Plant Dormancy Workshop, Plant Animal Genome XX
2012-1-17, San Diego

Functional Studies on Japanese apricot *PmDAM* genes

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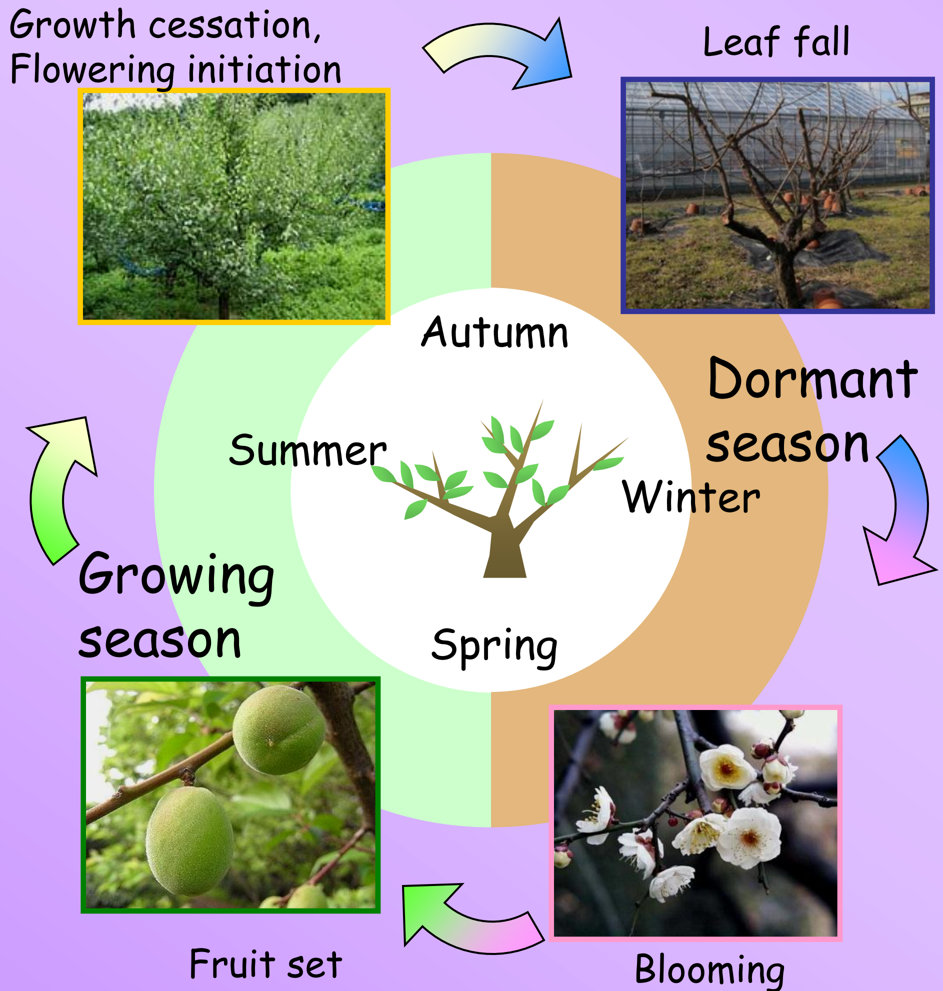
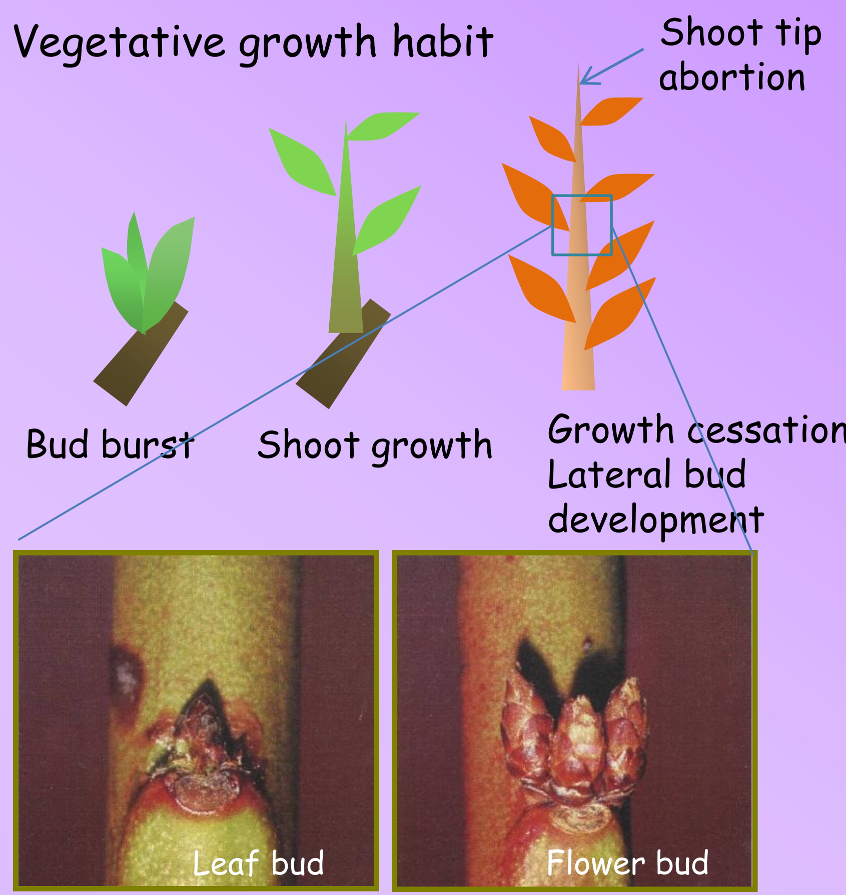
Annual growth and flowering cycle of Japanese apricot (*Prunus mume*)



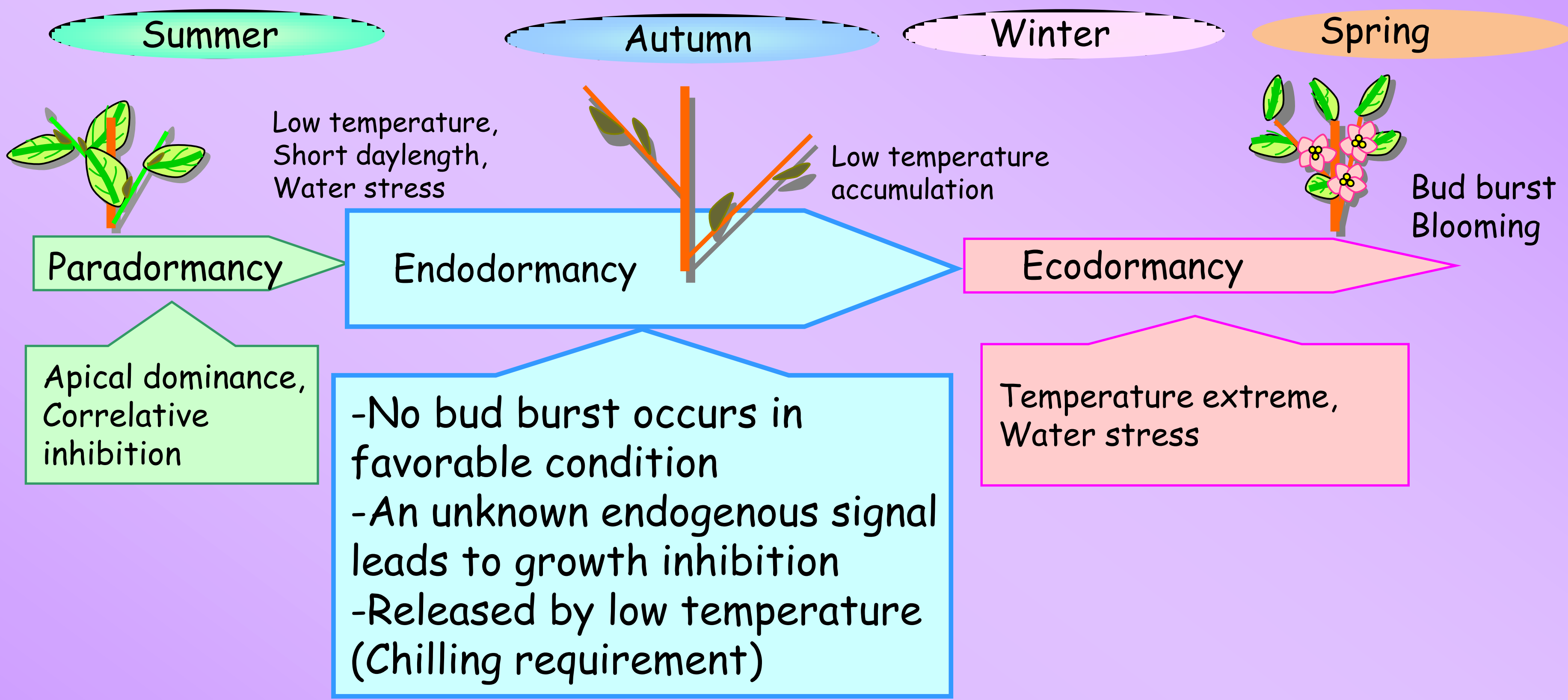


At leaf axil, a single leaf bud subtended by two or three flower buds was formed.

Leaf buds contain shoot apical meristem, whereas flower buds contain single flower meristem.

