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苦瓜花性之轉錄體分析

Transcriptomic Study on Flower Sexuality of *Momordica charantia* L.

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本論文係徐晧昇君（R02628111）在國立臺灣大學園藝暨景觀學系所完成之碩士學位論文，於民國 104 年 6 月 29 日承下列考試委員審查通過及口試及格，特此證明

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摘要

苦瓜 (*Mormordica charantia* L.) 為雌雄同株異花 (monoecious) 作物，外施激勃素 (gibberellin, GA) 可誘導其雌花生成，有別於其他葫蘆科 (Cucurbitaceae) 作物。次世代定序 (next generation sequencing, NGS) 不需序列資訊等，有利於非模式物種 (non-model organism) 之轉錄體 (transcriptome) 定序。外施 GA_3 於苦瓜品種'月華'六至八葉期苗株，會使花性雄雌比由 37 降至 23，促進雌花生成。經萃取激勃素處理苦瓜苗株之總 RNA，以次世代定序技術進行轉錄體分析，組成 106,057 條 contigs，其中 40,854 條可被註解 (annotate) 為推定基因 (putative gene)，其中 8,948 個開放解讀框架 (open reading frame) 註解的基因於處理組與對照組中表現量差異達兩倍以上。次世代定序結果顯示，外施激勃素會使苦瓜中激勃素生合成相關基因表現量下降；激勃素受體基因 *McGID1*s、激勃素訊息傳遞基因 *McXERICO*s、乙烯訊息傳遞基因 *McEIN3*/*EIL*s 及茉莉酸 (jasmonic acid, JA) 生合成基因 *McLOX1* 表現量上升；*McDELLA*s、*ARF6*s 及 *ARF8*s 等基因表現量下降，其中雄蕊 (stamen) 發育相關基因 *McMYB24.1* 於激勃素處理表現量較低，可能抑制雄蕊發育；而 *McXERICO*s 之表現量增加可能促進離層酸之生合成以改變花性。外施激勃素使 *ARF6*s 及 *ARF8*s 表現量降低，造成茉莉酸之生合成下降進而抑制 *McMYB24* 表現，抑制雄蕊發育；外施激勃素可能藉由誘導 *McXERICO*s 之表現進而促進離層酸生合成關鍵基因 *McNCED*s 之表現而累積離層酸，並誘導乙烯生合成共同促進植株雌性化。

關鍵詞：植物荷爾蒙、激勃素、離層酸、轉錄因子、雄蕊發育

Abstract

Bitter gourd (*Momordica charantia* L.) is one of monoecious species belonging to the family Cucurbitaceae. Unlike other Cucurbitaceae crops, gibberellin (GA) promotes female flower formation in bitter gourd. Next generation sequencing (NGS) provides a platform to analyze transcriptome for non-model organisms. Application of 100 $mg \cdot L^{-1}$ GA_3 on seedlings of bitter gourd 'Moon Light' at 6~8-leaf stage reduced the ratio of male to female flower from 37 to 23. RNA isolated from GA-treated bitter gourd was conducted for comparative transcriptome analysis using NGS technology. 40,854 among 106,057 contigs were annotated as putative genes. Approximately 8,948 open reading frame-annotated genes showed a 2-fold differential expression between RNA of control and treatment. Gene expression of *McGID*s, *McXERICO*s, *McEIN3*/*EIL*s, and *McLOX1*s were up-regulated and that of *McDELLA*, *McARF6*s, and *McARF8*s were down-regulated after GA treatment. *McMYB24.1* showed down regulated following GA application and it might play the role in inhibition of stamen development. Up regulation of *McXERIO*s might play a crucial role in GA-induced femaleness by promoted ABA accumulation in bitter gourds. *McARF6*s, and *McARF8*s were down-regulated after GA application which caused the reduction of jasmonate biosynthesis and repressed the expression level of *McMYB24*. These might inhibit the stamen development. *McXERIO*s might be induced following GA treatment which promoted the expression of *McNCED*s and ABA accumulation. It also induced thylene biosynthesis in bitter gourds following GA application. These might promote the femaleness level of bitter gourd together.

Key words: phytohormones, gibberellin, abscisic acid, transcription factor, stamen development

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壹、 前言

苦瓜 (*Momordica charantia* L.) 為葫蘆科 (Cucurbitaceae) 一年生蔓性草本作物，於臺灣可周年生產，為夏季重要之果菜類蔬菜，其未成熟之果實富含營養價值，並具有預防糖尿病、高血壓、人類免疫缺陷病毒 (Human Immunodeficiency Virus) 等附加功能 (Fang and Ng, 2011)。苦瓜常見栽培品種的花性為雌雄同株異花 (monoecy)，如同其他葫蘆科作物，雄花始花節位較低且佔總花數比例高為主要的問題之一，平均花性雄雌比可高達 50:1，限制果實產量 (Rasco and Castillo, 1990; Sumpoudlek and Abella 1974)。

激勃素為一重要植物荷爾蒙 (phytohormone)，參與植物各生長及發育時期，同時也調控營養生長轉換至生殖生長、花芽誘導、花器分化等 (Aya et al., 2009; Aya et al., 2011; Gocal et al., 2001; Olszewski et al., 2002; Plackett et al., 2013; Sun, 2010; Sun, 2011; Yu et al., 2004)。植物生長調節劑 (plant growth regulator) 對苦瓜花性改變之影響已被詳細探討，外施激勃素 (gibberellin, GA)、益收、萘乙酸、(1-naphthalene acetic acid, NAA)、吲哚乙酸 (indole-3-acetic acid, IAA) 及抑芽素 (maleic hydrazide, MH) 等均可誘導苦瓜雌花生成 (Akter and Rahman, 2010; Banerjee and Basu, 1992; Bisaria, 1974; Ghosh and Basu, 1982; Ghosh and Basu, 1983; Prakash, 1976; Thomas, 2008)。外施激勃素於稜角絲瓜 (*Luffa acutangula*)、葫蘆 (*Lagenaria siceraria*) 及胡瓜 (*Cucumis sativus*) 均促進雄花生成，不同於其他葫蘆科作物，激勃素會促進苦瓜雌花之生成、降低雌花始花節位及降低花性雄雌比 (Banerjee and Basu, 1992; Ghosh and Basu, 1982; Ghosh and Basu, 1983; Krishnamoorthy, 1972; Mitchell and Wittwer, 1962; Pike and Peterson, 1969; Rahman et al., 1992; Thomas, 2008)。

本研究為了進一步瞭解苦瓜花性相關基因，以次世代定序技術進行激勃素處理後的苦瓜之RNA定序 (RNA sequencing)，比較激勃素處理和未經處理之苦瓜植株間的基因差異性表現，並以即時定量反轉錄聚合酶連鎖反應 (real-time quantitative reverse transcription-polymerase chain reaction, qPCR) 偵測基因表現差異，藉此了解苦瓜花性分化之相關機制。

貳、 前人研究

一、 胡瓜及苦瓜花性分化

胡瓜 (*Cucumis sativus* L.) 之花原基 (floral primordia) 最初為兩性 (bisexual) 花，同時具雄蕊 (stamen) 及雌蕊 (pistil) 原基，且分化程度相當，隨發育階段漸增，選擇性停止雄蕊或雌蕊之發育而決定花性 (Malepszy and Niemirowicz-Szczytt, 1991)。於雄花，雌花器之發育受抑制，雄蕊則正常發育；於雌花，雌器官之發育受正常，雄蕊則停止發育 (Malepszy and Niemirowicz-Szczytt, 1991)。苦瓜之花原基最初亦為兩性花，雌花器之發育受阻，雄蕊則正常發育，生成雄花；雌花器之發育正常，雄蕊之發育停滯，生成雄花 (汪和曾，1997)。

二、 胡瓜花性相關基因

(一) 花性主控基因

胡瓜花性主要由三對非連鎖基因座 (locus) 調控，*F* (female) 為顯性基因座，調控植株雌性程度；*M* (male) 為顯性基因座，調控發育成單性花或兩性花，當 *M* 基因為同質隱性 (*mm*) 時，發育成兩性花；*A* (androecious) 基因座於 *F* 基因座同質隱性 (*ff*) 時，調控花器發育為雄花 (Mibus and Tatlioglu, 2004)。其中 *F* 基因座編碼 CsACS1G (Mibus and Tatlioglu, 2004; Trebitsh, 1997)，為 *CsACS1* 基因之第二拷貝 (copy) 與另一無性別特異性 (non-sex-specific) 之 *branched-chain amino acid transaminase* (*CsBACT*) 基因重組之產物 (Knopf and Trebitsh, 2006)。*M* 基因座編碼 CsACS2，其隱性等位基因 (allele) *m*，突變於一保守 (conserved) 區域，其第 33 個胺基酸由甘氨酸 (Glycine, Gly) 轉變為半胱氨酸 (Cysteine, Csy)，導致喪失酵素活性 (Li et al., 2009)。*A* 基因座可能與 *response to antagonist* (*CsRAN1*) 連鎖 (linked)，RAN1 參與銅離子 (copper ion) 傳遞至乙烯受體，而銅離子傳遞途徑為乙烯受體活化之必要過程 (Terefe et al., 2005)。

雌雄同株異花及雄花、兩性花同株 (andromonoecious) 胡瓜之乙烯生成量相近，外施益收能促進雌雄同株異花胡瓜生成雌花，但不生成兩性花；促進雄花、兩性花同株胡瓜生成兩性花，但不生成雌花；施用硝酸銀 ($AgNO_3$) 誘導全雌株胡瓜雄花生成但不生成兩性花，由其基因型推測 *M* 基因座會受乙烯之誘導表現而抑制

雄蕊發育 (Yamasaki et al., 2001)。*CsACS2* 之啟動子 (promoter) 於菸草 (*Nicotiana tabacum*) 轉殖株無活性，外施益收可促使 *CsACS2* 啟動子於花器表達；以益收處理野生型 (wild type) 胡瓜亦可誘導 *CsACS2* 表現，施以乙烯抑制劑則降低其表現量，推測 *CsACS2* 受乙烯誘導且具正回饋調控機制，進而生成更大量乙烯以抑制雄蕊之發育 (Li et al., 2012)。

全雌株具 *F* 基因座，故有足夠乙烯生成，誘導雌蕊發育及 *M* 基因座抑制雄蕊；雌雄同株異花不具 *F* 基因座，故乙烯可能不足以誘導 *M* 基因座抑制雄蕊且無法促進雌蕊生成，生成雄花，但乙烯於植物體內尚有其他基因座生成，若生成量足夠則誘導 *M* 基因座抑制雄蕊生成雌花；兩性株為 *m* 基因座同質隱性，故無法抑制雄蕊發育，且其具 *F* 基因座，可生成足夠乙烯促進雌蕊發育而得兩性花；雄花、兩性花同株具同質隱性 *f* 及 *m* 基因座，雄蕊生成無法受抑制，植物體乙烯的生成量不同而產生兩性花或雄花 (Yamasaki *et al*., 2001)。

(二) 雌花分化相關基因

胡瓜之乙烯受體 *ethylene response 1* (*CsETR1*) 於胡瓜雌花之雄蕊表現量下降；以 *APETALA3* (*AP3*) 之啟動子驅動反義 (antisense) *CsETR1* 降低 *ETR1* 於轉殖阿拉伯芥 (*Arabidopsis thaliana*) 雄蕊之表現量，造成雄蕊萎縮，推測乙烯感應 (ethylene perception) 參與抑制雄蕊發育之途徑 (Wang et al., 2010)。*CsETR1*、*CsETR2* 及 *ethylene response sensor* (*CsERS*) 之 messenger-RNA (mRNA) 於全雌株胡瓜之莖頂累積量大於雌雄同株異花胡瓜，可能由於全雌株產生較多之內生 (endogenous) 乙烯 (Yamasaki, 2000)。

利用抑制性扣減雜合技術 (suppression subtractive hybridization, SSH) 分析胡瓜雄花或雌花發育時期六之雄蕊，分離一個與阿拉伯芥 *calcium dependent nuclease* (*AtCAN*) 同源之表現序列標籤 (expressed sequence tag, EST)，命名為 *CsCaN*；*CsCaN* 具鈣依賴性及乙烯誘導性，並於雌花發育時期六之雄蕊大量表現，可能參與雌花之雄蕊原基特異性 DNA 損傷 (primodial anther-specific DNA damage) (Gu et al., 2011)。

外施 500 μM 益收於十一日齡之胡瓜雌雄同株異花品種‘Shimoshirazu-jibai’，可

誘導雌花於低節位發生，以差異展示法 (differential display method) 篩選差異性表現基因；選殖系#17 為一具 MADS-box 之基因，並命名為 *ethylene-responsive gene associated with the formation of female flower 17* (*ERAF17*)；*ERAF17* 會受乙烯所誘導，其表現時機與表現量與雌花之生成相符，乙烯可能藉由調控 *ERAF17* 之表現而影響胡瓜花性 (Ando et al., 2001)。選殖系#16 為編碼甲基轉移酶 (methyltransferase) 之基因，並命名為 *ERAF16*，*ERAF16* 會受乙烯所誘導，其表現量與雌花發育呈正相關；*ERAF16* 於全雌株胡瓜之未成熟花芽表現量高於雌雄同株，顯示 *ERAF16* 參與乙烯誘導雌花之形成 (Ando and Sakai., 2002)。

以次世代定序技術分別對雌雄同株異花‘Csg-M’及全雌株胡瓜‘Csg-G’進行 RNA 定序分析，‘Csg-G’相較於‘Csg-M’約 600 個基因負向調控 (down-regulation)、400 個基因正向調控 (up-regulation)；差異性表現基因主要功能為 biogenesis、transport and organization of cellular component 、 macromolecular and cellular biosynthesis、localization、establishment of localization、translation 及 other processes；許多荷爾蒙相關基因 *1-aminocyclopropane-1-carboxylic acid synthase* (*ACC synthase, ACS*)、*abscisic acid stress ripening 1* (*Asr1*)、*carotenoids-associated protein* (*CHRC*)、*indole-3-acetic acid inducible 2* (*CsIAA2*)、*auxin resistant 1* (*CS-AUX1*)、*transthyretin-like protein* (*TLP*) 、*sterol 4-α-methyl-oxidase* (*SMO*)與轉錄因子 *translation releasing factor 2* (*rf2*)、*Populus nigra late elongated hypocotyl 2* (*PnLHY2*)、*ethylene-responsive element binding protein 9* (*EREBP-9*)、*MYB*、*initiation factor-2* (*if-2*)、*bhlh*、*basic transcription factor 3* (*BTF3*)、*WAKY*、*dehydration response element-binding protein 3* (*DREB3*)、*TGACG sequence-specific binding protein 2* (*TGA2*) 可能參與胡瓜花性分化之調控 (Wu et al, 2010)。

(三) 雄花分化相關基因

胡瓜 *CsGAIP* 為阿拉伯芥 *Arabidopsis DELLA* [*GA insensitive* (*GAI*)、*repressor of ga1-3* (*RGA*)、*RGA-like1* (*RGL1*)、*RGL2* 及 *RGL3*] 之同源基因，主要於莖及雄花器表現；轉殖 *CsGAIP* 於阿拉伯芥 *rga-24* / *gai-t6* 雙突變株可部分回復雄蕊發育及雄稔性；過量表現 (overexpression) *CsGAIP* 於野生型阿拉伯芥造成雄蕊數量減少且抑制 *AP3* 及 *PISTILLATA* (*PI*) 表現，*CsGAIP* 可能藉由抑制 B 群基因而抑制雄花

器發育 (Zhang et al., 2014b)。胡瓜 *CsGAMYB1* 主要於雄花器表現，外施 GA_3 可誘導其表現；轉殖 *CsGAMYB1* 於阿拉伯芥 *myb33myb65* 雙突變株可部分回復雄稔性 (male fertility)；於胡瓜抑制 (knockdown) *CsGAMYB1* 表現促使雌花生成量增加，但 *ACS* 表現量、乙烯生成量皆不受影響，推測 *CsGAMYB1* 與乙烯不相關的途徑 (independent pathway) 調控胡瓜花性 (Zhang et al., 2014a)。

三、 苦瓜全雌性基因座 *gy-1*

苦瓜全雌株品系 (gynoecious line) 'Gy263B'與雌雄同株異花品系'Pusa Do Mosami'雜交之 F1，分別進行自交及回交全雌株品系'Gy263B'，其中 F2 子代雌雄同株異花及全雌花植株之分離比，以卡方檢定 (chi-squared test) 符合 3:1 之比例；回交'Gy263B'之子代雌雄同株異花及全雌花植株分離比，以卡方檢定符合 1:1 之比例，故推測苦瓜全雌株性狀由單一隱性基因座控制，並命名為 *gy-1* (Ram et al., 2006)。苦瓜全雌株品系'DBGy-201'及'DBGy-202'分別與雌雄同株異花品系'Pusa Do Mosami'、'Pusa Vishesh'及'Pusa Vishesh'，其 F1 子代均為雌雄同株異花，F1 自交子代 F2 雌雄同株異花及全雌花植株之分離比，以卡方檢定均符合 3:1 之比例，推測'DBGy-201'及'DBGy-202' 全雌株性狀由單一隱性基因座所調控，與 Ram 等人 (2006) 結論相同 (Behera et al., 2009)。苦瓜全雌株品系'OHB61-5'雜交雌雄同株異花品系'OHB95-1A'之 F1 子代，自交而得 F2 子代，以 restriction-associated DNA tag sequencing (RAD-seq) 分析，並以 522 個共顯性標記 (co-dominant marker) 建構一個連鎖圖譜 (linkage map)，發覺數個單一核苷酸多形性 (single nucleotide polymorphism, SNP)，其中 GTFL-1 為最靠近推斷 *gy-1* 基因座之 SNP，可作為分子標記 (molecular marker) 以輔助全雌株苦瓜之育種 (Matsumura et al., 2013)。

四、 苦瓜花性之誘導

(一) 浸種處理誘導改變花性

經 100 mg·L^{-1} 萘乙酸、激動素 (kinetin, KN) 及形態素 (morphactin, CFl) 浸種 24 小時長成之植株，可有效降低雌花始花節位並降低雄雌比；經 100 mg·L^{-1} GA_3 或 25 mg·L^{-1} 離層酸 (abscisic acid, ABA) 浸種 24 小時長成之植株，可降低雄花始花節位、增加雄花量及增加雄雌比；以 6℃低溫浸種處理苦瓜種子 5、10、15 日或

同時處理 6℃低溫及 100 mg·L^{-1} 萘乙酸、激動素或 25 mg·L^{-1} 益收、形態素，隨處理日數增加，雌花量增加、雌花始花節位下降、雄雌花比漸減；以 6℃低溫及 100 mg·L^{-1} GA_3 或 25 mg·L^{-1} 離層酸，隨處理日數增加，雄花量漸增，使花性雄雌比上升 (Prakash, 1976)。

(二) 外施生長調節劑誘導雌花

以 100 mg·L^{-1} 萘乙酸噴施於苦瓜二葉期苗株，可誘導苦瓜平均雌花量由 10 朵上升至 36 朵，雌花始花節位由第 17 節降低至第 5 節，雄雌比由 15.5 降低至 5.1 (Bisaria, 1974)。外施 40 mg·L^{-1} GA_3 於三到五葉期苦瓜'Karala'可增加平均雌花量由 10.66 朵至 21.33 朵，降低花性雄雌比由 5.28 至 2.75 (Banerjee and Basu, 1992)。四葉期苦瓜處理 10 mg·L^{-1} 吲哚乙酸可誘導平均雌花量由 12.3 朵上升至 16.5 朵，雌花始花節位由第 28.5 節降低至第 16.0 節，雄雌比由 3.5 降低至 2.2 (Akter and Rahman, 2010)。施用 500 mg·L^{-1} 益收於六至八葉期苦瓜植株，使雌花百分率由 12 增加至 27 (Thomas, 2008)。外施 150 mg·L^{-1} 抑芽素、100 mg·L^{-1} 2-chloroethyl trimethyl ammonium chloride (CCC)、40 mg·L^{-1} 苄氨基嘌呤 (6-benzylaminopurine, BA) 或 35 mg·L^{-1} 吲哚乙酸、3-hydroxymethyl oxindole (HMO) 於八至十葉期苦瓜，有效促進雌花量，降低雌花始花節位並使雄雌比下降 (Ghosh and Basu, 1982; Ghosh and Basu, 1983)。

五、 激勃素促進葫蘆科作物雄花之生成

外施 GA_3、GA_{4+7}、GA_5 或 GA_9 於稜角絲瓜 (*Luffa acutangula*) 會增加雄花量，增加雌花始花節位，其中以激勃素 GA_3 效果最佳 (Krishnamoorthy, 1972)。外施 10、50、75 mg·L^{-1} GA_3 於葫蘆 (*Lagenaria siceraria*)，均使雄花量上升，花性雄雌比由 12 上升至約 23 (Rahman et al., 1992)。噴施 50 mg·L^{-1} GA_{4+7} 於全雌株胡瓜能顯著誘導雄花之生成 (Pike and Peterson, 1969)。激勃素會誘導全雌株胡瓜雄花之形成 (Mitchell and Wittwer, 1962)。

六、 激勃素促進苦瓜雌花之生成

外施 40 mg·L^{-1} GA_3 於三到五葉期苦瓜'Karala'可增加平均雌花量由 11.33 朵至

24.0 朵，降低花性雄雌比由 5.11 至 3.22 (Banerjee and Basu, 1992)。四葉期苦瓜苗株處理 2.5、5、10 mg·L^{-1} GA_3 促進苦瓜生成雌花、降低雌花始花節位及降低花性雄雌比 (Akter and Rahman, 2010)。於苦瓜六至八葉期苗株施用 10、100、500、1000 mg·L^{-1} GA_3 皆使雌花百分率較控制組增加，其中以 100 mg·L^{-1} GA_3 效果最佳，使雌花百分率由 15 增加至 23 (Thomas, 2008)。外施 20、40、80 mg·L^{-1} GA_3 於八到十葉期苦瓜‘Uchche’或‘Karela’植株可增加總雌花量、增加雄花始花節位、降低雌花始花節位及降低花性雄雌比 (Ghosh and Basu, 1982)。於未處理之苦瓜，其內生激勃素含量隨發育天數逐漸升高，而花性雄雌比也隨之降低，顯示內生激勃素含量與雌花之生成相關 (Ghosh and Basu, 1983)。

