Journal of Cotton Research

RESEARCH Open Access

Effects of NaCl stress on the biochemical substances in Bt cotton as well as on the growth and development and adult oviposition selectivity of *Helicoverpa armigera*

LUO Junyu[1,2], ZHANG Shuai[1,2], ZHU Xiangzhen[1,2], JI Jichao[1,2], ZHANG Kaixin[1,2], WANG Chunyi[1,2], ZHANG Lijuan[1,2], WANG Li[1,2] and CUI Jinjie[1,2*]

Abstract

Background: Recently, due to the development of food security strategies, cotton has been planted in inland saline-alkali dry soils or in coastal some saline-alkali soils in China. Under the condition, to comprehensively prevent and control *Helicoverpa armigera* in cotton fields with saline-alkali soils, it is important to study the larval growth and development of *H. armigera* and to study adult oviposition selectivity in *H. armigera* adults that feed on NaCl-stressed cotton plants.

Results: In this study, Bt cotton GK19 was used for the experimental group and its nontransgenic parent Simian 3 was used for the control to study the effects of biochemical substances in cotton as well as larval growth and development and adult oviposition selectivity of *H. armigera*. The experiments were performed by growing cotton indoors under NaCl stress at concentrations of 0 mmol·L^{-1}, 75 mmol·L^{-1} and 150 mmol·L^{-1}, respectively. The results showed that the expression of Bt protein was significantly inhibited for NaCl-stressed Bt cotton. The content of soluble protein and K^+ in the leaves of cotton were decreased, while the content of gossypol and Na^+ were increased. In addition, the 5th instar *H. armigera* larvae exhibited shorten the life span in a 13-day trial period. Under enclosure treatments and at different female densities, the adult oviposition of *H. armigera* decreased on high NaCl-stressed nontransgenic cotton, while the oviposition on Bt cotton tended to first increase but then decrease under low, moderate and high NaCl stress treatments.

Conclusions: Under certain content ranges of NaCl stress treatments, larval of *H. armigera* growth and development, and adult oviposition were no significant difference in the change for a certain period. However, under high NaCl stress, larval growth, development and adult oviposition were affected, which may provide insights for the prevention and control of *H. armigera* for Bt cotton in saline-alkali soils.

Keywords: NaCl stress, Bt cotton, Biochemical substance, *Helicoverpa armigera*, Growth and development, Oviposition selectivity

* Correspondence: aycuijinjie@163.com
[1]Research Base, Anyang Institute of Technology, Anyang 455000, Henan, China
[2]State Key Laboratory of Cotton Biology / Institute of Cotton Research, Chinese Academy of Agricultural Sciences, Anyang 455000, Henan, China

Background

Saline-alkali soils are arable land resources; however, soil salinization severely restricts the sustainable development of agricultural production (Kahlown and Azam 2002; Barrett-Lennard 2003). Recently, due to the development of food security strategies, cotton has been planted in inland dry saline-alkali soils or coastal some saline-alkali soils in China. Ninety-eight percent of cotton currently planted in the Yellow River and the Yangtze River basins is Bt cotton. Many studies have investigated the effects of salt stress on both cotton growth (Yao et al. 2011; Zhang et al. 2016) and on Bt protein expressed in the cotton (Jiang et al. 2006; Luo et al. 2008; Luo et al. 2017). There are also many studies about the content of Bt proteins, soluble proteins, gossypol as well as about the concentrations of Na^+ and K^+ in salt-stressed cotton (Meloni et al. 2003; Wu 2004; Yao et al. 2010). However, there are no studies about the effects of salt-stressed Bt cotton on growth and development of *Helicoverpa armigera* larvae, and on the effects of NaCl-stressed Bt cotton on the oviposition selectivity of *H. armigera* adults.

In this study, GK19, a Bt cotton variety was used as the experimental plant and its nontransgenic parent Simian 3 were grown indoors under NaCl stress; the changes were measured for the main biochemical substances in cotton leaves and the effects of the larvae growth and development and oviposition selectivity of *H. armigera* adults. The results may provide insights for the comprehensive prevention and control of cotton-feeding insects in saline-alkali soils by elucidating the growth and development as well as the adult oviposition selectivity of *H. armigera* in Bt cotton in saline-alkali soils.

Materials and methods

Experimental materials

Cotton cultivars

The Bt cotton variety GK19 and its nontransgenic parent variety Simian 3 were provided by the Institute of Plant Protection and Soil Science, Hubei Academy of Agricultural Sciences, China.

Helicoverpa armigera

H. armigera larvae and adults were provided by the Laboratory of Plant Protection, Institute of Cotton Research of Chinese Agricultural Academy of Sciences (CAAS). *H. armigera* insects were reared on artificial food (Wu and Gong, 1997) in a light incubator under 60 ± 5% relative humidity, at 25 ± 1 °C and with a 14:10 (light: darkness) photoperiod (Wu and Gong, 1997).

NaCl stress treatment

The seeds of Bt cotton GK19 and nontranshenic cotton Simian 3 were sown in germination boxes. After two cotyledons of the cotton seedlings had opened and spread flat, the cotton roots were washed, and each seedling was transferred to a hydroponic growth box containing a nutrient solution. The growth boxes were subsequently placed in a plant culture room (temperature 25 ± 2 °C, humidity 50%–60%) until four true leaves had grown. NaCl stress treatments were then applied (by adding the NaCl into the nutrient solution). The NaCl stress treatments consisted of 3 concentration gradients, namely, 0 mmol $\cdot L^{-1}$, 75 mmol $\cdot L^{-1}$ and 150 mmol $\cdot L^{-1}$, respectively, with 3 replicates each. The cotton nutrient solutions containing NaCl levels were replaced every 2 days in stress duration. The nutrient solution was prepared, and the cotton seedlings were cultivated in accordance with the reported methods (Jiang et al. 2006). Tests were performed at the 7th days after the NaCl stress treatments.