Bud dormancy in temperate fruit trees

Dormancy is a temporary suspension of visible growth of any plant structure containing a meristem (Lang et al., 1987)



- Endodormancy is regulated by an endogenous signal within (i.e., "endo") the buds
- Endodormant buds require a certain amounts of chilling accumulation for dormancy release

Agricultural problem related to bud dormancy

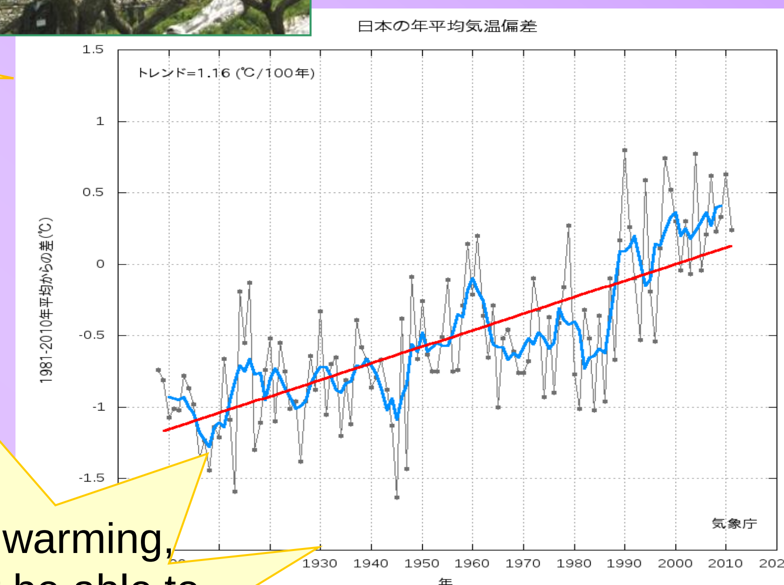
dormant

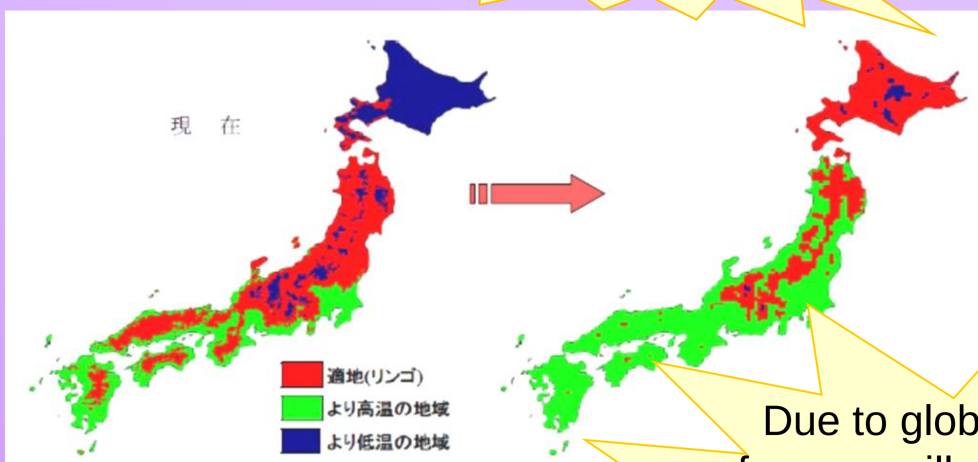
blooming

fruit set

bud abortion caused by incomplete dormancy release

Uniform flowering is not achieved





Due to global warming, farmers will not be able to grow temperate fruits in southern area of Japan!?

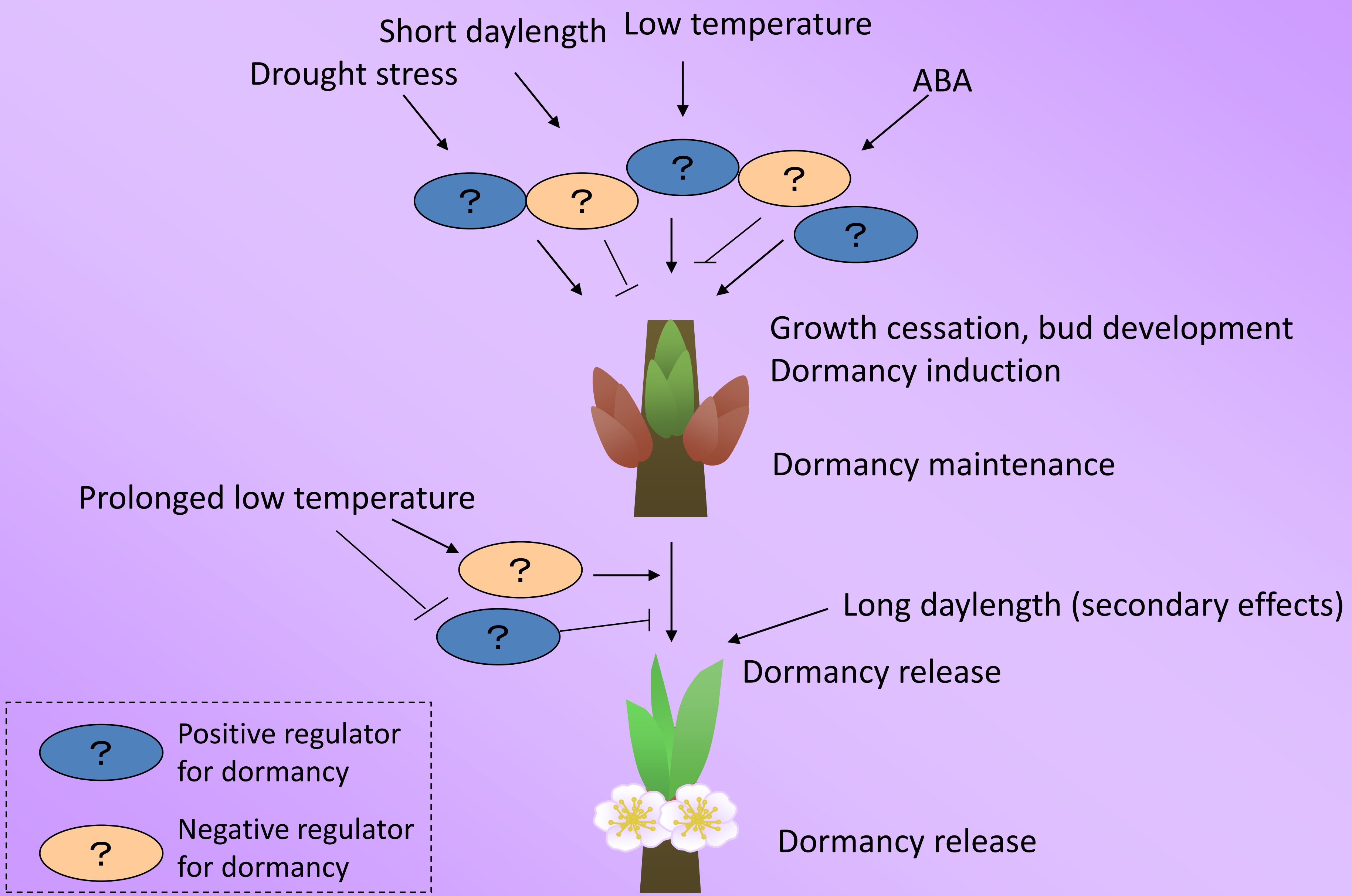
Studies on endodormancy regulation will help to maintain uniform blooming of temperate fruit trees

Why Japanese apricot for dormancy study?

- One of the transformable plants among stone fruits
- Kyoto is located near one of the large Japanese apricot production areas in Japan.
- There are genetic resources that vary with chilling requirements
- Small genome size (~220Mbp)
- Peach (close relatives) genome has been sequenced (Apr. 2010)

We started Japanese apricot dormancy studies since 2005.

Many factors are involved in dormancy induction, maintenance and release



Searching for genetic factors controlling endodormancy

First, we intended to isolate the genes specifically expressed in endodormant buds among para-, endo-, and eco-dormant buds using RNA subtraction technique (Yamane et al., 2008)