七、 激勃素訊息傳遞於花器分化之研究

激勃素訊息傳遞起始於激勃素與其受體 GA insensitive dwarf 1 (GID1) 之結合，並促進 GID1 與 DELLA 蛋白之交互作用，而 DELLA 蛋白為下游激勃素反應基因 (GA responsive genes) 之轉錄抑制子 (transcriptional repressor)，GID1 與 DELLA 之複合物 (complex) 會被 SLEEPY1 (SLY1) 辨識並泛素化 (ubiquitinated)，藉由 26S 蛋白酶體 (26S proteasome) 途徑降解之，以啟動下游激勃素反應基因 (Dill et al., 2004; Ito and Sun, 2005; McGinnis et al., 2003; Murase et al., 2008)。阿拉伯芥激勃素缺陷突變體 (GA-deficient mutant) *ga1-3* 之花器發育遲緩，尤其是雄蕊敗育，導致雄不稔性 (male sterility) (Hou et al., 2008; Yu et al., 2004)。*gid1a gid1b gid1c* 三重突變體 (triple mutant)，花絲 (filament) 及花葯 (anther) 因缺乏分化而導致雄蕊敗育 (abortion) (Griffithsa et al, 2006)。

(一) 激勃素藉由 GAMYB 及 *micro RNA* 調控花器發育

AtMYB33、AtMYB65 及 AtMYB101 具 GAMYB-like 之活性，並具有結合於 *LEAFY* (*LFY*) 啟動子上 8 個特異性 (specific) 核苷酸序列之能力，可能參與調控激勃素誘導開花之訊息傳遞途徑 (Gocal et al., 2001)。*myb33 myb65* 雙突變株花粉母細胞萎縮，導致花葯發育缺陷 (Millar and Gublera, 2005)。水稻 *Oryza sativa GAMYB* (*OsGAMYB*) 主要表現於糊粉層 (aleurone)、花序莖頂組織 (inflorescence shoot apical region)、雄蕊原基及絨氈層 (tapetum)，顯示 *OsGAMYB* 為花器及發粉發育之

重要因子 (Kaneko et al., 2004)。*microRNA 159* (*miR159*) 會導致編碼 GAMYB 之 mRNA 降解，而 *miR159* 會受激勃素抑制 DELLA 蛋白所調控；增加 *miR159* 之表現量會導致 *LFY* 表現量下降、延緩開花及花葯發育受阻 (Achard et al., 2004)。

(二) 激勃素抑制花器同源異型基因

突變 *rga-t2* 及 *rgl2-1* 可大幅恢復 *ga1-3* 突變體之花器發育，外施激勃素可迅速提升 *ga1-3* 突變體之 *AP3*、*PI* 及 *AGAMOUS* (*AG*) 的轉錄量；花器同源異型 (homeotic) 基因會受 *RGA* 之表現所抑制，但 *LFY* 及 *AP1* 則不受影響；過量表現 *AG* 可部分回復 *ga1-3* 突變體之花器發育，顯示激勃素藉由拮抗 DELLA 蛋白之作用，使花器能持續發育 (Yu et al., 2004)。

(三) 激勃素藉由茉莉酸調控雄蕊發育

阿拉伯芥 R2R3-MYB 轉錄因子 MYB21、MYB24 及 MYB57 為雄蕊發育必要之轉錄因子，*myb21-t1 myb24-t1 myb57-t1* 三重突變會導致雄蕊萎縮、雄不稔等性狀，而 DELLA 蛋白會抑制 MYB21、MYB24 及 MYB57 之表現 (Cheng et al., 2009; Mandaokar et al., 2006)；*MYB21*、*MYB24* 及 *MYB57* 於茉莉酸 (jasmonate, JA) 不足突變體 (JA-deficient mutant) *oxophytodienoate reductase 3* (*opr3*) 表現量降低，而外施茉莉酸能成功誘導三者之表現 (Mandaokar et al., 2006)。外施激勃素於 *opr3* 無法誘導 *MYB21*、*MYB24* 及 *MYB57* 之表現，而外施茉莉酸於 *ga1-3 gai-t6 rgat2 rgl1-1 rgl2-1* 五重突變體 (penta mutant) 能成功誘導 *MYB21*、*MYB24* 及 *MYB57* 之表現，其中激勃素反應基因 *GA3ox1*、*GA20ox1* 及 *GA2ox1* 表現量無顯著變化，顯示外施茉莉酸並非藉由誘導激勃素之生成調控 *MYB21*、*MYB24* 及 *MYB57* 之表現 (Cheng et al., 2009)。*lipoxygenase 1* (*LOX1*) 於 *ga1-3* 表現量顯著降低，而於 *ga1-3 gai-t6 rga-t2 rgl1-1* 四重突變體 (quadruple mutants) 則恢復其表現量，顯示 *LOX1* 為激勃素誘導型基因；*defective anther dehiscence 1* (*DAD1*) 於 *ga1-3* 及 *ga1-3 gai-t6 rga-t2 rgl1-1* 之表現量約為 20%野生型表現量，而於 *ga1-3 gai-t6 rgat2 rgl1-1 rgl2-1* 之表現量約為 60%野生型表現量，顯示激勃素可能藉由抑制 RGL2 調控 *DAD1* 之表現 (Cheng et al., 2009)。

Coronatine insensitive1 (COI1) 接受茉莉酸訊號後，結合至 jasmonate-ZIM

domain proteins (JAZs)，並誘導 26S 蛋白酶體途徑降解之，進而調解各種茉莉酸反應 (Katsir et al., 2008)。以酵母菌雙雜合系統 (yeast two-hybrid system) 篩選阿拉伯芥中與 JAZs 具交互作用之蛋白，其中 MYB21、MYB24 及 MYB57 與 JAZ1、JAZ8 及 JAZ11 具交互作用 (Song et al., 2011)。*myb21 myb24* 雙突變株 (double mutant) 於花粉成熟、花葯開裂 (anther dehiscence) 及花絲伸長 (filament elongation) 具缺陷，因此導致雄不稔性；過量表現 *MYB21* 於茉莉酸受體突變株 *coi1-1*，可部分回復雄稔性，但無法回復其他茉莉酸所調控之性狀，推測 JAZ 與 MYB21、MYB24 及 MYB57 相互作用以降低 MYB21、MYB24 及 MYB57 之調控功能 (Song et al., 2011)。

施用 1 μM 激勃素或 1 μM 巴克素 (paclobutrazol, PAC) 於阿拉伯芥 *35S:JAZ1-GUS* 轉殖株，顯示茉莉酸誘導之 JAZ1-GUS 並無受到激勃素或巴克素之影響，而激勃素或巴克素卻會影響茉莉酸反應基因 (JA-responsive genes) 於 *coi1-1* 突變株之表現；*in vitro* pull-down assay 顯示 His-MYC2 與 GST-JAZ1 之交互作用會受 GST-RGA 之影響，而 His-MYC2 亦降低 GST-RGA 與 His-JAZ1 之交互作用，顯示 DELLA 蛋白會與 MYC 競爭結合至 JAZ 而調控茉莉酸訊息傳遞 (Hou et al., 2010)。

參、 材料與方法

一、 植物材料

苦瓜品種‘月華’(*Momordica charantia* L. cv. Moon Shine) 種子係購得自農友種苗公司 (KNOWN-YOU SEED CO., LTD.)。將種殼去除後，以 50℃溫湯處理約 1 小時，播種介質為等體積混合之根基旺及泥炭苔，栽培於國立臺灣大學轉殖溫室及精密溫室。

二、 試驗方法

(一) 外施荷爾蒙處理

將苦瓜‘月華’種子去除種殼後，以 50℃溫湯處理約 1 小時，播種於以根基旺及泥炭苔一比一混合之介質，栽培於國立臺灣大學轉殖溫室及精密溫室。葉面噴施 100 mg·L^{-1} 之 GA_3 或 500 mg·L^{-1} 益收於六至八葉期苦瓜苗株，每三天噴施一次，共施用 3 次，於第 9 天採取植株地上部，以液態氮急速冷凍，凍存於-80℃備用。

(二) 低溫及荷爾蒙浸種處理

將苦瓜種子浸種於 100 mg·L^{-1} GA_3、離層酸、萘乙酸、激動素或 25 mg·L^{-1} 益收於 4℃處理 15 天，取出以二次蒸餾水潤洗，再以擦手紙拭乾，去除種殼後，播種於以根基旺及泥炭苔一比一混合介質，栽培於臺灣大學轉殖溫室及精密溫室，待其長至至六至八片葉時，採取植株地上部，以液態氮急速冷凍，凍存於-80℃備用。

(三) 苦瓜總 RNA 萃取

預先將 15 ml RNA 萃取緩衝液 [3% hexadecyl trimethyl-ammonium bromide (CTAB)、3% polyvinylpyrrolidone-40 (PVP-40)、25 mM ethylenediaminetetraacetic acid disodium (Na_2EDTA) pH 8.0、2M sodium chloride (NaCl)、0.1M Tris-HCl pH8.0、0.05% spermidine)] 與 600 μL 2-mercaptoethanol 混勻，取苦瓜地上部組織約 2 g 以液態氮研磨至細粉狀，加入 RNA 萃取緩衝液中混勻，於 65℃水浴 10 分鐘，加入 15 ml CI (chloroform/isoamyl alcohol = 24:1) 震盪混勻，以 Beckman J2-MC 離心機、轉子

JS13.1，於 4℃以 8,800 rpm 離心 10 分鐘，上清液再重複上步驟以 CI 萃取一次。取上清，加入 1/3 體積之 8 M 氯化鋰 (lithium chloride, LiCl)，於 4℃過夜沉澱。以 Beckman J2-MC 離心機、轉子 JS13.1，於 4℃以 8,800 rpm 離心 30 分鐘，倒除上清，以 500 μL 之 0.5% sodium dodecyl sulfate (SDS) 回溶 pellet，取至 1.5 ml 微量離心管，加入 500 μL CI 震盪混勻，於 4℃以 13200 rpm 離心 10 分鐘，取上清，加入兩倍體積之絕對酒精，貯存於-20℃沉澱備用。取用時，以 13,200 rpm 於 4℃離心 30 分鐘，倒除上清，以 200 μL 之 70%酒精洗鹽，再以 13,200 rpm 於 4℃離心 10 分鐘，pellet 以 200 μL 絕對酒精潤洗，以 13,200 rpm 於 4℃離心 10 分鐘，去除上清，風乾後回溶於 1% diethyl pyrocarbonate (DEPC) 處理過之無菌水 (1% DEPC-treated water) 約 100-200 μL，取 2 μL 以微量分光光度計 (nanodrop) ACTGene ASP-3700 定量，並將 RNA 樣品稀釋至 1,000 ng·μL^{-1}，凍存於-80℃備用。

(四) DNase I 處理

取 60 μL RNA 樣品加入 27.5 μL 1% DEPC- treated water、10 μL 10 X buffer 及 2.5 μL DNase I (Qiagen RNase-Free DNase Set. Cat. no. 79254)，於 37℃反應 30 分鐘；再加入 400 μL 1% DEPC- treated water，使總體積增加體積至 500 μL，再以 500 μL 之 CI 萃取，終止 DNase I 反應，取上清，加入 1/10 體積 5 M NaCl 及兩倍體積絕對酒精，於-20℃沉澱備用。取用時以 13,200 rpm 於 4℃離心 30 分鐘，倒除上清，以 200 μL 之 70%酒精潤洗，以 13,200 rpm 於 4℃離心 10 分鐘，再以絕對酒精潤洗，以 13,200 rpm 於 4℃離心 10 分鐘，去除上清，風乾後以 40-50 μL 1% DEPC-treated water 回溶，取 2 μL 以微量分光光度計 ACTGene ASP-3700 定量，並將 RNA 樣品稀釋至 1,000 ng·μL^{-1}，凍存於-80℃備用。

(五) RNA 電泳品質檢測

取 4 μg RNA 樣品加至 2.5 μL 0.2 M pH 7.0 磷酸緩衝液 (phosphate buffer)、7.1 μL 7 M deionized glyoxyal、25 μL dimethyl sulfoxide (DMSO)，並以 1% DEPC-treated water 定量至 50 μL，於 50℃水浴 1 小時，再加入 10 μL RNA loading dye 混勻，並取 35 μL 於 10 mM pH 7.0 磷酸緩衝液中電泳。取出膠體浸於 50 mM 氫氧化鈉 (sodium hydroxide, NaOH) 以 50 rpm 搖晃 30 分鐘、200 mM 醋酸鈉 (sodium acetate,

NaOAc) 10 分鐘，在取出膠體浸於 TBE buffer 並以每 10 mL 加入 1μL 溴化乙錠 (Ethidium bromide, EtBr)，以 50 rpm 搖晃外染 10 分鐘，照膠判斷品質。

(六) 轉錄體分析流程

苦瓜總 RNA 由普渡大學 (Purdue University) 以 Illumina 平台進行 RNA 定序後，獲得原始序列 (raw reads)，將定序品質不佳之序列移除 (filter) 後，混合兩組序列並藉由 Trinity (Grabherr et al., 2010) 重新組裝 (*de novo* assembly) 成 contigs (圖 1)。組裝後的所有 contigs 均由 ContigViews (Liu et al., 2014) 平台以 Basic Local Alignment Search Tool (BLAST) 與 The *Arabidopsis thaliana* Information Resource (TAIR) 解碼序列 (coding sequence, CDS) 資料庫 (TAIR 10) 及 European Molecular Biology Laboratory (EMBL) CDS 資料庫進行比對，預測開放解讀框架 (open reading frame, ORF) 並註解 (annotate) 之 (圖 1)。其中 contigs 能比對到 TAIR 或 EMBO CDS 資料庫所發表之序列，但無法完整界定其開放解讀框架，被歸類為其他 (others)，其他以及未註解之 contigs 共同歸為目前未定義 (undefined) 之 contigs (圖 1)。各基因之表現量以 fragments per kilobase of transcript per million mapped reads (FPKM) 估計，並以 DESeq2 package 鑑別激勃素處理與未處理苦瓜苗株之差異表現基因，以表現倍率大於二及 p-value ≤ 0.05 視為差異表現基因。

(七) 即時定量反轉錄聚合酶連鎖反應

使用 BioLab cDNA kit 進行反轉錄反應，取 1 μg 總 RNA 加入 2 μL $d(T)_{23}VN$，並以 Nuclease-free water 定量至 8 μL，置於 65℃ 反應 5 分鐘，解開 RNA 之二級結構，反應後立即置於冰上，加入 10 μL ProtoScript II Reaction Mix (2 X) 及 2 μL ProtoScrip II Enzyme Mix (10 X)，42℃ 反應 1 小時，接著於 80℃ 5 分鐘終止酵素反應，將合成之 cDNA 稀釋 10 倍，凍存於-20℃ 備用。以 LabStar SYBR qPCR Kit 進行即時定量反轉錄聚合酶連鎖反應偵測基因表現量，取 2 μL cDNA 加入 12.5 μL 2 X LabStar SYBR qPCR master mix、10 μM 引子對各 1.25 μL 及 8 μL RNase free water。以 Thermocycler® Real-time PCR machine 進行 qPCR，循環參數為：95℃ 3 分鐘；接著以 95℃ 5 秒、60℃ 20 秒、72℃ 8 秒，循環 40 次，最後再以 60℃ 至 95℃ 每 6 秒升溫 1℃，建立 melting curve 確認單一產物之合成。本論文所使用之

引子列表於表 1 至表 3。苦瓜 *Actin* 基因以引子對 McACT-5 (5’-ACA TGC CAT TCT TCG ACT TGA CCT TGC TG-3’) 及 McACT-3 (5’-GCC CAT CAG GCA ACT CAT AGC TCT TTT CAA C-3’) 擴增 (amplified) 做為內部控制 (internal control)，並以 qPCRsoft 3.0 計算相對表現量。

表 1. 用於即時定量反轉錄聚合酶連鎖反應之苦瓜 ACC 合成酶基因專一性引子

Table 1. Primers used for real-time quantitative reverse transcription-PCR analysis of ACC synthase genes.

Gene	Product size (bp)	Tm (℃)	Sequence (5'→3')
McACS1	230	61.2	CCT TTT CAC CCC ACT CTC AAC CCT C
		62.7	CGG TCG CCT CTC ACT TTT CCC ATG A
McACS8.1	163	61.0	CGT TTC TCC TCC CAA CTC CAT ACT ATC CAG
		63.3	GCG TAG GTT TCG GGT TTG GGC TTC T
McACS8.2	168	61.6	TCG TTC CCA CCC CAT ACT ATC CAG G
		60.9	CCC CTT TCA CTC TCA GTT TCA GCT CC
McACS9.1	203	61.6	CGG ACG AGA AGT TCA GGG GGA GTT A
		60.6	CAC AAT CTT CCT CCA CAG CTC CAT CTC
McACS9.3	133	62.0	TAA GTG CCT GAA CAG CAA TGC GGG
		61.8	GTT CAG TGC AGT GGC ATG AGG ATC C
McACS10	187	61.3	CCT TCG GAT GGA TTG GCG GAT TTG A
		61.4	GGT AAT ATG GAG TAG GGA CGA GAA ACG CC
McACS11	160	62.8	TCT TTT CTG CTG GGT GGA CAT GAG GC
		62.6	GGC AAA GCA CAT TCT GAA CCA ACC CG
McACS12	136	61.4	CCA ACT CCG ACT CCT CCT CCT CCA ATC
		64.2	CGA GAA GAG AGC CGA ACC CGT CCA A

表 2. 用於即時定量反轉錄聚合酶連鎖反應之苦瓜 ACC 氧化酶基因專一性引子

Table 2. Primers used for real-time quantitative reverse transcription-PCR analysis of ACC oxidase genes.

Gene	Product size (bp)	Tm (℃)	Sequence (5'→3')
McACO1	191	63.7	GCT GAT GTG CGA GAA TCT GGG CCT G
		62.8	CCG GAA CTT GGT CGT CTT GGA GGA G
McACO4.1	187	62.0	GCG GAA GAG TTG TTG GAG TTG CTG TG
		62.6	AGA GAA GGA TGA TGC CAC CAG CGT C
McACO4.2	203	63.9	AAC TGG CGG AGC TGG TAC TGG ACT T
		62.4	GTG GTC TTG GAA GAG GAG GAT GAG CC
McACO4.3	158	62.5	CAG TGG CCT ACA GCT CCT CAA AGA TGG
		63.2	GGC CGG TTC CGT TCG CTT GAG TTA T
McACO5	240	61.9	AAC AAG AGC GGT GAG AAG CTG GAG A
		63.3	GAA GAG CAA GAT GAC ACC GCC AGC A

表 3. 用於即時定量反轉錄聚合酶連鎖反應之苦瓜花性相關基因專一性引子

Table 3. Primers used for real-time quantitative reverse transcription-PCR analysis of flower sexuality related genes.

