Silencing of a lipase maturation factor 2-like gene by wheat-mediated RNAi reduces the survivability and reproductive capacity of grain aphid (*Sitobion avenae*)

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1.1 Wheat is the third grain crop in China.

- In 2016, the total area was near 24.2 million hectares, and the annual output was about 128 million tons.
- Northern Chinese are used to eat wheat flour food, such as noodles, steamed bread, and dumpling.
1.2 Wheat aphids are economically important and most destructive pest of wheat in China.

- Aphid feeding affects photosynthesis and the absorption and transfer of nutrients, resulting in reduced wheat yields and lower quality wheat.

- In 2016, the cumulative area (above occurrence level) was 17.3 million hectares.
The grain aphid, *Sitobion avenae* F., is the dominant wheat aphid species, and is mainly distributed on wheat spikes during the filling stage.

Grain aphids have a short life cycle with high fecundity, and outbreaks occur with great frequency.
1.3 Chemical pesticides have been used heavily for the control of agricultural pests in China. However, long-term and unmanaged overuse of single pesticides has resulted in increased levels of insect resistance.
1.4 Plant-mediated RNAi technology has become a major research focus in crop genetic engineering for aphid control.

Table 1.3: partial samples of Grain aphid control by RNAi

<table>
<thead>
<tr>
<th>Aphids</th>
<th>Method</th>
<th>Target gene</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Grain Aphid</td>
<td>Transgenic wheat</td>
<td>carboxylesterase gene</td>
<td>Xu et al., 2014</td>
</tr>
<tr>
<td>Grain Aphid</td>
<td>Transgenic wheat</td>
<td>Hpa1</td>
<td>Fu et al., 2014</td>
</tr>
<tr>
<td>Grain Aphid</td>
<td>Transgenic wheat</td>
<td>salivary sheath protein</td>
<td>Abdellat ef et al., 2015</td>
</tr>
<tr>
<td>Grain Aphid</td>
<td>Artificial diet</td>
<td>Cytochrome P450 gene (CYP6AE14)</td>
<td>Hui et al., 2012</td>
</tr>
</tbody>
</table>
1.5 Lipase maturation factor family proteins are involved in the maturation of specific proteins in the endoplasmic reticulum.

Figure: the Conserved domains of LMF family

Mutations in Lmf1 were associated with combined lipase deficiency and resulted in severe hyper-triglyceridemia in mice as well as human subjects (Peterfy et al., 2007). However, the role(s) of LMF2 in insect systems remain unknown.
Research objective:

to explore

1) the effects of the loss-of-function of a *lmf2*-like gene

2) possible use of this gene in the development of novel aphid control strategies
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2.1 Clone of *lmf2*-like fragment

Based on the *lmf2*-like sequences of pea aphid (XM_001950737.4 and FF314537), the specific primer pair for RT-PCR:

**SaLmf-s1:**
5’-CCTGTTCCTGAGAGGCGTCT-3’

**SaLmf-a1:**
5’-GCAACACCAGCTGAAAACGCTACTC-3’
2.2 Construction of the RNAi Vector

Fig: Sketch map and restriction sites of pBAC-RNAi-\textit{Lmf2}
2.3 Production of Transgenic Plants

biolistic transformation
2.4 Aphid numbers and molting Bioassays

Standard condition at 21 ± 1 °C and relative humidity 50-60%.
Photoperiod 16L:8D.
Non-choice Assay
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3.1 Expression profile of aphid Imf2-like gene

Figure 3.1: aphid Imf2-like gene expression at different growth stages

The Imf2-like expression levels did not change significantly.
3.2 Clone of *Imf2*-like fragment

Figure 3.2A: Grain aphid *Imf2*-like fragment by RT-PCR (0.5 kb)

M: Marker; 1: amplicons from aphid cDNA
It was 86.79%, 75.37%, and 78.86% identical to the pea aphid sequences, XM_001950737.4, XM_015511500.1, and FF314537, respectively.

Figure 3.2B  Sequence alignment of *lmf2* between grain aphid and pea aphid.
A: target gene. B, C and D are pea aphid sequences, B: XM_001950737.4; C: XM_015511500.1; D: FF314537.

It was 86.79%, 75.37%, and 78.86% identical to the pea aphid sequences, XM_001950737.4, XM_015511500.1, and FF314537, respectively.
3.3 Screening for Positive Transgenic Plants

The T3 lines 117-1-1 and 125-2-5 (designed dsLPL1-1 and dsLPL2-5, respectively), derived from two independent T0 plants, were positive for AtFAD2int1 and negative for Bar.
3.4 Influence of transgenic wheat on expression of the grain aphid Lmf2-like gene

Fig 3.4: aphid Lmf2-like expression level after feeding the transgenic lines
5d: feeding after five days; 10d: feeding after ten days.
3.5 Aphid numbers after feeding on transgenic lines

Fig 3.5A: Aphid numbers feed on transgenic line dsLPL1-1 and CK. The difference became significant by day 10.
Fig3.5B: Aphid numbers feed on transgenic line dsLPL2-5 and CK

On day 15, there were significantly fewer aphids on dsLPL2-5 plants than on control plants.
3.6 molting numbers of grain aphid after feeding on transgenic

Fig 3.6: effect of feeding transgenic line dsLPL2-5 on grain aphid molting number
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Discussion

1  the expression and loss function of Imf2-like gene

We infer the Imf2-like gene is needed throughout the entire aphid lifecycle.

And the Imf2-like is was necessary for grain aphid survival, growth and reproduction.

2  Imf2-like gene can be used as the target for aphid control by plant-mediated RNAi methods.

The molting numbers on transgenic line dsLPL2-5 on day 9 and 12 were reduced by 20% and 19%. These decreases were not statistically significant.
Conclusion

1 The Imf2-like genes may have potential as a target gene for the control of grain aphids.

2 Feeding aphids with wheat expressing Imf2-like RNAi resulted in reductions in target expression, growth and reproduction.
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