 0.2 mL leaf suspension in 4.8 mL distilled water was pipetted into a new glass tube. Then 0.1 mL liquid phenol and 6 mL H_2SO_4 (conc.) was added and the solution was suspended for 30 min. After 30 min, the absorbance value of sample solutions were measured by a spectrophotometer at a wavelength of 490 nm. Standard curve was made with various concentrations of glucose solutions. These standards were prepared by mixing solution of 2 mL distilled water, 0.1 mL liquid phenol and 6 mL H_2SO_4 (conc.) with 2 mL of glucose solutions at

various concentrations from 0.05 to 0.25 µmole/mL are these the final concentrations. The total soluble sugar content in the sample was calculated as percent of dry weight (Dubios, 1956).

Determination of starch concentrations

The residue was oven-dried overnight. Then, 2 mL of distilled water was added and incubated in water bath at 100 °C for 15 min. After incubation, 2 mL $HClO_4$ (9.2 N) was added and incubated for 15 min, and finally, 6 mL distilled water was added. The subsequent solutions were centrifuged at 4,000 rpm for 10 min and filtered through membrane cloth (pore size). The 0.1 mL of solution was transferred into a new glass tube with the addition of 1.9 mL distilled water, 0.1 mL liquid phenol, and 6 mL H_2SO_4 and put aside for 30 min. The glucose standard was prepared the same way as the total soluble sugars to generate a standard curve. The absorbance values of sample solutions were measured at a wavelength of 490 nm by spectrophotometer. The starch content was calculated as in percentages dry weight.

Determination of total nitrogen (Micro-Kjeldahl method)

Using the same method to prepare a dry sample powder of each mango leaf (see Analysis of total soluble sugar and starch), 0.2 g was weighed out and packed in Whatman No.1 filter paper then placed in digestion tube. One g Se mixture powder (Merck 8030) and 4.5 mL H_2SO_4 were added to the tube. The digestion tubes were placed in the digestion apparatus, then heated at 410 °C for about 3 hours until solution turned light blue. The digestion tubes were cooled down and 15 mL distilled water was added. Next, 20 mL of 12 N NaOH was added and the sample solution was placed into a Micro-Kjeldahl apparatus and measured into a 20 mL solution of 2% boric acid, which contained 9 µM bromocresol and 25 µM methyl red indicator solution. When total volume increased to 25 mL, 1/14 N H_2SO_4 was titrated and volumes recorded. The nitrogen (%) was calculated (AOAC, 1995).

Statistical analysis

The data of the experiment underwent statistical analysis using SAS 9.2 (Institute Inc, 2002) and was subjected to one-way analysis of variance (ANOVA) for a completely randomized design (CRD) statistical model. Mean values among treatments, when significant, were compared by least significant difference tests at the 5% ($P \leq 0.05$) level of significance.

Results

Flower initiation and development

The date of inflorescence removal of 'Irwin' mango (*Mangifera indica* L.) is referred to as day 0 for flower initiation. Successive samples were taken after 2, 4, 6, 8, 10, 12, 14, 16, 18, 20,

22, 24, 26, 28 and 30 days. Dormant lateral flower buds developed from 24 days until 30 days after inflorescence removal. At 30 days after inflorescence removal, the elongation and loosening of scales made these lateral buds enter the flower bud sprouting stage (Fig. 3).