Gene	Product size (bp)	Tm (℃)	Sequence (5'→3')
McGID1B	208	62.5	GTC TCA GTT AAC TAC CGC CGA TCG CC
		61.9	ATA CCT CGA CGT CTT CTT CAG CTG CC
McXERICO.1	249	62.1	CGT CTG AAG GTG TAC TGG GTG TGA TCC
		62.1	GCC ATT TGC AGC TGT GAA CTT TGT CGT
McXERICO.2	139	62.0	CTA GTC TCC CAG CTC CAT CCG AAG G
		61.1	TGG CGA TGA GAG ACG GAT TCC TAC A
McMYB33	233	60.0	AGC CGT TCC CAC CTT CTC TTA TCT C
		61.0	GGC GAC CCA TAT GAC TGA GCA ACT T
McMYB24.1	137	61.8	GTG GAC GGT GGA GGA GGA CAT AAC T
		61.4	GGA GAT AAT TCA GCC ACC GCA GAC G
McERAF16	131	60.9	TTC ATG GGT AGT ACA AGT CCG AGG AGT G
		60.7	CTC TTC CAC AAT TCC CTC AAC CAC CA
McERAF17	234	62.6	CCT ACC AGT TCT CCA GCC ACG ACA TG
		61.6	TCT CCT CAT CTC CTC CAT CTC ACT CTT CC

肆、 結果

一、 外施激勃素對苦瓜花性之影響

以100 mg·L^{-1} GA_3噴施於苦瓜品種'月華'六至八葉期苗株，可促使植株平均總雌花量由22朵上升至38朵，花性雄雌比則由37降至23，雄花量及全株總花量則無顯著差異 (表4)。顯示外施100 mg·L^{-1} GA_3能提升苦瓜'月華'植株之雌性程度。

二、 全轉錄體 (whole-transcriptome) 次世代定序分析

苦瓜總RNA定序共獲得406,003,512條原始序列 (raw reads)，其中225,603,242條序列來自未處理苦瓜苗株，180,400,270條序列來自激勃素處理苦瓜苗株 (圖 1)。濾除 (filter) 定序品質不佳之序列後，未處理與激勃素處理苦瓜苗株分別獲得223,173,640條及178,786,712條序列；總長度分別為22,334,848,879 nucleotides (nt)及 17,905,123,646 nt (圖 1)，平均序列長度約為 99 nt (表 5)。重新組裝 (*de novo* assembly) 後，共獲得106,057條contigs，其中 N_{50} 長度為2,463 nt (表 5)。總共有66,639條contigs (62.8%) 能比對 (aligned) 到資料庫之序列，並被註解為推定基因(putative genes)；39,418條contigs無法比對到資料庫之序列，約佔37.2%之總contigs數量 (圖 2A)。開放解讀框架預測結果顯示21,511條contigs具完整開放解讀框架(complete ORF)，約20.3%之總contigs數量；19,343條contigs僅具部分開放解讀框架 (partial ORF)，約18.2%之總contigs數量；其中25,785條contigs被歸類為其他 (others)。其他以及未註解之contigs共同歸為目前未定義 (undefined) 之contigs，約佔61.4%之總contigs數量 (圖 1、圖 2A)。各基因之表現量以FPKM估計，並以DESeq2 package鑑別激勃素處理與未處理苦瓜苗株之差異表現基因，所有開放解讀框架註解之推斷基因中，約8,948條contigs表現差異達兩倍以上，其差異表現分布情形如圖 2B；目前未定義之contigs中有26,181條表現差異達兩倍以上。將胡瓜花性相關基因於苦瓜之同源基因整理如表 5 及本實驗室分離之苦瓜 cDNA與其對應contigs整理如表 7。

經由 Gene Ontology (GO) 分析所有推定基因，其中生物過程 (biological process) 分類中，差異性表現基因多分布於cellular process、biosynthetic process、metabolic process，許多差異性表現基因分布於感興趣的類別 signal transduction、

post-embryonic development、reproduction、flower development、embryo development、regulation of gene expression, epigenetic (圖 3)；分子功能 (molecular function) 分類中，差異性表現基因多分布於 binding、nucleotide binding、catalytic activity、hydrolase activity，許多差異性表現基因分布於感興趣的類別 transferase activity、kinase ativity、transport activity、signal transducer activity、receptor activity (圖 4)；細胞組成 (cellular component) 分類中，差異性表現基因多分布於 nucleus、plastid、plasma membrane、membrane (圖 5)。

三、 激勃素生合成及訊息傳遞相關基因之差異表現

將激勃素生合成相關基因整理如表 8。除了 *ent-kaurenoic acid oxydase* (*McKAO1.1*, BG20689.1)、*McKAO2.2* (BG38639.1)、*McKAO2.3* (BG38636.1)、*GA 20-OXIDASE* (*McGA20ox2.1*, BG14156.1) 及 *McGA20ox3.1* (BG14156.1) 之外，其餘激勃素生合成相關基因 *CPP synthetase*s (*McCPS*s) (BG16911.1、BG54075.1)、*McKAO*s (BG17490.2、BG38638.1、BG13077.1) 及 *McGA20ox*s (BG9228.1、BG17029.1、BG6412.1、BG53175.1)，皆於處理後表現量降低 (圖 6)。除了 *McGA2ox2.1* (BG33874.1) 及 *McGA2ox6* (BG19309.1) 外，編碼激勃素失活酶 (GA-inactivating enzyme) GA2ox 之基因 (BG8254.1、BG20162.1、BG20174.1、BG49090.1、BG48466.1)，均於外施激勃素後表現量增加 (圖 6)。

激勃素訊息傳遞相關基因整理如表 8。兩個編碼激勃素受體 *GID1* 的基因，*McGID1B* (BG35789.1、BG35789.2) 及 *McGID1C* (BG16828.1、BG16828.2) 於激勃素處理後表現量增加。DELLA 蛋白為下游激勃素反應基因之轉錄抑制子，包含 GAI、RGA、RGL1、RGL2 及 RGL3，其中 *RGL3* 於苦瓜無相對應之序列。除了 *McRGA1.1* (BG16044.1) 及 *McRGA1.2* (BG43062.1) 表現量於激勃素處理後上升外，*McGAI*s (BG35379.1、BG36311.1、BG19427.1)、*McRGL1*s (BG19426.1、BG51724.1、BG16733.1)、*McRGL2* (BG22846.1) 均於激勃素處理後表現量不同程度下降 (圖 7)。

四、 乙烯生合成及訊息傳遞相關基因之差異表現

將乙烯生合成相關基因整理如表 9。如所有推定基因中，有八個 ACC 合成酶 (*ACC SYNTHASE, ACS*) 基因和六個 ACC 氧化酶 (*ACC OXIDASE, ACO*) 基因 (圖 8、表 10)。其中編碼 ACC 合成酶的基因中只有 *McACS8.1* (BG10149.1)、*McACS10* (BG32424.1)、*McACS11* (BG51419.1) 及 *McACS12* (BG19736.1) 於激勃素處理後表現量上升，其他 ACC 合成酶基因 (BG29132.1、BG9684.1、BG58303.1、BG4821.1、BG47803.1、BG4822.1、BG47992.1) 之表現量則下降 (圖 8)。*McACS8.2* (BG58303.1) 及 *McACS11* 核苷酸序列與 National Center for Biotechnology Information (NCBI) 已發表之核苷酸序列 FB801118 編碼相同，*McACS8.2* 位於 FB801118 序列上游，而 *McACS11* 位於 FB801118 序列下游，且 *McACS8.2* 及 *McACS11* 核苷酸序列有二十一個核苷酸相同且重疊 (圖 9)。BG42288.1 與 BG42284.12 序列完全相同但較短；BG42289.1 與 BG42284.12 序列相似度高，僅 BG42289.1 於 5'端及中間部分不盡相同，可能為 *de novo* assembly 之錯誤組裝，故共同歸類為 *McACO5* (BG42284.12、BG42288.1、BG42289.1) (圖 10)。ACC 氧化酶的基因表現情形如同 ACC 合成酶，僅有 *McACO5* 表現量於處理後微量上升，其餘表現量均下降 (圖 8、表 10)。

將乙烯訊息傳遞相關基因整理如表 10。大部分參與乙烯訊傳遞之基因 *McETR1* (BG37469.1、BG37469.2、BG37469.4)、*McETR2* (BG18654.1)、*ethylene-insensitive3*s (*McEIN3*s) (BG41424.1、BG41424.2、BG41424.3、BG21121.1)、*EIN3-binding F Box proteins* (*McEBF*s) (BG2025.1、BG2025.1、BG7310.1、BG8936.1、BG49442.1、BG44947.1)、*McEIN3-like 3* (*McEIL3*) (BG17837.1、BG17837.3、BG17837.4)、*ethylene response factor 1*s (*McERF1*s) (BG35672.1、BG41903.1、BG1913.1) 均受激勃素所誘導而表現量增加，僅 *constitutive triple response 1.3* (*McCTR1.3*) (BG25150.1) 表現量於處理後些微下降 (圖 11、表 11)。編碼 EIN5 之基因於苦瓜沒有比對到開放解讀框架註解序列，與其同源之序列均被歸類於其他，其中 BG25369.1 及 BG25369.2 分別於 5'及 3'端解碼序列中含數個終止密碼子且胺基酸相似度甚差；而 *ETP1* 及 *ETP2* 則於苦瓜無相對應之序列。

五、 離層酸生合成及訊息傳遞相關基因之差異表現

將類胡蘿蔔素及離層酸生合成相關基因分別整理如表 11 及表 12。大部分參與

類胡蘿蔔素 (carotenoids) 生合成之基因於激勃素處理後表現量無明顯差異，僅 *phytoene synthase.2* (*McPYS.2*)、*McPSY.4* 及 *lutein deficient 5* (*McLUT5*) 表現量下降，而 *phytoene desaturase 1* (*McPDS1*)、*beta carotenoid hydroxylase 1*s (*McCHY1*s) (BG33129.1、BG33129.5、BG22514.1、BG22514.2) 表現量上升。離層酸關鍵生合成基因 *nine-cis-epoxycarotenoid dioxygenase 1.2* (*McNCED1.2*)、*McNCED3.2* 於激勃素處理後表現量下降，而 *McNCED2*、*McNCED3.1*、*McNCED5* 則於處理後表現量增加 (圖 12、表 13)。

將離層酸訊息傳遞相關基因整理如表 14。大部分編碼離層酸受體 pyrabactin resistance (PYR) 及 PYR1-like (PYL) 之基因 (BG17593.1、BG34497.1、BG41684.1、BG56387.1、BG9941.1、BG9941.2) 於外施激勃素後表現量下降，僅 *McPYL6.2* (BG6137.1) 表現量上升 (圖 13、表 14)。蛋白磷酸酶 2C (protein phosphatases type 2C, PP2C) 會抑制下游 sucrose non-fermenting 1 (SNF1)-related protein kinase (SnRK) 進而抑制離層酸反應，大部分編碼 PP2C 之苦瓜同源基因 (BG39020.1、BG24497.1、BG24497.3、BG52143.1、BG41965.1) 表現量受外施激勃素而增加，僅 *McHAB1.3* (BG52931.1) 及 *McABI2* (BG56165.1) 表量降低；大部分 *McSnRK*s 於激勃素處理後無明顯表現差異，只有 *McSnRK3.10.1* (BG23645.1)、*McSnRK3.10.2* (BG15498.1)、*McSnRK3.10.3* (BG46261.1)、*McSnRK3.11.2* (BG56645.1)、*McSnRK3.15* (BG23267.1)、*McSnRK3.22.3* (BG44895.1) 於激勃素處理後表現量降低，而 *McSnRK1.1.2* (BG1335.1、BG1335.2)、*McSnRK3.10.4* (BG47884.1)、*McSnRK3.14.3* (BG53261.1) 表現量增加 (圖 13、表 14)。

六、 植物生長素訊息傳遞相關基因之差異表現

將植物生長素訊息傳遞相關基因整理如表 15。激勃素處理後，編碼植物生長素受體 TRANSPORT INHIBITOR RESPONSE 1 (TIR1) 及 AUXIN SIGNALING F-BOX (AFB) 之基因僅 *McAFB5.1* (BG38467.1) 及 *McAFB5.2* (BG38470.1) 表現量下降，其餘 *TIR1* 及 *AFB* 之苦瓜同源基因 (BG40740.1、BG45375.1、BG45375.3、BG38869.1、BG38869.3、BG28788.1、BG28788.4、BG28788.5) 表現量與未處理苦瓜苗株近似；ENDOPLASMIC RETICULUM AUXIN BINDING PROTEIN 1 (ABP1) 為植物生長素之輔助受體 (coreceptor)，其苦瓜同源基因 *McABP1* (BG36497.1) 於

激勃素處理後表現量亦無明顯差異 (圖 14、表 15)。AUX/IAA 為下游植物生長素反應基因 (auxin responsive gene) 之轉錄抑制子，其中 *IAA1*、*IAA2*、*IAA3*、*IAA6*、*IAA10*、*IAA11*、*IAA15*、*IAA16*、*IAA18*、*IAA20*、*IAA28*、*IAA30*、*IAA31*、*IAA34* 於苦瓜無相對應之序列。*McIAA19.2* (BG12525.1)、*McIAA19.3* (BG9910.1)、*McIAA26.1* (BG39164.1)、*McIAA26.2* (BG27519.1)、*McIAA27.1* (BG37209.1) 表現量受激勃素處理而上升，反之，*McIAA 4.1* (BG18450.1)、*McIAA5* (BG31366.1)、*McIAA12* (BG23742.1)、*McIAA14.1* (BG19521.1)、*McIAA14.2* (BG15663.1)、*McIAA14.3* (BG27213.1)、*McIAA17* (BG27211.1)、*McIAA33* (BG9656.1) 於激勃素處理後表現量降低 (圖 14、表 15)。AUXIN RESPONSE FACTORs (ARFs) 為一轉錄因子家族會結合至 auxin-response elements (AuxREs) 以啟動下游初期植物生長素反應，其中 *ARF11*、*ARF12*、*ARF13*、*ARF14*、*ARF15*、*ARF20*、*ARF21*、*ARF22*、*ARF23* 於苦瓜無同源序列，且大部分 ARFs 表現量與未處理苦瓜苗株相近，僅 *McARF7* (BG35524.1)、*McARF9.1* (BG34798.1)、*McARF19.1* (BG34597.1)、*McARF19.2* (BG35525.1、BG35525.2、BG35525.3) 表現量於激勃素處理後些微上升，*McARF5* (BG20685.1、BG20685.3)、*McARF6.1* (BG42471.1)、*McARF6.3* (BG19620.1)、*McARF8.1* (BG42468.1) 表現量則於處理後稍微降低 (圖 14、表 15)。

七、 茉莉酸訊息傳遞相關基因之差異表現

將茉莉酸訊息傳遞相關基因整理如表 15。JASMONATE RESISTANT 1 (JAR1) 會將茉莉酸催化成茉莉酯異亮胺酸 (jasmonyl-isoleucine, JA-Ile)，而 *JAR1* 之苦瓜同源基因 (BG20637.1、BG20920.1、BG20920.2、BG20920.4、BG20919.1、BG58230.1) 表現量皆於激勃素處理後增加 (圖 15、表 16)。編碼茉莉酸受體 COI1 之基因於苦瓜沒有比對到完整或部分開放解讀框架註解序列，與其同源之序列均被歸類於其他，其中 BG13730.2 之 5'端缺乏起始密碼子 (start codon)；BG13730.1 於解碼序列中含數個終止密碼子 (stop codon)；BG13730.3 則於解碼序列中含數個終止密碼子，且亦缺乏 5'端起始密碼子。除了 *McJAZ3.2* (BG27121.1) 之外，其餘 JAZ 之苦瓜同源基因 (BG24597.1、BG24597.2、BG27121.1、BG28497.1、BG21290.1、BG29943.1) 均於激勃素處理後表現量下降 (圖 15、表 16)。

八、 雄蕊發育相關基因之差異表現

將雄蕊發育相關基因整理如表 17。*XERICO* 之苦瓜同源基因 *McXERICO.1* (BG17271.1、BG17271.2、BG17271.3) 及 *McXERICO.2* (BG15458.1、BG15458.2) 於外施激勃素後表現量上升 (圖 16、表 17)。*LOX1* 為茉莉酸生合成相關基因，其苦瓜之同源基因 *McLOX1.2* (BG19644.1)、*McLOX1.3* (BG8248.1) 及 *McLOX1.4* (BG11124.1) 表現量同樣受激勃素處理而上升 (圖 16、表 17)。茉莉酸訊息傳遞相關基因 *McJAZ8* (BG28497.1) 與 *McMYB24.1* (BG46078.1) 皆於激勃素處理後表現量下降。編碼 ARF6 之基因 (BG42471.1、BG42465.1、BG19620.1、BG19619.1、BG19618.1) 及 ARF8 之基因 (BG42468.1、BG42473.1)，於激勃素處理後表現量下降；編碼核轉錄因子 (nuclear transcription factors) EIN3/EILs 之基因於外施激勃素後表現量則增加 (圖 16)。*McAP3*、*McPI*、*McAG* 為 MADS-box 之花器發育同源異型基因，其中 *McAP3* (BG1411.1) 及 *McAG* (BG30120.1) 於外施激勃素後表現量下降，而 *McPI* (BG11587.1) 則於處理後表現量增加 (圖 16、表 17)。*MYB33*、*MYB65* 及 *MYB101* 為 *GAMYB* 同源基因，其中 *MYB65* 及 *MYB101* 於苦瓜無同源序列，而 *McMYB33* (BG34956.1、BG34956.3、BG34956.4、BG34956.5) 則於激勃素處理後表現量無顯著差異 (圖 16、表 17)。*DAD1*、*MYB65*、*MYB101*、*MYB108*、*RGL3*、*JAZ1*、*JAZ11* 於苦瓜沒有比對到完整或部分開放解讀框架註解序列。

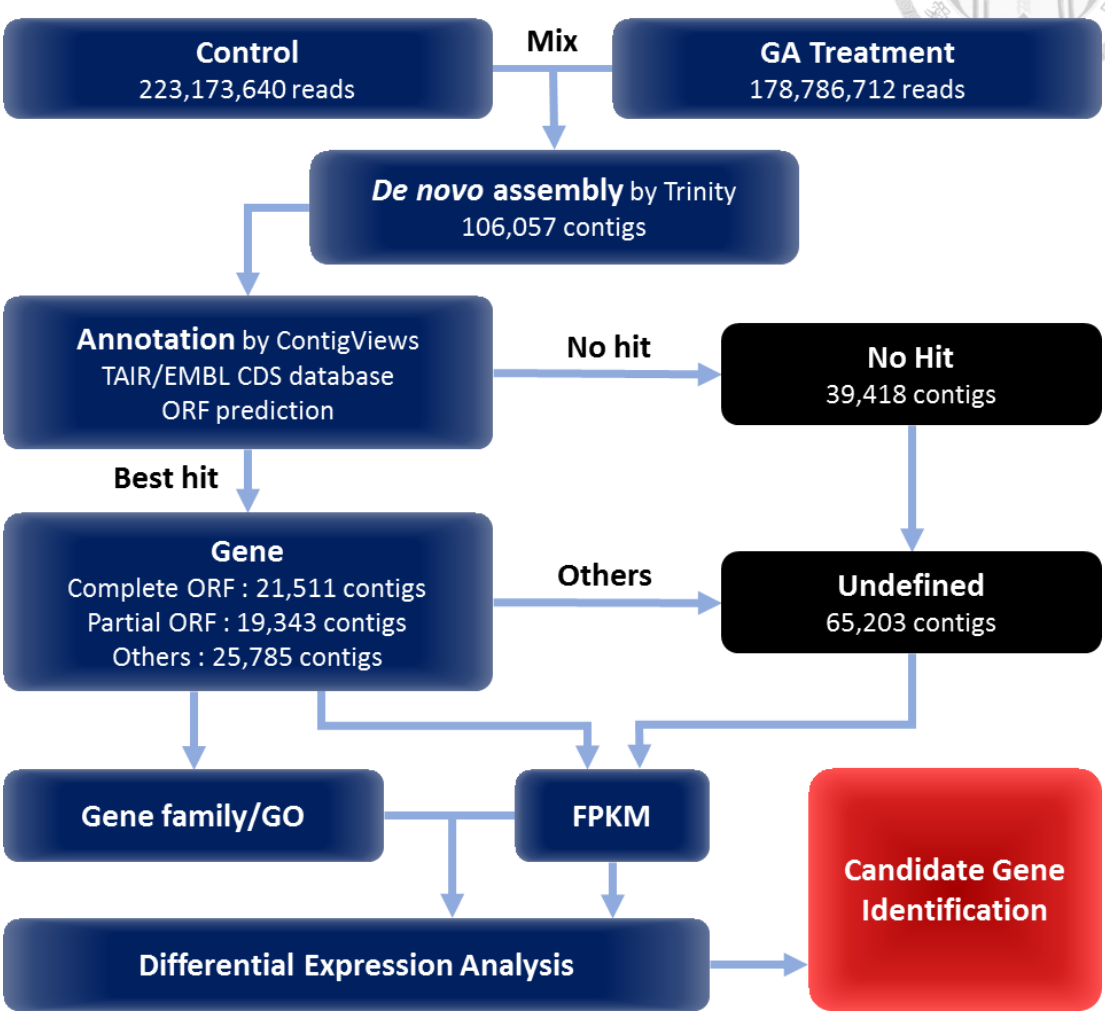

圖 1. 轉錄體次世代定序分析流程

Fig. 1. The NGS analysis workflow for the whole-transcriptome analysis pipeline. Control and GA treatment samples from *Momordica charantia* 'Moon Shine' were selected for whole-transcriptome analysis using Illumina. The 106,055 de novo assembled *M. charantia* contigs sequences were compared with the TAIR and EMBL coding sequence (CDS) databases through BLAST for open reading frame (ORF) annotation. FPKM, Fragments per kilobase of transcript per million mapped reads. GO, Gene ontology.

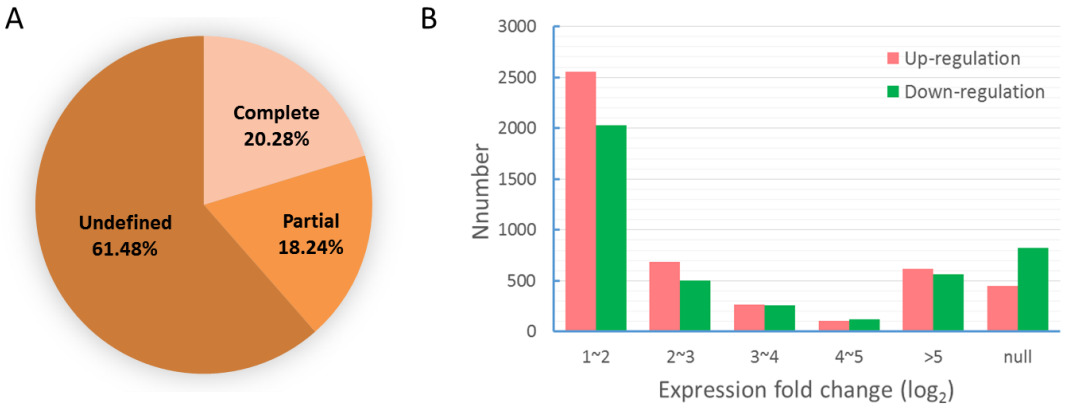

圖 2. 開放解讀框架註解情形

Fig. 2. The characterization of open reading frame (ORF) annotations. (A) Pie chart showing the percentages of complete genes, partial genes and undefined contigs. (B) Fold change distribution between expression levels of control and GA treated bitter gourd seedlings for complete and partial ORF contigs.