Detection of Bt protein

Unfolded leaves were collected from the tops of Bt cotton GK19 plants under different levels of NaCl stress. For each treatment, 5 leaves were collected. The leaves were then mixed evenly, frozen and ground in liquid nitrogen, after which the Bt protein was measured using the QuantiPlate™ Kit for *Cry1Ab/Cry1Ac* (EnviroLogix, Portland, ME, USA) according to the manufacturer's instructions.

H. armigera larval growth and development

The *H. armigera* tested were 5^{th}instar larvae. Leaves were collected from the tops of the GK19 and the Simian 3 plants that had been subjected to different levels of NaCl stress and then placed into clean bioassay boxes (10 cm × Φ5 cm). *H. armigera* 5^{th} instar larvae were weighed and cultured individually in the bioassay boxes; cotton leaves were changed every day until pupation. Fifteen larvae were reared for one replicate, and 3 replicates were performed per treatment. During the larval stage, the insects were weighed every 2 days.

Host selection of *H. armigera* adult oviposition

Bt cotton GK19 and nontransgenic cotton Simian 3 were placed in 500 mL triangular flasks with different levels of NaCl solutions, and two cotton seedlings were placed to each triangular flask. One triangular flask for each GK19 and Simian 3 plants that had been subjected to different treatments was randomly placed in a mesh enclosure with a diameter of 40 cm. A bottle with 15% honey was placed in the enclosure to provide energy for *H. armigera* adult oviposition. One, two, or three *H. armigera* female adults, which had emerged and mated, were placed into the mesh enclosure to oviposit on cotton plants that had been subjected to different treatments for 7 days. The number of *H. armigera* eggs on each treated cotton plant in the mesh

enclosure was counted 2 days after oviposition; each treatment test was repeated 5 times.

Data analysis

SPSS 17.0 statistical analysis software (SPSS, Chicago, IL, USA) was used to analyze the significant differences between the treatments. The Bt insecticidal protein expression as well as the growth and development and oviposition host selectivity of *H. armigera* were analyzed via one-way ANOVA with SPSS 17.0. Significant differences between the treatments were tested via LSD tests.

Results

Effects of NaCl stress on the Bt protein in Bt cotton leaves

Compared with the 0 mmol· L^{-1} NaCl stress treatment, the 75 mmol ·L^{-1} and 150 mmol ·L^{-1} NaCl stress treatments caused 53.76% ($F = 73.066$, $P < 0.001$) and 57.90% ($F = 62.210$, $P < 0.001$) reduction in Bt protein in the Bt cotton leaves, respectively (Fig. 1). These results showed that NaCl stress could significantly inhibit the expression of exogenous Bt protein in Bt cotton.

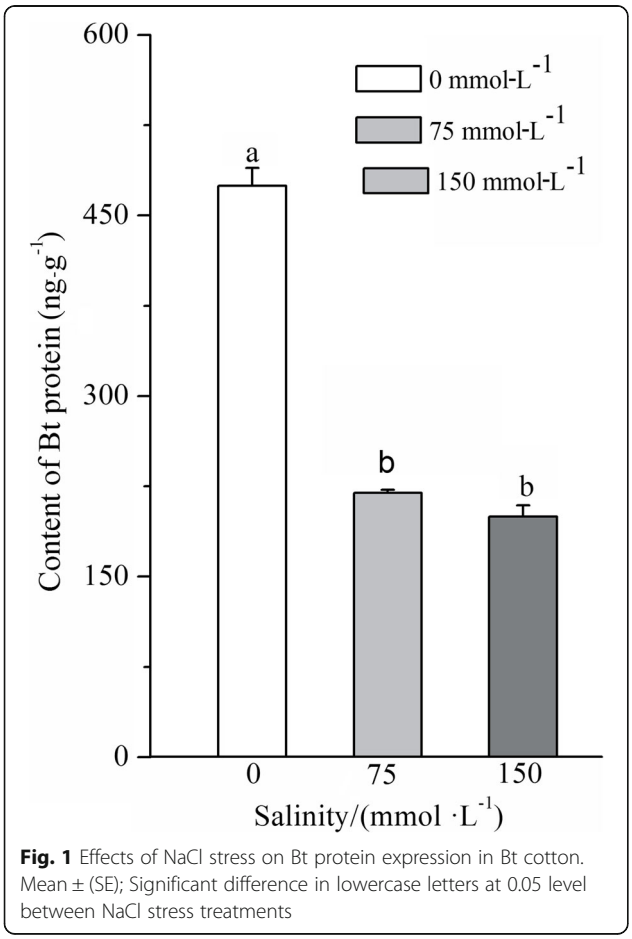

Fig. 1 Effects of NaCl stress on Bt protein expression in Bt cotton. Mean ± (SE); Significant difference in lowercase letters at 0.05 level between NaCl stress treatments

Effects of NaCl stress on main biochemical substances in Bt cotton

Na^+ and K^+

For both Bt and non-Bt cottons, the Na^+ content were increased, whereas the K^+ content were decreased in the cotton leaves under NaCl stress (Fig. 2). Compared with the 0 mmol·L^{-1} NaCl stress treatment, the 75 mmol· L^{-1} and 150 mmol· L^{-1} NaCl stress treatments caused 5.65% ($F = 0.101$, $P = 0.762$) and 86.87% ($F = 10.642$, $P = 0.017$) increase in Na^+ content in the Simian 3 nontransgenic cotton leaves, respectively, and caused 5.66% ($F = 0.393$, $P = 0.554$) and 27.87% ($F = 18.671$, $P = 0.005$) increase in Na^+ content in the Bt cotton leaves, respectively.