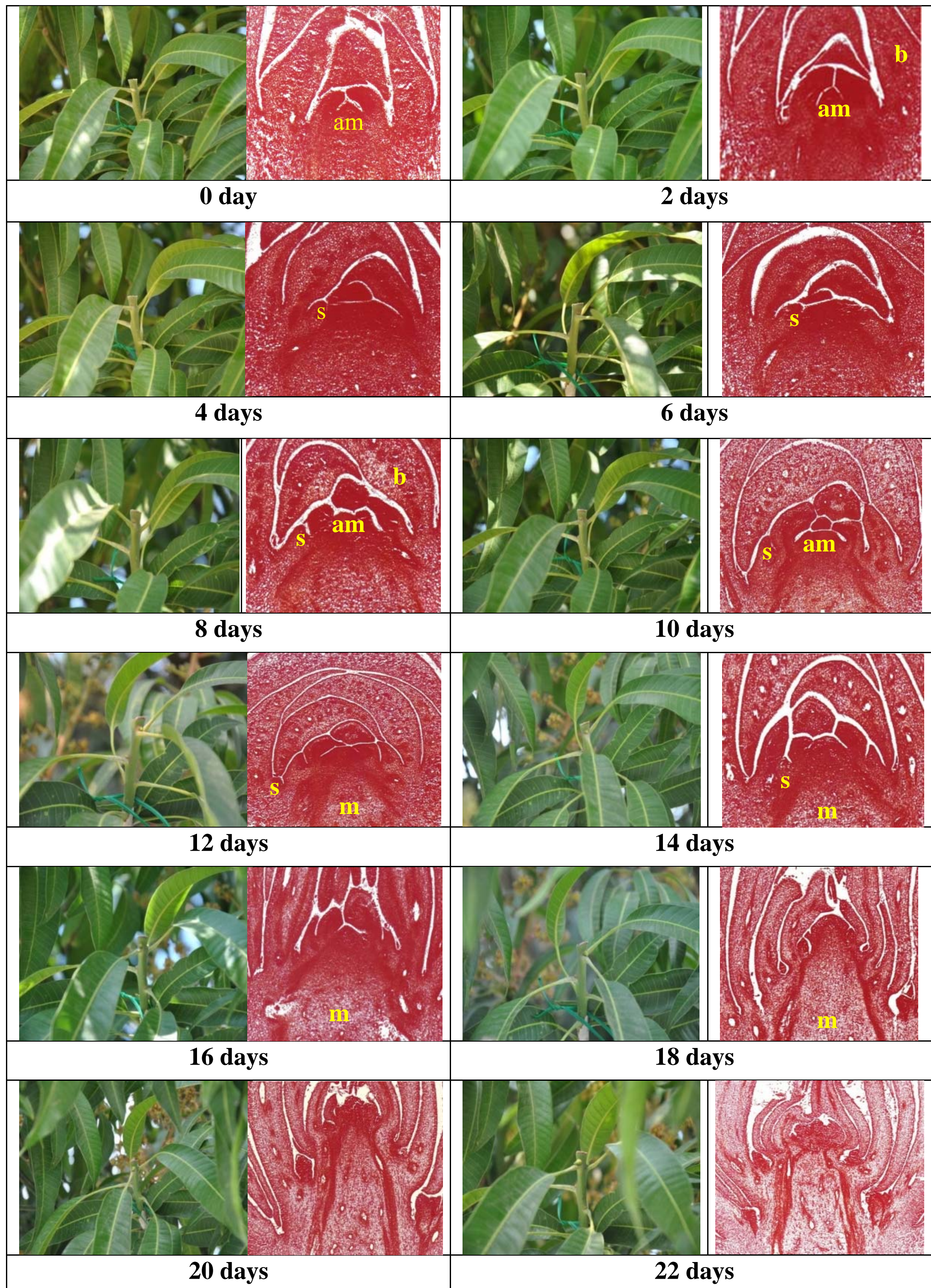
Longitudinal cross sections of the mango bud after inflorescence removal are illustrated in Fig. 4. Starting at day 0, axillary bud showed no prominent conical shaped bud meristems (Fig. 4A). From day 4 to 14 the bract axial elongated slightly and rounded off assuming a dome shape. As early as day 4, the terminal bud axis (primary inflorescence axis) elongated which is the first microscopic sign of bud activation and continued until day 14 with the appearance of bud meristems in which the axis elongated and became multi-lobed due to development of primary inflorescence of flower panicle (Fig. 2C, D, E and F). Finally day 16 to 30, these bud meristems continued to elongate and develop secondary inflorescence including lobes which is necessary to form flower cluster (Fig. 2G, H, I, J, K, L and M).

Source composition of mango leaves

Inflorescence removal affected nutritional composition of 'Irwin' leaves mango flower bud. A nitrogen levels in leaves were higher 20 days after inflorescence removal at 1.93%. At 22 days after inflorescence removal, nitrogen levels slowly decreased to 1.52% (Fig. 4). However, the total soluble sugar concentrations in leaves on the day of inflorescence removal (day 0) was 9.29%. It increased gradually and reached a peak of total soluble sugar of 10.91% at 16 days after inflorescence removal (Fig. 5). The starch level in leaves was lower at the beginning of inflorescence removal but thereafter, its content was slightly increased. At 22 days after inflorescence removal, starch concentrations increased and spiked at 4.89% and slightly decreased to 3.84% at 24 days after inflorescence removal. On 28 to 30 days after inflorescence removal, starch concentrations increased again (Fig. 6).