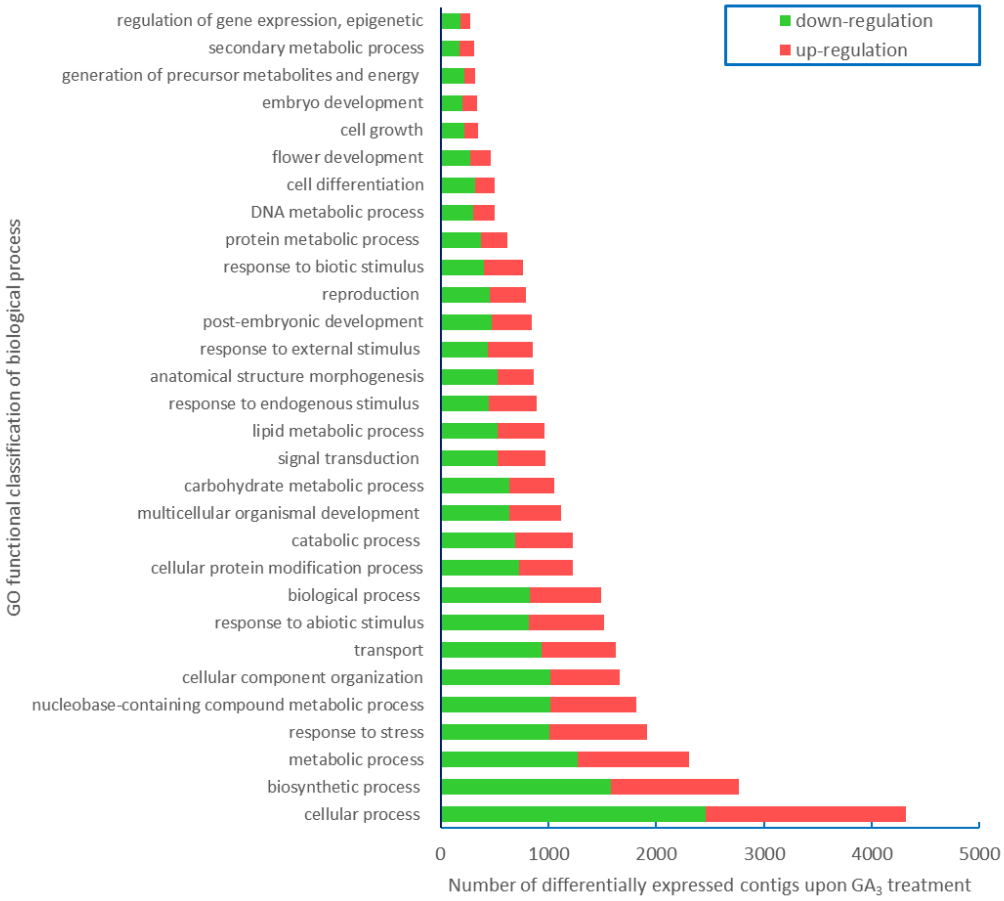

圖 3. 差異性表現基因之生物過程功能分類

Fig. 3. Functional categorization of differentially expressed contigs after GA_3 treatment based on the biological process of Gene Ontology.

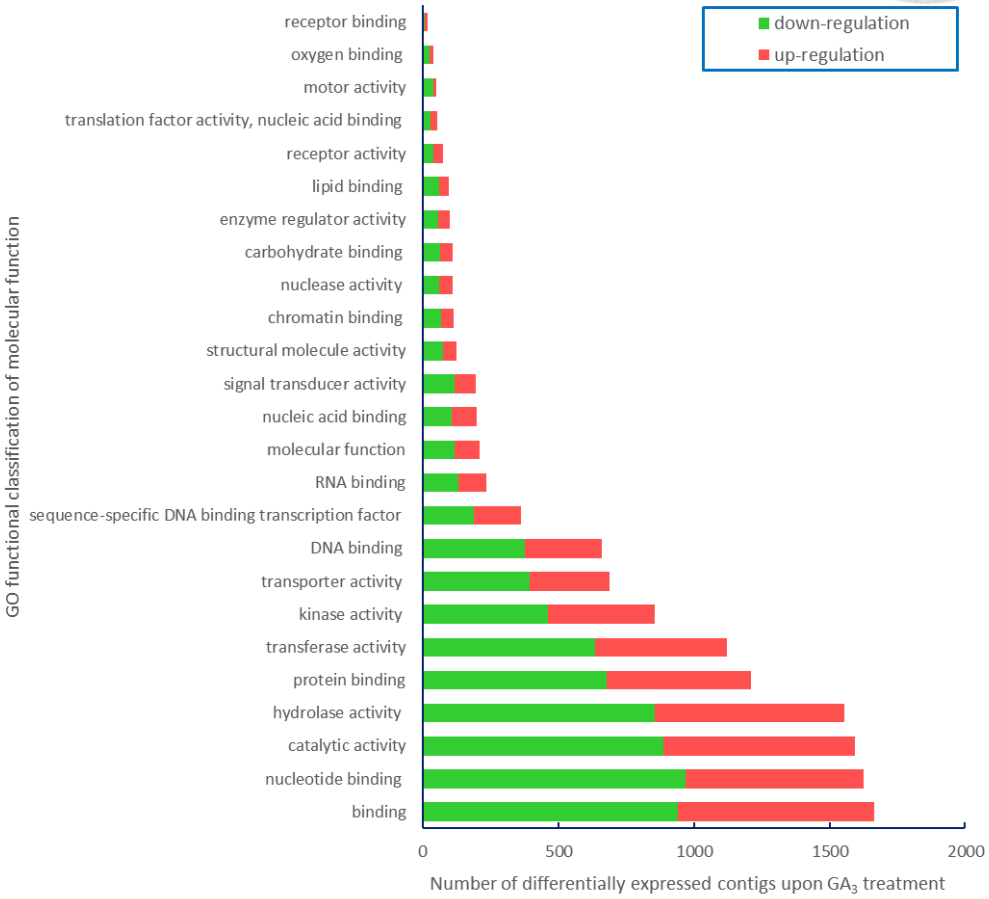

圖 4. 差異性表現基因之分子功能分類

Fig. 4. Functional categorization of differentially expressed contigs after GA_3 treatment based on the molecular function of Gene Ontology.

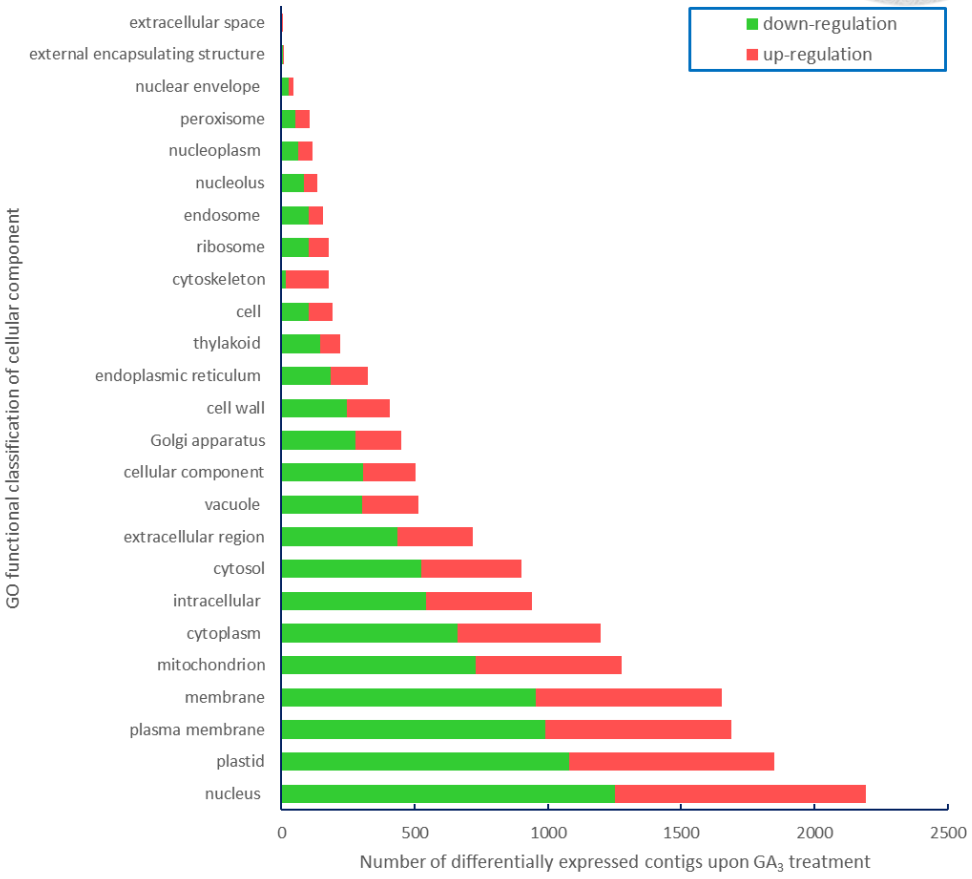

圖 5. 差異性表現基因之細胞組成分類

Fig. 5. Functional categorization of differentially expressed contigs after GA_3 treatment based on the cellular component of Gene Ontology.

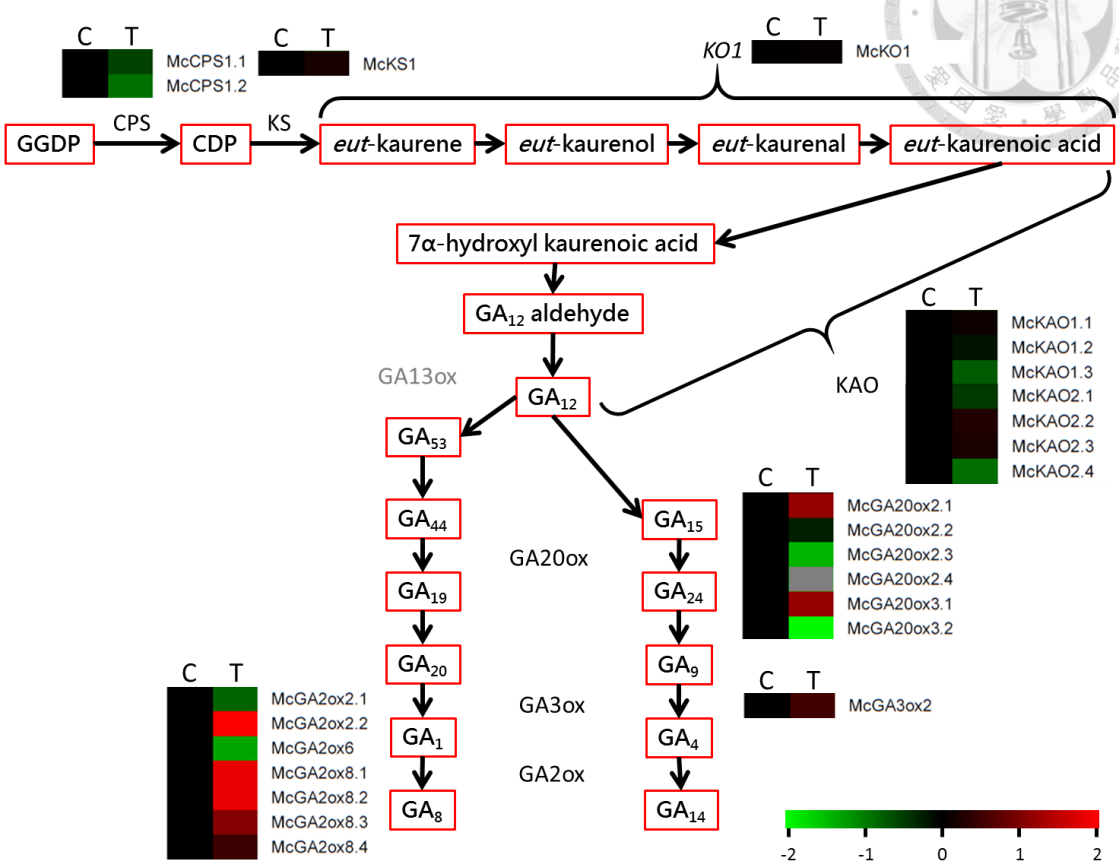

圖 6. 激勃素生合成途徑基因表現差異

Fig. 6. Representative gene expression profiles of the gibberellin biosynthesis pathway in *Momordica charantia*. The two color boxes represent gene expression level in control (C) and GA treated (T) bitter gourd. Log_2 fold change was correlated to control. The color scale is shown at the bottom. Red and green colors indicate higher and lower expression, respectively. Gray color indicates that no transcript was detected in GA_3-treated samples. The gibberellin biosynthesis pathway was referenced to the pathway in *Arabidopsis* (Fleet et al., 2003). Gray words mean no contigs align to the reference genes. *CPS, CPP synthetase*; *KS, ent-kaurene synthase*. *KO1, ent-kaurene oxidase 1*; *KAO, ent-kaurenoic acid oxydase*; *GA13ox, gibberellin 13-oxidase*; *GA20ox, gibberellin 20-oxidase*; *GA3ox, gibberellin 3-oxidase*; *GA2ox, gibberellin 2-oxidase*.

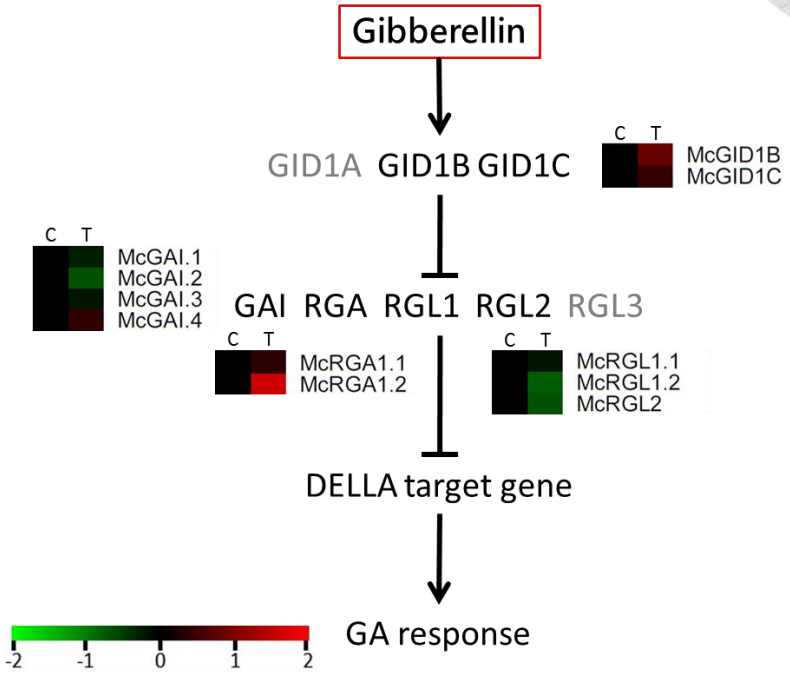

圖 7. 激勃素訊息傳遞途徑基因表現差異

Fig. 7. Representative gene expression profiles of the gibberellin signal transduction pathway in *Momordica charantia*. The two color boxes represent gene expression level in control (C) and GA treated (T) bitter gourd. Log_2 fold change was correlated to control. The color scale is shown at the bottom. Red and green colors indicate higher and lower expression, respectively. The gibberellin signal transduction pathway was referenced to the pathway in *Arabidopsis* (Wang and Deng, 2011). Gray words mean no contigs align to the reference genes. *GID1*, *GA insensitive dwarf 1*; *GAI*, *GA insensitive*; *RGA*, *repressor or gai-3*. *RGL*, *RGA-like*.

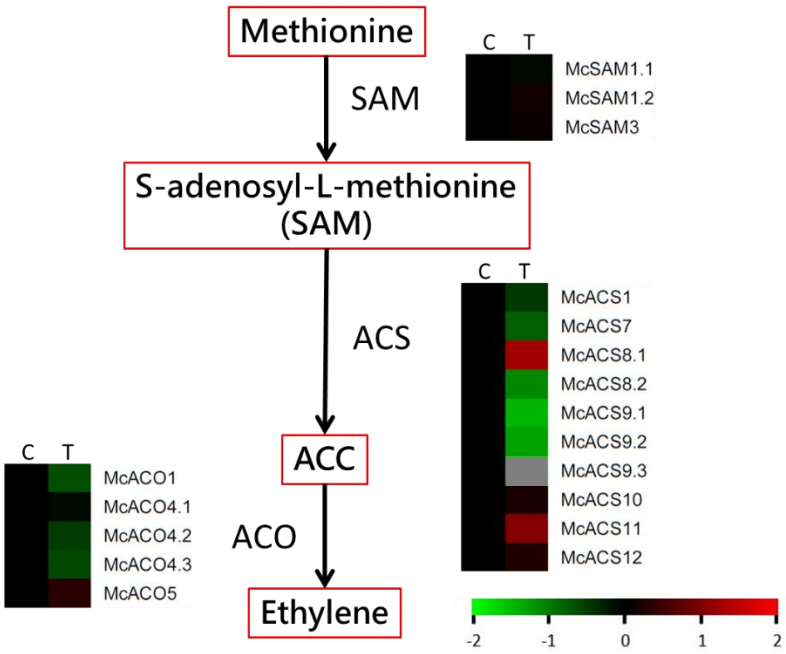

圖 8. 乙烯生合成途徑基因表現差異

Fig. 8. Representative gene expression profiles of the ethylene biosynthesis pathway in *Momordica charantia*. The two color boxes represent gene expression level in control (C) and GA treated (T) bitter gourd. Log_2 fold change was correlated to control. The color scale is shown at the bottom. Red and green colors indicate higher and lower expression, respectively. Gray color indicates that no transcript was detected in GA_3-treated samples. The ethylene biosynthesis pathway was referenced to the pathway in *Arabidopsis* (Sauter et al., 2013). *SAM, S-adenosylmethionine synthetase*; *ACS, ACC stnthase*; *ACO, ACC oxidase*.

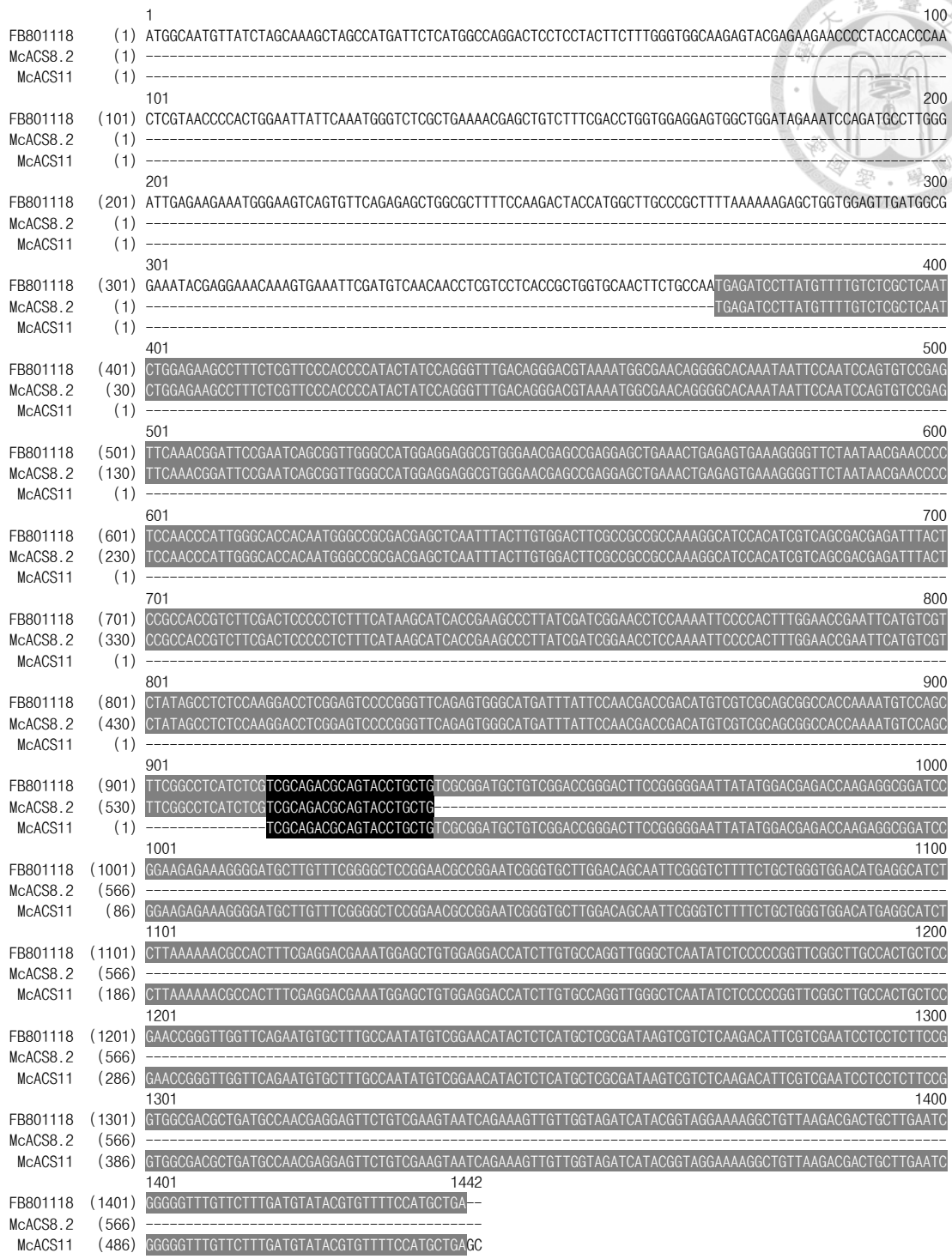

圖9. 苦瓜*McACS8.2*及*McACS11*與FB801118之核苷酸序列比對

Fig. 9. Alignment of *Momordica charantia McACS8.2*, *McACS11* and FB801118 nucleotide sequence. The black and gray boxes represent identical and conserved nucleotides, respectively.