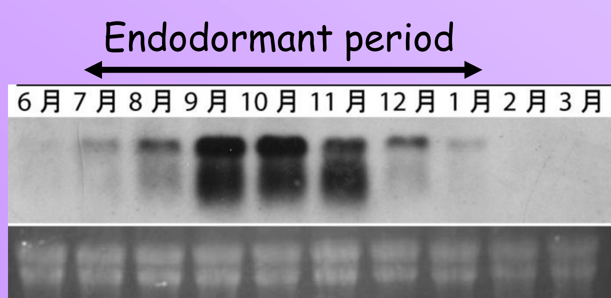
<u>RNA subtraction 1</u>

Endo minus Para

<u>RNA subtraction 2</u>

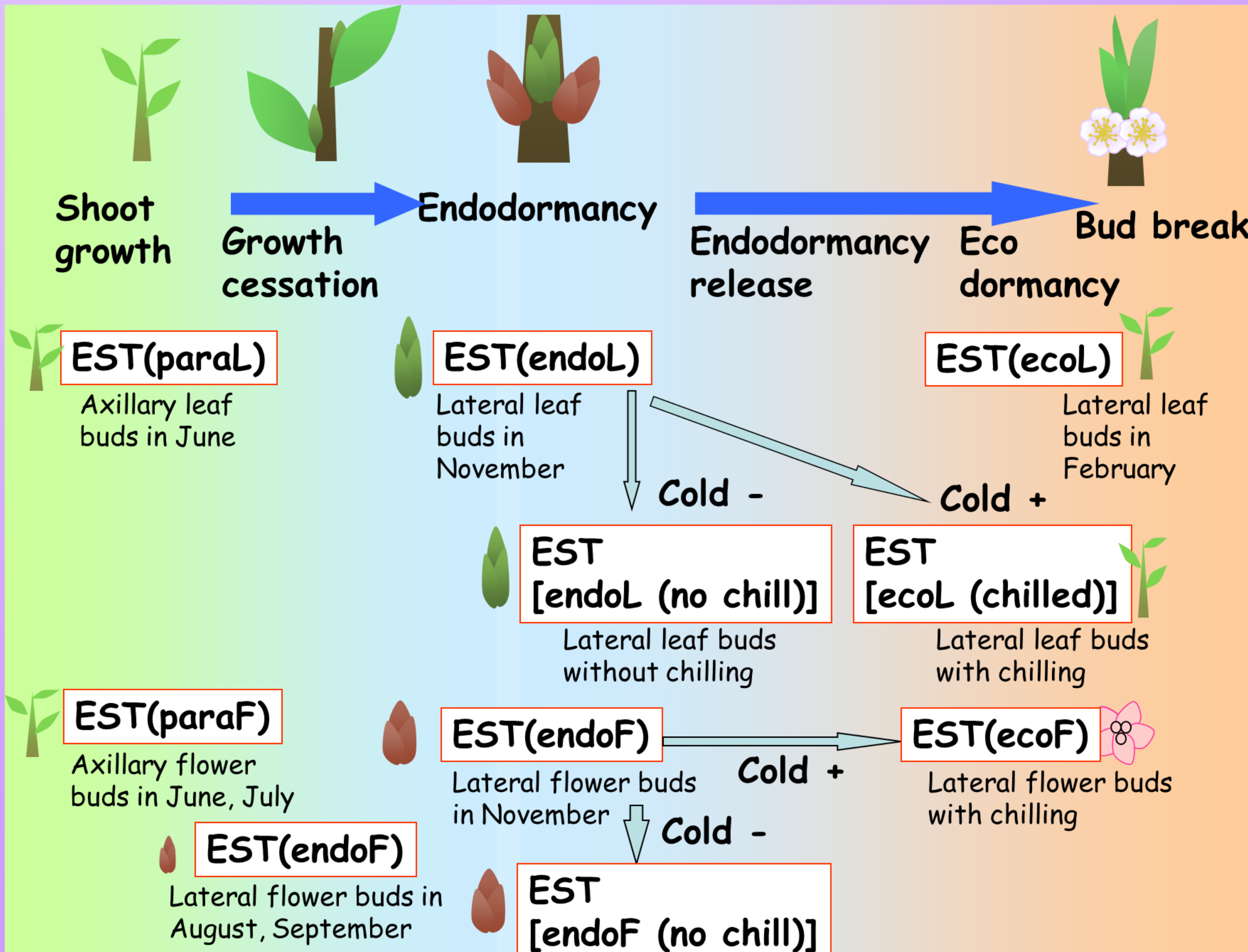
Endo minus Eco

Only one clone was commonly isolated from two libraries.
Because this gene was similar to peach *DORMANCY-ASSOCIATED MADS6*, we named this gene as *Prunus mume DAM6* (*PmDAM6*).



Specifically expressed in endodormant leaf buds!

Second, we conducted EST analysis to isolate the genes specifically expressed in endodormant buds among para-, endo-, and eco-dormant buds.



Total 485,376 raw reads

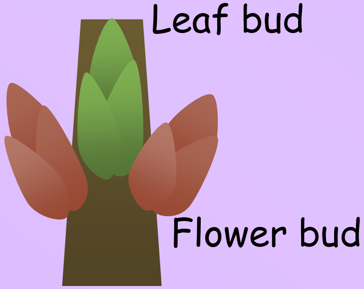
↓ Assembly

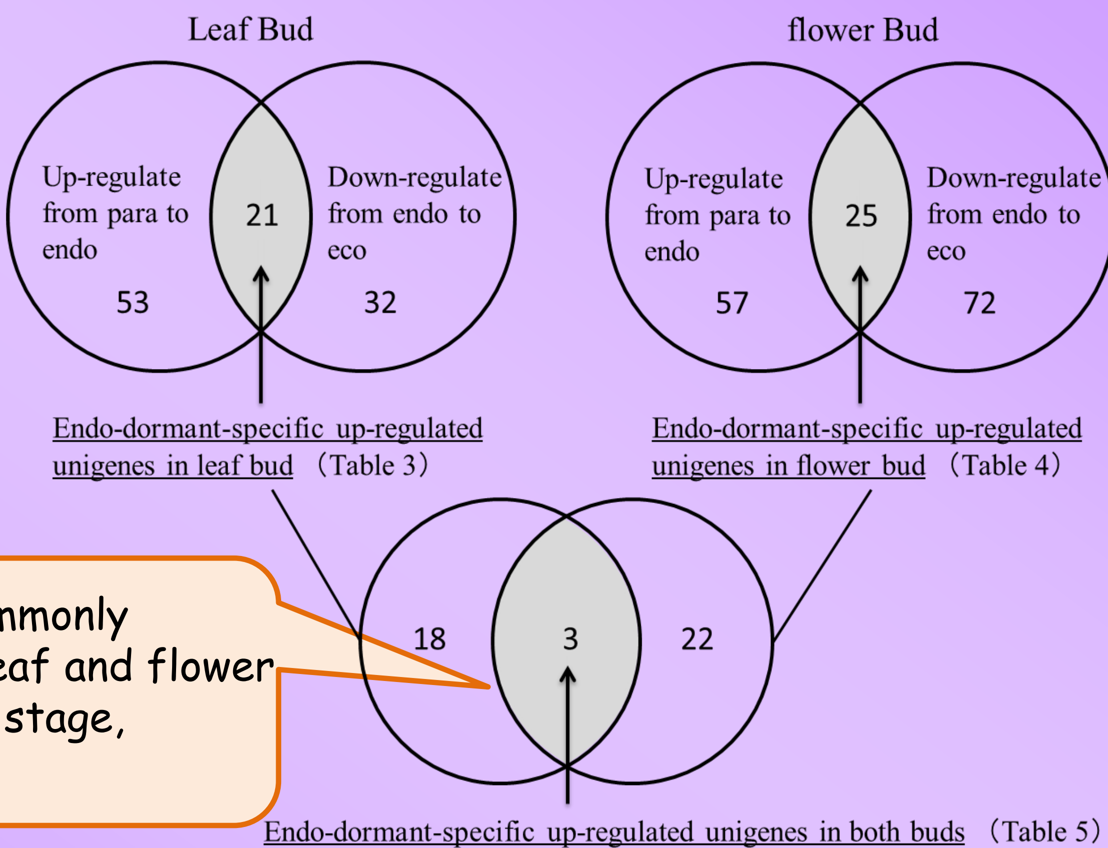
113,629 unique sequences

28,382 contigs and 85,247 singletons

(Habu et al., submitted)

Summary of Japanese apricot dormant bud ESTs



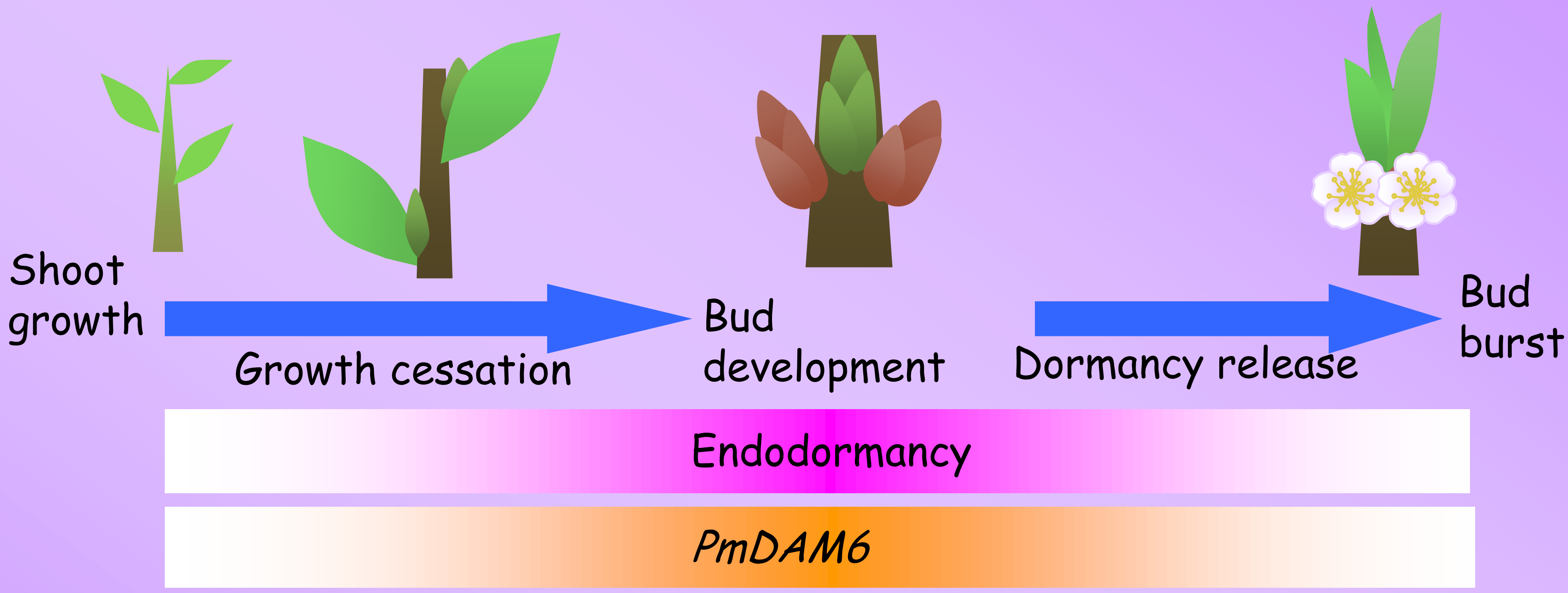


Three genes were commonly upregulated in both leaf and flower buds at endodormant stage, including *PmDAM6*.

Table 5. Endo-dormant-specific up-regulated unigenes in both buds

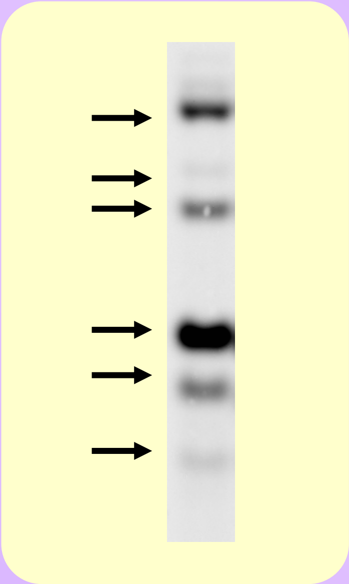
	EST number						Similar genes in GenBank	
Unigene name	paraL	endoL	ecoL	paraF	endoF	ecoF	Accession Number	Description
PmC015135	6	59	5	13	94	4	CBI15083.3	unnamed protein product [Vitis vinifera]
PmC016164	1	94	2	6	120	0	BAH22477.1	dormancy-associated MADS-box transcription factor 6 [Prunus mume]
PmC016193	23	109	13	28	207	12	ACU16624.1	unknown [Glycine max]

PmDAM6 showed endodormancy-associated expression



We focused on PmDAM6 as a candidate for controlling dormancy.