The changes in the ratio of the C:N ratio in 'Irwin' leaves fluctuate after inflorescence removal. The C:N ratio was higher from 6 to 12 days after inflorescence removal with a mean value of 10.16. Thereafter, its concentrations slightly decreased until 20 days after inflorescence removal. At day 22, C:N ratio increased again (Fig. 7).

The changes in total soluble sugar to starch ratio in 'Irwin' was significantly different during the study period (Fig. 7). The minimum total soluble sugar to starch ratio (1.76) was found 28 days after inflorescence removal whereas, the maximum total soluble sugar to starch ratio (3.83) as found on the day of inflorescence removal (day 0).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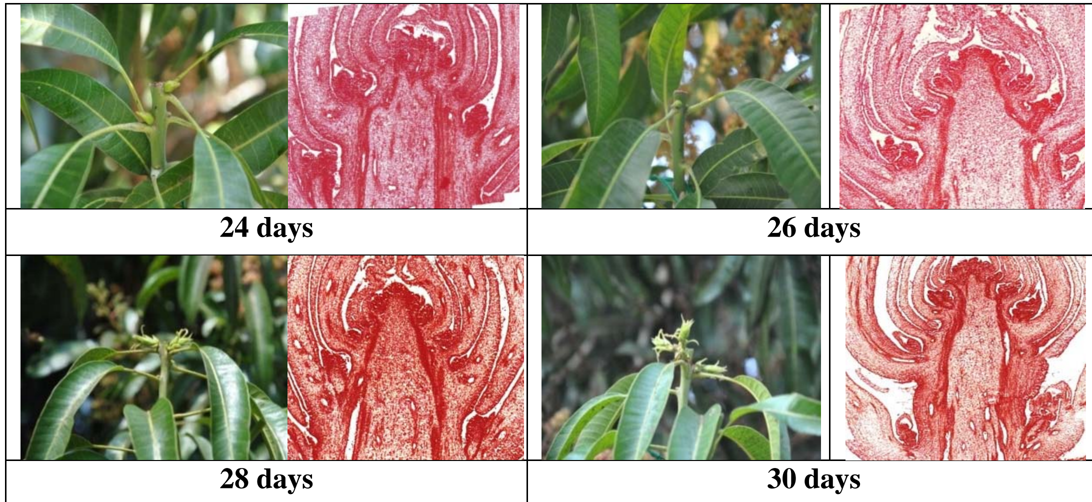

Fig. 3. During blossom, the inflorescences of 'Irwin' mango trees were detached on the same trees. The lateral buds beneath the terminal inflorescence were sampled every 2 days. Longitudinal sections of buds under light microscopy every 2 days after inflorescence removal. Ten buds were removed for each of the 16 time points for a total of 160 buds examined. Apical meristem (am), youngest leaf primordia (p), scars of outer bud scales (o), inner bract scars (b), elongating primary inflorescence axis (m). Scale bars: 100x.

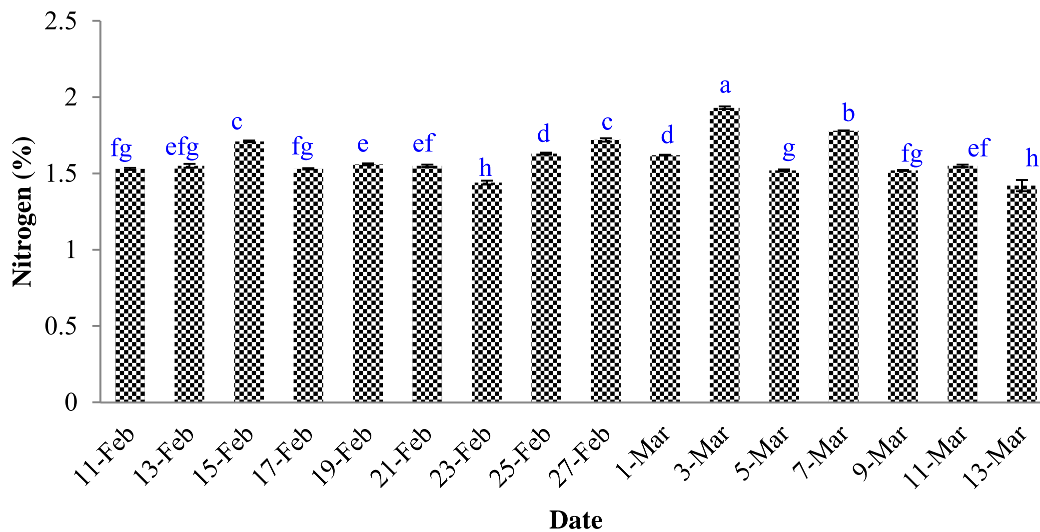

Fig. 4. Effect of inflorescence removal on total nitrogen in leaves of 'Irwin' mango trees. (Date of inflorescence removal: February 11th, 2013) Means at a time point followed by the same letter are not significantly different by LSD test at $P \leq 0.05$. at 1 cm position on branch are each of these leaves, moving down the branch as sample.

