13th International Wheat Genetics Symposium
23rd-28th April 2017, Tulln / Austria

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

Dr. LIANG Rongqi
liangrq@cau.edu.cn
China Agricultural University

Contents

1 Background

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.

Sitobion avenae ***Schizaphis graminum*** ***Rhopalosiphum padi*** ***Diuraphis noxia***

English Grain Aphid **Greenbug** **Russian wheat aphid**

- **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 resu lted 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.

Table1.3: partial samples of Grain aphid control by RNAi

Aphids	Method	Target gene	Reference
Grain Aphid	Transgenic wheat	carboxylesterase gene	Xu *et al*., 2014
Grain Aphid	Transgenic wheat	Hpa1	Fu *et al*., 2014
Grain Aphid	Transgenic wheat	salivary sheath protein	Abdellatef *et al*., 2015
Grain Aphid	Artificial diet	Cytochrome P450 gene (CYP6AE14)	Hui *et al*., 2012

1.5 Lipase maturation factor family proteins are involved in the maturation of specific proteins in the endoplasmic reticulum.

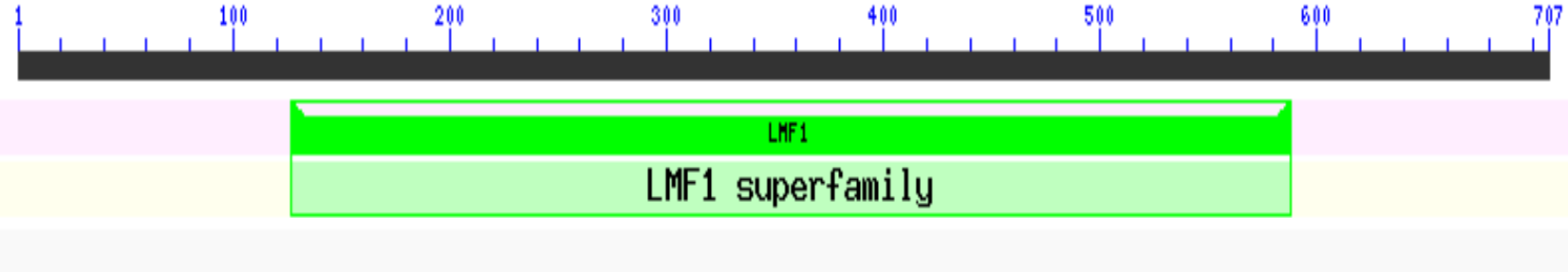


Figure: the Conserved domains of LMF family

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

Contents

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

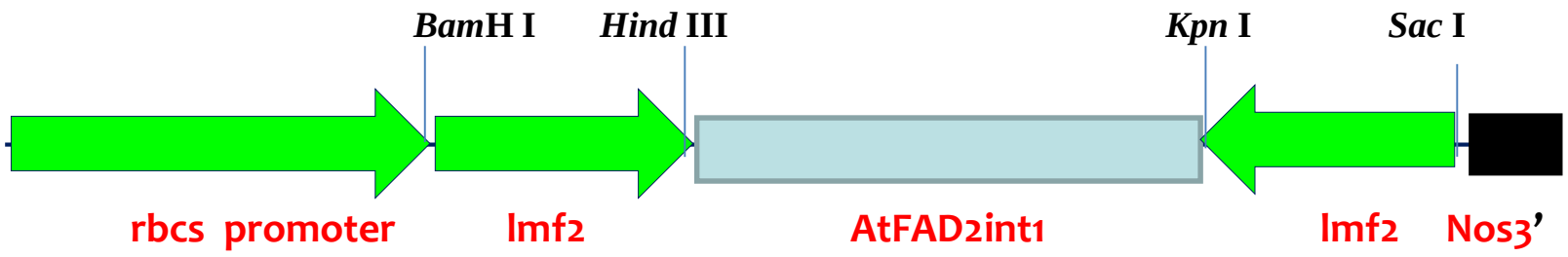
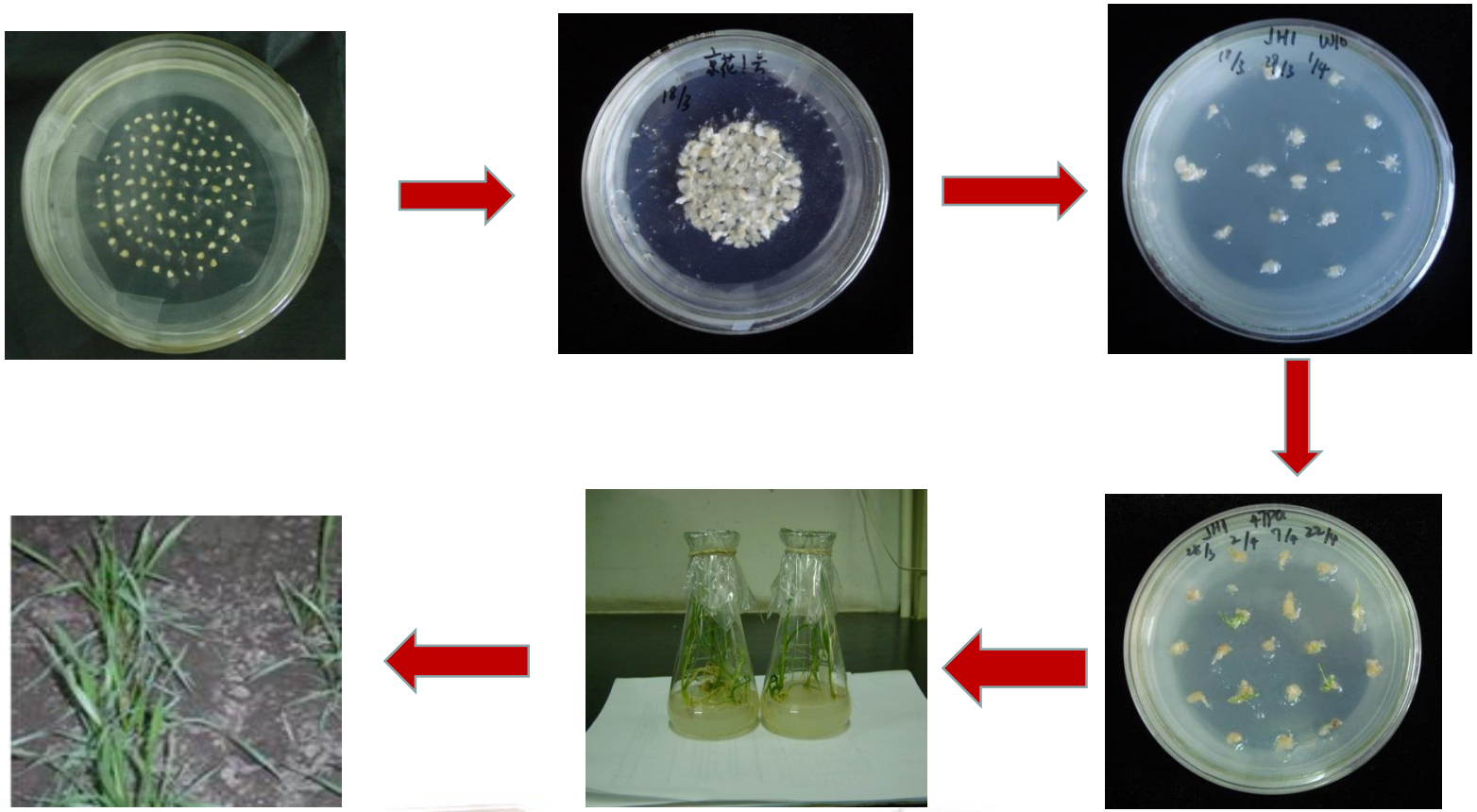