Genomic DNA blot analysis

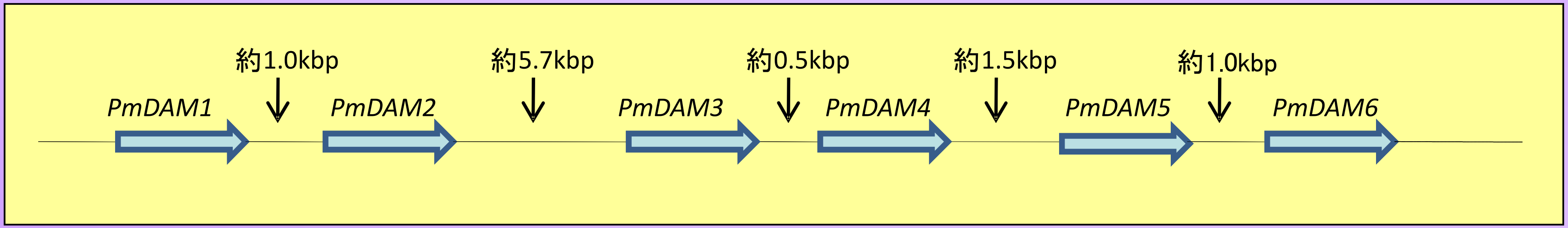


Japanese apricot genome has several sequences similar to PmDAM6.

↓

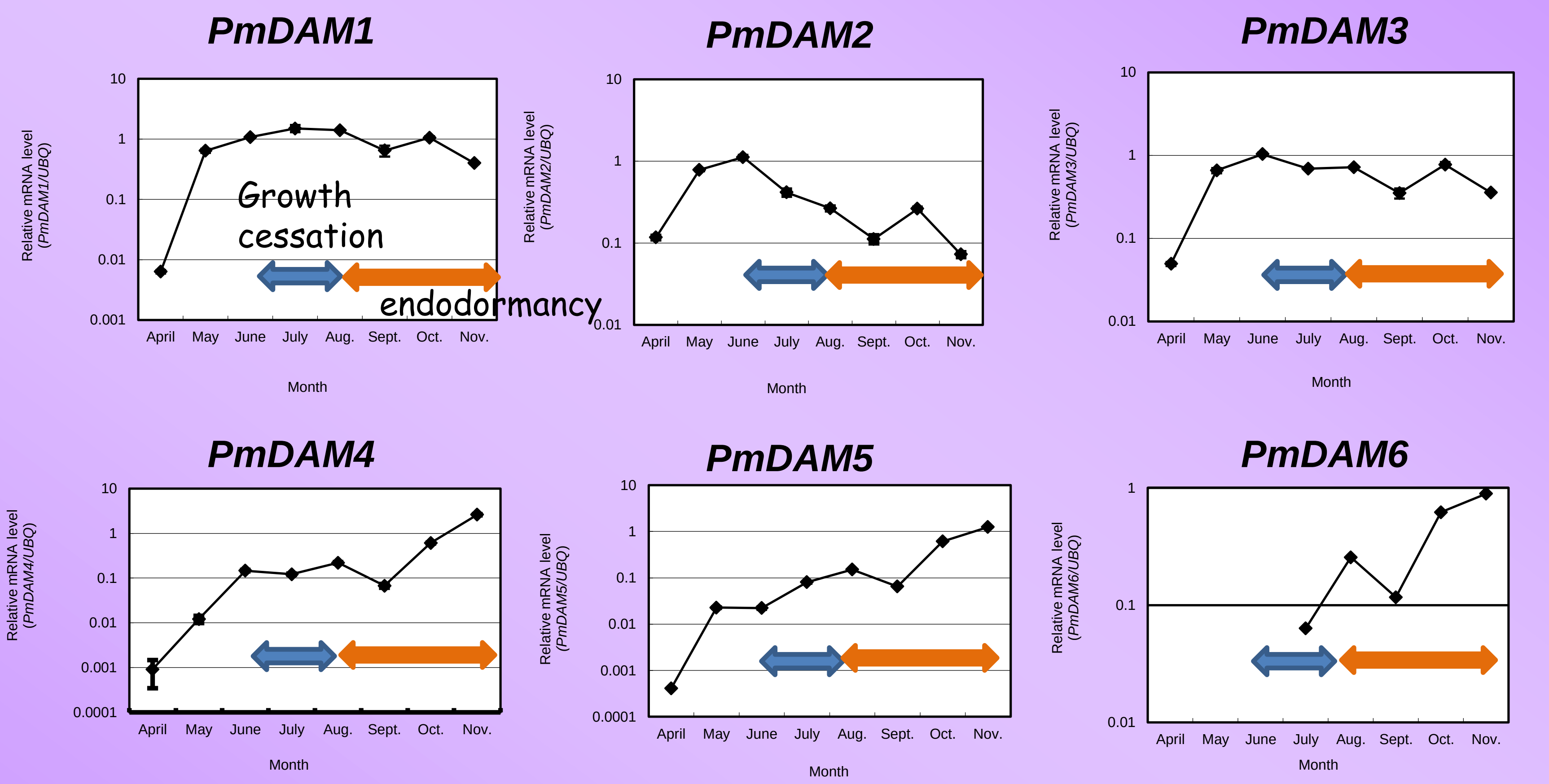
Genomic library screening and shotgun sequencing

↓



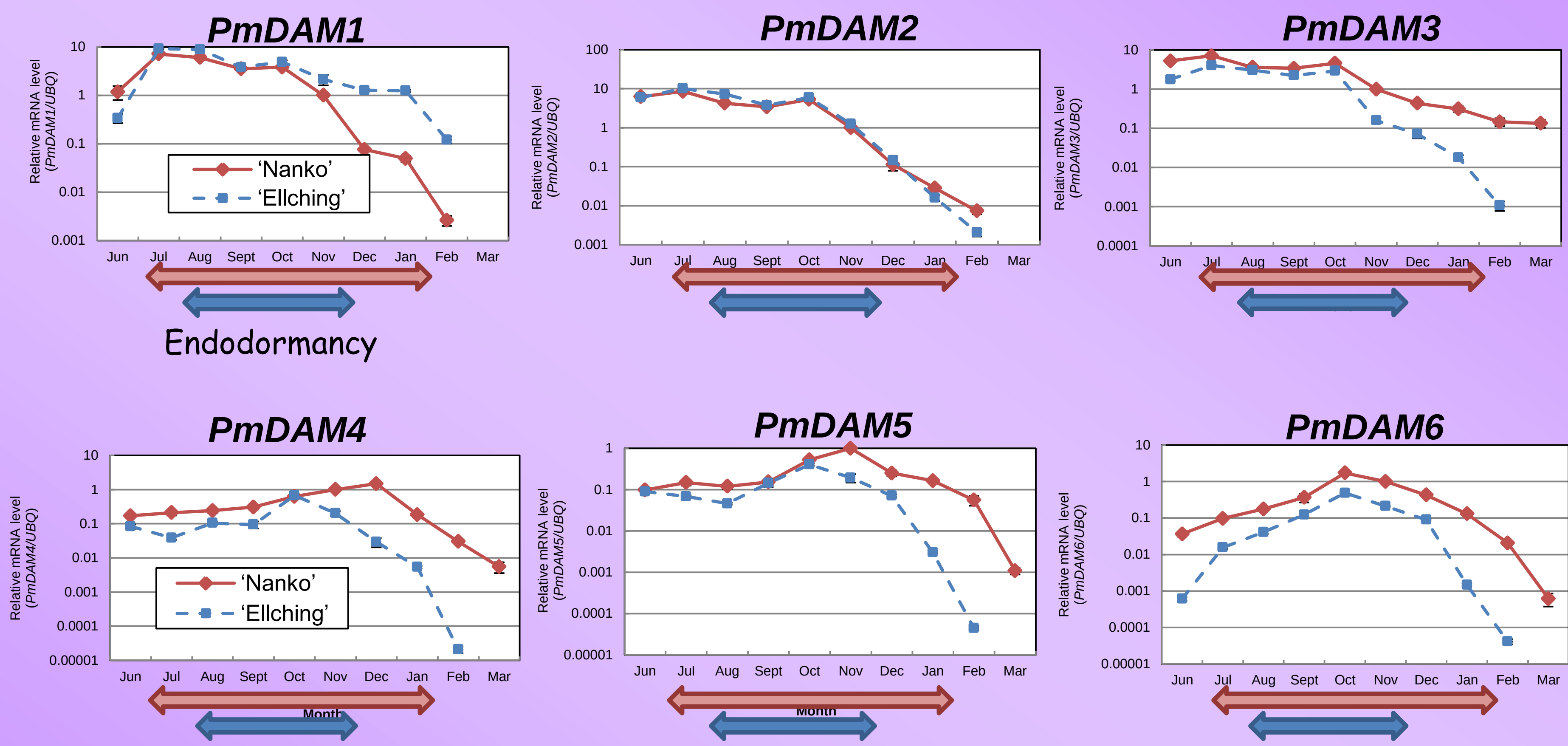
Japanese apricot contains six tandemly arrayed MADS-box genes (SVP/AGL24 clade) as is the case in peach (Bielenberg et al., 2008).

