Biotechnology and Bioprocess Engineering 2008, 13: 624-631
DOI/10.1007/s12257-008-0049-9

Proteome Analysis on Differentially Expressed Proteins of the Fat Body of Two Silkworm Breeds, *Bombyx mori*, Exposed to Heat Shock Exposure

S. Hossein Hosseini Moghaddam[1,2], Xin Du[1], Jun Li[1], Jinru Cao[3], Boxiong Zhong[1], and YuYin Chen[1]*

[1] College of Animal Sciences, Zhejiang University, Hangzhou 310029, China
[2] Agriculture Faculty, University of Gilan, Rasht, Iran
[3] Sericultural Research Institute, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China

Abstract Proteomes of heat tolerant (multivoltine) and heat susceptible (bivoltine) silkworms (*Bombyx mori*) in response to heat shock were studied. Detected proteins from fat body were identified by using MALDI-TOF/TOF spectrometer, MS/MS, and MS analysis. Eight proteins, including small heat shock proteins (sHSPs) and HSP70, were expressed similarly in both breeds, while 4 protein spots were expressed specifically in the bivoltine breed and 12 protein spots were expressed specifically in the multivoltine breed. In the present proteomics approach, 5 separate spots of sHSP proteins (HSP19.9, HSP20.1, HSP20.4, HSP20.8, and HSP21.4) were identified. Protein spot intensity of sHSPs was lower in the multivoltine breed than in the bivoltine breed after the 45°C heat shock treatment, while the difference between two breeds was not significant after the 41°C heat shock treatment. These results indicated that some other mechanisms might be engaged in thermal tolerance of multivotine breed except for the expression of sHSP and HSP70. There were visible differences in the intensity of heat shock protein expression between male and female, however, differences were not statistically significant. © KSBB

Keywords: proteome analysis, heat shock proteins, silkworm, 2D electrophoresis, mass spectrometry

INTRODUCTION

The se ic l e in s as con i e significan l o e economic e elo men of man co n ies. This as e s l e in en ic e sil o m ge m lasm eso ces e o e effo s of sil o m ee e s in e esea c of gene ics an ee ing of sil o m *Bombyx mori*. *B. mori* is se i el in asic esea c in io ec nolog an as a mo el insec . n e s of geog a ical aces an gene icall im o e s ains a e main aine in iffe en co n ies w e e se ic l e was/is in og e. These aces an s ains a e no onl in well c a ac e i e en elian ai s also in no so well s ie an i a i e ai s s c as o si e fee ing a ion e mal ole ance an isease esis ance 1. The mo ole ance is a ic la l em asi e in sil o m ee ing og ams fo e selec ion of sil o m a ie ies i e e a a a ion o a ie en i onmen al con i ions es eciall in o ical egions s c as So e n n ia o s mme an a mn season in China.

A se of o eins no n as ea s oc o eins (s) a ea s o e in ol e in ole ance agains a e se g o con i ion in man o ganisms 2 . s a e in ce a a i o s em e a es in iffe en o ganisms an e n m e of o eins an e ela i e im o ance of eac famil in s ess ole ance a f om o ganism o o ganism . The concen a ion of in ci le s an o e molec la c a e ones i in cells a 4. as een well es a lis e a io e os e o s ess in ces ole ance an c oss ole ance o s se en s ess 5 . e ession can e co ela e i esis ance o s ess . Wi in e s small ea

*Corresponding author
Tel: +86-571-86971657 Fax: +86-571-86971657
e-mail: chenyy@zju.edu.cn

s oc o eins (s) ange f om o Da. T e a e s n esi e i i ousl in e a o ic an o a o ic cells in es onse o ea an o e s esses in ce e mo ole ance in some o ganisms 7 . A s ong co ela ion as een foun e een e ession an e mo ole ance 4. e e ession an in o c ion of e ogenous inc eases e e mo ole ance of a ious es of mammal ian cells in c l e o ec s cells agains l a iole a ia ion o ec s ole mammalian ea s agains os isc emic a ma an inc eases e in ci le e mo ole ance of *Drosophila* cells in c l e em os an la ae 4 10. n ce e mo ole ance is me ia e inc ease e ession of ea s oc o eins in a i e a ie of cells an o gan isms 7 11. Wang an ang 10 s ie e gene ic asis of e mo ole ance in o ical an em e a e o la ions of e mig a o loc s *Locusta migratoria* meas ing e ession of 7 an m A a lo (C) an ig em e a es (4 C) an sugges e a e mo ole ance of loc s eggs a a com le gene ic asis an ea s oc o eins mig e in ol e in iffe ences in e mo ole ance e een loc s o la ions. s o l e men ione a e con i ioning insec s can confe e mal ole ance o a s se en ig e e mal ea men s c an effec is no necessa il ela e o s o e fac o s ma a ici a e in e mal ole ance .