Compared with the 0 mmol ·L^{-1} NaCl stress treatment, the 75 mmol· L^{-1} and 150 mmol· L^{-1} NaCl stress treatments caused 27.36% ($F = 0.753$, $P = 0.419$) and 91.46% ($F = 19.887$, $P = 0.004$) decrease in K^+ content in the Simian 3 nontransgenic cotton leaves, respectively, and caused 16.13% ($F = 3.831$, $P = 0.098$) and 26.75% ($F = 11.703$, $P = 0.014$) decrease in K^+ content in the Bt cotton leaves, respectively.

Soluble protein and gossypol

Compared with the 0 mmol· L^{-1} NaCl stress treatment, the 75 mmol· L^{-1} and 150 mmol ·L^{-1} NaCl stress treatment showed 15.39% ($F = 2.879$, $P = 0.141$) and 17.23% ($F = 3.726$, $P = 0.102$) lower soluble protein contents in the Simian 3 nontransgenic cotton leaves, respectively, and showed 30.38% ($F = 22.191$, $P = 0.003$) and 23.64% (F = 13.540, $P = 0.010$) lower soluble protein contents in the Bt cotton leaves, respectively (Fig. 3).

Compared with 0 mmol· L^{-1} NaCl stress treatment, the 75 mmol· L^{-1} and 150 mmol ·L^{-1} NaCl stress treatments showed 90.69% ($F = 14.944$, $P = 0.008$) and 67.66% ($F = 8.843$, $P = 0.025$) higher gossypol contents in the Simian 3 nontransgenic cotton leaves, respectively, and showed 2.82% ($F = 0.035$, $P = 0.857$) and 7.83% ($F = 295$, $P = 0.606$) higher gossypol contents in the Bt cotton leaves, respectively (Fig. 3).

Effects of NaCl-stressed Bt cotton on *H. armigera* larval growth and development

H. armigera larval weight

The 5th instar *H. armigera* larvae were fed with leaves of GK19 and Simian 3 plants that had been subjected to different levels of NaCl stress. During the larval stage before pupating, the larval weight tended to first increase but then decrease (Fig. 4). The weight of the 5th instar *H. armigera* larvae fed with leaves of Simian 3 nontransgenic cotton did not significantly differ during the first 2 days of NaCl treatment ($P > 0.05$); compared with the 0 mmol· L^{-1} NaCl stress treatment, the 75 mmol· L^{-1} NaCl stress treatment caused no significant differences in larval weight during the entire 5th instar

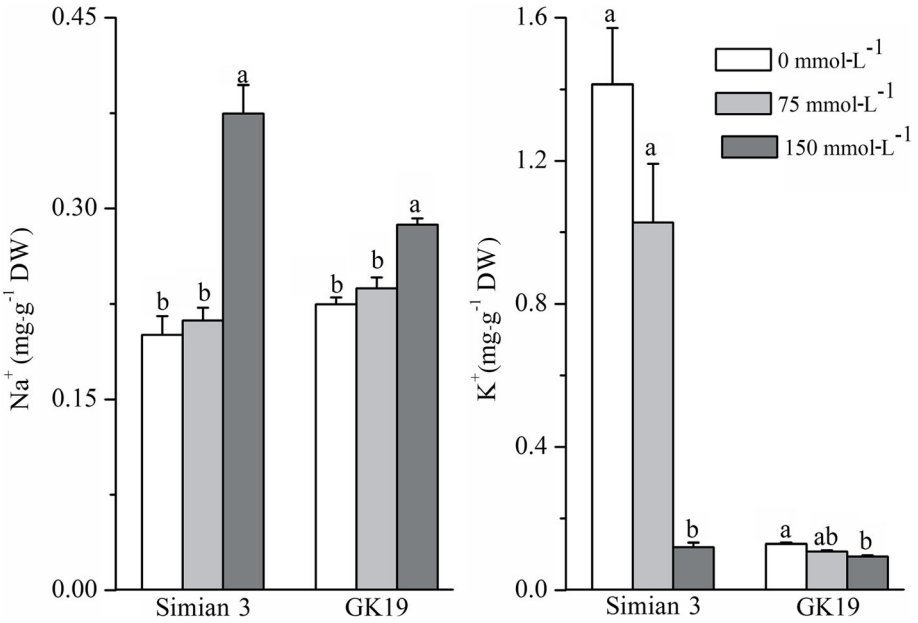

Fig. 2 Effects of NaCl stress on Na and K in GK19 and Simian 3. Mean ± (SE); Significant difference in lowercase letters at 0.05 level between NaCl stress treatments

stage. However, from the 4th day, the *H. armigera* larvae fed with leaves of Simian 3 plants that had been subjected to the 150 mmol $\cdot L^{-1}$ NaCl stress treatment showed a significant decrease in weight, namely, 19.17% ($F = 24.143$, $P = 0.008$), 12.55% ($F = 16.000$, $P = 0.016$) and 17.24% ($F = 23.273$, $P = 0.008$) lower than those under the 0 mmol$\cdot L^{-1}$ NaCl stress treatment on days 4, 6, and 8, respectively. Bt cotton GK19 showed a similar trend as that of Simian 3 but caused no significant differences in weight at the larval stage.