Seasonal expression changes of *PmDAM1-PmDAM6*



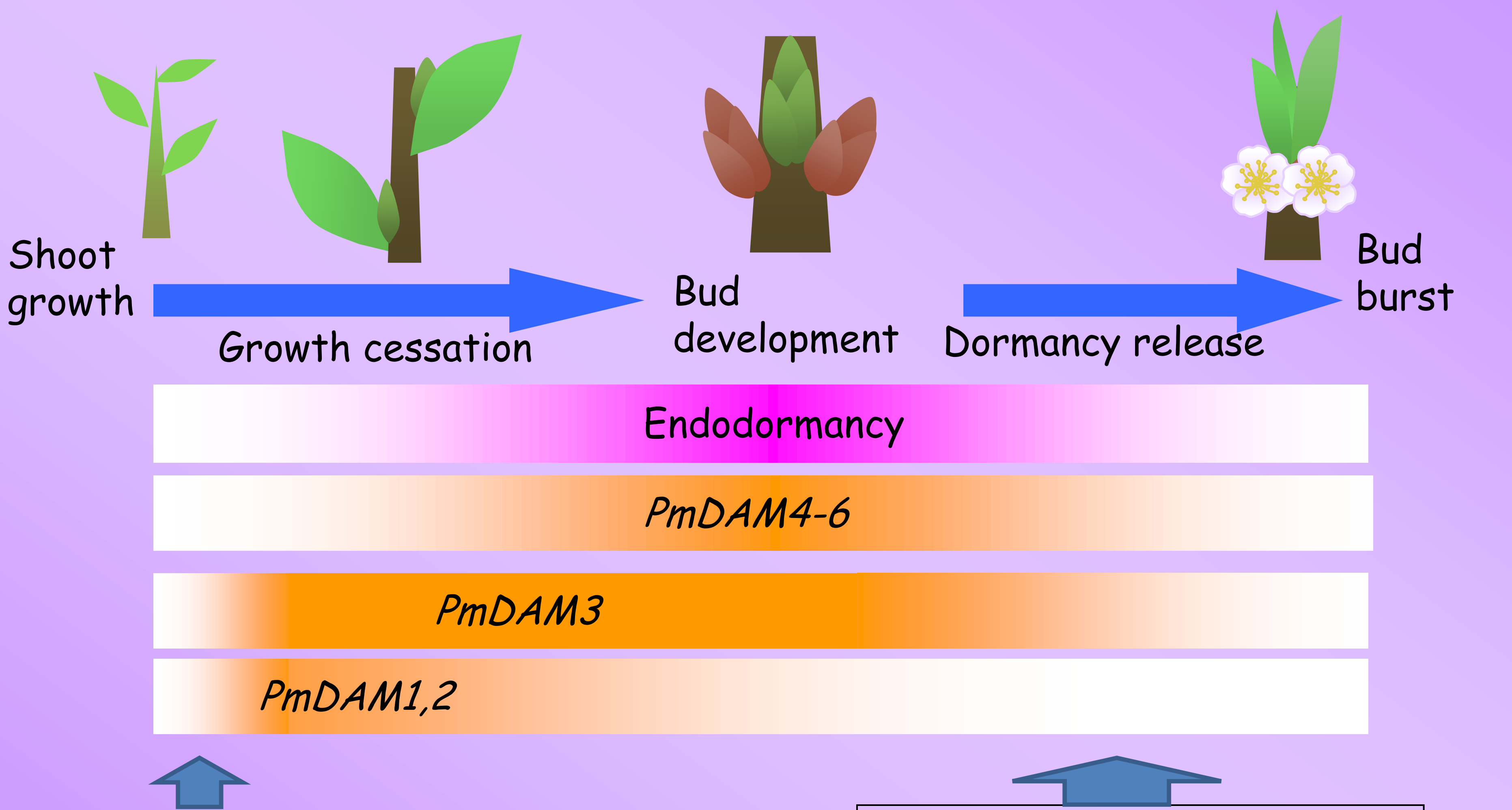
All six PmDAM expressions were lower during active shoot growth, then up-regulated during growth cessation.

Seasonal expression changes of *PmDAM1-PmDAM6* in high-chill, Nanko (long and deep) and low-chill, Ellching (short and shallow)



- All six PmDAMs were down-regulated toward spring.
- Negative correlations between *PmDAM3-6* expressions and endodormancy release

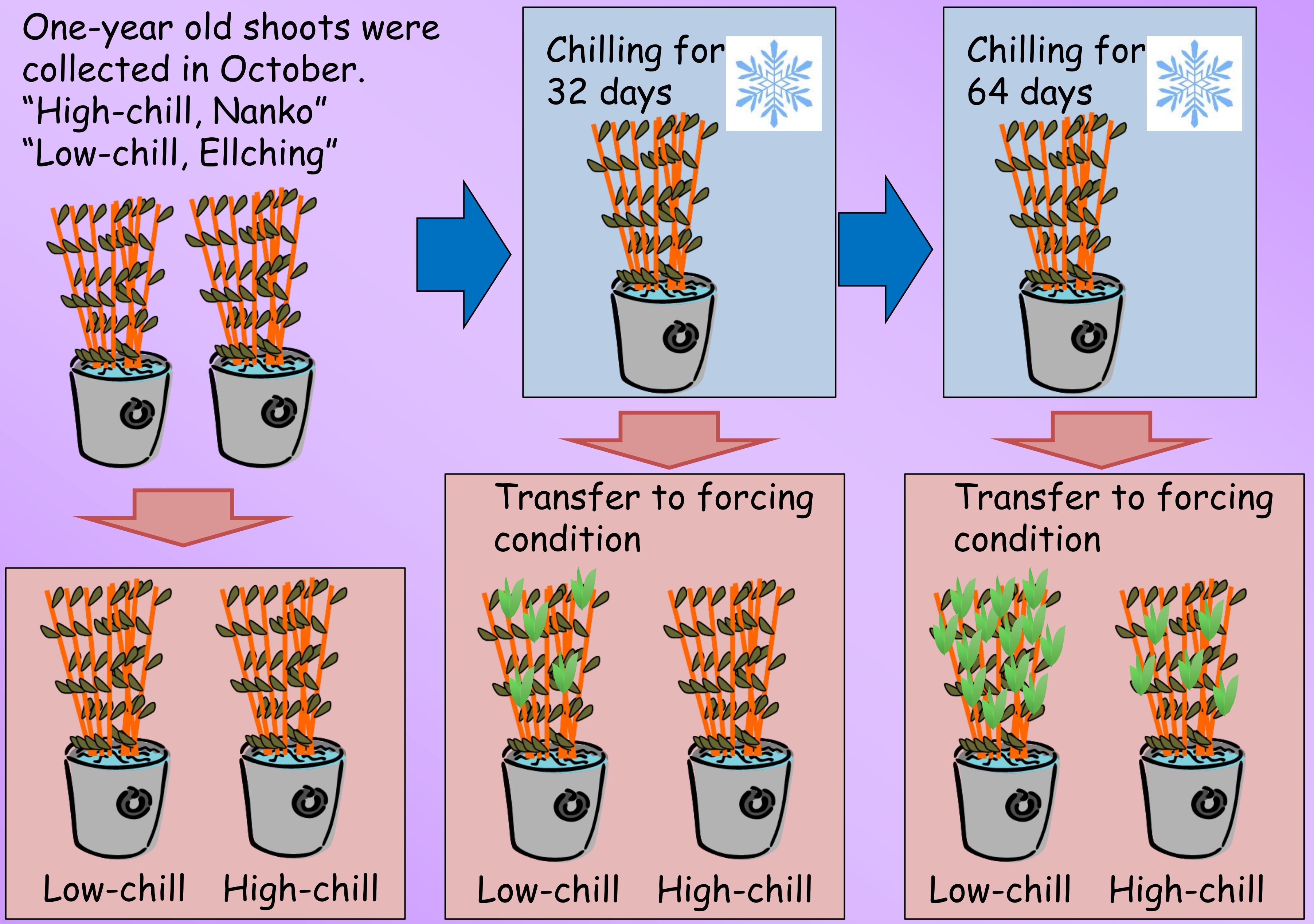
Seasonal growth habit and expressions of *PmDAM1-6*



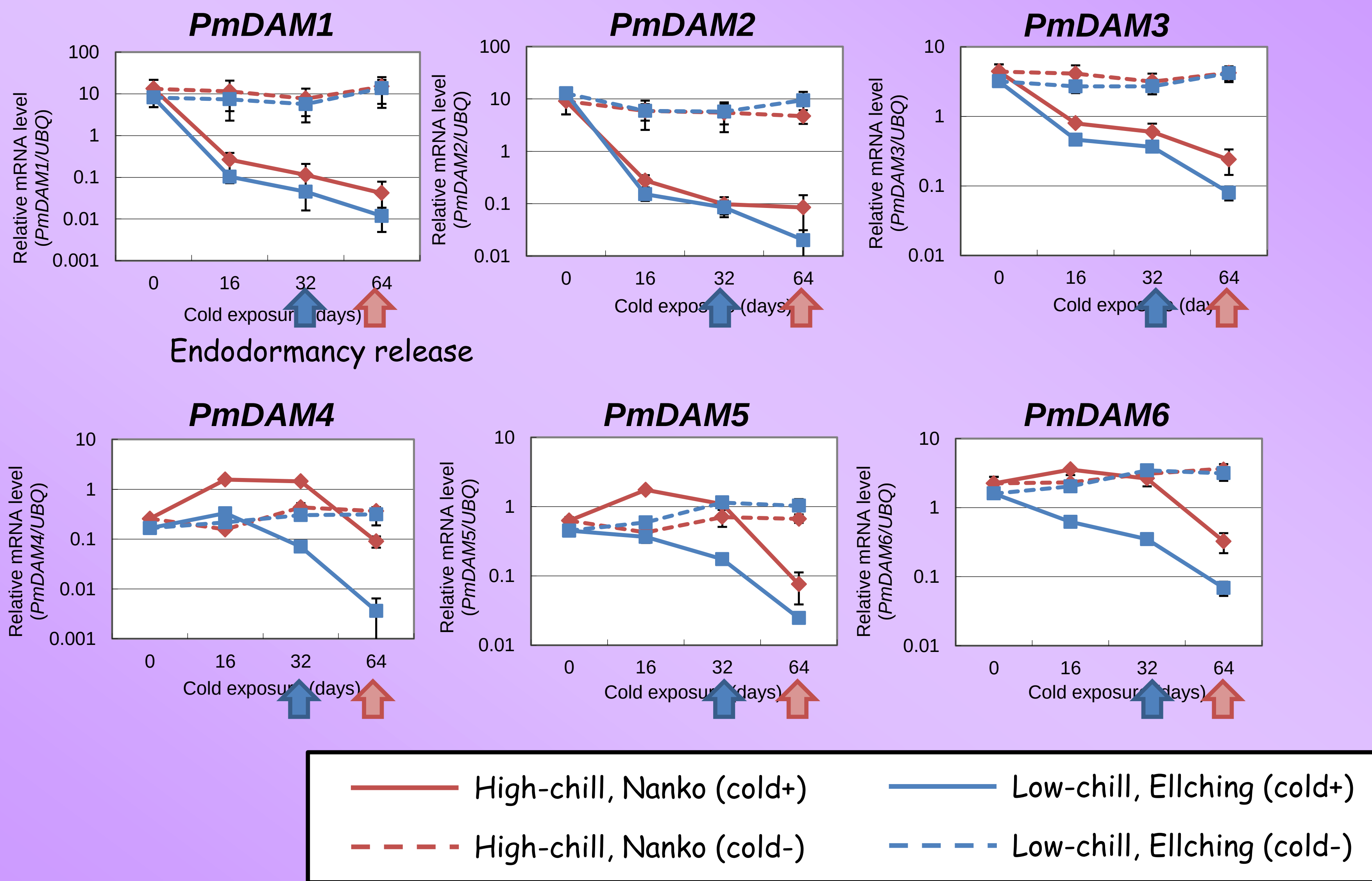
PmDAMs were up-regulated during growth cessation.