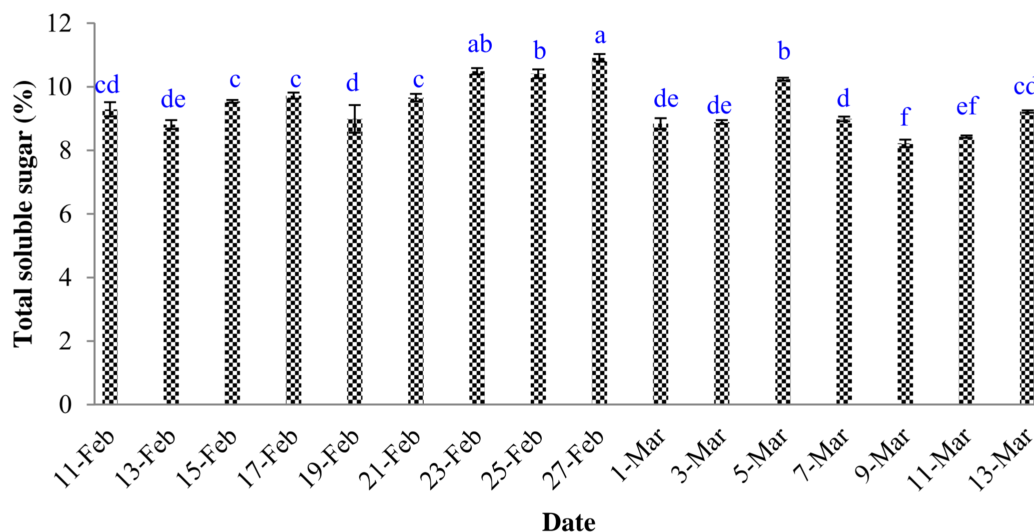

Fig. 5. Effect of inflorescence removal on total soluble sugar in leaves of 'Irwin' mango trees. (Date of inflorescence removal: February 11th, 2013) Means within a column followed by the same letter are not significantly different by LSD test at $P \leq 0.05$.

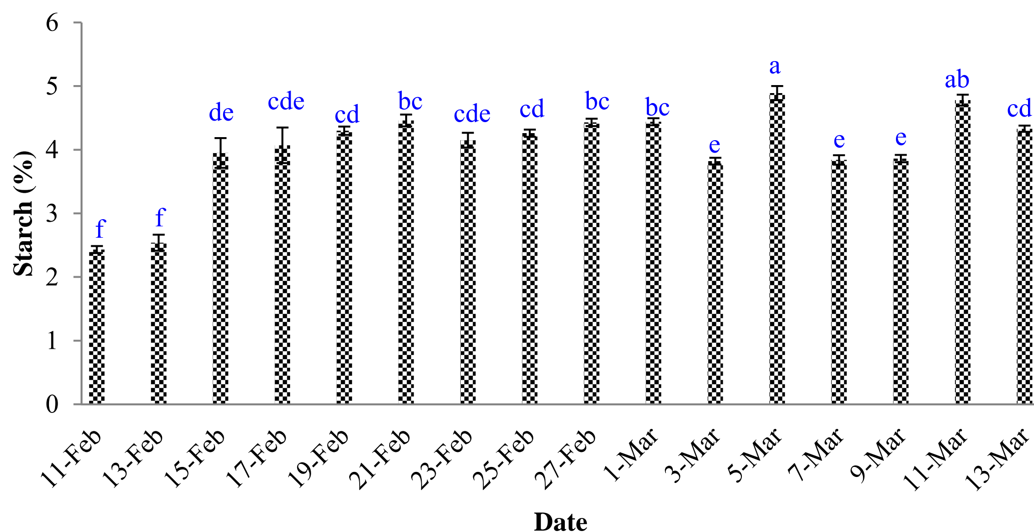

Fig. 6. Effect of inflorescence removal on starch content in leaves of 'Irwin' mango trees. (Date of inflorescence removal: February 11th, 2013) Means from 10 samples, within a column followed by the same letter are not significantly different by LSD test at $P \leq 0.05$.