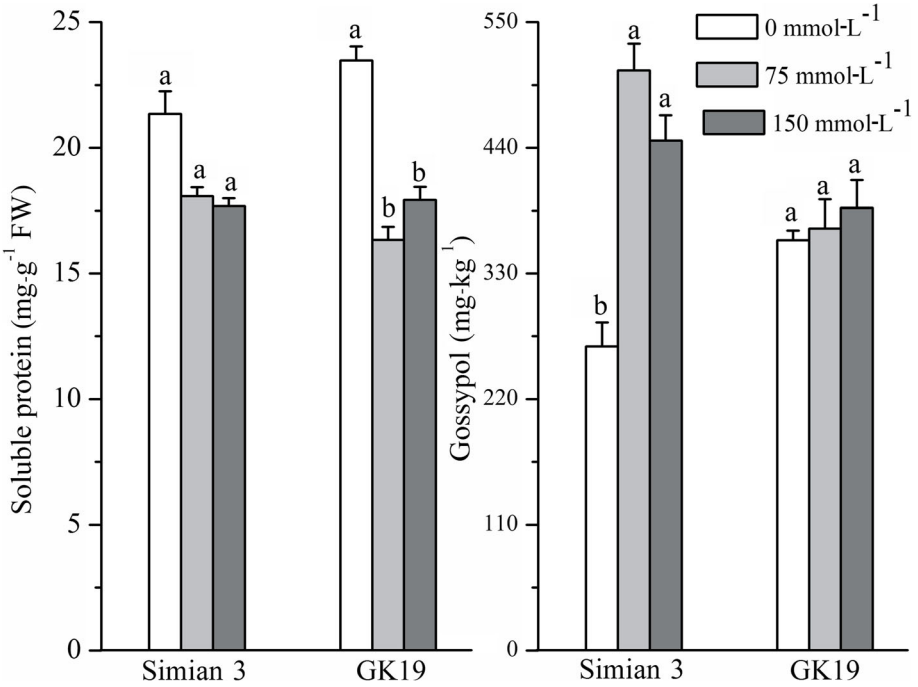

Fig. 3 Effects of NaCl stress on the soluble protein and gossypol contents in GK19 and Simian 3. Mean ± (SE); Significant difference in lowercase letters at 0.05 level between NaCl stress treatments

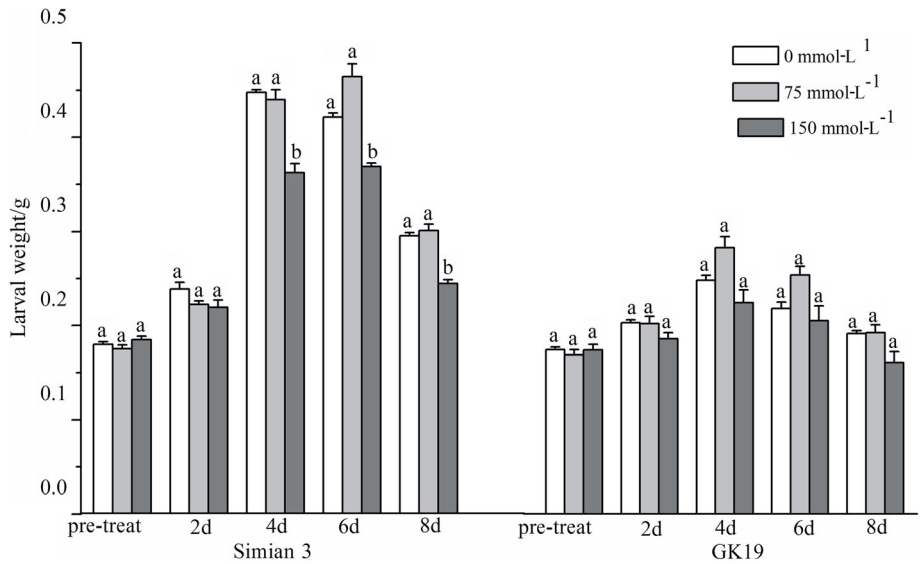

Fig. 4 Larval weight of *H. armigera* larvae fed GK19 and Simian 3 grown under NaCl stress. Mean ± (SE); Significant difference in lowercase letters at 0.05 level between NaCl stress treatments

Life span in a 13-day trial period and mortality rate

For NaCl-stressed Bt cotton, the 5th instar the life span in a 13-day trial period and mortality rate of *H. armigera* fed with cotton leaves are shown in Fig. 5. Compared with the 0 mmol $\cdot L^{-1}$ NaCl stress treatment, the 75 mmol$\cdot$ L^{-1} NaCl stress treatment caused no significant differences in the life span in a 13-day trial period in the 5th instar larval of *H. armigera*. However, the 5th instar larval life span in a 13-day trial period of *H. armigera* under the 150 mmol$\cdot$ L^{-1} NaCl stress treatment decrease by 12.34% ($F = 41.63$, $P = 0.003$) in Simian 3 cotton. The 5th instar life span in a 13-day trial period of *H. armigera* larvae fed with GK19 leaves was the longest under the 75 mmol$\cdot$ L^{-1} NaCl stress treatment. Compared with the 0