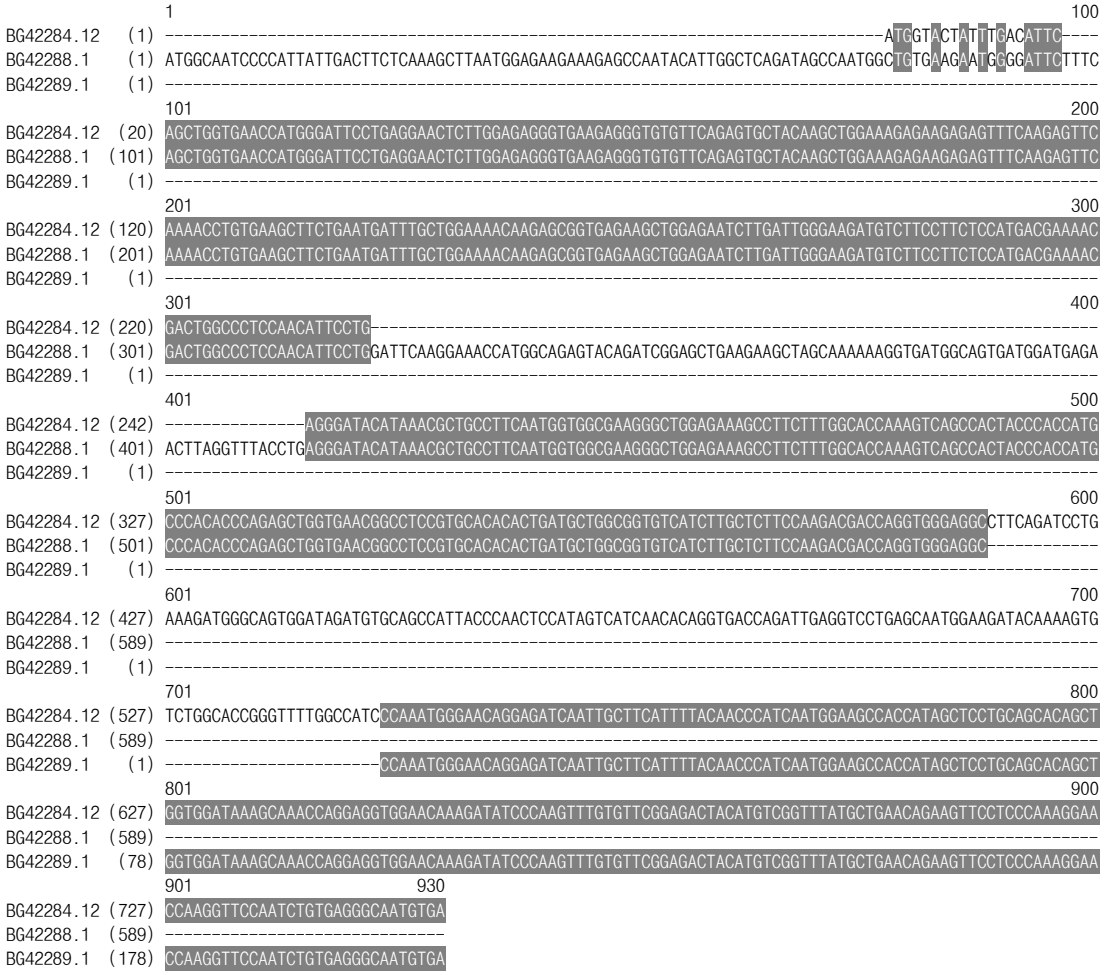

圖10. 阿拉伯芥*AtACO5*同源之苦瓜contigs核苷酸序列比對

Fig. 10. Alignment of *Momordica charantia* contigs which are homologus to *AtACO5* nucleotide sequence. The gray boxes represent conserved nucleotides, respectively.

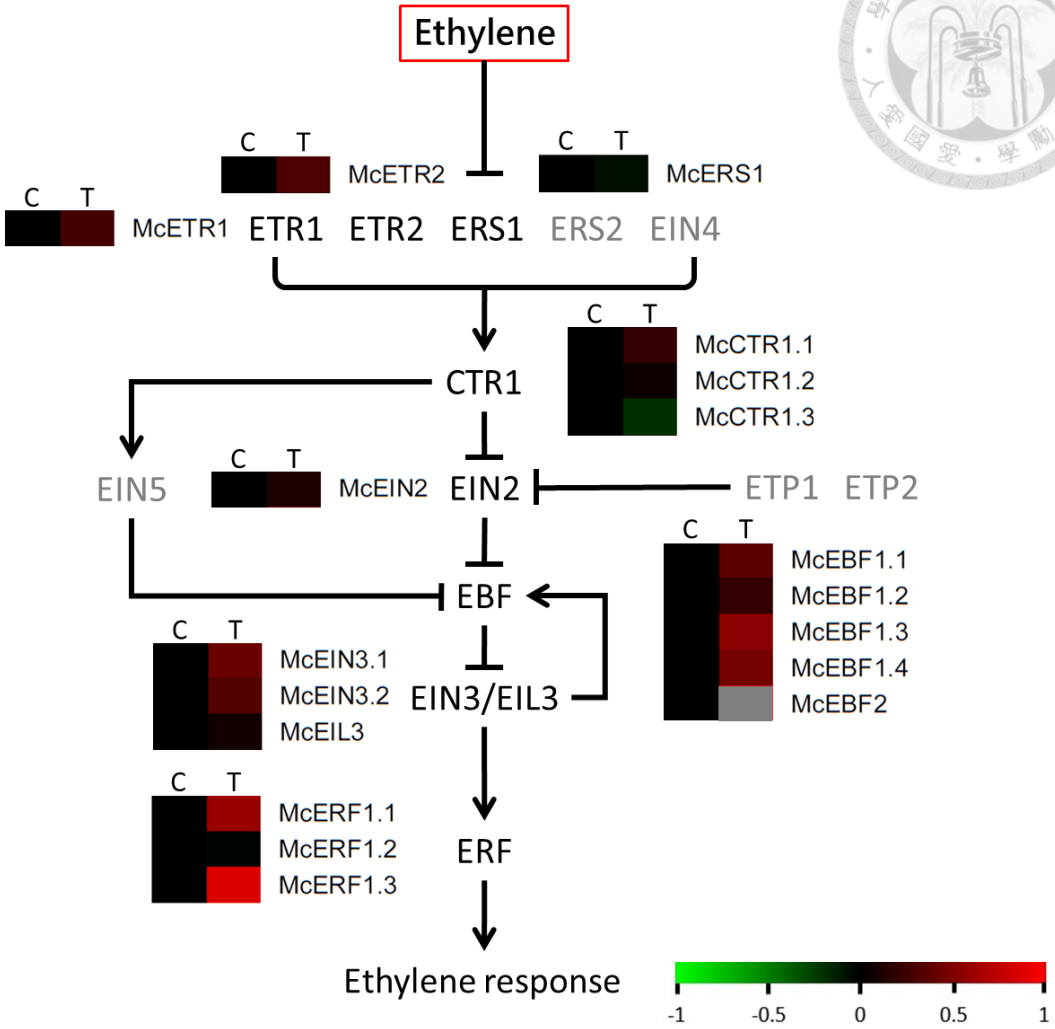

圖 11. 乙烯訊息傳遞途徑基因表現差異

Fig. 11. Representative gene expression profiles of the ethylene signal transduction pathway in *Momordica charantia*. The two color boxes represent gene expression level in control (C) and GA treated (T) bitter gourd. Log_2 fold change was correlated to control. The color scale is shown at the bottom. Red and green colors indicate higher and lower expression, respectively. Gray color indicates that no transcript was detected in untreated samples. The ethylene signal transduction pathway was referenced to the pathway in *Arabidopsis* (Zhao and Guo, 2011). Gray words mean no contigs align to the reference genes. *ETR1/2*, *ethylene response 1/2*; *ERS1*, *ethylene response sensor 1*; *CTR1*, *constitutive triple response 1*; *EIN2*, *ethylene insensitive 2*; *ETP1*, *EIN2 targeting protein*; *EIN3*, *ethylene-insensitive3*; *EBF*, *EIN3-binding F box protein 1*; *EIL*, *EIN3-like*; *ERF1*, *ethylene response factor 1*.

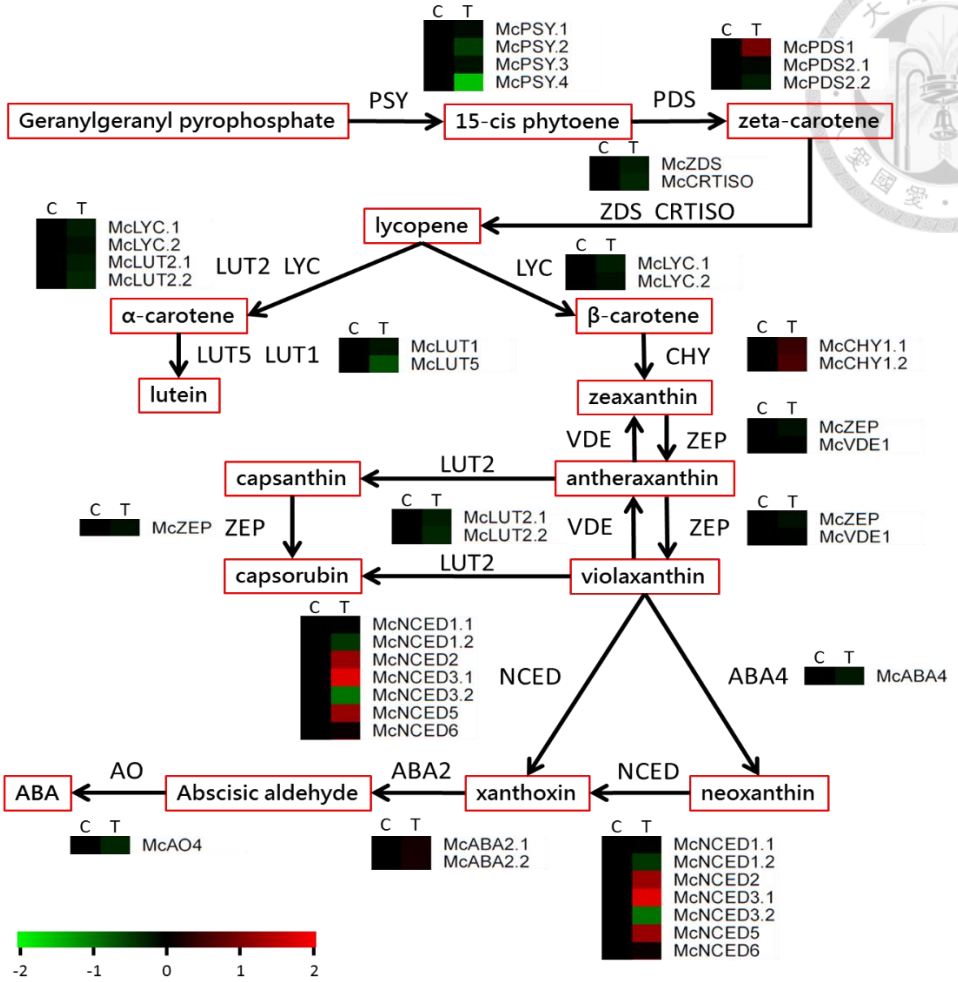

圖 12. 類胡蘿蔔素及離層酸生合成途徑基因表現差異

Fig. 12. Representative gene expression profiles of the carotenoids and abscisic acid biosynthesis pathway in *Momordica charantia*. The two color boxes represent gene expression level in control (C) and GA treated (T) bitter gourd. Log_2 fold change was correlated to control. The color scale is shown at the bottom. Red and green colors indicate higher and lower expression, respectively. The carotenoids and abscisic acid biosynthesis pathway was referenced to the pathway in *Arabidopsis* (Davison et al., 2002; Kim and DellaPenna, 2006; Seo and Koshiba, 2002). *PSY, phytoene synthase*; *PDS, phytoene desaturase*; *ZDS, zeta-carotene desaturase*; *CRTISO, carotenoid isomerase*; *LYC, lycopene cyclase*; *LUT1/2/5, lutein deficient 1/2/5*; CHY, *beta carotenoid hydroxylase*; *VDE, violaxanthin de-epoxidase*; *ZEP, zeaxanthin epoxidase*; *ABA2/4, abscisic acid-deficient 2/4*; *AO, aldehyde oxidase*; *NCED, nine-cis-epoxycarotenoid dioxygenase*.

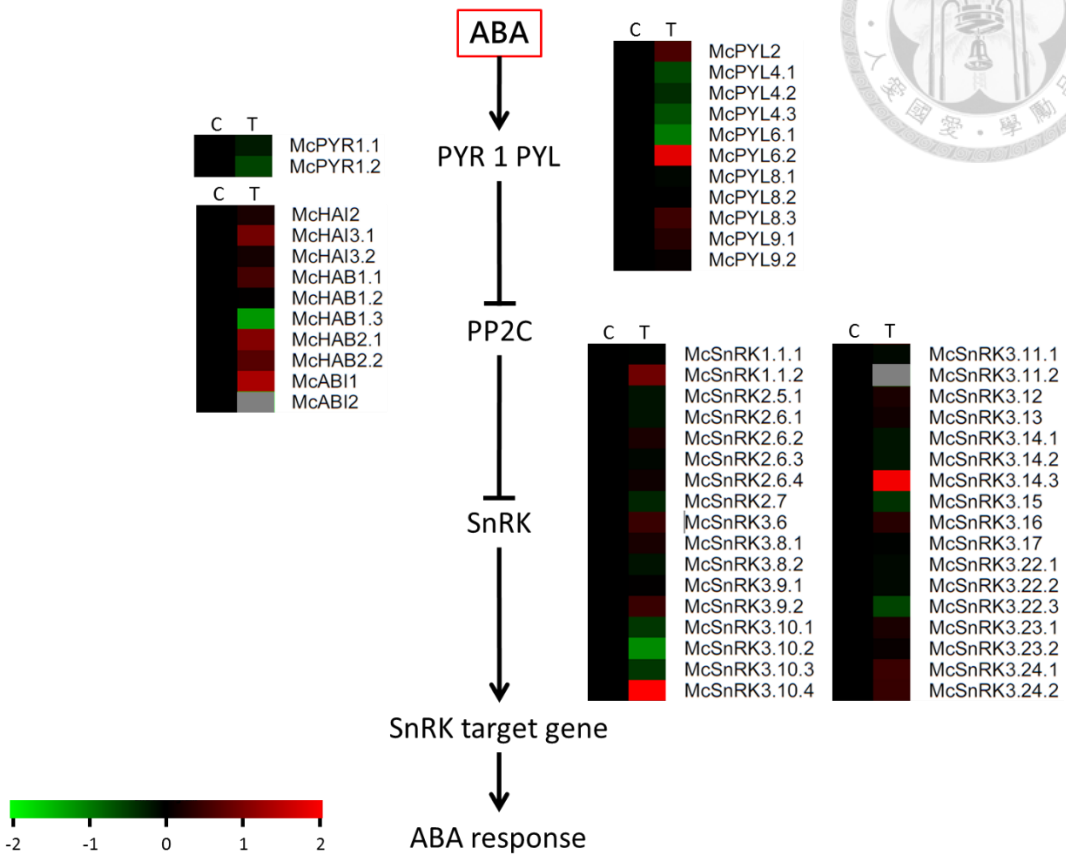

圖 13. 離層酸訊息傳遞途徑基因表現差異

Fig. 13. Representative gene expression profiles of the abscisic acid signal transduction pathway in *Momordica charantia*. The two color boxes represent gene expression level in control (C) and GA treated (T) bitter gourd. Log_2 fold change was correlated to control. The color scale is shown at the bottom. Red and green colors indicate higher and lower expression, respectively. Gray color indicates that no transcript was detected in GA_3-treated samples. The abscisic acid signal transduction pathway was referenced to the pathway in *Arabidopsis* (Hubbard et al. 2010). *PYR*, *pyrabactin resistance*; *PYL*, *pyrabactin resistance 1-like*. PP2C; protein phosphatase 2C; *HAI*, *highly ABA-induced PP2C gene*; *HAB*, *hypersensitive to ABA*; *ABI1/2*, *ABA insensitive 1/2*; *SnRK*, *SNF related kinase*.

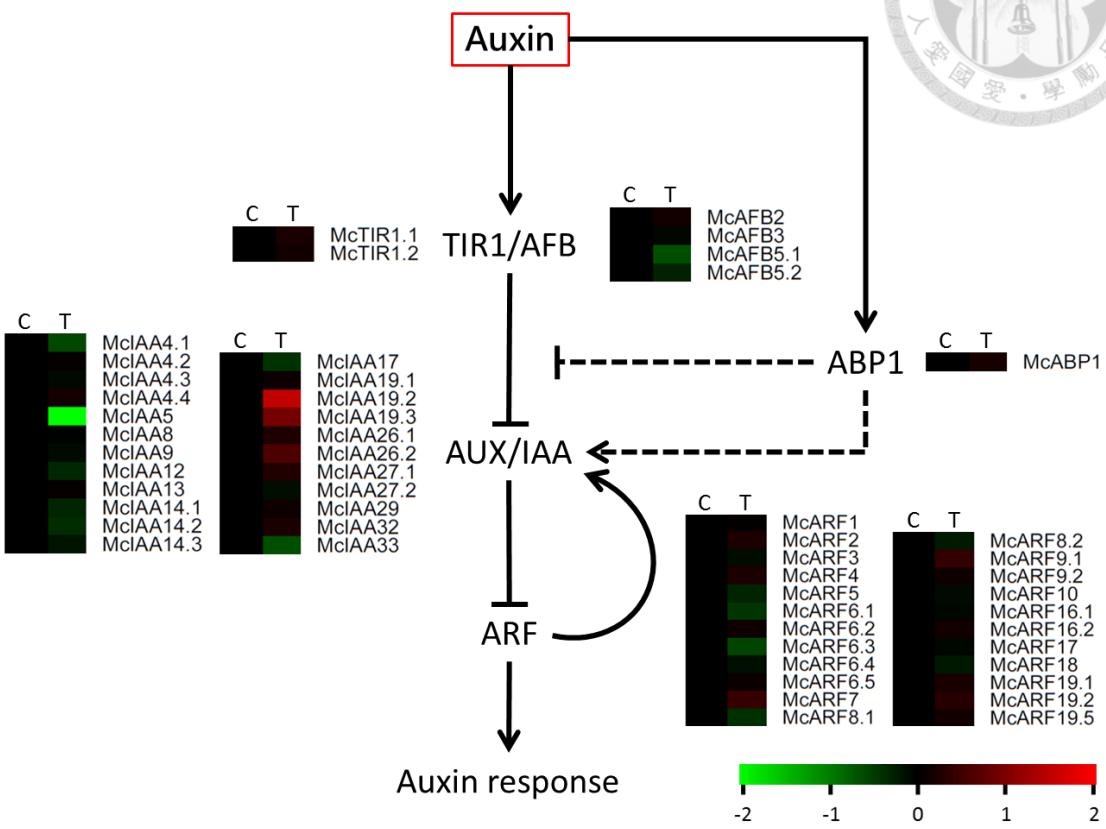

圖 14. 植物生長激素訊息傳遞途徑基因表現差異

Fig. 14. Representative gene expression profiles of the auxin signal transduction pathway in *Momordica charantia*. The two color boxes represent gene expression level in control (C) and GA treated (T) bitter gourd. Log_2 fold change was correlated to control. The color scale is shown at the bottom. Red and green colors indicate higher and lower expression, respectively. The auxin signal transduction pathway was referenced to the pathway in *Arabidopsis* (Woodward, and Bartel, 2005; Tromas et al., 2013). Gray words mean no contigs align to the reference genes. *TIR1*, *transport inhibitor response 1*; *AFB*, *auxin signaling F-box*; *ABP1*, *auxin binding protein 1*; *IAA*, *indole-3-acetic acid inducible*; *ARF*, *auxin response factor*.

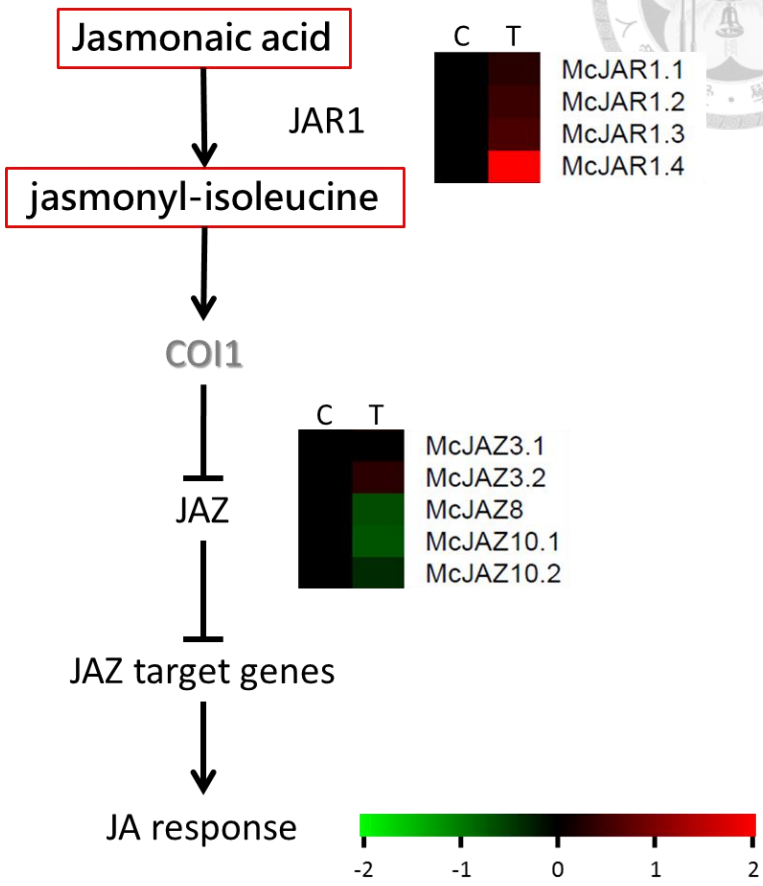

圖 15. 茉莉酸訊息傳遞途徑基因表現差異

Fig. 15. Representative gene expression profiles of the jasmonic acid signal transduction pathway in *Momordica charantia*. The two color boxes represent gene expression level in control (C) and GA treated (T) bitter gourd. Log_2 fold change was correlated to control. The color scale is shown at the bottom. Red and green colors indicate higher and lower expression, respectively. The jasmonic acid signal transduction pathway was referenced to the pathway in *Arabidopsis* (Song et al., 2013). Gray words mean no contigs align to the reference genes. *JAR1*, *jasmonate resistant 1*; *COI1*, *coronatine insensitive 1*; *JAZ*, *jasmonate-ZIM-domain protein*.

