

1    **Supplementary Information**

2

3    **Expression regulation of malate synthase involved in glyoxylate cycle during**  
4    **protocorm development in *Phalaenopsis aphrodite* (Orchidaceae)**

5

6    Wan-Lin Wu<sup>1,2§</sup>, Yu-Yun Hsiao<sup>3§</sup>, Hsiang-Chia Lu<sup>1,2,4,</sup>, Chieh-Kai Liang<sup>2</sup>, Chih-Hsiung  
7    Fu<sup>6</sup>, Tian-Hsiang Huang<sup>3</sup>, Ming-Hsiang Chuang<sup>5</sup>, Li-Jun Chen<sup>1</sup>, Zhong-Jian Liu<sup>4\*</sup>, and  
8    Wen-Chieh Tsai<sup>2,3,5,\*</sup>

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

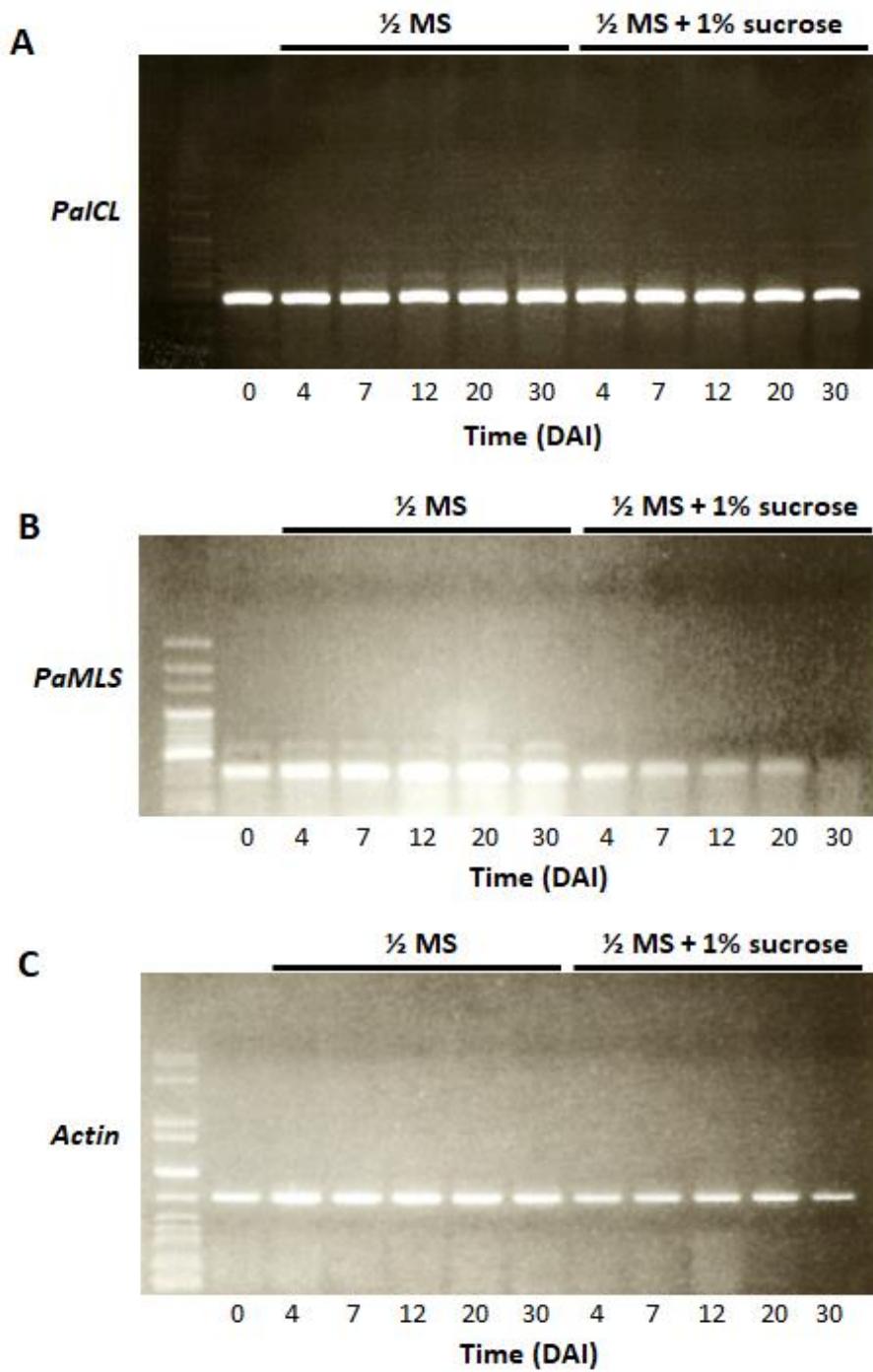
35

36

## Content

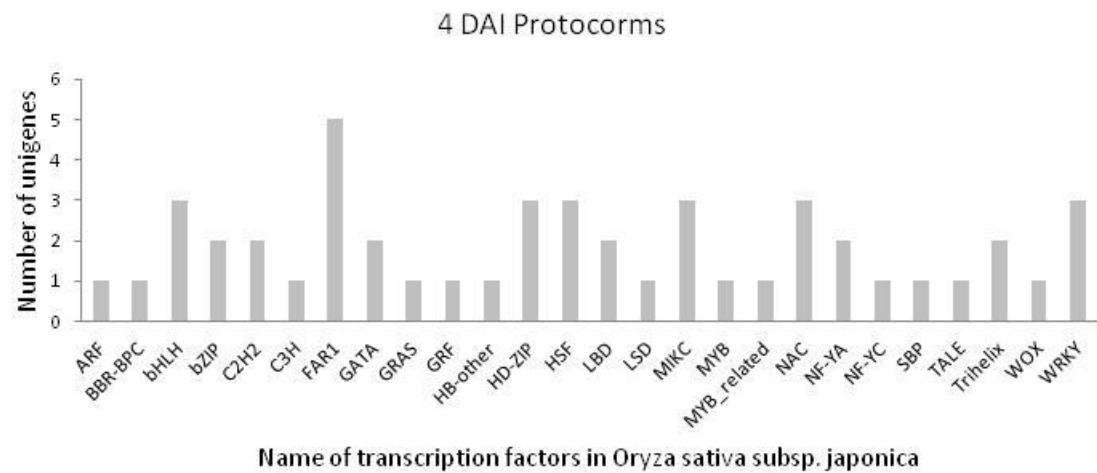
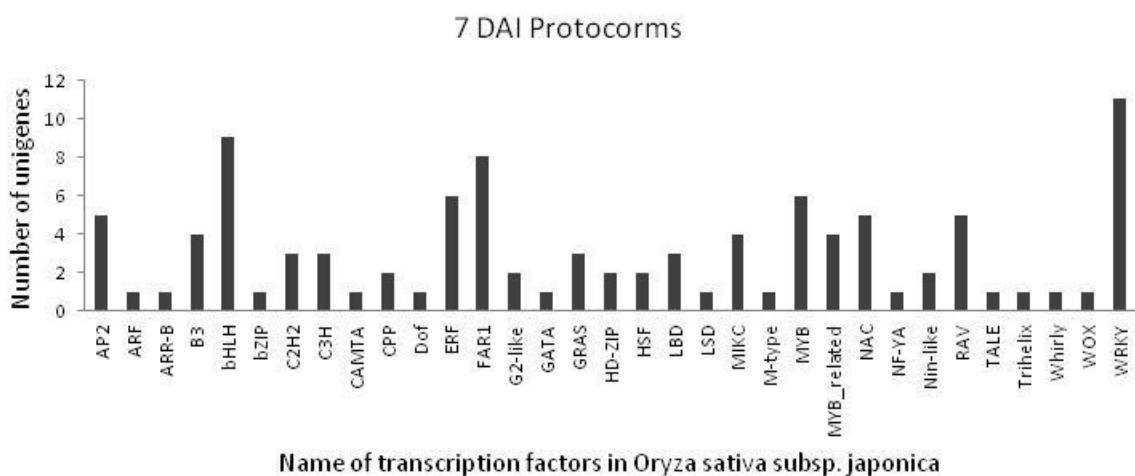
37

38	Supplementary Figures.....	3
39	Figure S1. Expression profiles analysis of <i>PaICL</i> and <i>PaMLS</i> of different	
40	developmental protocorm stage in <i>P. aphrodite</i> by RT-PCR.....	3
41	Figure S2. Number of unigenes related to transcription factors in each transcription	
42	factor family.....	4
43	Supplementary Tables.....	5
44	Table S1 List of primers used in this study.....	5
45	Table S2 Prdicted ATHB5 binding sites on 2000 bp upstream sequence of <i>PaMLS</i> .....	6



**Figure S1. Expression profiles analysis of *PaICL* and *PaMLS* of different developmental protocorm stage in *P. aphrodite* by RT-PCR.**

*P. aphrodite* seeds after resuspended in  $\frac{1}{2}$  MS liquid medium for 48 hr which were defined as 0 DAI protocorms. 4 to 30 DAI protocorms incubated in the absence of sucrose or in the presence of 1% sucrose of  $\frac{1}{2}$  MS medium. (A) *PaICL* (B) *PaMLS* (C) *Actin*. (DAI, days after incubation).