ani la ion o enginee ing of genes ela e o e mo ole ance as e o e fo e co n m e of 7 ic as sufficien o affec in ci le e mo ole ance a some life s ages of *Drosophila melanogaster* . T e *Drosophila* ea s oc o ein 7 (7) omo e as in o ce as i e fo in ci le e ession of e ogenous genes in insec s an successf l ansfec ion i en e *Drosophila* 7 omo e as ca ie o fo e elo ing a ea s oc in ci le an an in e i a le A in e fe ence (Ai) s s em in e sil o m (*B. mori*) 14.

o mann an i ifo in ica e a e ea s oc es onse of *B. mori* as simila o a of o e insec s in ic o ce e e ee g o s of ea s oc o eins incl ing e 7 an s acco ing o mo lec la eig ma e s (one imensional gel elec o o esis). T e also concl e a e ea s oc es onse of *B. mori* as iffe en an a of *Drosophila* in ic e e es sion of non ea s oc o ein s n esis ing ea s oc as no a ominen fea e of e es onse. s ing e ea s oc es onse of e iffe en aces of sil o m in cl ing e m l i ol ine ee s C. ic i an e so e an e i ol ine ee o an o ina an s o e a e esence of ne o eins in es onse o ea s oc as iffe en among iffe en iss es an a o m l i ol ine an i ol ine sil o ms es on e o ea s oc as e i ence e esence of a i ional o eins. as e o e a e ession of ea s oc o eins in sil o m mig a in iffe en e elo men al s ages ase on e e imen s sing SDS A elec o o esis . i *et al.* anal e e e ession of e small ea s oc gene m in sil o ms T C an fo n a ing le els of is o ein in iss es. as mos a n an in es is o a sil glan an ae. Song *et al.* fo n a e

ea s oc 7 Da o ein cogna e as one of e eg la e emoc ic o eins en sil o m la ae e s on e o e inoc la ion of ea inac i a e ac e ia (*Bacillus megaterium*).

S c al o eomics ma s o e s c e of o ein com le es o e o eins esen ing in a s ecific cell la o ganelle f nc ional o eomics is a oa e m fo man s ecific i ec e o eomics a oac es an e ession o eomics is e an i a i e s of o ein e ession in sam les a iffe in some a ia le . T e e a e een no lica ions o a e e o ing e o eomics a oac o ea s oc o eins in sil o m. n e esen o o eome a e ns of fa o f om ea s oc e sil o ms as com a e i con ol sil o ms in o esis an an s s ce i le ee s an e ession a e ns of e iffe en ial o ein e e a ge e fo i en ifica ion. T e fa o iss e of insec s (a omolog e of mammalian li e) as im o an f nc ions as a s o age iss e an as a e cen e of me a o lism an ioc emis .

MATERIALS AND METHODS

e ic a eria s a d k orm eari

T o ee s of sil o m (*B. mori* .) e ea ole an is a i (a m l i ol ine ee) an e ea s sce i le ing song (a i ol ine ee f om C ina) e e selec e ase on e io s e al a ions fo ea ole ance e Se ic l al esea c Cen e of C ina. o ee s e e o i e e Sil o m ene ics an ee ing a o a o College of Animal Sciences iang Uni e si is a i is f om o i cal egions e e e fiel em e a es of en eac es 4 C o ig e in s mme an i as e i e ig ole ance o ig em e a es .

e imen al sil o m la ae e e ea e sing s an a ec ni es an con i ions in a . To com a e e effec of e os e o ele a e em e a e on males an fe males in e en en l se i en ifica ion as ca ie o o se ing sil o m la al se ma s (imaginal s on os e io a ominal sec ion of sil o m la a) efo e ea s oc . l i ol ine females m l i ol ine males i ol ine females an i ol ine males e e se in e esen e e imen . To e al a e s i al a e of e ea e ose sil o ms e a la ae e e e ose o ea in eac ea men an en e a no mal ea ing con i ion. T e amo n an a ion of e os e in o e e imen e e i e same as i *et al.* an o an o ina an .

ermal rea me s a d am i

eca se e fa o is loca e n e e c icle in la ae ea s ess can easil eac e iss e en e la a is e ose o ea . n e fo a of e fif ins a sil o m la ae of eac gen e e e ose o ei e 5°C fo 5 min o 4 C fo in con olle g o c am e s. Af e e ea e os e e sil o ms e e e ne o e s an a ea ing em e a e (4 C) an allo e o eco e . T e fa 'o y was emo e 2 af e ea e osu e an lace in ice col insec siological sal sol ion (7 aCl) 7 . T ee fa 'o y sam les we e oole o minimi e a ia ion an o ge eno g issue fo anal sis. Wa e was emo e f om sam les 'y s o ime cen ifuga ion. Con ol sam les of e fa 'o y we e e a e f om la ae a we e no e ose o ea . All la ae we e gene icall simila (f om a single mo famil). All sam les we e s o e a 2°C un il anal sis.