PmDAM3-6 were down-regulated during endodormancy release.

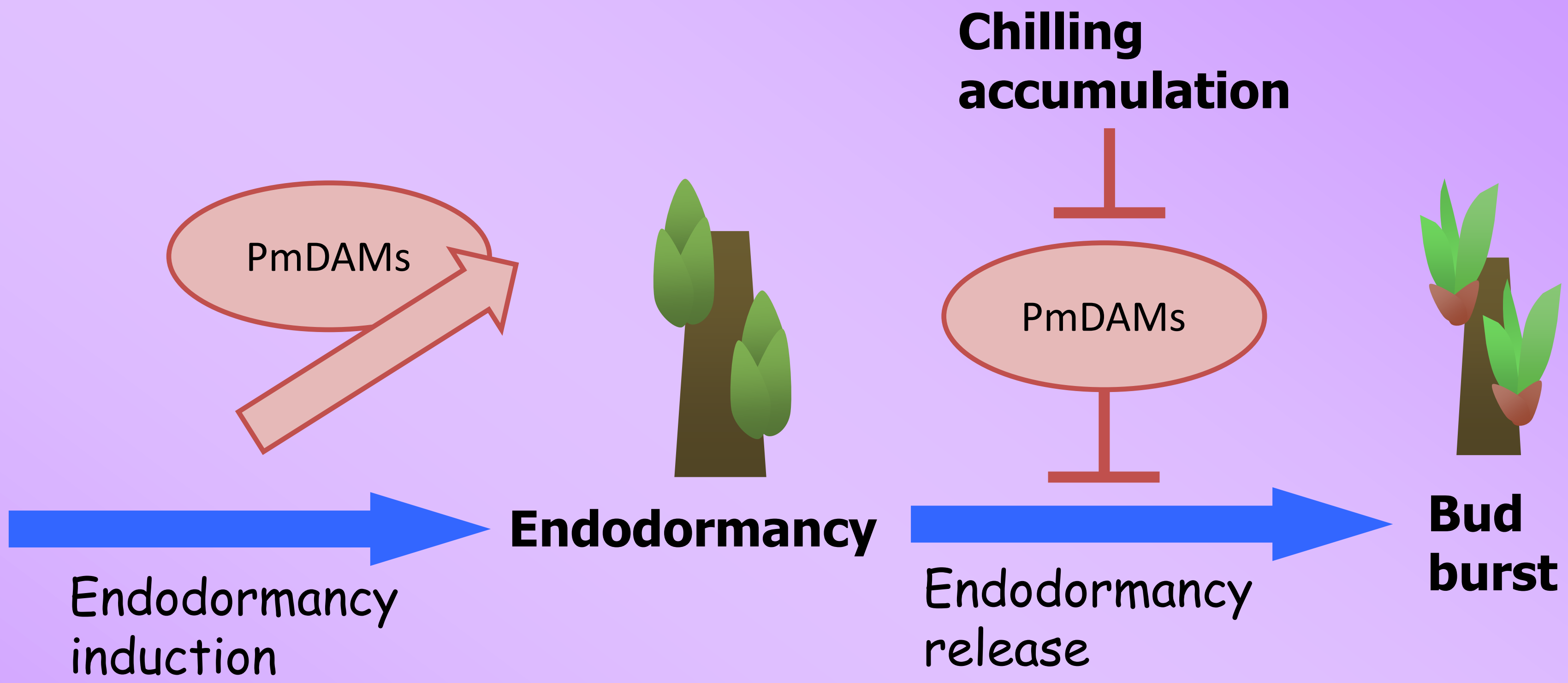
Cold treatment to induce endodormancy release



Decrease in *PmDAM4-6* expressions coincided very well with endodormancy release



Summary of expressional analyses of PmDAMs



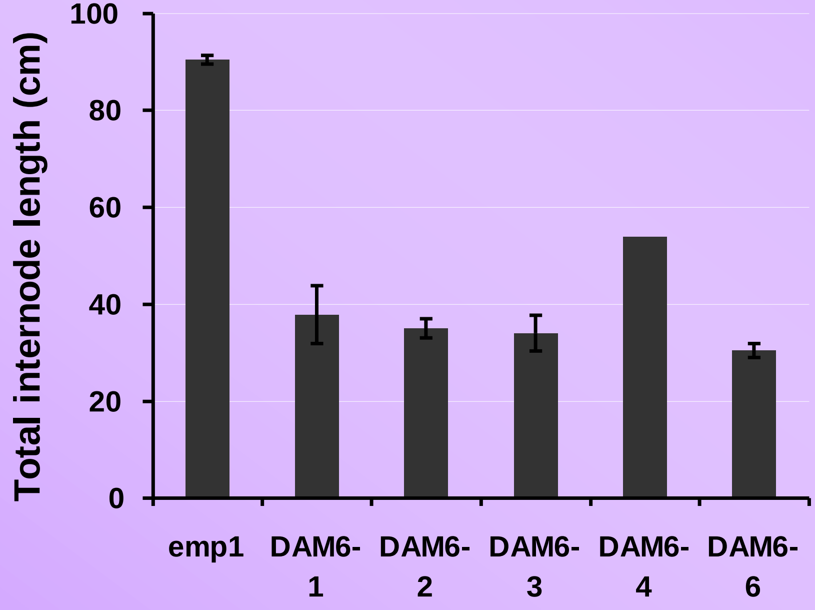
Downregulation of DAM-like genes during dormancy release has been reported in other temperate fruit trees including;
kiwifruit (Wu et al., 2011),
Japanese pear (Ubi et al., 2010),
Peach (Jimenez et al., 2010; Yamane et al., 2011)
Raspberry (Mazzitelli et al., 2007)

What are the biological functions of PmDAMs?

Are PmDAMs directly involved in bud endodormancy?
Endodormancy induction? Maintenance?
If so, How? Which pathway?

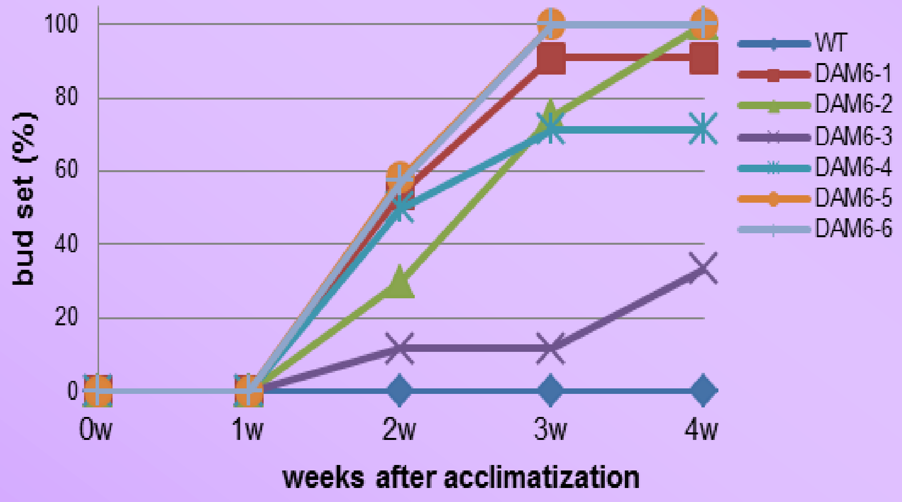
Growth of 35S:PmDAM6 was retarded

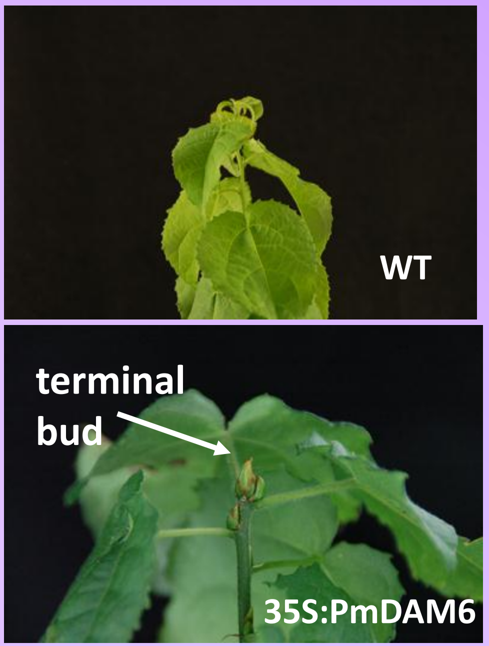
Sasaki et al.,
Plant Physiol. (2011)



Shoot growth was suppressed in all six transgenic *35S:PmDAM6* lines.