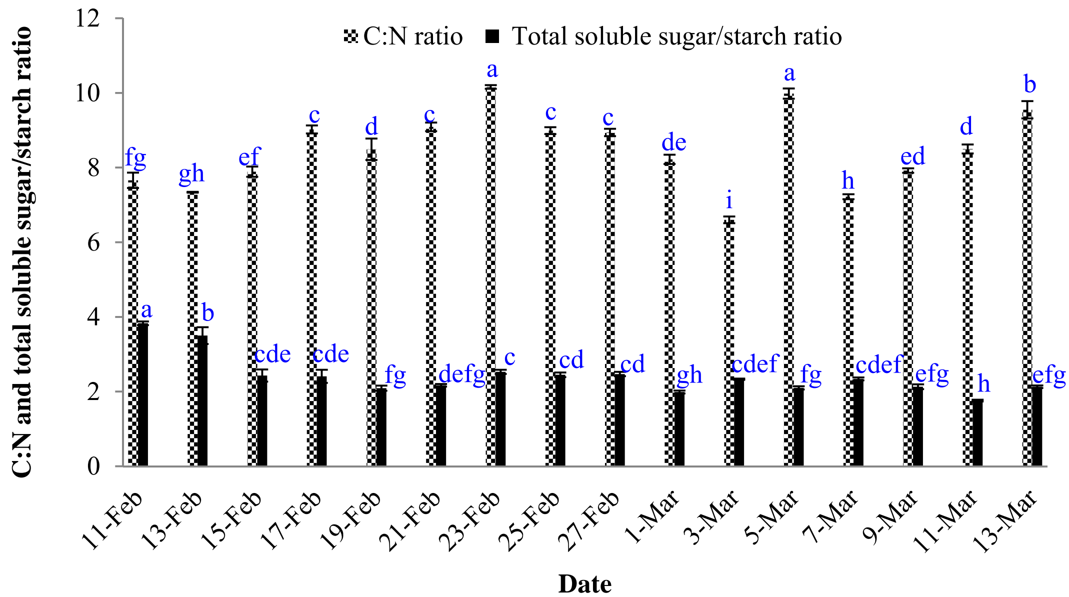

Fig. 7. Changes in the organic C:N ratio and total soluble sugar/starch ratio in leaves of 'Irwin' mango trees. (Date of inflorescence removal: February 11th, 2013) Means are from 10 samples within a column followed by the same letter are not significantly different by LSD test at $P \leq 0.05$.

Discussion

The physiological and biochemical changes indicate that flower initiation could be enhanced by de-inflorescence in 'Irwin' mango shoots. The date of inflorescence removal is referred to as day 0 for flower initiation. Paraffin sectioning observation showed the complex nature of the mango bud. At 0 day, axillary bud showed no prominent conical shaped bud meristems. Four days after inflorescence removal, the primary inflorescence axis has enlarged and three sepals could be distinguished in the primary bud of the axillary bud complex. From 12 days after inflorescence removal, the bud meristem continued to elongate and develop secondary inflorescence including lobing which is necessary to form flower cluster (Fig. 3). Flower initiation could have occurred even when it is delayed, since non-systematic observations of younger buds showed floral dome swelling in the primary bud. This result coincides with Soler and Cuevas's (2009) findings that axillary buds of cherimoya have already formed the sepals when the subtending leaf has just begun unfolding (0 week), while the petals are clearly visible in 1-week-old of cherimoya buds. At 24 days after inflorescence removal, bud sprouting appeared and the bud meristem elongated and developed secondary inflorescence including lobes which is necessary to form flower cluster.

Soler and Cuevas (2009) also observed the development of the floral organs before bud break and sepals, petals and stamen initiation before sprouting, although the individual carpels could not be recognized in 32-week-old 'Fino de Jete' buds.

The days following inflorescence removal showed that it affected the nutritional composition of mango flower bud. Although total nitrogen concentrations in leaves were higher 2 days after inflorescence removal, at 4 days after inflorescence removal, total nitrogen concentration slowly decreased. At 6 to 14 days after inflorescence removal, total nitrogen was increased again (Fig. 4). In agreement with Urban *et al*. (2004), leaf nitrogen concentration per unit mass was lower in leaves close to developing inflorescences than in vegetative shoot leaves. The leaf nitrogen content per unit mass during the floral period was more evidently decreased in vegetative shoot leaves than in leaves close to inflorescences indicating that nitrogen was mobilized from leaves distant from the developing inflorescences, possibly to meet the increased demand for nitrogen during panicle development.