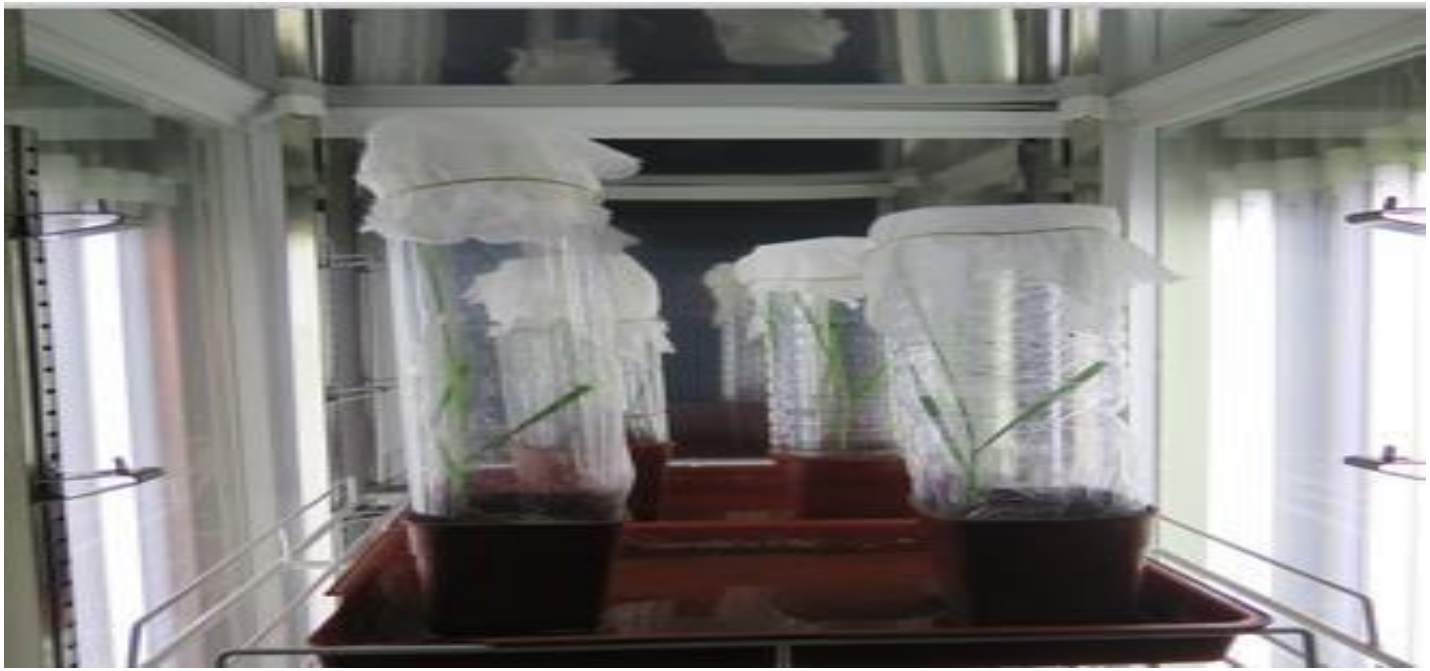
Fig: Sketch map and restriction sites of pBAC-RNAi-*Lmf2*

2.3 Production of Transgenic Plants

biolistic transformation



2.4 Aphid numbers and molting Bioassays



Standard condition at 21 ± 1 ℃ and relative humidity 50-60%.

Photoperiod 16L:8D.

Non-choice Assay

Contents

3.1 Expression profile of aphid lmf2-like gene

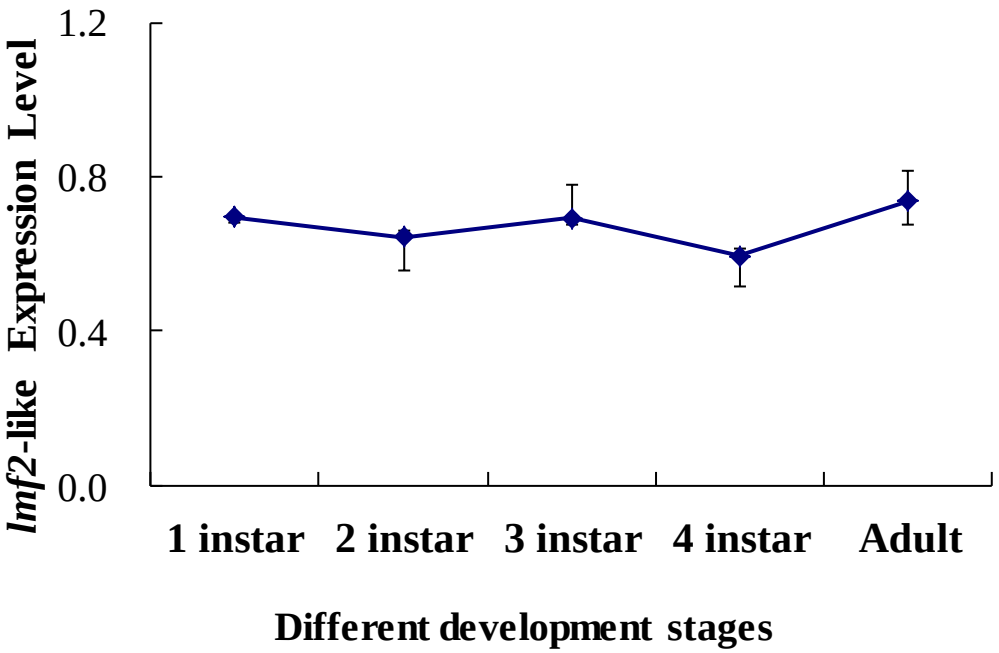


Figure3.1: aphid *lmf2-like* gene expression at different growth stages

The *lmf2*-like expression levels did not change significantly.