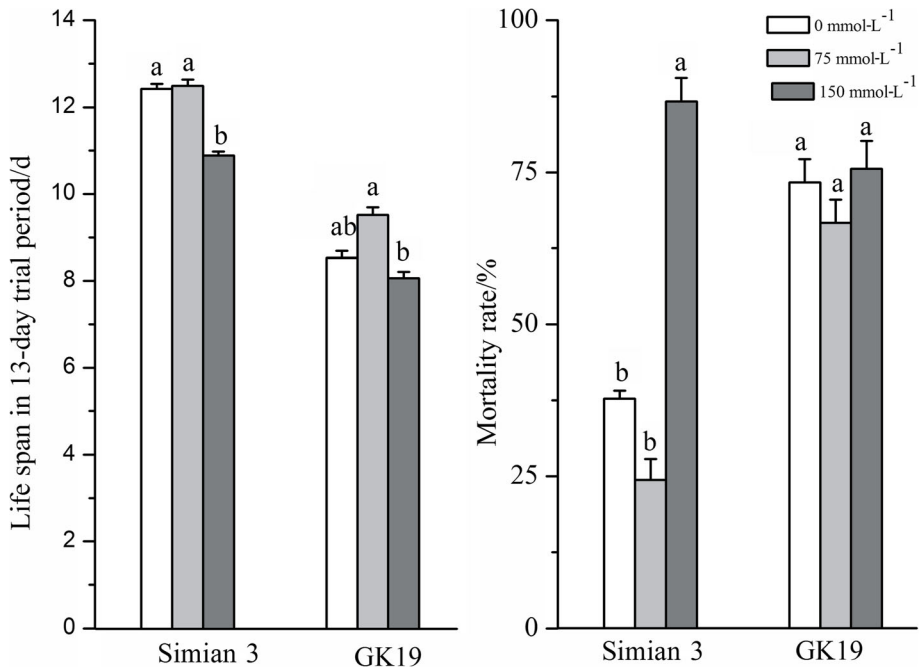

Fig. 5 Life span in a 1-day trial period and mortality rate of larvae fed leaves of GK19 and Simian 3 plants grown under NaCl stress. Mean ± (SE); Significant difference in lowercase letters at 0.05 level between NaCl stress treatments

mmol ·L^{-1} NaCl stress treatment, the 75 mmol ·L^{-1} NaCl stress treatment caused a 11.51% ($F = 5.546$, $P = 0.078$) longer 5th instar larval duration of *H. armigera,* which was not a significant difference but it was close. However, the 5th instar larval life span in a 13-day trial period of *H. armigera* did not significantly decrease under the 150 mmol ·L^{-1} NaCl stress treatment compared with the 0 mmol ·L^{-1} NaCl stress treatment.

Compared with the 0 mmol ·L^{-1} NaCl stress treatment, the 150 mmol ·L^{-1} NaCl stress treatment caused a 129.41% ($F = 48.391$, $P = 0.002$) higher mortality rate in 5th instar *H. armigera* larvae fed with Simian 3 nontransgenic cotton leaves. The mortality rate for NaCl-stressed Bt cotton tended to first decrease but then increase; however, in general, there were no significant differences.

Effects of NaCl-stressed Bt cotton on *H. armigera* adult oviposition host selectivity

For NaCl-stressed GK19 and Simian 3, the *H. armigera* adult oviposition selectivity at different densities is shown in Fig. 6. One, two, or three female adults were used for oviposition when both Bt cotton and nontransgenic cotton plants were placed randomly under different levels of NaCl stress. For nontransgenic cotton, the amount of *H. armigera* oviposition under high NaCl stress was less than that under low NaCl stress ($P > 0.05$). For Bt cotton, the trends of the amounts of *H. armigera* oviposition tended to first increase but then decrease under the 0 mmol ·L^{-1}, 75 mmol ·L^{-1} and 150 mmol ·L^{-1} NaCl stress treatments; with respect to the oviposition of one, two and three female adults, compared with the 0 mmol• L^{-1} NaCl stress treatment, the 75 mmol ·L^{-1} NaCl stress treatment caused 368.57% ($F = 13.093$, $P = 0.007$), 91.78% ($F = 2.888$, $P = 0.128$) and 32.67% ($F = 0.536$, $P = 0.485$), respectively, more *H. armigera* oviposition in Bt cotton; but under the 150 mmol ·L^{-1} NaCl stress treatment, the amount of *H. armigera* oviposition fell again. Under closed conditions, the *H. armigera* oviposition selectivity is somewhat related to insect population density and cotton plant treatment.