ro ei rac io a d ec ro oresis

A mg sam le of fa 'o y was omogeni e 'y g in ing i in 5 µ l sis sol ion (7 u ea 2 iou ea 4 C A S an 1 m SF). T e sam le mi u e was o e e an en incu'a e fo min in ice. Si m i io ei ol (DTT) an 2 'uffe (ange 1 was en a e . Af e cen ifuga ion (min a g C) e solu'le o ein f ac ion was emo e an e o ein con cen a ion was e e mine using e a fo me o 2 1. soelec ic focusing was ca ie ou wi µg of o ein sam le in 5 µ sol ion (u ea 2 C A S 2 /m DeS ea TM eagen an 5 'uffe 1 . o ein was loa e on o D S i s (ange 1 'y e in gel e a ion me o an su'jec e o elec o o esis using an an o F uni (Ame s am a macia io ec) a fo 2 5 fo 1 2 fo 1 4 fo 1 an fo 1 . Af e F se a a ion e s i s we e imme ia el e uili'a e 2 × 5 min in 5 m T is Cl 'uffe (.) con aining u ea 2 SDS an gl ce ol. Fo e sam le wi ou e uc ion an al la ion DTT (1) was a e in e fis e uili'a ion se an 2.5 io oace ami e was a e in e secon e uili'a ion se . T e s i s we e su'jec e o e secon imensional elec o o esis using an an DA Tsi mul i le gel elec o o esis uni (eal ca e) on o of 2.5 ol ac lami e gels fo SDS A . T e elec o o ese o eins we e s aine wi a sil e s ain. ig gel e lica es of eac 'ee (ea e ose g ou an con ol g ou) we e e ea e wice.

DeS ea TM eagen 'uffe s an D S i s we e u c ase f om eal ca e io sciences A (Swe en) C A S an DTT we e u c ase f om US co o a ion (Cana a) io oace ami e was u c ase f om eal ca e (uc ing ams i e U) an u ea an iou ea we e u c ase f om Ames am iosciences (U) an Sigma es ec i el . lec o o esis we e u c ase f om Am esco (US). Deioni e wa e (illi o e F ance) wi esis ance of 1 .2 Ω cm was use ou ou .

ma e c isi io a a a sis a d ro ei de i ica io

S o s we e scanne using a ig esolu ion image scanne (Ame s am ioscience i els/gel) an anal e 'y mage ase 2D sof wa e (e sion). olecula mass an we e calcula e f om igi i e 2 D images using s an a molecula mass ma e o eins. ac selec e s o wic me e c i e ion a i was e ea e l esen in wo gels was com a e in 'o ea men s an se es. n o e o measu e o ein e ession le els e s o olume was cal cula e as a e cen age ela i e o e o al olume of all e s o s in e gel as no mali e a a o uan if gel s o s an use o e alua e o ein e ession iffe ences 'e ween gels. o mali e olumes of some s o s we e anal e using anal sis of a iance (A A) 'y S SS sof wa e wi ee fac o s inclu ing e mal ea men 'ee an se .

o ein sam les we e is aine an sin iges e an e i es we e e ac e as esc i'e elsew e e 22 . S an S/ S s ec a we e o'aine using e A 47 o eomics Anal e A D T F/T F mass s ec ome e (A lie ios s ems). o eins we e anal e using S/ S o F anal sis an we e i en ifie wi e a a'ase sea c og am ASCOT Daemon (a i Science) agains C n /Swiss o a a'ase using e following a ame e s: en me sin fi e mo ifica ion ca'ami ome l (C) a ia'le mo ifica ions o i a ion ( ) no es ic ion on o ein mass one misse clea age e i e c a ge + 1 monoiso o ic a e i e mass ole ance of 1 m. o ein i en ifi ca ion wi a confi ence in e al (C. .) o ein sco e g ea e an 5 (< 5) was acce e in 'o S/ S an F esul s. iological an molecula func ions we e foun 'y using Uni o nowle ge'ase (Swiss o an T) (://www.e as .o g/s o).

RESULTS

i a i e om ariso s o ro ei a er s

Com a ison of e fa 'o y o eomes of 'o se es of ea e ose mul i ol ine an 'i ol ine 'ee s of sil wo m an con ols is s own in Fig. 1. n ese o eome ofiles 5 4 an 7 44 s o s we e e ec e in 'i ol ine females an males es ec i el an 5 2 an 25 s o s we e e ec e in mul i ol ine females an males es ec i el 'y igi al im age anal sis an using e same e ec ion a ame e s (Smoo 2 in A ea 5 an Salienc 5). T e num'e of s o s was ig e in mul i ol ine sil wo ms an in 'i ol ine sil wo ms an was ig e in males an in females (Ta'le 2). Fig. 1 s ows 2 iffe en iall e esse o ein s o s in 'i ol ine sil wo ms an 2 iffe en iall e esse o ein s o s in mul i ol ine sil wo ms in es onse o ea e osu e. T e e a e wo egions in e 2D gels wic s ow al e e ession of o eins in 'o mul i ol ine an 'i ol ine sil wo ms ea s oc a e ns. T ese a e num'e e 1 o 4 (egion 1) an 5 o (egion 2) in Fig. 2. T ese e esse s o s a e e simila fo 'o 'ee s an gi e e o ucic'le s aining a e ns (common es onse s o s). Al oug o ein s o is i'u ion a e ns iffe 'e ween e wo 'ee s e a e simila wi in eac 'ee 'e ween e se es an ea ea men s (5 an 4°C). T e efo e se an e wo ea ea men s can 'e oole fo is e e imen . esi es e common es onse s o s (s o s 1) e e a e 4 s o s (s o s 11 14) in e 'i ol ine sil wo ms an 2 s o s (s o s 1 2) in mul i ol ine sil wo ms in uce 'y ea s oc an