The total soluble sugar content in leaf on the day of inflorescence removal (day 0) and thereafter increased gradually and reached a peak at 24 days after inflorescence removal. Total soluble sugar content was increased on 0 to 4 days after inflorescence removal thereafter its content was slightly decreased from 6 until 14 days after inflorescence removal. At 16 to 24 days after inflorescence removal, total soluble sugar was increased again (Fig. 5). This coincides with Perilleux and Bernier (1997), who reported that sucrose in leaf exudates of *Lolium temulentum* L. increased when flowering was induced. Sucrose levels were increased during the flower induction period in mango (Adil *et al*., 2011). In short, although sucrose concentrations increased during floral transition, it did not suffice to trigger the complete sequence of floral evocation (Houssa *et al*., 1991; Ulger *et al*., 2004).

The starch content in leaves showed higher levels at the beginning of inflorescence removal thereafter its content slightly decreased. At 2 days after inflorescence removal, starch content showed increase and slowly decreased at 16 days after inflorescence removal. On day 18 to day 24, starch content increased again (Fig. 6). In agreement with Urban *et al*. (2004), leaf starch concentration was lowest in leaves close to inflorescences. However, leaf starch was globally higher during the floral period. Starch concentrations are higher in all parts of flowering lychee trees than in nonflowering but vegetative flushing trees (Menzel *et al*., 1995). Similarly, starch is present in excess in avocado leaves during flower development (Thorp *et al*., 1993). The observed increase in starch may be the result of a regulatory process linked to floral initiation (Eimert *et al*., 1995).

The carbohydrate to nitrogen ratio (C:N) is considered as an important factor in the regulation of flowering in fruit crops. The change in the ratio of C:N in leaves starting the day

after inflorescence removal was significantly different. C:N ratio was higher on the day on de-inflorescence (day 0) until 4 days after inflorescence removal thereafter its content was slightly decreased from 6 to 14 days after inflorescence removal. At 16 to 24 days after inflorescence removal, C:N ratio was increased again (Fig. 7). In agreement with Ito et al. (2004), the increase in C:N ratio is ascribed to the consequence of increased carbohydrate availability as suggested, which is necessary for the induction of flowering. A high C:N ratio has been postulated promotory to flowering whereas opposite beneficial for vegetative growth (Corbesier *et al*., 2002).

Conclusion

Inflorescence removal of 'Irwin' mango shoots delayed its fruit season and reproductive cycle: flower initiation, development of lateral flower buds, sprouting of flower buds, first flowering, and full bloom. Light microscopy recorded the changes (elongation and formation of dome shape) in the primary inflorescence axis determining that flower initiation occurred 4 days after inflorescence removal. Microscopy also confirmed the development of a normal bud meristem changed into a secondary inflorescence axis (elongation, multi-lobed lateral buds, bearing of flowers) 12-24 days inflorescence removal). Lab analysis of leaf found the total soluble sugar and C:N ratio increased during the days after inflorescence removal, which is necessary for the induction of mango flowering. Inflorescence removal successfully delayed the flowering season of 'Irwin' mango shoots by 30 days (March 16).

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'愛文'芒果(*Mangifera indica* L.)除花穗對側芽花芽分化及開花之影響

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關鍵字：芒果(*Mangifera indica*)、移除花序、花芽創始

摘要：本研究目的為探討剪除頂端花序對促使'愛文'芒果在非產季開花之效果。為調查花芽創始日期與側芽完成花芽前期分化的時間，在花期剪除花穗(2013 年 2 月 11 日)後，每隔 2 天採集側芽加以固定，並持續調查 30 天。以顯微鏡觀察石蠟切片之芽體，在花穗移除後 4 天，有多數初生花序發育，顯示這些側芽的分生組織是次級花序軸的前身，並且於花穗移除 12 天後開花。與花穗移除相關的生化因素顯示，花穗移除後側芽活化的期間，全可溶性糖和碳氮比增加，全可溶性糖和碳氮比增加對花芽的形成有正面的效果。由切片和營養分析結果證實：芽體內部的生化因子改變與花芽的創始與開花有關。

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