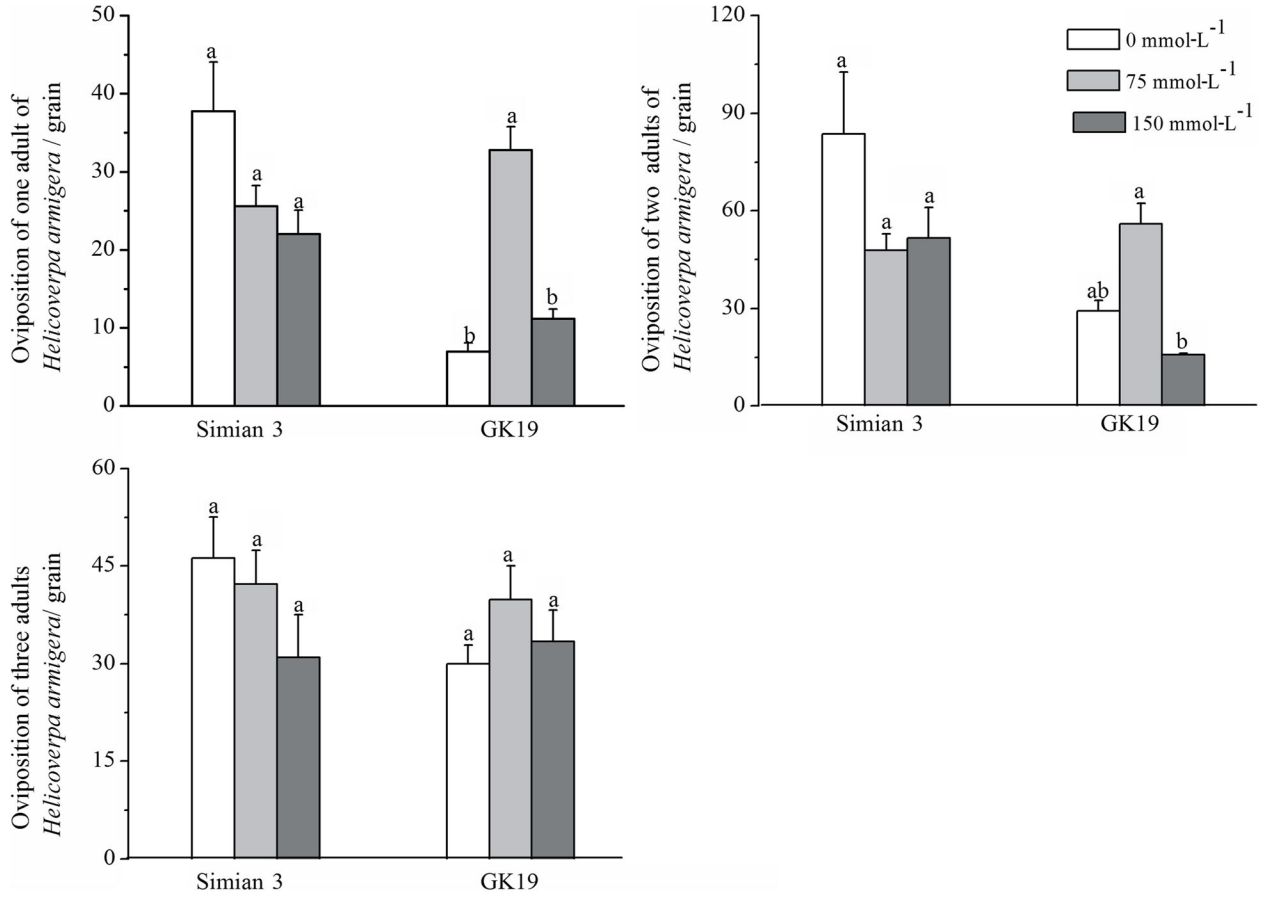

Fig. 6 Adult oviposition host selectivity of *H. armigera* fed leaves of GK19 and Simian 3 plants grown under NaCl stress. Mean ± (SE); Significant difference in lowercase letters at 0.05 level between NaCl stress treatments

Discussion

Effects of NaCl stress on the expression of Bt protein in Bt cotton leaves

Bt cotton has a high degree of commercialization, and there are many studies on major pest and natural enemy population dynamics in cotton fields (Deng et al. 2002; Hussain et al. 2014; Kalkal et al. 2014; Asiimwe et al. 2014), which show that Bt cotton can effectively control *H. armigera* (Ullah et al. 2014). Exogenous Bt protein is the basis for the insect resistance of Bt cotton, and the expression of exogenous Bt protein exhibits a spatiotemporal dynamic nature during cotton growth. Moreover, environmental conditions such as temperature (Chen et al. 2005), drought and waterlogging (Martins et al. 2010), salt and alkali concentrations (Jiang et al. 2006; Luo et al. 2017), CO_2 concentrations (Coviella et al. 2002), seasonal changes (Adamczyk and Sumerford 2001) and other nonbiological factors may affect Bt protein expression. The results of present study showed that the expression of Bt protein in the leaves of NaCl-stressed Bt cotton was clearly inhibited. Predecessors measured the contents of Bt protein by using Bt cotton under quantified NaCl stress indoors; their results showed that the Bt protein expression in cotton seedling leaves tended to decrease as the salt concentration increased (Jiang et al. 2006; Iqbal et al. 2013). Li (2014) reported the same conclusion by conducting NaCl stress tests in a salt pond within a dry shed. These results are consistent with the conclusion of that was obtained via field tests. The inhibition of Bt protein expression in cotton is related to the degree and duration of NaCl stress (Luo et al. 2017).

Effects of NaCl stress on main biochemical substances in Bt cotton

External adversity stress or exogenous gene introduction may cause an unanticipated potential pleiotropic effect or a change in mutagenic effects for plants and may cause a change in the physiological properties of plants (Reddy et al. 2004; Pommerrenig et al. 2007; Guha et al. 2010). In the present study, we measured the contents of soluble proteins, secondary metabolites, such as gossypol, as well as the concentrations of Na^+ and K^+. The results showed that the content of soluble protein in the leaves of NaCl-stressed cotton decreased, which is the same as the result of Luo et al. (2008); the content of gossypol increased, which is consistent with the results of Luo et al. (2008) and Li (2014). The concentration of Na^+ increased significantly and that of K^+ decreased significantly, which confirms the results of Akhtar et al.(2010), Munis et al. (2010) and Saleh (2011).