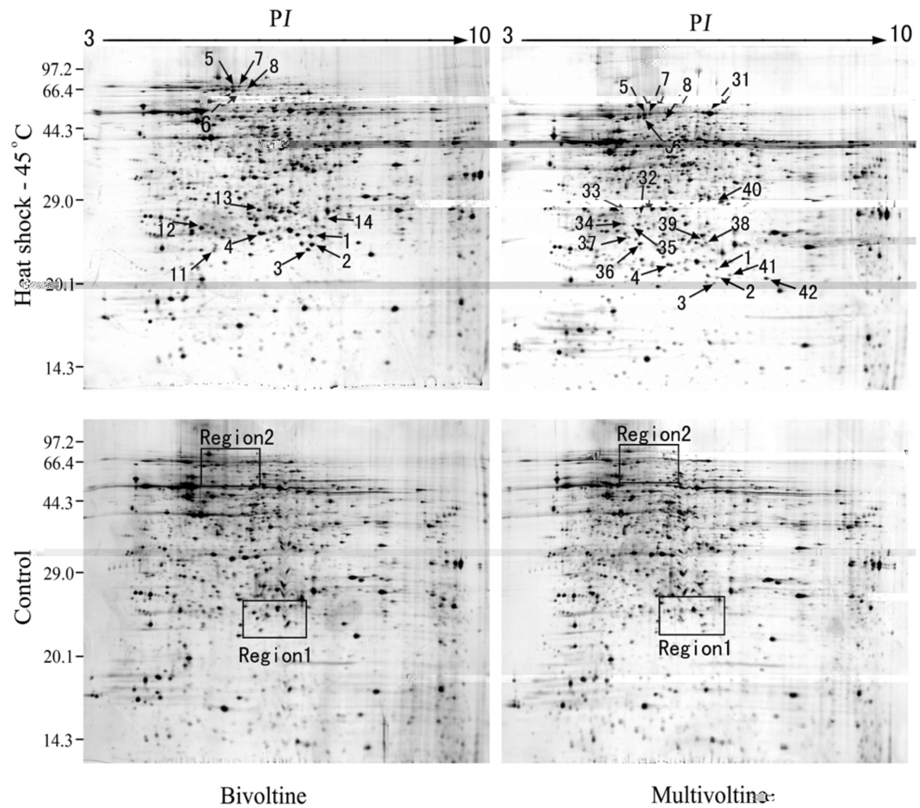

Fig. 1. 2D electrophoresis protein profiles of fat body of the control and heat exposed silkworm larvae from the thermo-susceptible

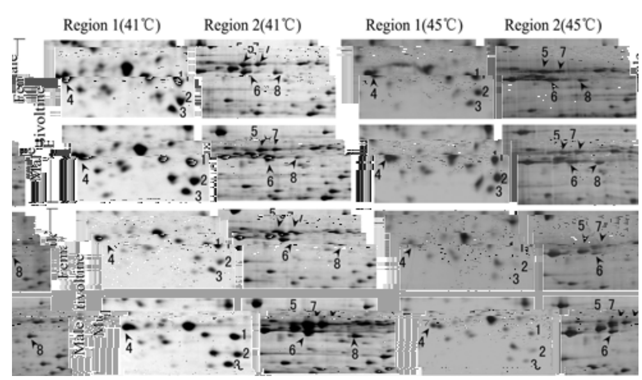

Table 1. List of identified silkworm fat body proteins in responses to high heat exposure