Growth cessation and bud set occurred in 35S:PmDAM6 poplars at non-dormancy-inducing condition (16-h daylength, 22C)





Under LD condition, growth cessation was promoted and terminal bud set was induced in 35S:PmDAM6, whereas control plants showed continuous shoot growth.

Sasaki et al. (2011)

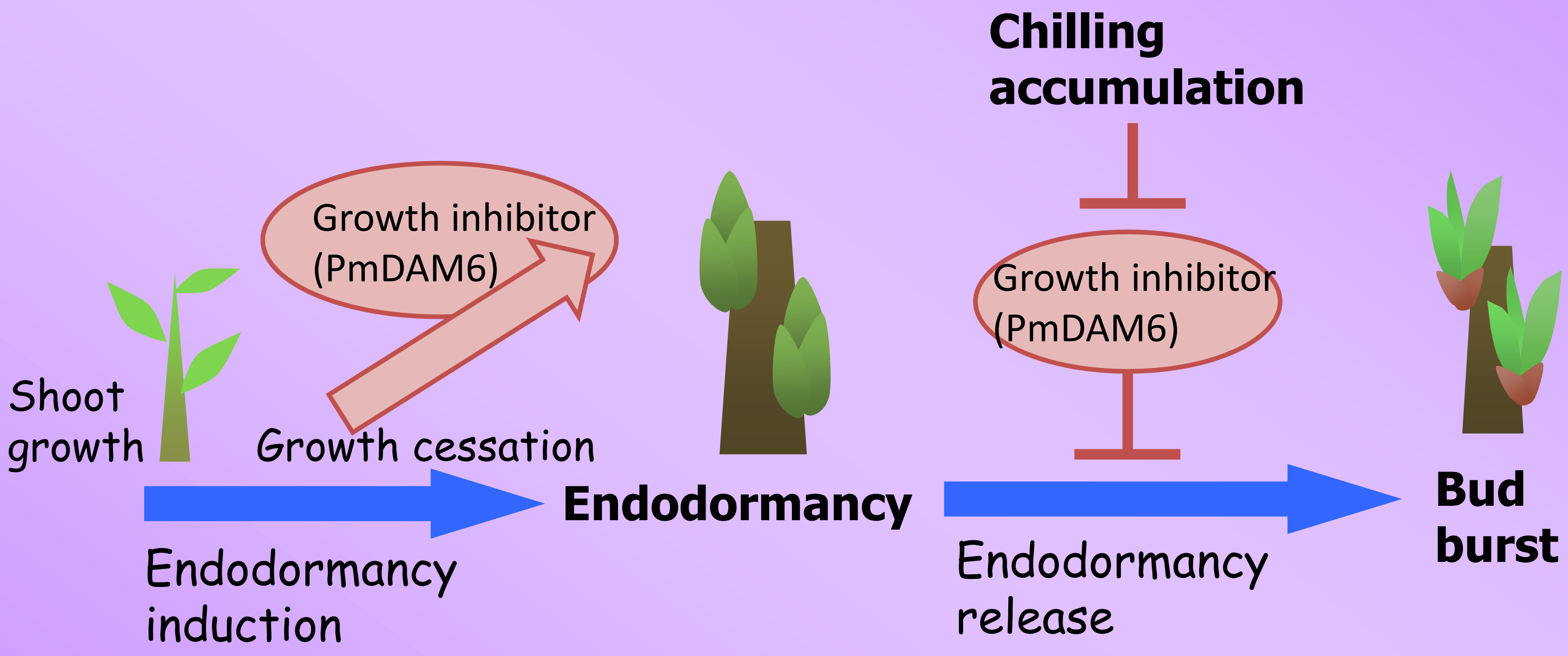
Endodormancy was induced in *35S:PmDAM6* poplars at non-dormancy-inducing condition (16-h daylength, 22C)

Leaf removal

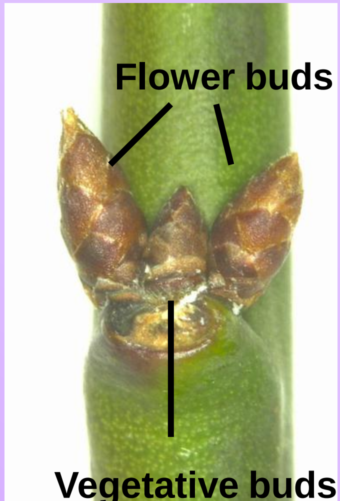
35S:PmDAM6 poplars did not resume their growth, whereas the control poplars showed bud burst.

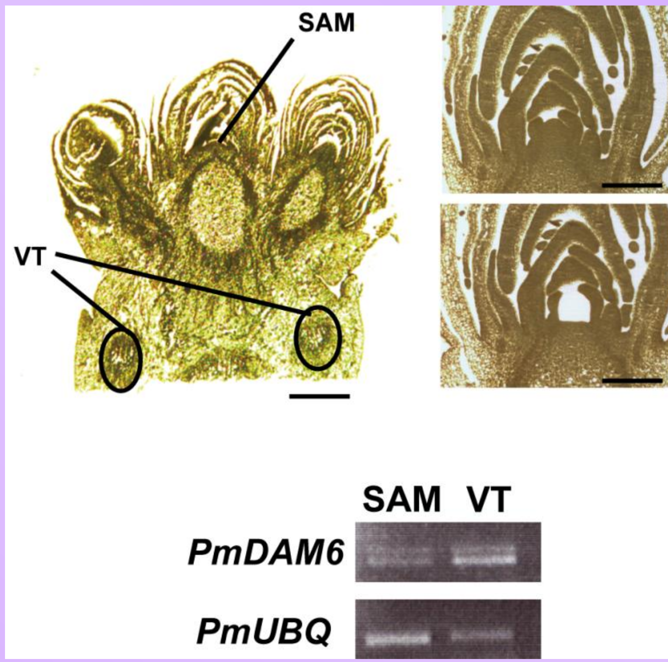
Sasaki et al. (2011)

PmDAM6 positively regulates endodormancy through its growth inhibitory effect



How is *PmDAM6* involved in dormancy regulation?





PmDAM6 is expressed not only in SAM but also in vascular tissues of shoots (leaves, stems and bud scales).

Does PmDAM6 act within meristem?

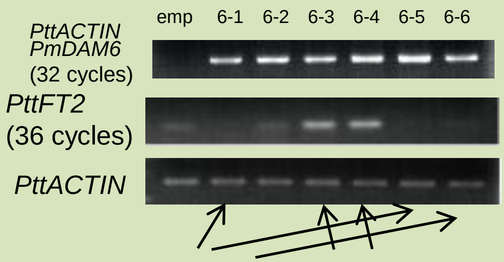
According to Lang et al. (1987) nomenclature, factors involved in endodormancy should work within meristem (not only subtending tissues).

What is the target gene of PmDAM6 ?

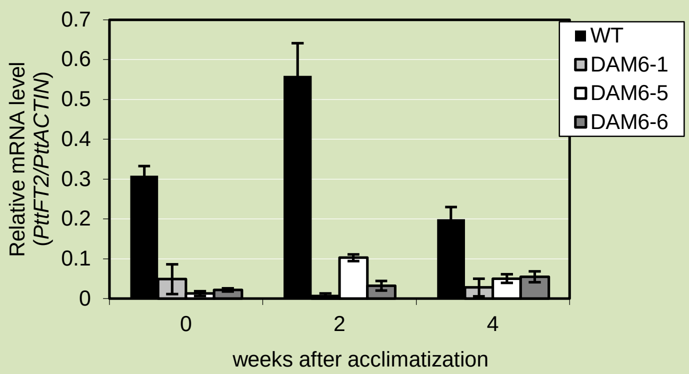
What is the target of PmDAM6 for dormancy regulation?

PtFT RNAi poplars showed bud set in LD condition (Bohlenius et al., 2006), which seems to be similar to the phenotype observed in our *35S:PmDAM6* poplars.

PtFT2 expressions in *35S:PmDAM6* poplars



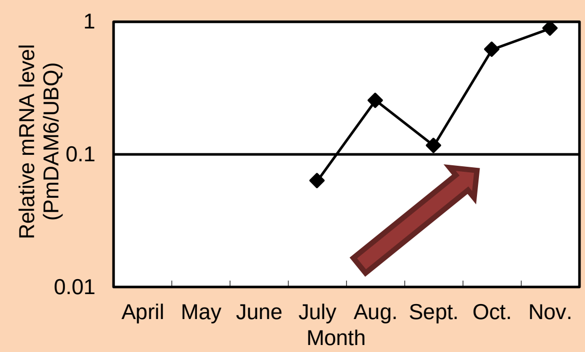
Down-regulated? Up-regulated?



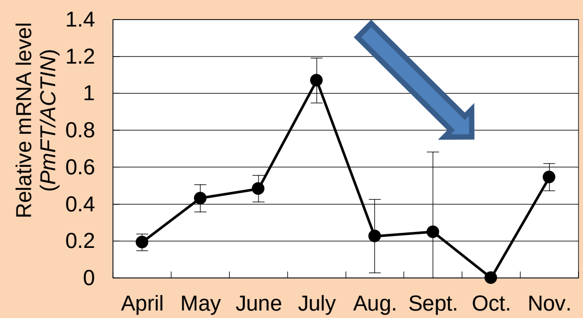
PtFT2 is down-regulated in some DAM6-1,5,6 lines.