Effects of NaCl-stressed Bt cotton on *H. armigera* larval growth and development

The introduction of exogenous genes into cotton plants can ultimately inhibit the growth and development of *H. armigera* (a target pest) to some extent (Olsen et al. 2000; Arshad et al. 2009; Basavaraja et al. 2011). Under NaCl stress, in our test, there were different changes in the content of biochemical substances and Bt protein expression in cotton, which had a slight inhibitory effect on *H. armigera* larval weight during early feeding, and the inhibitory effect increased as the feeding time progressed (there were significant differences in the 4-8 days treatments of non-Bt cotton variety). However, nontransgenic cotton caused a greater effect on larval weight than Bt cotton, which may be due to the Bt protein in Bt cotton; this protein inhibits pest growth in the beginning, then after a certain concentration of salt stress, the Bt protein and the inhibition decreased. Under high salt stress condition, although Bt protein content decreased, because of other factors, such as leaf senescence or inactivation, etc. it might affect the larvae of *H. armigera*, yet had less effect than nontransgenic cotton. The Bt protein produced by Bt cotton had strong toxic effect under low NaCl stress. The content of the Bt protein decreased with an increase in stress concentrations, and the mortality rate decreased in a certain concentration of salt stress, but excessive high salt stress led to poor growth of cotton in the first place, and cotton leaves also changed accordingly in terms of their morphology, physiology, the degree of tenderness or senility and palatability, etc. So under high salt stress, the mortality rate rised slightly, e.g., "fitness" selection. This phenomenon and result are similar to the conclusion of Luo et al. (2017) regarding a survey of natural saline fields; the amount of *H. armigera* oviposition and the numbers of *H. armigera* larvae were low under low NaCl conditions and were the highest under moderate NaCl conditions, but both decreased under high NaCl conditions (Luo et al. 2017). For NaCl-stressed cotton, the reason for such "fitness selectivity" may be, (1) under NaCl stress, especially high NaCl stress, cotton plants grew poorly and had senescent leaves that presented poor "palatability" for *H. armigera*; (2) the content of nutrients in the cotton leaves decreased, causing a less effective nutrition ratio for *H. armigera* between feeding and self-nutrition needs; and (3) *H. armigera* larvae were fed with cotton leaves with a high Na^+ concentration for a long period and were "thirsted" to death. This influence of the result is that ecological selection mechanism is greater than the physiological selection mechanism, or the physiological selection mechanism is greater than the ecological selection mechanism, or the combined action of the ecological selection mechanism and the physiological selection mechanism.

Effects of NaCl-stressed Bt cotton on *H. armigera* adult oviposition host selectivity

Insects have a certain selectivity to different hosts (Roslin et al. 2006; Gripenberg 2007; Jin et al. 2008;

Béguinot 2012), and at different stages, insects will select different hosts based on their purpose; e.g., breeding adults will select a suitable "nest"(Roslin et al. 2006; Gripenberg 2007; Béguinot 2012). Selection tests showed that the amount of *H. armigera* adult oviposition decreased on cotton plants stressed in high NaCl stress under closed conditions. Furthermore, the amount of oviposition tended to first increase but then decrease under low, moderate and high NaCl stress, which is consistent with the results of Luo et al. (2017) concerning natural oviposition in cotton fields on saline-alkali soil under open conditions. These results may have occurred because which, under different treatments, there were different changes in cotton "appearance states", such as senescent leaves in a poor state due to high NaCl stress, fresh leaves in a good state due to moderate rangs NaCl stress, etc. (Wang et al. 2014) and "internal states", such as changes in nutrient substances (Cui 2012; Xie et al. 2015), biochemical substances (Saleh 2013; Lu et al. 2018) and secondary metabolites (Wang et al. 2015; Ma et al. 2016) in cotton tissues) or changes in the content or percentage of plant volatiles (Martini et al. 2016).

However, the experiment was carried out in laboratory, the cotton bollworm can have more "freely activity" and "selective food" in natural field conditions. Therefore, pest monitoring should be strengthened in field for timely prevention and control.

Conclusion

After Bt cotton was stressed by NaCl solution, the expression of Bt protein was inhibited significantly, the soluble protein and K^+ contents in the leaves decreased, the gossypol and Na^+ content increased. Many factors may jointly reduce larval weight in non-Bt cotton of 5^{th} instar *H. armigera* larvae, and the changing trends of contents in nontransgenic cotton were consistent with those in Bt cotton.

Acknowledgements
We are grateful for the financial support from the National Natural Science Foundation of China (31501253).

Funding
We are grateful for the financial support from the National Natural Science Foundation of China (31501253).

Availability of data and materials
Data sharing not applicable to this article as no datasets were generated or analyzed duiring the current study.

Authors' contributions
Luo J, Zhang S, and Cui J conceived and designed the experiments; Luo J, Zhang S and Zhu X performed the experiments; Ji J, Zhang K and Wang C analyzed the data; Zhang L and Wang L evaluated the conclusions; Luo J and Cui J wrote the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The authors declare responsible for the submission and the BioMed Central License Agreement as detailed above.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 4 December 2018 Accepted: 18 March 2019
Published online: 22 April 2019