Spot no.	Protein name (Matched organism)	Accession GI no.	No. of peptides (coverage)	Protein score (C.I. %)	*Mr* calcd/obsd (P*I* calcd/obsd)	Ontology
	Common response spots					
1	Heat shock protein HSP20.4 (*Bombyx mori*)	49036077	9 (49.86%)	148 (100)	26 / 20.41 (7.10 / 6.54)	Response to stress
2	DNA-formamidopyrimidine glycosylase* (alpha proteobacterium BAL199)	163793016	7 (31.00%)	90 (99.42)	25 / 32.87 (7.05 / 8.57)	Zinc ion binding, DNA binding; catalytic activity
3	Heat shock protein HSP 19.9 (*Bombyx mori*)	56378317	7 (29.24%)	120 (100)	24 / 19.88 (6.23 / 6.53)	Response to stress
4	Heat shock protein HSP20.8 (*Bombyx mori*)	11120618	7 (46.09%)	177 (100)	26 / 20.79 (5. 80 / 5.98)	Response to stress
5	Heat shock protein HSP70 (*Antheraea yamamai*)	47232576	16 (40.28%)	98 (99.96)	85 / 69.55 (5.80 / 5.7)	Response to stress ATP binding
6	Heat shock protein HSP70 (*Antheraea yamamai*)	47232576	12 (25.87%)	110 (99.98)	80 / 69.55 (5.9 / 5.7)	Response to stress ATP binding
7	Heat shock protein HSP70 (*Antheraea yamamai*)	47232576	13 (29.65%)	102 (99.98)	85 / 69.55 (5.9 / 5.7)	Response to stress ATP binding
8	Heat shock protein HSP70 (*Antheraea yamamai*)	47232576	15 (45.40%)	86 (99.44)	79 / 69.55 (6.15 / 5.7)	Response to stress ATP binding
	Specific response spots (Bivoltine)					
11	Heat shock protein HSP20.1* (*Bombyx mori*)	112983134	7 (33.00%)	84 (97.80)	25 / 20.18 (5.51 / 5.46)	Response to stress
13	PRETICTED: similar to zinc finger protein 436 (*Canis familiaris*)	57048379	7 (20.54%)	80 (97.74)	30 / 55.30 (6.3 / 8.94)	Zinc ion binding
	Specific response spots (Multivoltine)					
34	PREDICTED: similar to CG10504-PA* (*Tribolium castaneum*)	91079909	14 (33.00%)	82 (96.20)	33 / 51.45 (5.45 / 7.77)	Transferase activity; protein amino acid phosphorylation
36	Heat shock protein HSP21.4 (*Bombyx mori*)	56378321	8 (60.43%)	120 (100)	29/2139 (5.74 / 5.79)	Response to stress
38	PREDICTED: similar to zinc finger protein 46* (*Canis familiaris*)	57048379	13 (27.00%)	96 (99.84)	29/55.30 (6.91 / 8.94)	Zinc ion binding
40	PREDICTED: similar to CG9935-PA isoform 1* (*Apis mellifera*)	66507549	11 (27.00 %)	86 (98.50)	37/61.90 (7.04 / 6.14)	Transferase activity; protein amino acid phosphorylation

C.I. %: confidence interval of protein score.
*Identification of protein by PMF analysis.

ge as e 2D sof a e. T ese anal ses e eale a e es sion ensi of some s o s e e iffe en in m l i ol ine an i ol ine ee s. Among e 4 i en ifie o eins of e i ol ine ee s o s 11 an 1 s o e a s ecific eg la ion n e o ea e os e ea men s. Among e 1 i en ifie o eins of m l i ol ine ee s o s 4 an 4 e e eg la e in es onse o o ea e os e ea men an s o s an 41 s o e eg la ion in a 4 C ea men alone. T ese iffe ences s o e a in e m l i ol ine ee e n m e of e esse o ein s o s inc eases in es onse o an inc ease in e ea e os e em e a e o e e in e i ol ine ee no iffe ences e e o se e e een e ea e os e ea men s. S o 7 as eg la e onl in females of e m l i ol ine ee . T e e a e iffe ences in e ol me of o ein e ession e een i ol ine an m l i ol ine ee s in o ea e os e ea men s as ell as e een se es. Ta le 1 esen s e means of e no mali e ol mes (ol me e cen age) of common es onse s o s incl ing 4s s (egion 1) an 4 7 (egion 2) se a a e o ea men ee an se . Significan iffe ences e een e o ee s an e em e a e e os es e e e e mine A A (Ta le 2). Da a in Ta le 1 an A A in Ta le 2 e eale a common es onse o ein s o s e e e esse in eac of e sam les. o e e e an i of s iffe e . o eo e o ein e ession in ensi of s s (egion 1) iffe e significan l (< 0.01) e een e o ea e os e ea men s an e sil o m ee s. T e e ession of s s in e m l i ol ine ee as lo e an in e i ol ine ee af e e 4 C ea e os e ea men ile e e as no significan iffe ence

Table 2. The mean of normalized volumes (%) of 8 protein spots, including 4 sHSP (region 1) and 4 HSP70 (region 2), in different treatments, breeds, and sexes

Breed	Sex	Heat treatment (45°C)			Heat treatment (41°C)	
		Number of spot*	sHSP	HSP70	sHSP	HSP70
Bivoltine	Female	534	0.353 (± 0.102)	0.215 (± 0.086)	0.322 (± 0.067)	0.218 (± 0.063)
	Male	744	0.332 (± 0.091)	0.225 (± 0.070)	0.332 (± 0.069)	0.221 (± 0.135)
Multivoltine	Female	582	0.072 (± 0.043)	0.151 (± 0.050)	0.282 (± 0.063)	0.278 (± 0.221)
	Male	825	0.077 (± 0.040)	0.225 (± 0.079)	0.235 (± 0.042)	0.302 (± 0.040)

*Total number of spots in 2D electrophoresis image pattern.

