Functional Studies on Japanese apricot PmDAM genes

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

**Growth cessation, Flowering initiation**

**Autumn**
- Leaf fall

**Dormant season**
- Leaf fall

**Winter**
- **Fruit set**

**Growing season**
- **Spring**
  - Bud burst
  - Shoot growth
  - Growth cessation
  - Lateral bud development

**Summer**
- **Blooming**

**Vegetative growth habit**
- **Shoot tip abortion**

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)

- **Apical dominance, Correlative inhibition**
- **Endodormancy**
  - No bud burst occurs in favorable condition
  - An unknown endogenous signal leads to growth inhibition
  - Released by low temperature (Chilling requirement)
  - Low temperature, Short daylength, Water stress

- **Paradormancy**
- **Ecodormancy**
  - Temperature extreme, Water stress

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

- **Summer**
- **Autumn**
- **Winter**
- **Spring**
Due to global warming, farmers will not be able to grow temperate fruits in the 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.

- Short daylength
- Low temperature
- Drought stress
- ABA

Growth cessation, bud development
Dormancy induction

Prolonged low temperature

- Dormancy maintenance

Long daylength (secondary effects)

Dormancy release

Positive regulator for dormancy

Negative regulator for dormancy
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).

RNA subtraction 1

RNA subtraction 2

Endo minus Para

Endo minus Eco

Still dormant but ready to bud burst

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).

Endodormant period

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. 113,629 unique sequences. Assembly: 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

<table>
<thead>
<tr>
<th>Unigene name</th>
<th>paraL</th>
<th>endoL</th>
<th>ecoL</th>
<th>paraF</th>
<th>endoF</th>
<th>ecoF</th>
<th>Accession Number</th>
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<td>5</td>
<td>13</td>
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<td>4</td>
<td>CBI15083.3</td>
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<td>94</td>
<td>2</td>
<td>6</td>
<td>120</td>
<td>0</td>
<td>BAH22477.1</td>
<td>dormancy-associated MADS-box transcription factor 6 [Prunus mume]</td>
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<tr>
<td>PmC016193</td>
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<td>109</td>
<td>13</td>
<td>28</td>
<td>207</td>
<td>12</td>
<td>ACU16624.1</td>
<td>unknown [Glycine max]</td>
</tr>
</tbody>
</table>
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*

During growth cessation, *PmDAMs* were up-regulated. During endodormancy release, *PmDAM3-6* were down-regulated.
One-year old shoots were collected in October. “High-chill, Nanko” “Low-chill, Ellching”

Chilling for 32 days

Chilling for 64 days

Transfer to forcing condition

Transfer to forcing condition
Decrease in *PmDAM*4-6 expressions coincided very well with endodormancy release.
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)

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

Seasonal expressions in Japanese apricot leaves

PmDAM6 in leaves

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

PmDAM6 possibly down-regulates FT in leaves?
What is the target of \textit{PmDAM6} for dormancy regulation?

Seasonal expressions in Japanese apricot buds

\textit{PmFT} in buds

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{ft_expression.png}
\end{figure}

\textit{PmDAM6} in buds

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{dam6_expression.png}
\end{figure}

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 \textit{PmDAM} genes highly expressed in Japanese apricot dormant buds.

\textit{PmDAM} genes were down-regulated during chilling-mediated dormancy release.

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

\textit{PmDAM6} affected endodormancy release of \textit{35S:PmDAM6} poplars.

We are currently searching for the genes under the control of \textit{PmDAM6} in bud meristem.
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