3.2 Clone of *lmf2*-like fragment

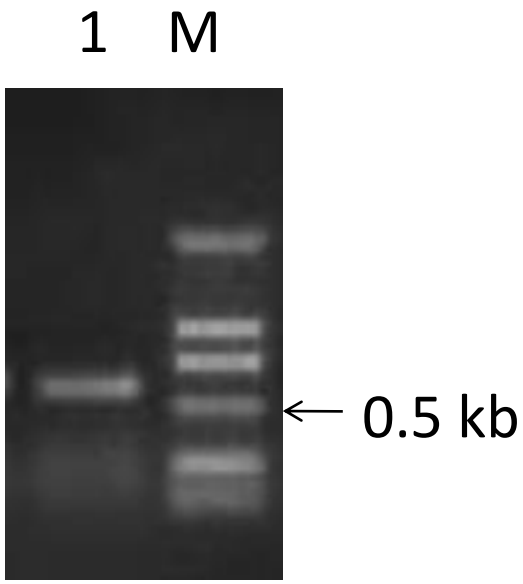


Figure3.2A： Grain aphid *lmf2-like* fragment by RT-PCR（ 0.5 kb）

M: Marker; 1: amplicons from aphid cDNA

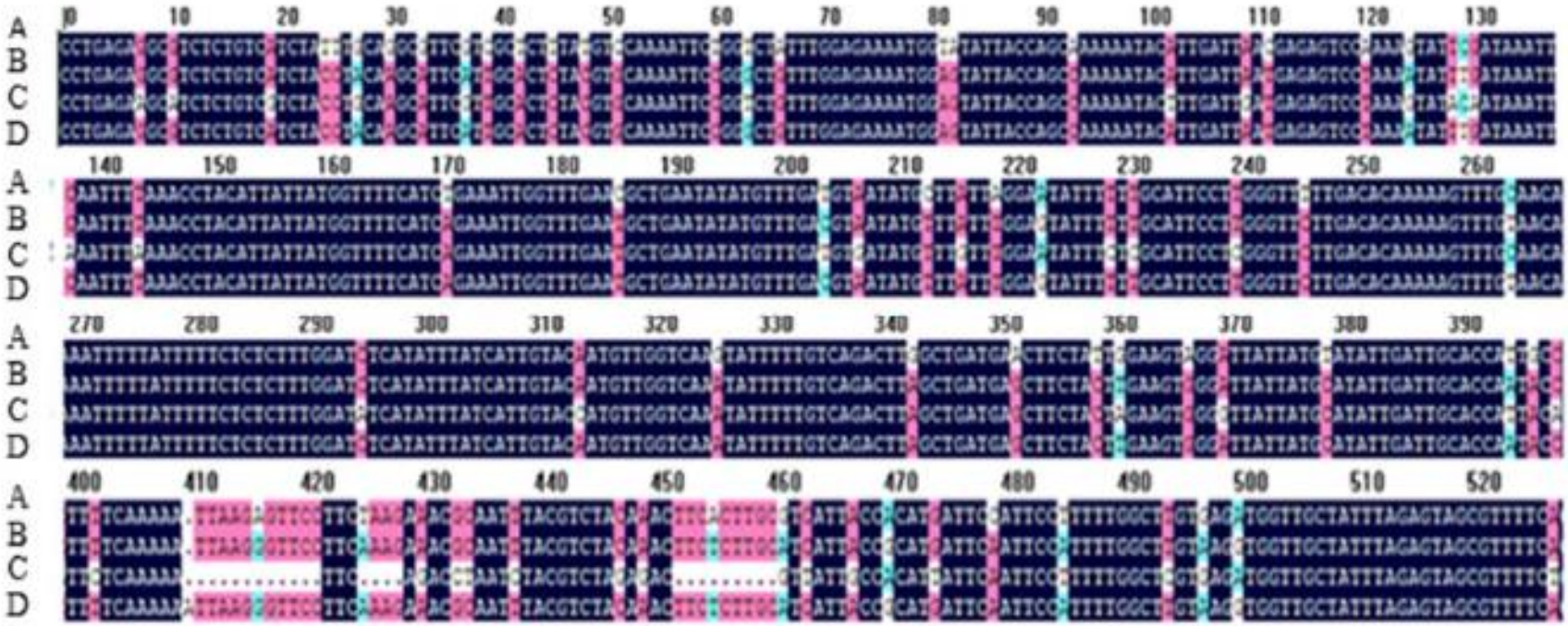


Figure 3.2B Sequence alignment of *lmf2* between grain aphid and pea aphid.