Table 3. ANOVA on normalized volumes of 8 protein spots including 4 sHSP (region 1) and 4 HSP70 (region 2)

Source	df	sHSP		HSP70	
		M.S.	P	M.S.	P
Heat treatment	1	0.057	0.008	0.021	0.125
Breed	1	0.226	0.000	0.003	0.554
Sex	1	0.001	0.657	0.006	0.400
Error	28	0.007	–	0.008	–

in e in ensi y of o ein e ession e een ee s af e e 41°C ea e os e ea men . n o e o s a lowe ea e os e ea men s sil o m ee s i no iffe significan l in ei es onse ile a ig e em e a e e os e ea men s e e mo ole an ee e esse significan l lowe s s (< 0.0). e iffe ences e een e o em e a es e e no significan fo 70 in e m l i ol ine ee . owe e ession of s s an s a le e ession of 70 sugges e a 70 an s ma la iffe en ole in e mal ole ance a ig e em e a e in e e mo ole an ee . Com a ison of e ol me e een e o se es in ica es a e e a e some iffe ences in o ein e ession al o g i as no signifi can (Ta le 2).

DISCUSSION

e mal sensi i i an ea s oc es onse of iffe en aces of *B. mori* can e meas e o se ing e s i al a e of la a a mo an egg an o se ing cocoon c a ac e is ics . e s of e ea s oc es onse on e molec la le el gi es mo e info ma ion a o ea s oc o eins an ioma e s. en ifica ion of o ein ma e s ill also o i e ee e s i a mean fo mo e effi cien an co ec selec ion of ea ole an ai s 27 . We i en ifie 14 o eins a a e iffe en iall e esse af e ea e os e of ic a e nown s an a e e ice o e in ol e in ea s oc es onses. e me o s e se in ese e e imen s incl ing ig esol ion 2D gel elec o o esis of fa o sing sil e s aining com ine i S/ S an S anal sis of mass s ec ome o e o e a s ccessf l s a eg in e s of s in iffe en sil o m a ie ies. e c anges in o ein e ession as a es l of ea s oc es onse e e no i en ical in e o ee s. is s g ges s some clea can i a e ma e o eins fo i en if ing ea ole an an ea s sce i le sil o m la ae. ene all e m l i ol ine ee as s own ig e s i al a es an e ol ine ee in es onse o ea s oc 2 . o n in a *et al.* 2 s owe a e is a i ee ic is e m l i ol ine ee se in e esen e imen is e mos ole an ee among 11 m l i ol ine ee s. S ecific es onse o eins incl ing s o s 11 14 an 1 2 ma se e as ma e o eins fo ea s sce i le an ea ole an es ec i el n a ic la o ein s o s 11 an in ol ine ee an s o s 4 an 4 in m l i ol ine ee can e consi e e as o ein ma e s ela e o ole ance. S las *et al.* 27 o se e a 7 o ein s o s e e e esse in a ea s oc ole an c l i a of ea af e ea s oc sing o eome anal sis. le *et al.* 2 also se o eomic anal sis (2D A S) o e ec e effec s of ea s oc on an a io ic s ess ole an an a io ic s ess s sce i le c l i a of ale . e fo n o o eins s o s ni e o e s ess s sce i le c l i a .

n is o e i en ifie 5 low molec la eig o eins (2 4 1 2 4 2 1 an 2 1 4) ic e e e esse af e ea e os e. Sa ano *et al.* e o e a *B. mori* a si s s incl ing e a o e esc i e s s an s 2 .7. S las *et al.* 27 e o e a e m o i of e ea s oc o eins in o e ea s sce i le an e ea ole an c l i a s of ea a low molec la eig . Fo o ein s o s in egion 2 of o ee s e e 70 nc eases in 70 can o ec in ac la ae agains e e mal inac i a ion of alco ol e ogenase an agains e mal in i ion of fee ing . 70 la s a cen al ole in s ess ole ance incl ing omo ing g o a mo e a el ig em e a es an o ec ing o ganisms f om mo ali a e eme em e a es c a e oning nfol e o eins . nce fol e o e l ese o eins a e less sensi i e o ena a ion an agg ega ion. ee e esse o eins ic a e simila o inc finge o ein i en ifie in is esea c a e li el in ol e in e fol ing ocess of o eins ecause inc finge s a e in ol e in fol ing of o eins.