**A****B**

**Figure S2. Number of unigenes related to transcription factors in each transcription factor family**

Number of unigenes related to transcription factors 10-fold up-regulated in (A) 4-DAI protocorms and (B) 7-DAI protocorms.

**Table S1 List of primers used in this study**

Primer name	Sequence
<b>For real-time quantitative PCR</b>	
QPaMals-F	CGTGGAACAAAGGTCGAGCTTAGG
QPaMals-R	TGGGGAACAAAGCTCCACAAACCTTC
QPaICL-F	GCAAATCAGGTCCGGGACGATG
QPaICL-R	ATCCATCCACTCCTAACATCGAATCC
QPaANT-F	AGTGGTGGAGTTGGAGGATTG
QPaANT-R	GCATCGAGACTCCAAGCA
QPaHB5-F	GATGAGGGGGATGGAATGTATG
QPaHB5-R	CAGCTCCATATTGTTATCACATTCC
QPaMADS2-F	AGGGAAACTTACCGCGCTCTA
QPaMADS2-R	GAGATGGGGATCTATTAGAGAGA
QPaMYB4-F	ATCGAGTGATGATGAGGCTATT
QPaMYB4-R	AACCCAAGTCATCCAAACTCC
QPaPIF3-F	GCGTGTGTTGGCGCAAGTGGCAT
QPaPIF3-R	AGGCGCCCAAATATCAGCACCGTTG
QPaRAV1-1-F	CAGTTATGGGGATTGATGATTTC
QPaRAV1-1-R	TTCTCCGCATCCAAATCCATC
QPaWRKY18-F	GAAGAACTCCGATAACTTGAACAGA
QPaWRKY18-R	AATGCAAATGAGTTCAAGGTCTAGT
QPaWRKY71-F_2	TCCCGCTTATCCGAAAACAT
QPaWRKY71-R	TCGAGCCGCAAGGTTCAA
<b>For RACE</b>	
3'PaMLS_F1	GTCCCCTCTTTACCTCCCCAA
3'PaMLS_F2	GCTCCGTTCTCACTCTGCTGG
5'PaICL_R1	GCCCCGAGAACATGAACTGGTGGTC
5'PaICL_R2	CCTCTGCTGCTGCGGTCGTGGTAG
<b>For <i>PaMLS</i> promoter-reporter plasmid construction</b>	
PaMLSp_1000_5'_BamH I	GGATCCTTCAGATAGGTTACAGGTAAATTATATAGA
PaMLSp_3'_Nco I	CCATGGCGTCACCACCGGAG
<b>For <i>PaHB5</i> plasmid construction</b>	
PaHB5_5'_Xba I	TCTAGAATGGAAGAAGAGAAGTCTCTGATCA
PaHB5_3'_BamH I	GGATCCTCATCCATCCAAACTCCC

**Table S2 Predicted ATHB5 binding sites on 2000 bp upstream sequence of *PaMLS***

Putative binding site	Strand <sup>a</sup>	Putative binding sequence
-1662	+	ttcTTATTg
-1328	+	ggcTTATTa
-1108	+	aacTTATTt
-800	+	caaTTATTt
-796	+	tatTTATTt
-792	+	tatTTATTt
-769	-	aAATAAaaa
-756	-	aAATAAgaa
-709	+	tatTTATTa
-706	+	ttaTTATTt
-702	+	tatTTATTt
-611	+	taaTTATTt
-598	+	aagTTATTa
-590	-	aAATAAtat
-559	+	aaaTTATTt
-499	+	aatTTATTt
-487	-	taATAAatt
-334	-	aAATAAata
-330	-	aAATAAtat
-296	-	aAATAAtat
-278	-	tAATAAaaa
-241	-	aAATAAcga

a: Forward or reverse directions of binding sequences