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

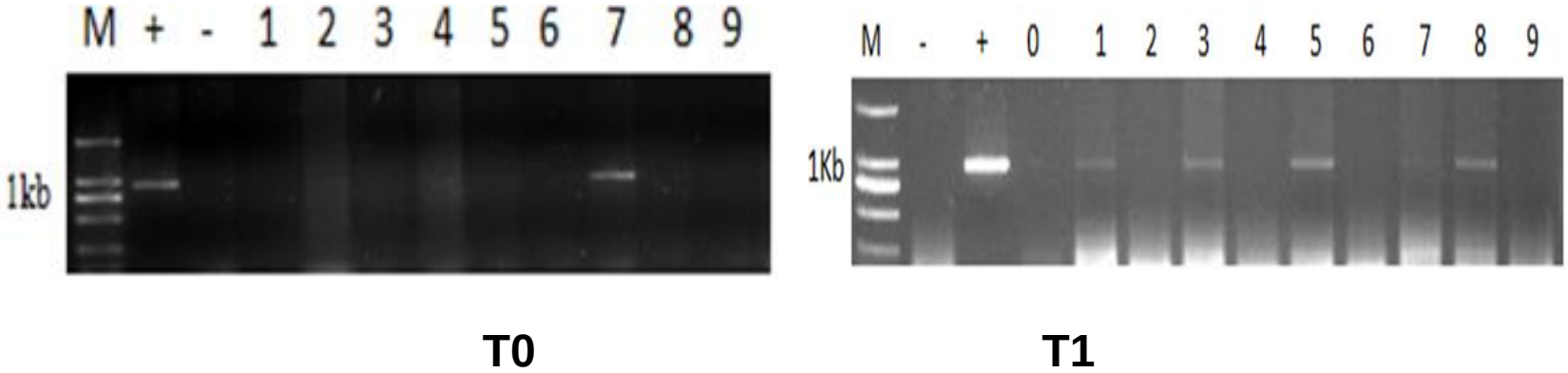


Figure3.3: Screening the positive transformants via PCR

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

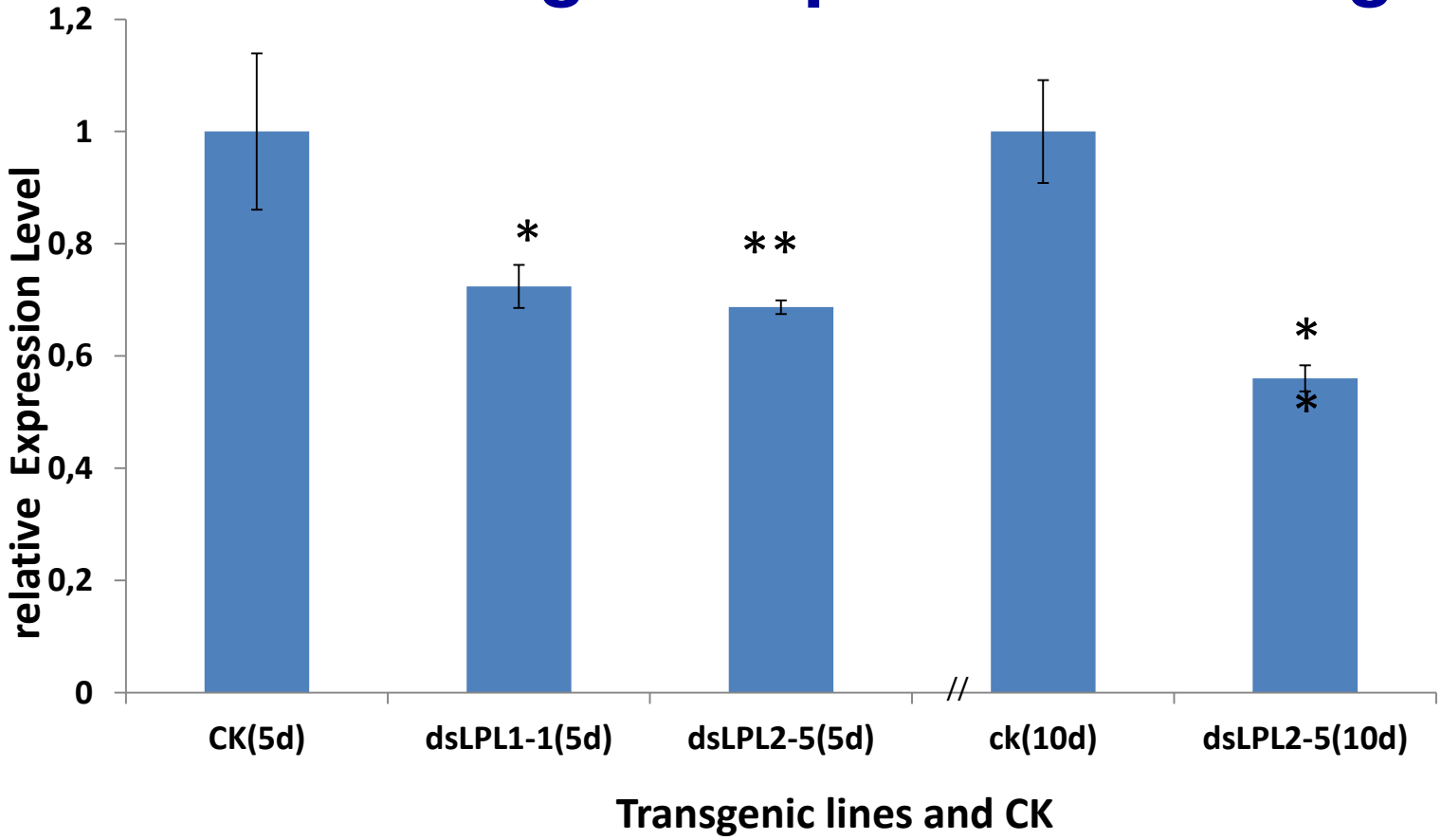


Fig3.4: aphid *lmf2*-like expression level after feeding the transgenic lines

5d: feeding after five days; 10d: feeding after ten days.