T e e ession of s s in e m l i ol ine ee is significan l (< 0.0) lo e an in e i ol ine ee en e ose o e 5°C ea men , e e is no iffe ence e een e ee s en e ose o e 41°C ea men (Ta le 2 an Fig. 2). T is emons a es a e e mo ole an sil o m ee as no c a ac e i e a ig e le el of s s n esis n e se e ea s oc as com a e o e e mosensi i e ee . 70 e ession as no significan l e ce a e ig e em e a e ea men . T is sugges s e s an 70 ma la iffe en ole in e mo ole ance of sil o ms. ase on e a aila le esea c , e a o s concl e a o e mec anisms mig e in ol e i e mo ole ance o e an e s s an e 70. T e n m e of s ecific o eins in ol e in e ole ance of m l i ol ine ee mig also a e an im o an ole as e a e ec e 2 s o s in e m l i ol ine ee com a e i 4 s o s in e i ol ine ee en e ose o ea . S ilo a *et al.* 7 concl e a e mo ole ance e i e se e al al e na i e molec la mec anisms an a 40 s s an o e ni en ifie fac o s la e an im o an ole in is ocess along i 70 in *D. melanogaster*. e io s esea c as s o n a in e mo ole an ee s of *D. melanogaster* 70 s n esis is main aine a lo le els. T e mos e mo ole an s ain T (isola e in Cen al Af ica) as a lo e le el of 70 s n esis n e mo e a e ea e os e (37.5°C) com a e o e less e mo ole an egon s ain 7 3 1.

Fig. 2 an Ta le 2 s o a male sil o m la a e esse slig l mo e 70 es eciall in e m l i ol ine ee , e iffe ence is no significan (< 0.0). T e n m e of o ein s o s e ec e image anal sis sof a e is also ig e in males an in females. To e es of o no le ge no o e lica ion isc sses e iffe ences e een female an male sil o ms in es on ing o ea ole ance. F e e e imen a ion is e i e o e e mine e iffe ence in e mo ole ance e een e se es of sil o m la ae.

n o e o i en if mo e o ein ma e s an o en ance o n e s an ing of e ela ions i e een sil o m ee s an ei iffe en e mal ole ances an ei e essions of iffe en in s of s i is necessa o sea c fo mo e iffe en ial s o s sing mo e e mo ole an an s sce i le sil o m ee s. A i ional me o s fo e o e sil o m iss es s o l also e e lo e . n e f e e efo e e ill in es iga e e effec s of e cessi e ea s oc on e o eome of iffe en ee s an se es.

F e mo e e e a e man successf l e e imen s on ansgenic sil o m. o e e i is onl ecen l a scien is s a e ec nicall ca a le of a ge ing en ogeno s genes en enginee ing ansgenic sil o m 14. T e efo e ma ni la ion of genes ela e o o s ness an e mo ole ance of sil o m is no oo fa a a . An n e s an ing of e molec la mec anisms of e mal ole ance is essen ial fo a aining an es l s in is i ec ion a ic la l in e n e s an ing e iffe en ial e ession a e n of a io s s in i ol ine an m l i ol ine ee s. T e im o ance of 70 ic as confi me fo sil o m la ae e mo ole ance in e esen esea c o l g ea l facili a e is esea c .

Acknowledgements We an ian ing i ong a o an Fang fo ei ec nical el in e e imen s. Financial s o e e a men of Science an Tec nolog iang o ince C ina (200C 2 5) an a ional asic esea c og am of C ina (2005C 2 100) is g a ef ll ac no le ge .

ecei e a c 25 200 acce e l 200

REFERENCES

1. aga . (200) A lica ion of gene ic inci les fo im o ing sil o c ion. *Curr. Sci.* : 40 414
2. Fe ei a A. S. . T ola . C. as a . F. A a o an A. C. o ges (2005) Small ea s oc o eins in e elo men of e mo ole ance in *Pisolithus* s . *J. herm. Biol.* 0 5 5 2.
3. a sell . A. an S. in is (1) T e f nc ion of ea s oc o eins in s ess ole ance: eg a a ion an eac i a ion of amage o eins. *Annu. e . enet.* 27: 47 4 .
4. e s . A. an . Fe e (1) 70 an la al e mo ole ance in *Droso hila melanogaster*: o m c is eno g an en is mo e oo m c . *J. nsect Ph siol.* 44 10 1 1101
5. e man A. . . Foe s e . . S oema e an . . o e son (200) S ess in ce e mo ole ance of en ila o mo o a e n gene a ion in e loc s *ocusta migratoria*. *J. nsect Ph siol.* 4 : 10 1047.
6. Fe e . . an . . ofmann (1) ea s oc o eins molec la c a e ons an e s ess es onse: e o l iona an ecological siolog . *Annu. e . Ph siol.* 1 2 4 2 2.
7. S ilo a . . . . a . . gen e an . . a se ina (200) Small ea s oc o eins an a a a ion of a io s *Droso hila* s ecies o e e mia. *ol. Biol.* 40 2 5 2 .
8. S n W. . an on ag an . e oggen (2002) Small ea s oc o eins an s ess ole ance in lan s. *Biochim. Bio h s. Acta* 1577: 1 .
9. e s . A. an . . e enco (1) ol ion of e mo ole ance an a ia ion in e ea s oc o ein 70. *Am. ool.* : 10 1 .
10. Wang . an . ang (2005) Diffe ences in egg e mo ole ance e een o ical an em e a e o la ions of e mig a o loc s *ocusta migratoriam* (o e a: Ac i ii ae). *J. nsect Ph siol.* 5 1 277 2 5.
11. o e son . . (2004) T e mal s ess an ne al f nc ion: a a i e mec anisms in insec mo el s s ems. *J. herm. Biol.* 2 : 5 1 5 .
12. e en . . (2000) siological es onses of insec s o ea . *Postharvest Biol. echnol.* 21: 10 111