Seasonal expressions in Japanese apricot leaves

PmDAM6 in leaves

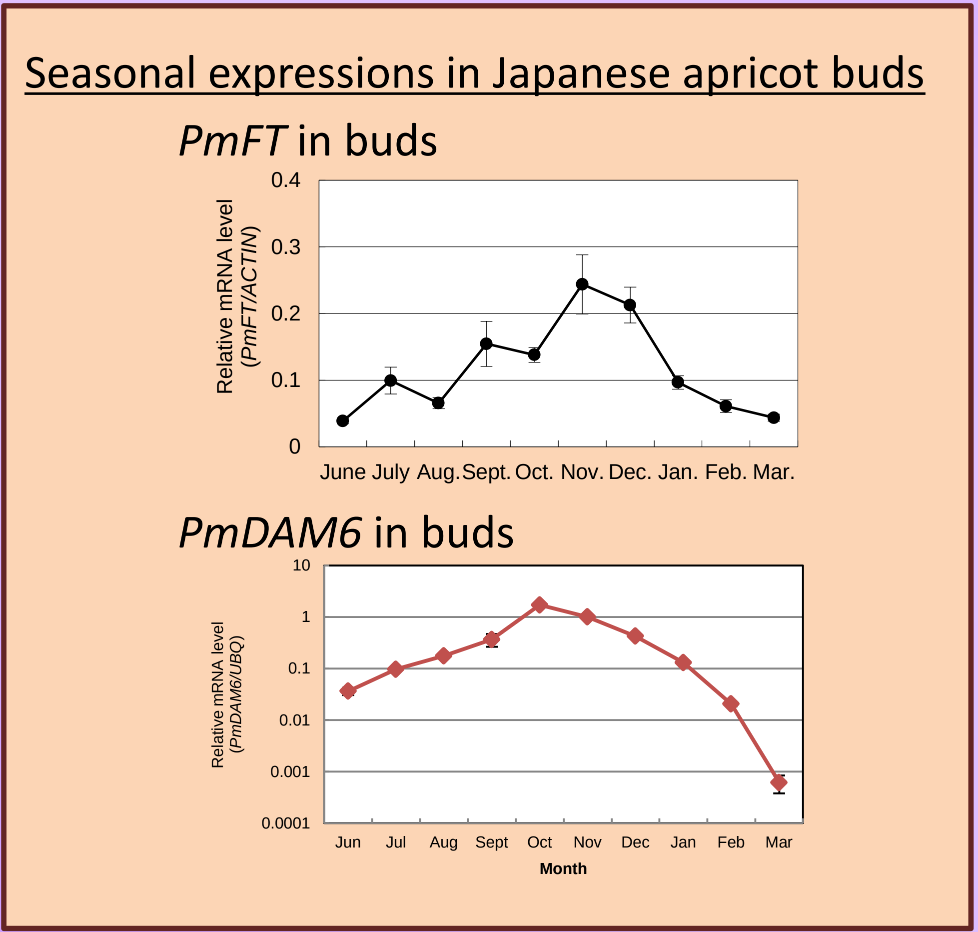


PmFT in leaves

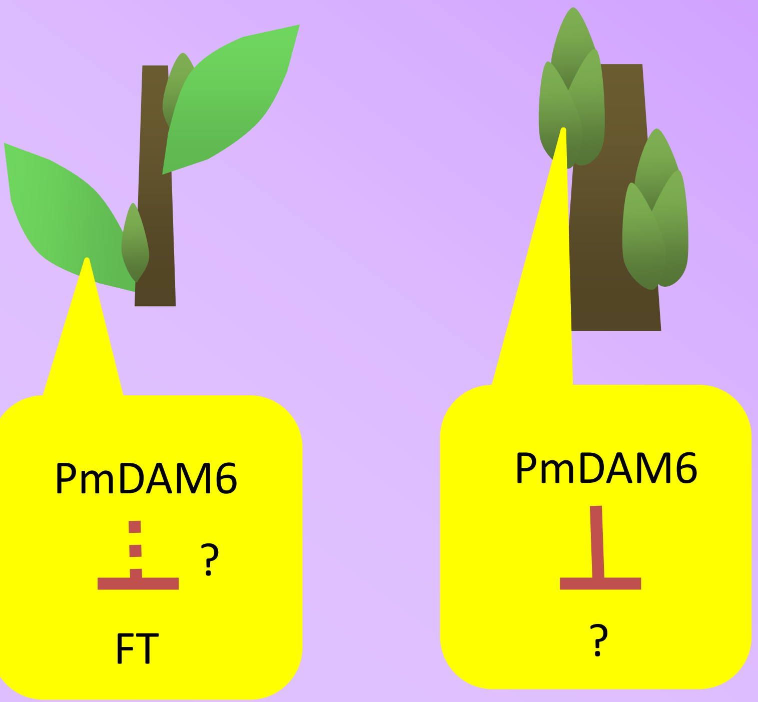


PmDAM6 possibly down-regulates *FT* in leaves?

What is the target of *PmDAM6* for dormancy regulation?



PmDAM6 appeared not to repress FT expression in buds.



We are now trying to identify the target gene of PmDAM6 in dormant buds.

Conclusion and prospects

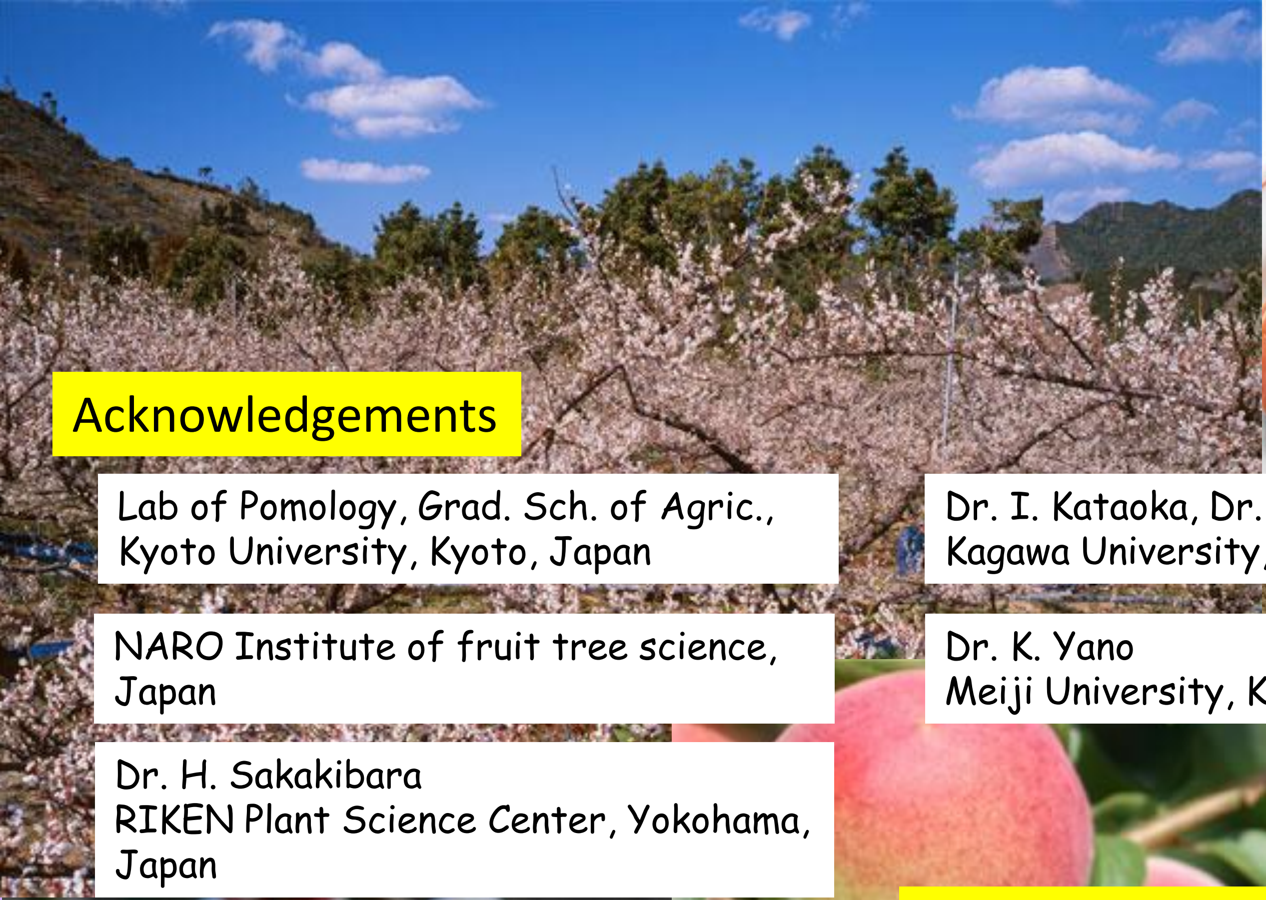
We identified *PmDAM* genes highly expressed in Japanese apricot dormant buds.

PmDAM genes were down-regulated during chilling-mediated dormancy release.

35S:PmDAM6 poplars showed growth cessation and bud set under non-dormancy-inducing condition.

PmDAM6 affected endodormancy release of *35S:PmDAM6* poplars.

We are currently searching for the genes under the control of *PmDAM6* in bud meristem.



Acknowledgements

Lab of Pomology, Grad. Sch. of Agric.,
Kyoto University, Kyoto, Japan

NARO Institute of fruit tree science,
Japan

Dr. H. Sakakibara
RIKEN Plant Science Center, Yokohama,
Japan

Dr. M. Takagi, Dr. M. Nakata
Bioproduction Research Institute,
Tsukuba, Japan

Dr. I. Kataoka, Dr. K. Beppu
Kagawa University, Miki, Kagawa, Japan

Dr. K. Yano
Meiji University, Kawasaki, Japan

Research funds

Program for the Promotion of Basic and Applied Researches for Innovation in Bio-oriented Industry, Japan.
(2008-2010)

Grant-in-Aid for Scientific Research (KAKENHI) from Japan Society for the Promotion of Science (JSPS), Japan.
(2005-2008, 2011-)