3.5 Aphid numbers after feeding on transgenic lines

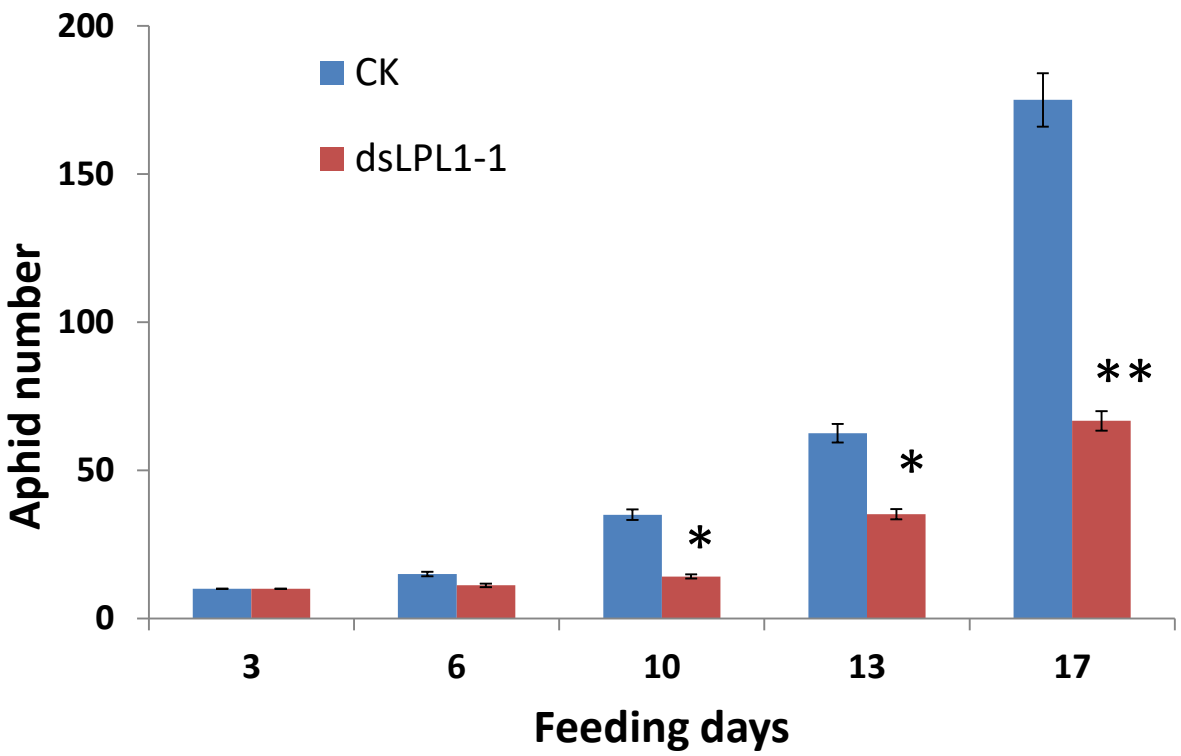


Fig3.5A: Aphid numbers feed on transgenic line dsLPL1-1 and CK

The difference became significant by day 10.