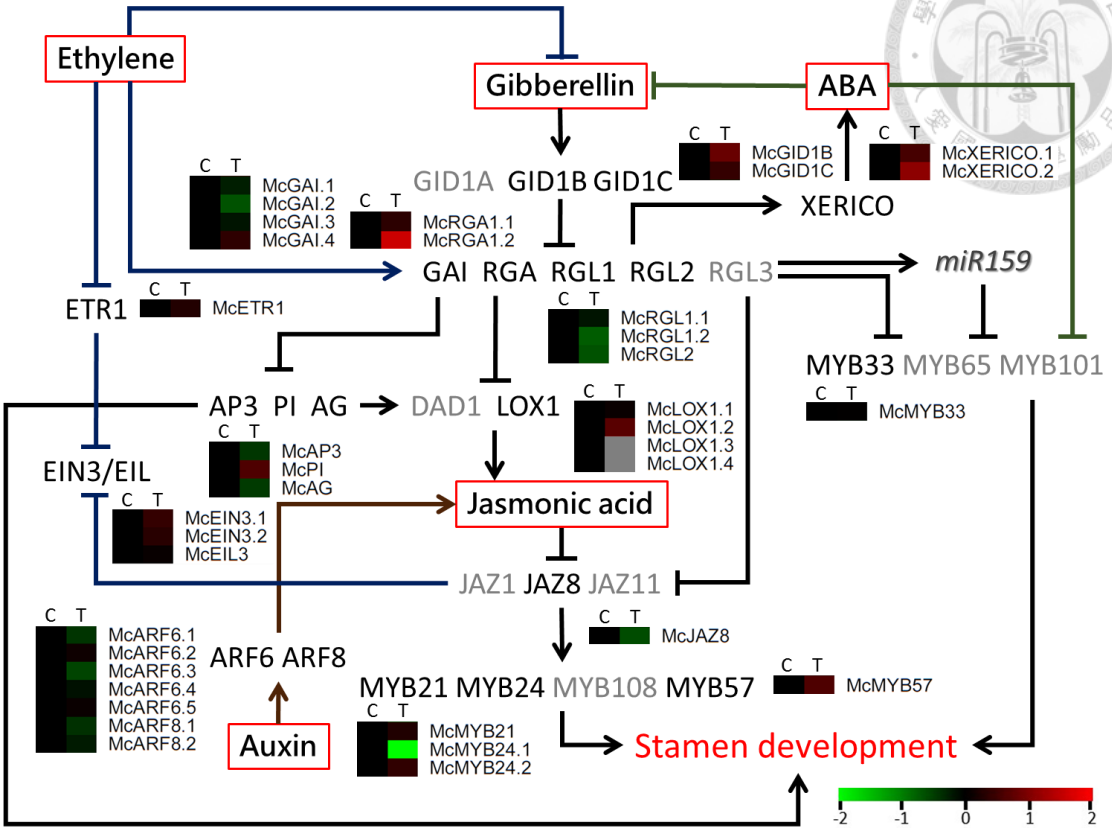

圖 16. 雄蕊發育調控路徑基因表現差異

Fig. 16. Representative gene expression profiles of the stamen development pathway in *Momordica charantia*. The two color boxes represent gene expression level in control (C) and GA treated (T) bitter gourd. Log_2 fold change was correlated to control. The color scale is shown at the bottom. Red and green colors indicate higher and lower expression, respectively. Gray color indicates that no transcript was detected in untreated samples The stamen development pathway was referenced to the pathway in *Arabidopsis* (Cheng et al., 2009 ; Ito et al., 2007 ; Nagpal et al., 2005 ; Peng, 2009 ; Song et. al., 2011； Sun, 2011 ; Yu et al., 2004; Zhua et al., 2011). Gray words mean no contigs align to the reference genes. *GID1, GA insensitive dwarf 1. GAI, GA insensitive. RGA, repressor or gai-3. RGL, RGA-like. LOX1, lipoxygenase 1. JAZ, jasmonate-ZIM-domain protein. MYB, MYB domain protein. AP3, APETALA3. PI, PISTILLATA. AG, AGAMOUS. ETR1, ethylene response 1. EIN3, ethylene-insensitive3. EIL, EIN3-like.*

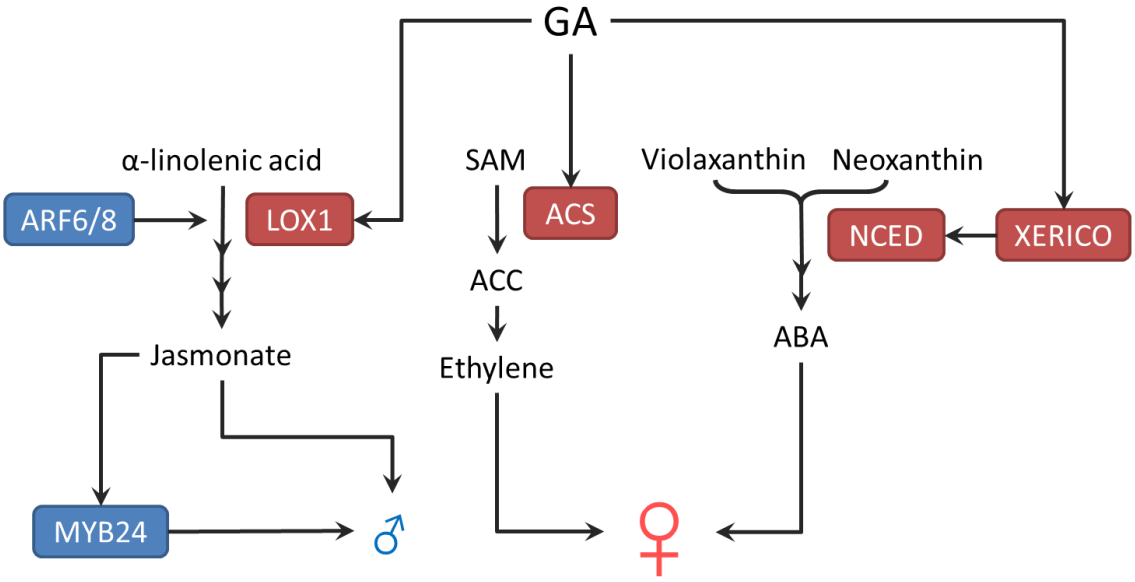

圖 17. 苦瓜花性決定之生物途徑示意圖

Fig. 17. Scheme of some of the biological pathways involved in Momordica charantia flower sex determination following GA application. Red boxes indicate genes that were up-regulated in GA treated sample compared with untreated sample, whereas blue boxes indicate genes that were down-regulated in GA treated sample compared with untreated sample. *NCED*, *nine-cis-epoxycarotenoid dioxygenase*; *ACS*, *ACC synthase*; *LOX1*, *lipoxygenase 1*; *ARF*, *auxin response factor*; *MYB24*, *MYB domain protein 24*.

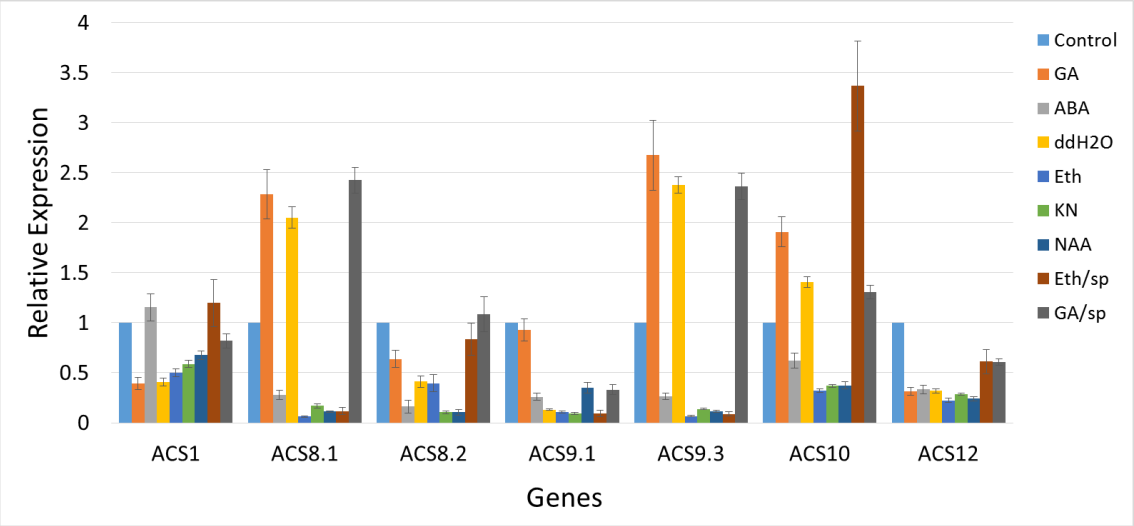

圖 18. 以 RT-PCR 分析不同處理之苦瓜 ACC 合成酶基因的表現量

Fig. 18. The qRT-PCR analysis of the ACC synthase genes in the *Momordica charantia* of different treatments. The gene expression level in control sample was normalized to 1. ddH_2O, GA, ABA, NAA, KN and Eth means the 6-8-leaf stage bitter gourd seedlings which germinated from seed treated with double distilled water, 100 mg•L^{-1} GA, ABA, NAA, KN and 25 mg•L^{-1} ethrel at 5℃ for 15 days, respectively. Eth/sp and GA/sp means the bitter gourd seedlings foliar sprayed of 500 mg•L^{-1} ethrel and100 mg•L^{-1} GA at 6-8-leaf stage, respectively. The *actin* was used as the internal control gene. Each value represents the mean ± SE of three replicates.

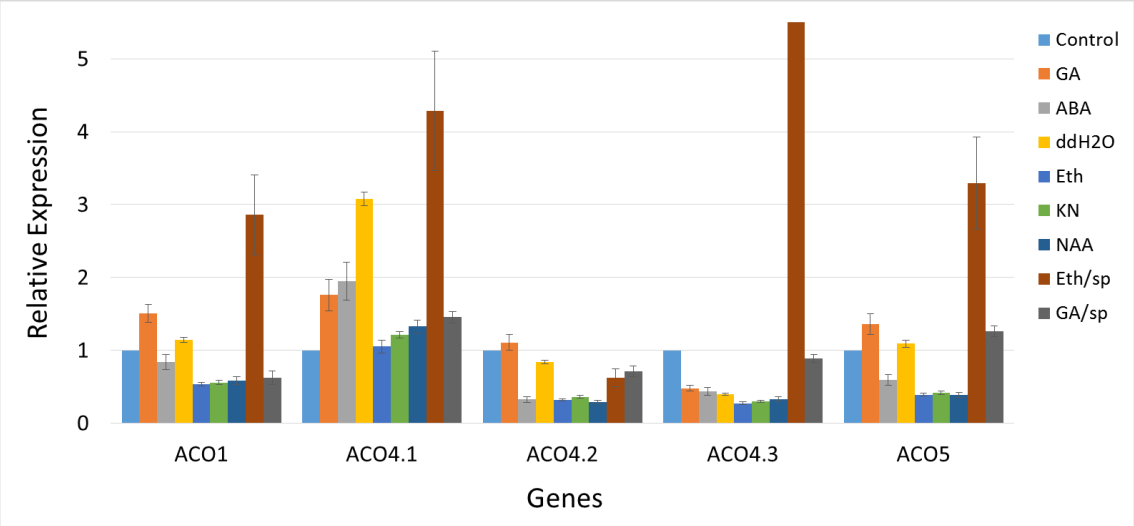

圖 19. 以 RT-PCR 分析不同處理之苦瓜 ACC 氧化酶基因的表現量

Fig. 19. The qRT-PCR analysis of the ACC oxidase genes in the *Momordica charantia* of different treatments. The gene expression level in control sample was normalized to 1. ddH_2O, GA, ABA, NAA, KN and Eth means the 6-8-leaf stage bitter gourd seedlings which germinated from seed treated with double distilled water, 100 mg•L^{-1} GA, ABA, NAA, KN and 25 mg•L^{-1} ethrel at 5℃ for 15 days, respectively. Eth/sp and GA/sp means the bitter gourd seedlings foliar sprayed of 500 mg•L^{-1} ethrel and100 mg•L^{-1} GA at 6-8-leaf stage, respectively. The *actin* was used as the internal control gene. Each value represents the mean ± SE of three replicates.

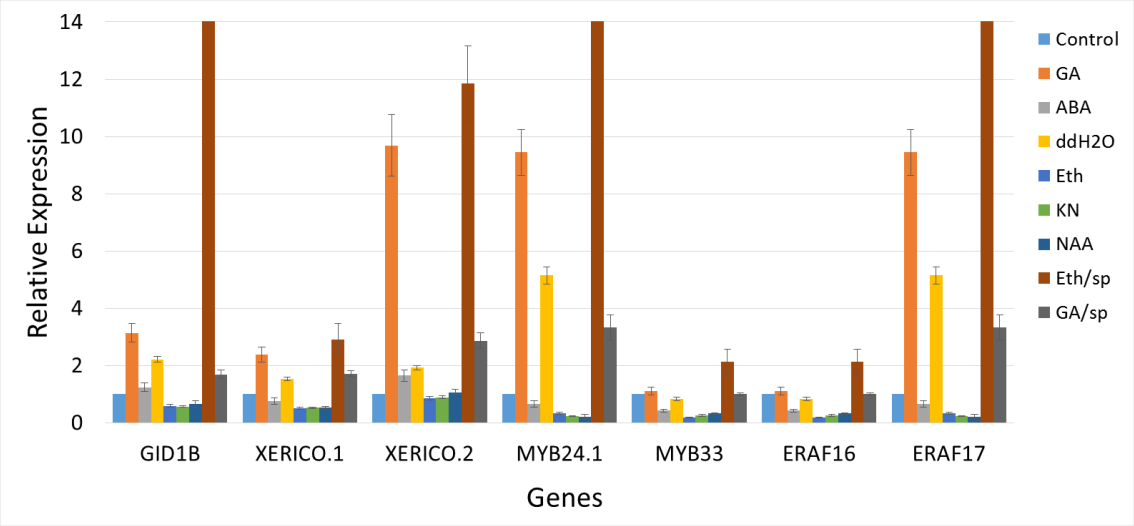

圖 20. 以 RT-PCR 分析不同處理之苦瓜花性相關基因的表現量

Fig. 20. The qRT-PCR analysis of the flower sexuality-related genes in the *Momordica charantia* of different treatments. The gene expression level in control sample was normalized to 1. ddH_2O, GA, ABA, NAA, KN and Eth means the 6-8-leaf stage bitter gourd seedlings which germinated from seed treated with double distilled water, 100 mg $\cdot L^{-1}$ GA, ABA, NAA, KN and 25 mg $\cdot L^{-1}$ ethrel at 5℃ for 15 days, respectively. Eth/sp and GA/sp means the bitter gourd seedlings foliar sprayed of 500 mg $\cdot L^{-1}$ ethrel and100 mg $\cdot L^{-1}$ GA at 6-8-leaf stage, respectively. The *actin* was used as the internal control gene. Each value represents the mean ± SE of three replicates.

表 4. 外施激勃素於六至八葉期苦瓜對其花性之影響

Table 4. Effect of GA_3 on number of male, female flowers and male to female ratio of *Momordica charantia* by foliar spray at 6-8 leaf stage.

	Number of flowers			Male / Female ratio
	Total	Male	Female	
Control	858±78.5[a]	837±77.8[a]	22±0.7[b]	37±2.3[a]
GA_3 treatment	901±139.3[a]	863±136.4[a]	38±2.8[a]	23±1.9[b]

Data are the mean values ± SE of two replicates. Numbers within a row followed by different letters are significantly different by ANOVA, P=0.05.

表 5. 苦瓜轉錄體次世代定序概要

Table 5. Summary of next generation sequencing results of the *Momordica charantia* transcriptome.

Total reads	406,003,512	reads
Total reads length	41,006,354,712	nt
Filtered reads	401,960,352	reads
Filtered reads length	40,239,972,525	nt
De novo assembly	106,057	contigs
N_{50}	2,463	nt

表 6. 胡瓜花性相關基因於苦瓜之同源基因及表現情形

Table 6. List of *Momordica charantia* contigs homologous to cucumber sex-associated genes.

Name	Contig ID	FPKM		Nearest cucumber gene	Description
		Control	Treated		
McACS8.1	BG10149.1	3.04	7.25	*CsACS1G*	Major sex-determined genes
McACS1	BG29132.1	3.23	2.35	*CsACS2*	
McRAN1	BG18281.1	11.41	10.56	*CsRAN1*	
McACO4.1	BG22617.1	640.85	607.91	*CsACO1*	Ethylene biosynthesis
McACO4.2	BG3185.1	79.71	57.53	*CsACO2*	
McACO4.3	BG20621.1	21.44	14.42	*CsACO3*	
McETR1	BG37469.4	31.67	38.21	*CsETR1*	Ethylene signal transduction
	BG37469.2				
	BG37469.1				
McETR2	BG18654.1	32.88	40.70	*CsETR2*	
McERS1	BG32732.1	46.19	44.32	*CsERS*	
McCTR1.1	BG42151.1	4.27	4.89	*CsCTR1*	
McRGA1.1	BG16044.1	38.08	48.65	*CsGAIP*	Gibberellin signal transduction
McGAI.1	BG35379.1	93.86	79.32		
McRGL1.1	BG19426.1	20.56	18.41		
McMYB33	BG34956.1	4.72	4.79	*CsGAMYB1*	Transcription factors
	BG34956.5				
	BG34956.4				
	BG34956.6				
CsERAF16	BG21294.1	2.29	2.55	*CsERAF16*	Ethylene-induced gene
CsERAF17	BG21769.1	0.57	1.58	*CsERAF17*	

RNA was extracted for untreated control and GA treated seedling of bitter gourd sprayed by 100 mg·L^{-1} GA for transcriptome analysis.

表 7. 本實驗室分離之苦瓜 cDNA 與其對應 contigs

Table 7. List of *Momordica charantia* cDNA isolated in our lab and their aligned contigs.

cDNA clone	Gene	Contig ID	FPKM	
			Control	Treated
pMACS1	*McACS1*	BG29132.1	3.23	2.35
pMACS7	*McACS11*	BG51419.1	0.09	0.18
	McACS8.2	BG58303.1	0.20	0.10
pMcER102	*McETR1*	BG37469.4	31.67	38.21
		BG37469.2		
		BG37469.1		
pMcER287-4	*McERS1*	BG32732.1	46.19	44.32
pMCTR16-1	*McCTR1.1*	BG42151.1	4.27	4.89
	AT5G03730	BG42151.2	15.86	19.24
pEIN2	*McEIN2*	BG26487.1	28.71	31.09
pMEIL324	*McEIN3.2*	BG21121.1	44.17	55.41
pMcEIL56	*McEIN3.2*	BG21121.1	44.17	55.41
pMcEDR111	AT1G18160	BG25544.1	15.84	18.23
		BG25544.3		
		BG25544.5		
		BG25544.4		
pMAEC28	*McPIN1.1*	BG35748.1	25.78	17.93
pMAEC43	*McPIN1.1*	BG35748.1	25.78	17.93
pMAEC93	AT1G70940	BG23381.1	13.36	15.79
	AT2G01420	BG23385.1	52.65	66.39
pMAIC11	*McAUX1.3*	BG30543.1	3.27	5.42
		BG30543.4		
		BG30543.3		
		BG30543.2		
pMAIC33	AT5G01240	BG31466.1	24.35	18.69
	McAUX1.4	BG31472.1	86.26	65.81
	McAUX1.1	BG31464.1	25.78	17.93
pMAIC23	*McAUX1.3*	BG30543.1	3.27	5.42
		BG30543.4		
		BG30543.3		
		BG30543.2		
pMcAR76	*McTIR1.1*	BG40740.1	48.66	56.44
	McTIR1.2	BG45375.1	0.87	0.97
		BG36497.3		
pMcPAP-76	AT4G04020	BG12789.1	49.30	57.26

RNA was extracted for untreated control and GA treated seedling of bitter gourd sprayed by 100 mg·L^{-1} GA for transcriptome analysis.

表 8. 苦瓜激勃素生合成途徑相關基因及表現情形

Table 8. *Arabidopsis* gibberellin biosynthesis-related genes and their orthologs in *Momordica charantia*.

Contig ID	Gene ID	FPKM		AGI	Annotation
		Control	Treated		
BG16911.1	*McCPS1.1*	0.65	0.44	AT4G02780	*CPP SYNTHASE*
BG54075.1	*McCPS1.2*	0.18	0.10		
BG35315.1	*McKS1*	11.8	13.44	AT1G79460	*ENT-KAURENE SYNTHASE 1*
BG35315.4					
BG35315.6					
BG35315.9					
BG17739.1	*McKO1*	12.51	12.96	AT5G25900	*ENT-KAURENE OXIDASE 1*
BG20689.1	*McKAO1.1*	13.89	14.96	AT1G05160	*ENT-KAURENOIC ACID OXYDASE 1*
BG17490.1	*McKAO1.2*	1.22	1.09		
BG17490.2	*McKAO1.3*	0.11	0.07		
BG38638.1	*McKAO2.1*	1.71	1.24	AT2G32440	*ENT-KAURENOIC ACID HYDROXYLASE 2*
BG38639.1	*McKAO2.2*	208.09	249.84		
BG38639.3					
BG38639.4					
BG38636.1	*McKAO2.3*	44.35	51.53		
BG13077.1	*McKAO2.4*	0.73	0.39		
BG14156.1	*McGA20ox2.1*	0.21	0.48	AT5G51810	*GIBBERELLIN 20 OXIDASE 2*
BG9228.1	*McGA20ox2.2*	2.80	2.33		
BG17029.1	*McGA20ox2.3*	1.07	0.39		
BG6412.1	*McGA20ox2.4*	0.00	0.32		
BG14156.1	*McGA20ox3.1*	0.21	0.48	AT5G07200	*GIBBERELLIN 20-OXIDASE 3*
BG53175.1	*McGA20ox3.2*	0.21	0.05		
BG9447.1	*McGA3ox2*	2.19	3.13	AT1G80340	*GIBBERELLIN 3-OXIDASE 2*
BG33874.1	*McGA2ox2.1*	3.95	2.24	AT1G30040	*GIBBERELLIN 2-OXIDASE 2*
BG8254.1	*McGA2ox2.2*	0.06	0.24		
BG19309.1	*McGA2ox6*	0.44	0.18	AT1G02400	*GIBBERELLIN 2-OXIDASE 6*
BG20162.1	*McGA2ox8.1*	0.06	0.21	AT4G21200	*GIBBERELLIN 2-OXIDASE 8*
BG20174.1	*McGA2ox8.2*	0.27	0.96		
BG20174.5					
BG49090.1	*McGA2ox8.3*	0.27	0.57		
BG48466.1	*McGA2ox8.4*	0.22	0.30		

表 9. 苦瓜激勃素訊息傳遞相關基因及表現情形

Table 9. *Arabidopsis* gibberellin biosynthesis-related genes and their orthologs in *Momordica charantia*.