1. Fe e . . an . A. e s (1) a al an gene ic enginee ing of e ea s oc o ein s 70 in *Droso hila melanogaster*: Conse ences fo e mo ole ance. *Am. ool.* : 50 5 7

14. ai . iang . Wang . . Cao . Wang an . Fei (2 07) e elo men of a ea s oc in ci le an in e i a le Ai s s em in sil o m. *Biomol. ng.* 24 25 0

15. o mann C. . F. an . . i ifo (1 2) T e ea s oc es onse an ea sensi i i y of *Bom mori*. *Sericologia* 2: 5 5 7.

1 . o . an . . o ina an (1 5) ea s oc e s onse in m l e sil o m aces i iffe en e mo ole ances. *J. Biosci.* 2 0 4 5 .

17. C a a i . . A. Sosalego a an . o ego a (2 00) m ac of ea s oc on ea s oc o eins e ession iological an comme cial ai s of *Bom mori*. *nsect Sci.* : 2 4 25 0

1 . i . . . ia F ii . anno an C. (2 05) ession of e small ea s oc o ein m s 1 gene in sil o m (*Bom mori*). *Chin. J. Agr. Biotechnol.* : 1 5 2 01

1 . Song . S. . ng . . Seo S. W. ang an S. S. an (2 00) en ifica ion of eg la e o eins in e emo l m of imm ni e *Bom mori* la ae. *Com . Biochem. Ph siol. Part D enomics Proteomics* 1 2 02 .

20. a es . . an T. A. a s ea (2 02) olec la iologis s g i e o o eomics. *icro iol. ol. Biol. e.* : .

21. a fo . . (1 7) A a i an sensi i e me o fo e an i a ion of mic og am ali ies of o eins ili ing e inci le of o ein e in ing. *Anal. Biochem.* 72: 2 4 25 4

22. in . . . . C en . iang an . . (2 00) o eome anal sis of e sil o m (*Bom mori*.) colle e ial glan ing iffe en e elo men s ages. *Arch. nsect Biochem. Ph siol.* 1 4 5 0

2 . Uma e i . an . . ao (2 05) al a ion an se lec ion of m l i ol ine ee ing eso ce ma e ials of sil o m *Bom mori* . fo ig em e a e an mi i con i ions. *J. ool.* : 2 .

24. ma . S. . asa a a . . al ana . . e an S. . an in (2 00) ffec of ig em e a e an ig mi i on e cocoon s a e an si e of a en s fo n a ion c osses single an o le o i s of i ol ine sil o m *Bom mori* . *n ian J. Sericulture* 4: 5 40

25. a o . . aga as W. a a an . inagi (1) ffec of e os e of e sil o m *Bom mori* o ig em e a e on s i al a e an cocoon c a ac e . *J n. Agr. es.* . 2: 1 4

2 . sie F. . S. . S. . S an S. . eng (1 5) S ies on e e mo ole ance of e sil o m *Bom mori*. *Chin. J. ntomol.* 5: 1 101

27. S las . . S. . Co ell . . ins . . a sen an . . asseal (2 02) ea s oc of ea ing g ain filling: o eins associa e i ea ole ance. *J. Cereal Sci.* 5: 175 1 .

2 . o n in a . . . ma esan . . Sin a an . T anga el (2 00) Sc eening of omising ge m lasm of ol ol ine sil o m (*Bom mori* .) fo e mo ole ance. *n ian J. Sericulture* 4: 7 7 0

2 . C le A. F. an o ae s . s . an ee men an . e eese (2 004) o eomic anal sis of small ea s oc o ein isofo ms in a le s oo s. *Ph tochemistr* 5: 1 5 1 .

0. Sa ano . . i . ia . amamo F ii an . Aso (2 00) enes enco ing small ea s oc o eins of e sil o m *Bom mori*. *Biosci. Biotechnol. Biochem.* 7 0 2 44 2 45 0

1. a se ina . . . . eli o o s aia . . olo so . . a . . e man . . e en co . . Fe e an . . gene (2 00) A *Drosohila melanogaster* s ain f om s e a o ial Af ica as e ce ional e mo ole ance ec ease s 70 e ession. *J. . Biol.* 2 04 1 1 1