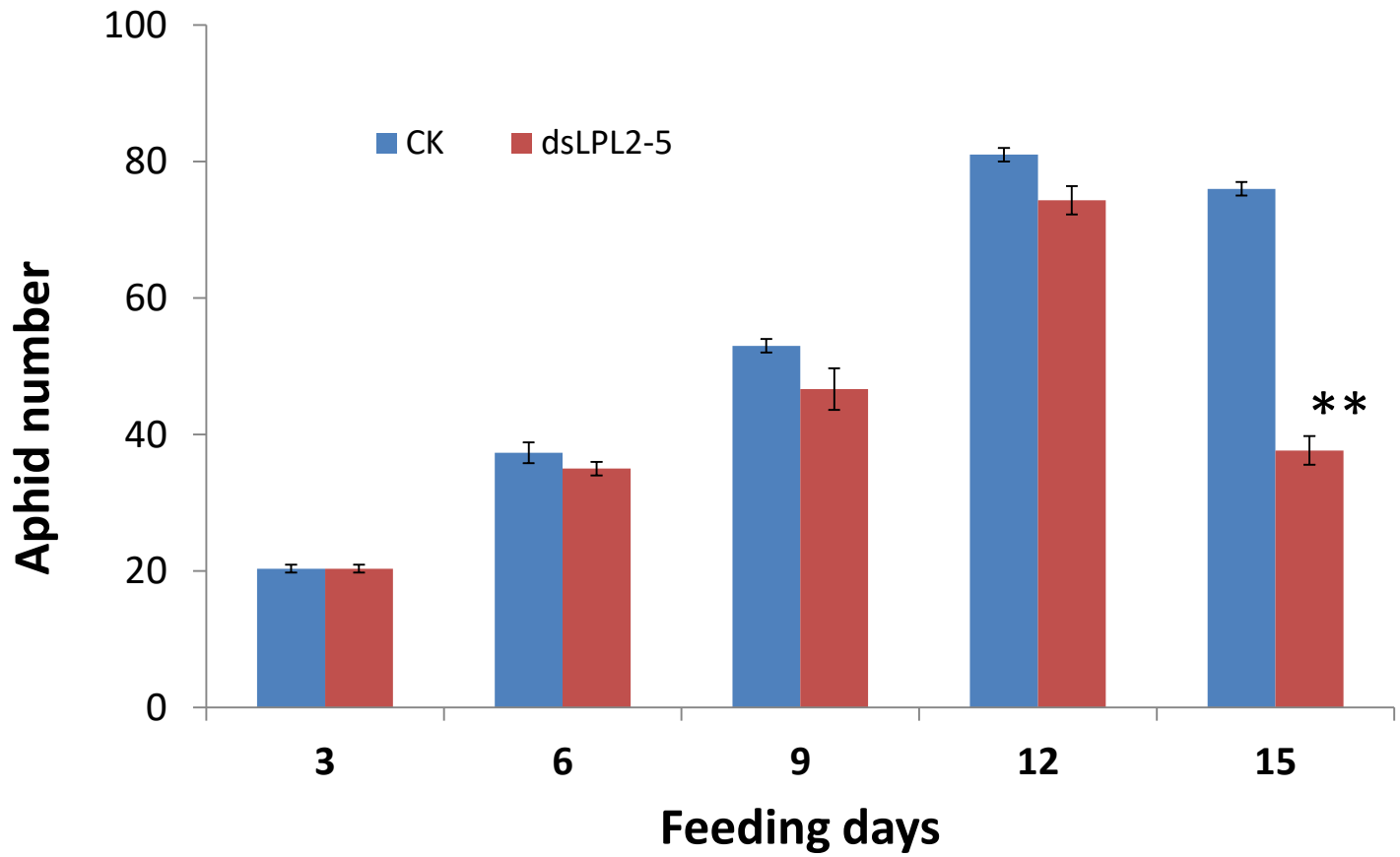


Fig3.5B: Aphid numbers feed on transgenic line dsLPL2-5 and CK

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

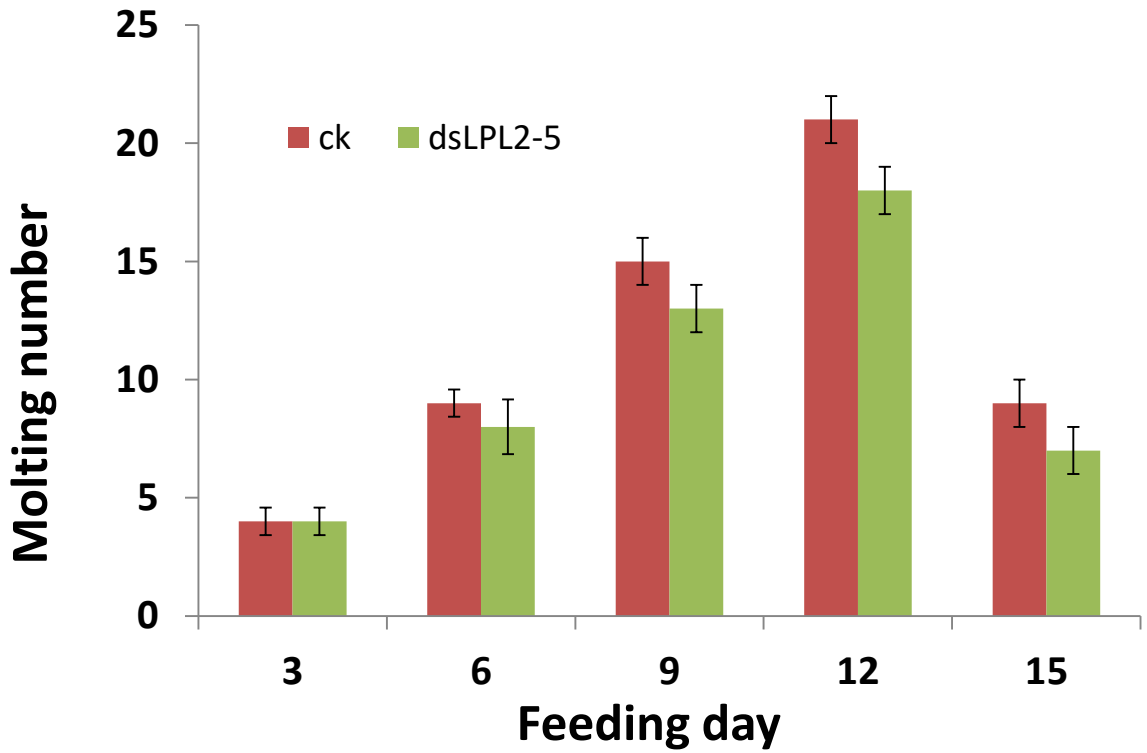


Fig 3.6: effect of feeding transgenic line dsLPL2-5 on grain aphid molting number

Contents

Discussion

1 the expression and loss function of lmf2-like gene

We infer the *lmf2*-like gene is needed throughout the entire aphid lifecycle.

And the *lmf2*-like is was necessary for grain aphid survival, growth and reproduction.

2 lmf2-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 lmf2-like genes may have potential as a target gene for the control of grain aphids.

2 Feeding aphids with wheat expressing lmf2-like RNAi resulted in reductions in target expression, growth and reproduction.

Acknowledgements

Dr. XU Lanjie
Mr. HOU Qiling
Ms. ZHAO Yanjie
Mr. LU Lihua
Prof. LI Baoyun
Prof. YOU Mingshan
Prof. NI Zhongfu
Prof. SUN Qixin

Chinese National Program on Research and Development of Transgenic Plants
(**2016ZX08002-001** and **2011ZX08002-001**)

Thank you for your attention!

Lanjie Xu, Qiling Hou, Yanjie Zhao, Lihua Lu, Baoyun Li, Zhongfu Ni, Rongqi Liang. Silencing of a lipase maturation factor 2-like gene by wheat-mediated RNAi reduces the survivability and reproductive capacity of grain aphid, *Sitobion avenae.* Archives of Insect Biochemistry and Physiology. Accepted