Contig ID	Gene ID	FPKM		AGI	Annotation
		Control	Treated		
BG35789.1	*McGID1B*	5.26	9.25	AT3G63010	*GA INSENSITIVE DWARF1B*
BG35789.2					
BG16828.1	*McGID1C*	63.66	84.99	AT5G27320	*GA INSENSITIVE DWARF1C*
BG16828.2					
BG35379.1	*McGAI.1*	93.86	79.32	AT1G14920	*GIBBERELLIC ACID INSENSITIVE*
BG36311.1	*McGAI.2*	14.74	9.37		
BG19427.1	*McGAI.3*	60.52	53.96		
BG16045.1	*McGAI.4*	77.33	99.63		
BG16044.1	*McRGA1.1*	38.08	48.65	AT2G01570	*REPRESSOR OF ga1-3 1*
BG43062.1	*McRGA1.2*	0.63	1.89		
BG19426.1	*McRGL1.1*	20.56	18.41	AT1G66350	*RGA-LIKE 1*
BG51724.1	*McRGL1.2*	1.63	0.98		
BG16733.1					
BG22846.1	*McRGL2*	1.29	0.82	AT3G03450	*RGA-LIKE 2*

表 10. 苦瓜乙烯生合成相關基因及表現情形

Table 10. *Arabidopsis* ethylene biosynthesis-related genes and their orthologs in *Momordica charantia*.

Contig ID	Gene ID	FPKM		AGI	Annotation
		Control	Treated		
BG18379.1	*McSAM1.1*	293.19	277.75	AT1G02500	
BG18379.2					*S-ADENOSYLMETHIONINE SYNTHETASE 1*
BG18377.1	*McSAM1.2*	1151.13	1272.47		
BG18377.2					
BG8105.1	*McSAM3*	0.19	0.20	AT3G17390	*S-ADENOSYLMETHIONINE SYNTHETASE 3*
BG29132.1	*McACS1*	3.23	2.35	AT3G61510	*ACC SYNTHASE 1*
BG9684.1	*McACS7*	0.23	0.14	AT4G26200	*ACC SYNTHASE 7*
BG10149.1	*McACS8.1*	3.04	7.25	AT4G37770	*ACC SYNTHASE 8*
BG58303.1	*McACS8.2*	0.20	0.10		
BG4821.1	*McACS9.1*	0.99	0.37	AT3G49700	
BG4822.1	*McACS9.2*	0.6	0.25		*ACC SYNTHASE 9*
BG47992.1	*McACS9.3*	0.04	0.00		
BG32424.1	*McACS10*	8.80	10.13	AT1G62960	*ACC SYNTHASE 10*
BG51419.1	*McACS11*	0.09	0.18	AT4G08040	*ACC SYNTHASE 11*
BG19736.1	*McACS12*	11.39	13.67	AT5G51690	*ACC SYNTHASE 12*
BG16781.1	*McACO1*	4.32	2.80	AT2G19590	*ACC OXIDASE 1*
BG22617.1	*McACO4.1*	640.85	607.91	AT1G05010	
BG3185.1	*McACO4.2*	79.71	57.53		*ACC OXIDASE 4*
BG20621.1	*McACO4.3*	21.44	14.42		
BG42284.12	*McACO5*	9.45	11.76	AT1G77330	*ACC OXIDASE 5*

表 11. 苦瓜乙烯訊息傳遞相關基因及表現情形

Table 11. *Arabidopsis* ethylene signal transduction-related genes and their orthologs in *Momordica charantia*.

Contig ID	Gene ID	FPKM		AGI	Annotation
		Control	Treated		
BG32732.1	*McERS1*	46.19	44.32	AT2G40940	*ETHYLENE RESPONSE SENSOR 1*
BG37469.1	*McETR1*	31.67	38.21	AT1G66340	
BG37469.2					*ETHYLENE RESPONSE 1*
BG37469.4					
BG18654.1	*McETR2*	32.88	40.70	AT3G23150	*ETHYLENE RESPONSE 2*
BG42151.1	*McCTR1.1*	4.27	4.89	AT5G03730	
BG25156.1	*McCTR1.2*	19.36	20.01		*CONSTITUTIVE TRIPLE RESPONSE 1*
BG25156.3					
BG25150.1	*McCTR1.3*	3.57	3.12		
BG26487.1	*McEIN2*	28.71	31.09	AT5G03280	*ETHYLENE INSENSITIVE 2*
BG2025.1	*McEBF1.1*	71.35	91.88	AT2G25490	
BG2584.1	*McEBF1.2*	74.53	85.50		
BG7310.1	*McEBF1.3*	47.71	69.65		*EIN3-BINDING F BOX PROTEIN 1*
BG8936.1	*McEBF1.4*	0.48	0.66		
BG49442.1					
BG44947.1	*McEBF2*	0.00	0.29	AT5G25350	*EIN3-BINDING F BOX PROTEIN 2*
BG41424.1	*McEIN3.1*	142.14	189.49		
BG41424.3					*ETHYLENE-INSENSITIVE3*
BG21121.1	*McEIN3.2*	44.17	55.41	AT3G20770	
BG17837.1	*McEIL3*	4.87	5.13	AT1G73730	
BG17837.3					*ETHYLENE-INSENSITIVE3-LIKE 3*
BG17837.4					
BG35672.1	*McERF1.1*	1.17	1.76	AT3G23240	
BG41903.1	*McERF1.2*	1.26	1.24		*ETHYLENE RESPONSE FACTOR 1*
BG1913.1	*McERF1.3*	6.17	11.18		

表 12. 苦瓜類胡蘿蔔素生合成相關基因及表現情形

Table 12. *Arabidopsis* carotenoids biosynthesis-related genes and their orthologs in *Momordica charantia*.

Contig ID	Gene ID	FPKM		AGI	Annotation
		Control	Treated		
BG29307.1	*McPSY.1*	7.08	6.70	AT5G17230	
BG29307.2					
BG15686.1	*McPSY.2*	31.70	22.88		*PHYTOENE SYNTHASE*
BG25066.1	*McPSY.3*	1.50	1.36		
BG44671.1	*McPSY.4*	0.51	0.18		
BG35782.1	*McPDS1*	20.81	39.69	AT1G06570	*PHYTOENE DESATURATION 1*
BG28119.1	*McPDS2.1*	17.80	17.18	AT3G11945	
BG28119.2					
BG28119.3					*PHYTOENE DESATURATION 2*
BG28119.4					
BG7864.1	*McPDS2.2*	1.14	0.98		
BG11213.1	*McZDS*	48.15	41.15	AT3G04870	*ZETA-CAROTENE DESATURASE*
BG25643.1	*McCRTISO*	21.31	17.18	AT1G06820	*CAROTENOID ISOMERASE*
BG16858.1	*McLYC.1*	30.28	25.76	AT3G10230	
BG16858.2					*LYCOPENE CYCLASE*
BG19705.1	*McLYC.2*	1.02	0.93		
BG4945.1	*McLUT2.1*	67.78	58.16	AT5G57030	*LUTEIN DEFICIENT 2*
BG54054.1	*McLUT2.2*	2.34	1.90		
BG25287.1	*McLUT1*	25.32	22.52	AT3G53130	*LUTEIN DEFICIENT 1*
BG24857.1	*McLUT5*	38.29	25.02	AT1G31800	*LUTEIN DEFICIENT 5*
BG33129.1	*McCHY1.1*	18.52	25.34	AT4G25700	
BG33129.5					*BETA CAROTENOID HYDROXYLASE 1*
BG22514.1	*McCHY1.2*	1.54	2.31		
BG22514.2					
BG24801.1	*McZEP*	15.26	13.91	AT5G67030	*ZEAXANTHIN EPOXIDASE*
BG21689.1	*McVDE1*	68.08	67.29	AT1G08550	*VIOLAXANTHIN DE-EPOXIDASE 1*

表 13. 苦瓜離層酸生合成相關基因及表現情形

Table 13. *Arabidopsis* abscisic acid biosynthesis-related genes and their orthologs in *Momordica charantia*.

Contig ID	Gene ID	FPKM		AGI	Annotation
		Control	Treated		
BG23638.1	*McABA4*	42.26	36.93	AT1G67080	*ABA-DEFICIENT 4*
BG13530.1	*McABA2.1*	52.19	58.52	AT1G52340	*ABA DEFICIENT 2*
BG2299.1	*McABA2.2*	15.48	17.60		
BG42579.1	*McAO4*	4.12	3.33	AT1G04580	*ALDEHYDE OXIDASE 4*
BG32342.1	*McNCED1.1*	28.3	27.83	AT3G63520	*NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 1*
BG56633.1	*McNCED1.2*	18.81	13.91		
BG56348.1	*McNCED2*	0.29	0.66	AT4G18350	*NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 2*
BG17329.1	*McNCED3.1*	1.79	6.00	AT3G14440	*NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 3*
BG8222.1	*McNCED3.2*	0.40	0.22		
BG2216.1	*McNCED5*	1.98	4.49	AT1G30100	*NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 5*
BG10227.1	*McNCED6*	0.68	0.73	AT3G24220	*NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 6*

表 14. 苦瓜離層酸訊息傳遞相關基因及表現情形

Table 14. *Arabidopsis* abscisic acid signal transduction-related genes and their orthologs in *Momordica charantia*.

Contig ID	Gene ID	FPKM		AGI	Annotation
		Control	Treated		
BG33935.1					
BG33935.2	*McPYR1.1*	53.13	46.10	AT4G17870	*PYRABACTIN RESISTANCE 1*
BG33935.4					
BG17593.1	*McPYR1.2*	4.42	3.04		
BG30373.1	*McPYL2*	0.51	0.76	AT2G26040	*PYR1-LIKE 2*
BG34497.1	*McPYL4.1*	9.34	6.35		
BG41684.1	*McPYL4.2*	7.44	5.86	AT2G38310	*PYR1-LIKE 4*
BG56387.1	*McPYL4.3*	5.24	3.38		
BG9941.1	*McPYL6.1*	1.80	0.94	AT2G40330	*PYR1-LIKE 6*
BG9941.2					
BG6137.1	*McPYL6.2*	0.10	0.34		
BG31899.1	*McPYL8.1*	12.37	11.95		
BG31901.1	*McPYL8.2*	20.50	20.34	AT5G53160	*PYR1-LIKE 8*
BG36966.1	*McPYL8.3*	24.17	33.65		
BG16891.1	*McPYL9.1*	130.21	159.28	AT1G01360	*PYR1-LIKE 9*
BG50757.1	*McPYL9.2*	2.56	2.66		
BG19080.1	*McHAI2*	17.75	20.49	AT1G07430	*HIGHLY ABA-INDUCED PP2C GENE 2*
BG39020.1	*McHAI3.1*	8.45	15.76	AT2G29380	*HIGHLY ABA-INDUCED PP2C GENE 3*
BG9843.1	*McHAI3.2*	0.22	0.25		
BG24497.1	*McHAB1.1*	20.49	29.66		
BG41775.1	*McHAB1.2*	33.92	34.63	AT1G72770	*HYPERSENSITIVE TO ABA1*
BG41775.2					
BG41775.3					
BG41775.5					
BG52931.1	*McHAB1.3*	1.11	0.49		
BG41965.1	*McABI1*	67.38	168.34	AT4G26080	*ABA INSENSITIVE 1*
BG56165.1	*McABI2*	0.14	0.00	AT5G57050	*ABA INSENSITIVE 2*
BG24497.3	*McHAB2.1*	1.17	2.39	AT1G17550	*HOMOLOGY TO ABI2*
BG52143.1	*McHAB2.2*	0.26	0.41		
BG24044.1	*McSnRK1.1.1*	38.71	38.12		
BG1335.1	*McSnRK1.1.2*	0.34	0.62	AT3G01090	*SNF1-RELATED PROTEIN KINASE 1.1*
BG1335.2					
BG37404.1	*McSnRK2.5.1*	105.1	94.88	AT5G63650	*SNF1-RELATED PROTEIN KINASE 2.5*
BG37404.8					

continued

Contig ID	Gene ID	FPKM		AGI	Annotation
		Control	Treated		
BG31402.1	*McSnRK2.6.1*	26.79	24.08	AT4G33950	*SNF1-RELATED PROTEIN KINASE 2.6*
BG19224.1	*McSnRK2.6.2*	26.57	30.71		
BG49850.1	*McSnRK2.6.3*	2.71	2.60		
BG19223.1	*McSnRK2.6.4*	48.85	53.26		
BG23506.1	*McSnRK2.7*	14.65	11.94	AT4G40010	*SNF1-RELATED PROTEIN KINASE 2.7*
BG2031.1	*McSnRK3.6*	0.12	0.17	AT5G45820	*SNF1-RELATED PROTEIN KINASE 3.6*
BG21059.1	*McSnRK3.8.1*	6.03	6.92	AT5G58380	*SNF1-RELATED PROTEIN KINASE 3.8*
BG21970.1	*McSnRK3.8.2*	16.44	14.79		
BG21970.2					
BG21970.3					
BG21970.4					
BG2481.1	*McSnRK3.9.1*	5.54	5.61	AT4G18700	*SNF1-RELATED PROTEIN KINASE 3.9*
BG48367.1	*McSnRK3.9.2*	0.36	0.50		
BG23645.1	*McSnRK3.10.1*	2.15	1.59	AT3G23000	*SNF1-RELATED PROTEIN KINASE 3.10*
BG15498.1	*McSnRK3.10.2*	0.55	0.26		
BG46261.1	*McSnRK3.10.3*	0.42	0.31		
BG47884.1	*McSnRK3.10.4*	0.05	1.05		
BG35904.1	*McSnRK3.11.1*	4.24	4.06	AT5G35410	*SNF1-RELATED PROTEIN KINASE 3.11*
BG35904.5					
BG35904.8					
BG35904.10					
BG56645.1	*McSnRK3.11.2*	0.57	0.00		
BG20703.1	*McSnRK3.12*	35.87	41.30	AT1G01140	*SNF1-RELATED PROTEIN KINASE 3.12*
BG36956.1	*McSnRK3.13*	16.74	18.42	AT4G24400	*SNF1-RELATED PROTEIN KINASE 3.13*
BG19481.1	*McSnRK3.14.1*	146.56	131.11	AT4G30960	*SNF1-RELATED PROTEIN KINASE 3.14*
BG19480.1	*McSnRK3.14.2*	37.54	33.42		
BG53261.1	*McSnRK3.14.3*	4.41	16.52		
BG23267.1	*McSnRK3.15*	3.42	2.64	AT5G01820	*SNF1-RELATED PROTEIN KINASE 3.15*
BG34511.1	*McSnRK3.16*	16.30	20.08	AT3G17510	*SNF1-RELATED PROTEIN KINASE 3.16*
BG38398.1	*McSnRK3.17*	83.87	82.75	AT2G26980	*SNF1-RELATED PROTEIN KINASE 3.17*
BG38398.3					
BG38398.4					
BG38398.9					
BG35684.1	*McSnRK3.22.1*	11.72	11.21	AT2G30360	*SNF1-RELATED PROTEIN KINASE 3.22*
BG46844.1	*McSnRK3.22.2*	0.77	0.73		
BG44895.1	*McSnRK3.22.3*	2.80	1.93		

continued

ID	Annotation	FPKM		AGI	Gene
		Control	Treated		
BG20838.1					
BG20838.3	*McSnRK3.23.1*	21.75	25.09		
BG20838.4					
BG37139.1				AT1G30270	*SNF1-RELATED PROTEIN KINASE 3.23*
BG37139.2	*McSnRK3.23.2*	7.82	8.17		
BG37139.3					
BG37139.6					
BG43044.1	*McSnRK3.24.1*	10.25	14.17	AT5G10930	*SNF1-RELATED PROTEIN KINASE 3.24*
BG53685.1	*McSnRK3.24.2*	42.84	57.66		

表 15. 苦瓜植物生長素訊息傳遞相關基因及表現情形

Table 15. *Arabidopsis* auxin signal transduction-related genes and their orthologs in *Momordica charantia*.

Contig ID	Gene ID	FPKM		AGI	Annotation
		Control	Treated		
BG40740.1	*McTIR1.1*	48.66	56.44	AT3G62980	*TRANSPORT INHIBITOR RESPONSE 1*
BG45375.1 BG36497.3	*McTIR1.2*	0.87	0.97		
BG38869.1 BG38869.3	*McAFB2*	143.21	158.96	AT3G26810	*AUXIN SIGNALING F-BOX 2*
BG28788.1 BG28788.4 BG28788.5	*McAFB3*	34.24	33.05	AT1G12820	*AUXIN SIGNALING F-BOX 3*
BG38467.1	*McAFB5.1*	8.71	5.70	AT5G49980	*AUXIN SIGNALING F-BOX 5*
BG38470.1	*McAFB5.2*	6.49	5.38		
BG36497.1	*McABP1*	22.30	25.37	AT4G02980	*ENDOPLASMIC RETICULUM AUXIN BINDING PROTEIN 1*
BG18450.1	*McIAA4.1*	20.45	13.8		
BG37209.6 BG37209.10	*McIAA4.2*	33.68	35.04		
BG34917.1 BG34917.2 BG34917.4 BG34917.5	*McIAA4.3*	215.29	204.75	AT5G43700	*INDOLE-3-ACETIC ACID INDUCIBLE 4*
BG34920.1	*McIAA4.4*	50.62	56.94		
BG31366.1	*McIAA5*	0.72	0.12	AT1G15580	*INDOLE-3-ACETIC ACID INDUCIBLE 5*
BG32466.1 BG32466.3	*McIAA8*	256.62	252.13	AT2G22670	*INDOLEACETIC ACID-INDUCED PROTEIN 8*
BG25978.1 BG25978.2 BG25978.3	*McIAA9*	83.75	78.77	AT5G65670	*INDOLE-3-ACETIC ACID INDUCIBLE 9*
BG23742.1	*McIAA12*	25.23	20.28	AT1G04550	*INDOLE-3-ACETIC ACID INDUCIBLE 12*
BG39506.1 BG39506.2 BG39506.3 BG39506.4 BG39506.7	*McIAA13*	74.69	78.66	AT2G33310	*INDOLE-3-ACETIC ACID INDUCIBLE 13*

continued

Contig ID	Gene ID	FPKM		AGI	Annotation
		Control	Treated		
BG19521.1 BG19521.4	*McIAA14.1*	267.72	217.18	AT4G14550	*INDOLE-3-ACETIC ACID INDUCIBLE 14*
BG15663.1	*McIAA14.2*	26.71	20.97		
BG27213.1	*McIAA14.3*	438.56	390.62		
BG27211.1	*McIAA17*	173.10	131.22	AT1G04250	*INDOLE-3-ACETIC ACID INDUCIBLE 17*
BG12469.1	*McIAA19.1*	16.72	17.97		
BG12525.1	*McIAA19.2*	1.25	3.55	AT3G15540	*INDOLE-3-ACETIC ACID INDUCIBLE 19*
BG9910.1 BG9910.2	*McIAA19.3*	4.85	9.14		
BG39164.1	*McIAA26.1*	83.57	98.45	AT3G16500	*INDOLE-3-ACETIC ACID INDUCIBLE 26*
BG27519.1	*McIAA26.2*	13.39	20.21		
BG37209.1	*McIAA27.1*	165.61	198.24	AT4G29080	*INDOLE-3-ACETIC ACID INDUCIBLE 27*
BG35757.1	*McIAA27.2*	72.59	66.92		
BG25815.1	*McIAA29*	4.18	4.536	AT4G32280	*INDOLE-3-ACETIC ACID INDUCIBLE 29*
BG21942.1 BG21942.2	*McIAA32*	3.08	3.58	AT2G01200	*INDOLE-3-ACETIC ACID INDUCIBLE 32*
BG9656.1	*McIAA33*	6.31	4.10	AT5G57420	*INDOLE-3-ACETIC ACID INDUCIBLE 33*
BG19394.1	*McARF1*	66.52	68.16	AT1G59750	*AUXIN RESPONSE FACTOR 1*
BG23967.1	*McARF2*	57.51	67.14	AT5G62000	*AUXIN RESPONSE FACTOR 2*
BG35238.1	*McARF3*	19.7036	18.27	AT2G33860	*AUXIN RESPONSE TRANSCRIPTION FACTOR 3*
BG35237.1	*McARF4*	33.85	39.40		
BG20685.1 BG20685.3	*McARF5*	1.42	1.16	AT5G60450	*AUXIN RESPONSE FACTOR 4*
BG42471.1	*McARF6.1*	11.68	8.79	AT1G30330	
BG42465.1	*McARF6.2*	79.85	86.64		
BG19620.1	*McARF6.3*	10.69	7.32		*AUXIN RESPONSE FACTOR 6*
BG19619.1	*McARF6.4*	4.14	3.79		
BG19618.1	*McARF6.5*	114.15	121.32		
BG35524.1	*McARF7*	96.12	127.78	AT5G20730	*AUXIN RESPONSE FACTOR 7*
BG42468.1	*McARF8.1*	10.04	7.73	AT5G37020	*AUXIN RESPONSE FACTOR 8*
BG42473.1	*McARF8.2*	59.38	51.48		
BG34798.1	*McARF9.1*	21.29	28.19	AT4G23980	*AUXIN RESPONSE FACTOR 9*
BG34799.1	*McARF9.2*	20.75	22.56		
BG34779.2	*McARF10*	7.08	6.67	AT2G28350	*AUXIN RESPONSE FACTOR 10*

continued

Contig ID	Gene ID	FPKM		AGI	Annotation
		Control	Treated		
BG20942.1	*McARF16.1*	28.54	27.54	AT4G30080	
BG34778.6	*McARF16.2*	15.02	16.57		
BG34778.7					*AUXIN RESPONSE FACTOR 16*
BG34778.10					
BG34778.13					
BG20943.1	*McARF17*	3.45	3.32	AT1G77850	*AUXIN RESPONSE FACTOR 17*
BG37835.1	*McARF18*	11.41	9.96	AT3G61830	*AUXIN RESPONSE FACTOR 18*
BG34597.1	*McARF19.1*	20.96	24.01	AT1G19220	
BG35525.1	*McARF19.2*	20.08	24.93		*AUXIN RESPONSE FACTOR 19*
BG35525.2					
BG35525.3					

表 16. 苦瓜茉莉酸訊息傳遞相關基因及表現情形

Table 16. *Arabidopsis* jasmonic acid signal transduction-related genes and their orthologs in *Momordica charantia*.

Contig ID	Gene ID	FPKM		AGI	Annotation
		Control	Treated		
BG20637.1	*McJAR1.1*	24.11	30.20	AT2G46370	
BG20920.1	*McJAR1.2*	31.24	42.89		
BG20920.2					*JASMONATE RESISTANT 1*
BG20920.4					
BG20919.1	*McJAR1.3*	87.32	131.06		
BG58230.1	*McJAR1.4*	0.38	3.67		
BG24597.1	*McJAZ3.1*	91.53	90.6	AT3G17860	
BG24597.2					*JASMONATE-ZIM-DOMAIN PROTEIN 3*
BG27121.1	*McJAZ3.2*	95.77	120.83		
BG28497.1	*McJAZ8*	1.64	1.09	AT1G30135	*JASMONATE-ZIM-DOMAIN PROTEIN 8*
BG21290.1	*McJAZ10.1*	2.70	1.70	AT5G13220	*JASMONATE-ZIM-DOMAIN PROTEIN 10*
BG29943.1	*McJAZ10.2*	16.04	12.75		

表 17. 苦瓜雄蕊發育相關基因及表現情形

Table 17. *Arabidopsis* stamen development-related genes and their orthologs in *Momordica charantia*.

Contig ID	Gene ID	FPKM		AGI	Annotation
		Control	Treated		
BG35789.1 BG35789.2	*McGID1B*	5.26	9.25	AT3G63010	*GA INSENSITIVE DWARF1B*
BG16828.1 BG16828.2	*McGID1C*	63.66	84.99	AT5G27320	*GA INSENSITIVE DWARF1C*
BG35379.1	*McGAI.1*	93.85	79.32	AT1G14920	*GA INSENSITIVE*
BG36311.1	*McGAI.2*	14.74	9.37		
BG19427.1	*McGAI.3*	60.52	53.96		
BG16045.1	*McGAI.4*	77.33	99.63		
BG16044.1	*McRGA1.1*	38.08	48.65	AT2G01570	*REPRESSOR OF ga1-3 1*
BG43062.1	*McRGA1.2*	0.63	1.89		
BG19426.1	*McRGL1.1*	20.56	18.41	AT1G66350	*RGA-LIKE 1*
BG51724.1 BG16733.1	*McRGL1.2*	1.63	0.98		
BG22846.1	*McRGL2*	1.29	0.82	AT3G03450	*RGA-LIKE 2*
BG17271.1					
BG17271.2	*McXERICO.1*	58.26	85.92		
BG17271.3				AT2G04240	*XERICO*
BG15458.1 BG15458.2	*McXERICO.2*	53.99	115.38		
BG15457.1	*McLOX1.1*	11.07	11.77		
BG19644.1	*McLOX1.2*	60.23	97.93	AT1G55020	*LIPOXYGENASE 1*
BG8248.1	*McLOX1.3*	0.00	0.2		
BG11124.1	*McLOX1.4*	0.00	0.13		
BG28497.1	*McJAZ8*	1.64	1.09	AT1G30135	*JASMONATE-ZIM-DOMAIN PROTEIN 8*
BG27807.1	*McMYB21*	12.23	14.7	AT3G27810	*MYB DOMAIN PROTEIN 21*
BG46078.1	*McMYB24.1*	0.62	0.16	AT5G40350	*MYB DOMAIN PROTEIN 24*
BG27805.1	*McMYB24.2*	0.8	1.03		
BG11347.1	*McMYB57*	0.31	0.47	AT3G01530	*MYB DOMAIN PROTEIN 57*
BG34956.1 BG34956.3 BG34956.4 BG34956.5	*McMYB33*	4.72	4.79	AT5G06100	*MYB DOMAIN PROTEIN 33*

continued

Contig ID	Gene ID	FPKM		AGI	Annotation
		Control	Treated		
BG1411.1	*McAP3*	0.44	0.32	AT3G54340	*APETALA 3*
BG11587.1	*McPI*	3.99	6.15	AT5G20240	*PISTILLATA*
BG30120.1	*McAG*	0.73	0.54	AT4G18960	*AGAMOUS*
BG42471.1	*McARF6.1*	11.68	8.79		
BG42465.1	*McARF6.2*	79.85	86.64		
BG19620.1	*McARF6.3*	10.69	7.32	AT1G30330	*AUXIN RESPONSE FACTOR 6*
BG19619.1	*McARF6.4*	4.14	3.79		
BG19618.1	*McARF6.5*	114.15	121.32		
BG42468.1	*McARF8.1*	10.04	7.73	AT5G37020	*AUXIN RESPONSE FACTOR 8*
BG42473.1	*McARF8.2*	59.38	51.48		
BG37469.1					
BG37469.2	*McETR1*	31.67	38.21	AT1G66340	*ETHYLENE RESPONSE 1*
BG37469.4					
BG41424.1	*McEIN3.1*	142.14	189.49	AT3G20770	*ETHYLENE-INSENSITIVE3*
BG41424.3					
BG21121.1	*McEIN3.2*	44.17	55.41		
BG17837.1					
BG17837.3	*McEIL3*	4.87	5.13	AT1G73730	*ETHYLENE-INSENSITIVE3-LIKE 3*
BG17837.4					

伍、 討論

一、 序列組裝之探討

經次世代定序得到的苦瓜contigs，具完整開放解讀框架註解之contigs與其相對應之阿拉伯芥解碼序列長度具高度正相關，相關係數 (correlation coefficient) 達0.989。N_{50}為評估*de novo* assembly效果優劣的指標之一，本研究將激勃素處理苦瓜苗株及未處理苦瓜苗株之RNA定序結果共同組裝，獲得之N_{50}為2,463 nt，即超過半數以上之定序量皆組成長度大於2,463 nt之contigs (表5)，而阿拉伯芥mRNA平均長度約為1,700 nt，其中解碼序列長度平均約為1,300 nt，5’及3’非轉譯區 (untranslated region, UTR) 平均分別約為125 nt及250 nt (Kawaguchi and Bailey-Serres, 2005; The *Arabidopsis* Genome Initiative, 2000; Wortman et al., 2003)。可能與本研究之定序深度 (sequencing depth) 較深有關，亦可能是許多之contigs包含內含子 (intron) 或組裝錯誤被歸類於其它，因而增加contigs之長度。由於定序之RNA樣本皆以DNase I處理，並以Agilent 2100 Bioanalyzer檢測亦無大片段核酸之殘留，故組裝到內含子之原因較可能為受到precursor mRNA之干擾，如BG13730.1、BG27974.2。

二、 激勃素生合成之負回饋抑制效應

外施激勃素造成苦瓜激勃素生合成相關基因*McCPS*s、*McKAO*s及*McGA20ox*s表現量降低，而解碼激勃素失活酶GA2ox之基因表現量增加，可能為一負回饋機制 (negative feedback mechanism) 調控內生激勃素含量。施用激勃素於葡萄品種‘巨峰’(*Vitis labrusca* × *vinifera* cv. Kyoho) 之花前12天花序，其激勃素生合成基因*KS*、*GA20oxs*、*GA3ox*表現量下降，而解碼激勃素失活酶GA2oxs之基因則表現量上升 (Cheng et al., 2015)，可能亦具負回饋調控機制。

已知阿拉伯芥之激勃素訊息傳遞途徑，下游相關基因之表現，受到具生物活性之激勃素 (bioactive GA) 及激勃素誘導 GID1-DELLA 複合物降解正向調控 (Dill et al., 2004; Fleet and Sun, 2005; McGinnis et al., 2003; Murase et al., 2008)。但本研究之苦瓜 *GID1* 同源基因 *McGID1B* 及 *McGID1C* 皆於施用激勃素後表現量上升，而 *DELLA* 同源基因除了 *McRGA*s 外，其餘均於激勃素處理後表現量下降。外施激勃素於葡萄‘巨峰’之花前 12 天花序及阿拉伯芥 14 日齡苗株，皆造成激勃素受體 *GID1*

基因之表現量下降，而誘導 *DELLA* 同源基因之表現量上升，顯示激勃素感應機制亦具負回饋調控機制 (Cheng et al., 2015; Ribeiro et al, 2012)。葡萄'巨峰'之花序於激勃素處理後 72 小時，由於其激勃素生合成基因 *KS*、*GA20oxs*、*GA3ox* 表現量下降，解碼激勃素失活酶 GA2oxs 之基因表現量上升，其激勃素含量顯著低於未處理之葡萄花序 (Cheng et al., 2015)。本研究之苦瓜苗株取樣時間亦為激勃素處理後三天，且激勃素生合成相關基因 *McCPS*s、*McKAO*s 及 *McGA20ox*s 表現量亦降低，解碼激勃素代謝相關基因 *GA2ox* 之基因表現量增加，故處理激勃素之苦瓜苗株內生激勃素含量可能已低於未處理之苦瓜苗株，造成激勃素訊息傳遞相關基因 *McGID1*s 表現量上升及 *McDELLA*s 之表現量下降，有待進一步測定激勃素含量。

三、 激勃素藉由誘導乙烯相關基因調控苦瓜之花性

McACS8.1 於激勃素處理後表現倍率約為未處理苦瓜苗株之 2.39 倍，此基因解碼的胺基酸序列與胡瓜顯性之雌花決定主控基因 (major gynoecy determining gene) (Trebitsh et al., 1997) *CsACS1G* 最為相似。激勃素處理可使 *McACS8.1* 表現量增加，可能會造成乙烯生合成量增加，進而促進苦瓜雌花之生成，故 *McACS8.1* 可能於苦瓜之花性決定扮演重要角色。噴施 500 mg·L^{-1} 益收於六至八葉期苦瓜苗株，確使雌花百分率由 12 增加至 27 (Thomas, 2008)。因此，外施激勃素可能藉由增加 *ACC synthase* 之表現，促進乙烯之生成以誘導雌花產生。

McACS10 及 *McACS12* 之表現量高於其他 *ACC synthase*，但 *McACS10* 及 *McACS12* 於處理前後之表現差異不明顯，阿拉伯芥之 *AtACS10* 及 *AtACS12* 解碼胺基酸轉移酶 (aminotransferase)，不作用於支鏈胺基酸 (branched chain amino acids)，亦不具 ACC 合成酶之活性 (Yamagami et al., 2003)，故 *McACS10* 及 *McACS12* 可能不參與乙烯之生合成，亦與花性之誘導較不相關。而 *AtACS1* 具轉錄活性 (transcriptional activity)，但其胺基酸序列缺失 (deletion) 於高度保守的三肽 (tripeptide)，導致蛋白產物缺乏酵素功能 (enzymatical inactivity) (Yamagami et al., 2003)，*McACS1* 是否具 ACC 合成酶活性尚待後續之研究。

McETR1 及 *McETR2* 於激勃素處理後表現量些微增加，乙烯訊息傳遞路徑下游之乙烯反應基因 (ethylene responsive genes) *McEIN3/EIL*s、*McERF*s 表現量亦於激勃素處理後上升。相同的調控模式亦發生於外施益收的胡瓜，乙烯受體 *CsETR1*、

CsETR2 及 *CsERS* 之基因表現量增加，而於全雌株胡瓜可能產生較多之內生乙烯，使之莖頂 *CsETR2*、*CsERS* mRNA 累積量大於雌雄同株異花胡瓜 (Yamasaki et al., 2000; Yamasaki et al., 2001)。阿拉伯芥中 *EIN3/EIL*s、*ERFs* 會受乙烯所誘導而表現量增加 (Zhao and Guo, 2011)，顯示苦瓜植株體內乙烯含量可能較未處理苦瓜苗株增加，進而促進苦瓜植株雌性化。

四、 激勃素藉由調控離層酸相關基因改變苦瓜之花性

NCED 為離層酸關鍵生合成酵素，過量表現 *NCED* 會導致離層酸生成量增加 (Wan and Li, 2006)。本研究中，*McNCED2* (BG56348.1)、*McNCED3.1* (BG17329.1)、*McNCED5* (BG2216.1) 表現量倍率皆增加兩倍以上，可能會增加苦瓜中離層酸之生合成。由於離層酸與胡瓜雌花之誘導相關 (Wang and Zhou, 1991; Rudich and Halevy, 1974)，且胡瓜雌性化程度與內生離層酸含量呈正相關 (Miao et al., 2011)，故顯示外施激勃素可能會藉由提高離層酸關鍵生合成相關基因 *McNCED*s 之表現而促進離層酸之生合成，進而促進苦瓜雌性化程度。另外，苦瓜 *McXERICO.1* 及 *McXERICO.2* 於外施激勃素後表現量上升，其中 *McXERICO.2* 於處理後之表現量高達 115 FPKM。XERICO 為一 RING-H2 zinc-finger motif 蛋白，而 RING-H2 domain 於蛋白質交互作用扮演重要角色，利用酵母菌雙雜合系統篩選，得知 XERICO 與阿拉伯芥離層酸訊息傳遞途徑相關蛋白 tubby-like protein 9 (AtTLP9) (Lai et al., 2004) 及 ubiquitin conjugating enzyme 8 (AtUBC8) 具交互作用 (Ko et al., 2006)。阿拉伯芥過量表現 *XERICO* 之轉殖株，其離層酸生合成相關基因 *AtNCED3* 表現量顯著高於野生型植株 (Ko et al., 2006)，以高效能液相層析 (high performance liquid chromatography, HPLC) 分析 *35S::XERICO* 轉殖株之離層酸含量，顯示轉殖株之離層酸含量較野生型植株高十倍以上，而 *xerico* 突變株種子之離層酸含量顯著低於野生型種子，證實 XERICO 會促進離層酸之累積 (Ko et al., 2006; Zentella et al., 2007)。阿拉伯芥中 *DELLA* 基因產物會誘導 *XERICO* 基因表現，並促進離層酸之生合成及累積 (Ko et al., 2006)，與苦瓜之反應相反，*McDELLA*s 表現量下降而 *McXERICO* 表現量仍增加，顯示 *McXERICO*s 於苦瓜之調控模式可能與阿拉伯芥不同。此調控模式可能為激勃素誘導苦瓜雌性化之重要途徑，激勃素於苦瓜可能藉由誘導 *McXERICO*s 表現量，間接促進 *McNCED*s 之表現，以增加離層酸之生合成及累積量，並促進植株雌性化程度。

五、 激勃素抑制雄蕊發育相關基因

外施激勃素於苦瓜會抑制*McDELLA*s基因表現量，蛋白質累積量可能降低，而誘導其下游茉莉酸生合成相關基因*McLOX1*s之表現。阿拉伯芥*ga1-3 gai-t6 rga-t2 rgl1-1*四重突變體可恢復*ga1-3*突變體之*LOX1*表現量，顯示DELLA蛋白活性會抑制*LOX1*之表現；*ga1-3 gai-t6 rgat2 rgl1-1 rgl2-1*突變體可部分回復*ga1-3*及*ga1-3 gai-t6 rga-t2 rgl1-1*之*DAD1*表現量，顯示RGL2可能抑制*DAD1*之表現，故激勃素會藉由抑制DELLA蛋白進而誘導*DAD1*及*LOX1*表現，增加茉莉酸之生合成量 (Cheng at al., 2009; Peng, 2009)。

外施茉莉酸於阿拉伯芥會誘導茉莉酸訊息傳遞相關基因*AtJAZ*s表現量增加 (Chini et al., 2007)，故茉莉酸之生合成若增加，會促進*McJAZ8*表現，然而於本研究處理激勃素苦瓜苗株內，*McJAZ8*基因表現量較未處理組低，其下游的*McMYB24.1*於激勃素處理後表現量亦降低，由於阿拉伯芥R2R3-MYB轉錄因子MYB21、MYB24及MYB57為雄蕊發育必要之轉錄因子，會受茉莉酸所誘導並促進雄蕊之發育 (Cheng et al., 2009; Song et al., 2011)，故*McMYB24.1*可能於抑制雄蕊發育促成雌花之形成扮演重要角色。

茉莉酸之生合成尚受植物生長素藉由ARF6及ARF8進行調控，阿拉伯芥*arf6-2 arf8-3*雙重突變株於花器發育各階段皆無法偵測到茉莉酸之含量，顯示茉莉酸之生合成需要ARF6及ARF8之功能，ARF6及ARF8可能直接結合至茉莉酸生合成相關基因*AtLOX2*、*allene oxide synthase* (*AtAOS*) 與*oxophytodienoate-reductase 3* (*AtOPR3*)之啟動子序列皆中的AuxREs，調控其表現程度 (Nagpal et al., 2005)，然而*McARF6*及*McARF8*於激勃素處理後表現量均下降，可能進而降低茉莉酸之生成量，且影響力可能較*McLOX1*s表現量上升顯著。

苦瓜MADS-box同源異型蛋白AG之同源基因，於激勃素處理後表現量降低，阿拉伯芥之AG會藉由調控*DAD1*之表現而調節茉莉酸之生合成 (Ito et al., 2007)，然而*DAD1*於苦瓜並無相對應之完整或部分解讀框架註解序列。於阿拉伯芥中以*DAD1* promoter::glucuronidase (GUS) 進行表現部位分析，結果顯示GUS活性侷限於花絲部分，而以反轉錄聚合酶連鎖反應 (RT-PCR) 於其他器官亦無法偵測到*DAD1*之訊號，故得知*DAD1*之表現侷限於花絲部分 (Ishiguro et al., 2001)。本研究

所組裝之contigs無與*DAD1*相對應之序列可能原因有二，其一為阿拉伯芥與苦瓜之親緣性甚遠，而DAD1可能非高度保守之蛋白，故難以比對到同源序列；其二為*DAD1*僅限於花絲表現，本研究取樣之苦瓜幼苗分生組織可能尚未進行花芽分化，*DAD1*於此狀況下無mRNA之產出。

*McAP3*及*McAG*於激勃素處理後之苦瓜苗株表現量下降，已知阿拉伯芥*AP3*與*PI*為MADS-box B群之同源異型基因，會受DELLA蛋白活性而抑制基因表現 (Yu et al., 2004)。藉由*DELLA*突變體分析阿拉伯芥之五個DELLA蛋白，係以RGA及RGL2於抑制雄蕊發育中扮演主要角色，而RGL1之效果最差 (Peng et al., 1997; Silverstone et al., 1997; Pysh et al., 1999; Cheng et al., 2004)。於阿拉伯芥中，誘導RGA之表現，會抑制*AP3*、*PI*及*AG*之表現量 (Yu et al., 2004)。過量表現胡瓜*DELLA*同源基因*CsGAIP*於阿拉伯芥，導致B群 (B class) 之花器發育同源異型基因之表現量顯著下降，進而抑制雄蕊生長及發育 (Zhang, 2014b)。苦瓜之DELLA同源基因除了*McRGA*s表現量上升之外，其餘基因之表現量皆下降，因此推測McRGAs可能為抑制*McAP3*及*McAG*效果最佳之DELLA蛋白。綜觀以上，外施激勃素可能會藉由抑制花器發育同源異型基因之表現，誘導雌花生成。

外施激勃素使 *ARF6*s 及 *ARF8*s 表現量降低，*ARF6*s 及 *ARF8*s 調控茉莉酸生合成相關基因，造成茉莉酸之生合成下降進而抑制 *McMYB24* 表現，抑制雄蕊發育；外施激勃素同時藉由誘導 *McXERICO*s 之表現進而促進離層酸生合成關鍵基因 *McNCED*s 之表現而累積離層酸，並誘導乙烯生合成，共同促進植株雌性化 (圖 17)。

陸、 結語

本研究藉由外施激勃素於苦瓜品種‘月華’六至八葉期苗株，誘導其成株花性組成之改變，並藉由轉錄體之分析，了解植物體內在之反應與花性轉變之關聯。利用次世代定序不需基因體資訊等優點，運用於非模式物種苦瓜之 RNA 定序，並以 ContigViews (Liu et al., 2014) 平台進行分析，獲得概觀之差異表現情形，了解外施激勃素對苦瓜植株內生植物荷爾蒙之影響。未來可針對不同處理之苦瓜植株進行 RNA 定序或藉由 RNA 定序所獲得之序列資訊設計探針 (probe)，針對不同誘導苦瓜花性之處理，進行微陣列 (microarray) 分析，並建立網路分析 (network analysis)，以釐清各基因間之相互關係，並聚焦於重點基因。轉錄體分析僅提供 RNA 層級之資訊，而生物體內主要具功能之產物為蛋白質，然而生物體之調控錯綜複雜，受轉錄後調控 (post-transcriptional regulation)、轉譯後調控 (post-translational regulation)、細胞自噬 (autophagy) 等機制共同作用，其蛋白質表現情形無法由轉錄體得知，故仍須仰賴後續蛋白質體之分析。

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