References
Adamczyk JJ Jr, Sumerford DV. Potential factors impacting season-long expression of *Cry1Ac* in 13 commercial varieties of Bollgard cotton. J Insect Sci. 2001;1:1–6.
Akhtar J, Saqib ZA, Sarfraz M, et al. Evaluating salt tolerant cotton genotypes at different levels of NaCl stress in solution and soil culture. Pak J Bot. 2010;42:2857–66.
Arshad M, Suhail A, Arif MJ, Khan MA. Transgenic-Bt and non-transgenic cotton effects on survival and growth of *Helicoverpa armigera*. Int J Agric Biol. 2009;11:473–6.
Asiimwe P, Naranjo SE, Ellsworth PC. Effects of irrigation levels on interactions among *Lygus hesperus* (Hemiptera:Miridae), insecticides, and predators in cotton. Environ Entomol. 2014;43:263–73.
Barrett-Lennard EG. The interaction between waterlogging and salinity in higher plants: causes, consequences and implications. Plant Soil. 2003;253:35–54.
Basavaraja H, Chhillar BS, Singh R. Effect of transgenic Bt cotton mature top leaves on consumption-utilization indices of *Helicoverpa armigera* (Hubner) and *Spodoptera litura* (Fabricius) larvae. J Cotton Res Dev. 2011;25:252–7.
Béguinot J. Would mothers relax their degree of selectivity for supports suitable for egg-laying when the local density of conspecific females increases? A case study with three common lepidopteran leaf miners. Int Scholarly Res Notices. 2012;2012:1–6.
Chen D, Ye G, Yang C, et al. The effect of high temperature on the insecticidal properties of Bt cotton. Environ Exp Bot. 2005;53:333–40.
Coviella CE, Stipanovic RD, Trumble JT. Plant allocation to defensive compounds: interactions between elevated CO_2 and nitrogen in transgenic cotton plants. J Exp Bot. 2002;53:323–31.
Cui Y. Analysis of differential proteins of cotton leaves under salt stress. Molecular Plant Breeding. 2012;13:455.
Deng S, Xu J, Zhang Q, et al. Effect of transgenic Bt cotton on population dynamics of the non target pests and natural enemies of pests. Acta Entomol Sin. 2002;46:1–5.
Gripenberg S. Spatial ecology of a specialist insect herbivore : the leaf-mining moth *Tischeria ekebladella* on the pedunculate oak *Quercus robur*. Helsingin Yliopisto. 2007:1–22.
Guha A, Sengupta D, Rasineni GK, Reddy AR. An integrated diagnostic approach to understand drought tolerance in mulberry (*Morus indica* L.). Flora-morphology, distribution. Funct Ecol Plants. 2010;205:144–51.
Hussain M, Akram M, Abbas Q. Impact of environmental factors on the population dynamics of leafhopper *Amrasca biguttula biguttula* ishida (Homoptera:Jassidae) on various transgenic cotton genotypes in Multan. Acad J Entomol. 2014;7:27–31.
Iqbal A, Ali S, Zia MA, et al. Comparative account of *Bt* gene expression in cotton under normal and salt affected soil. Int J Agric Biol. 2013;15:1181–6.
Jiang L, Duan L, Tian X, et al. NaCl salinity stress decreased *Bacillus thuringiensis* (Bt) protein content of transgenic Bt cotton *(Gossypium hirsutum* L.) seedlings. Environ Exp Bot. 2006;55:315–20.
Jin J, Pan Y, Wang X, Lei C. Studies on the selectivity of maruca testulalis for host plants and comparison of the insect-resistance of different cowpea varieties. Plant Prot. 2008;34:81–5.
Kahlown MA, Azam M. Individual and combined effect of waterlogging and salinity on crop yields in the indus basin. Irrig Drain. 2002;51:329–38.
Kalkal D, Lal R, Dahiya KK, et al. Effect of abiotic factors on population dynamics of pink bollworm in relation to genetically modified cotton. J Cotton Res Dev. 2014;28:280–5.
Li MY. Effect of NaCl on growth and the resistance efficiency of transgenic Bt cotton (*Gossypium hirsutum* L.) and application plant growth regulator alleviate the adverse effects. Doctoral Dissertation. Beijing: China Agricultural University.2014.
Lu P, Magwanga RO, Lu H, et al. A novel g-protein-coupled receptors gene from upland cotton enhances salt stress tolerance in transgenic arabidopsis. Genes. 2018;9:209.

Luo JY, Zhang S, Peng J, et al. Effects of soil salinity on the expression of Bt toxin (Cry1Ac) and the control efficiency of *Helicoverpa armigera* in field-grown transgenic Bt cotton. PLoS One. 2017;12:e0170379.
Luo Z, Dong H, Li W, et al. Individual and combined effects of salinity and waterlogging on *Cry1Ac* expression and insecticidal efficacy of Bt cotton. Crop Prot. 2008;27:1485–90.
Ma H, Wang Q, Zhao M, et al. Effects of abiotic stress on cotton secondary metabolism and cotton aphid population dynamics. Cotton Science. 2016;28:324–30.
Martini X, Willett DS, Kuhns EH, Stelinski LL. Disruption of vector host preference with plant volatiles may reduce spread of insect-transmitted plant pathogens. J Chem Ecol. 2016;42:357–67.
Martins CM, Beyene G, Hofs JL, et al. Effect of water-deficit stress on cotton plants expressing the *Bacillus thuringiensis* toxin. Annals of Applied Biology. 2010;152:255–62.
Meloni DA, Oliva MA, Martinez CA, Cambraia J. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ Exp Bot. 2003;49:69–76.
Munis MFH, Tu L, Ziaf K, et al. Critical osmotic, ionic and physiological indicators of salinity tolerance in cotton (*Gossypium hirsutum* L.) for cultivar selection. Pak J Bot. 2010;42:1685–94.
Olsen KM, Daly JC. Plant-toxin interactions in transgenic Bt cotton and their effect on mortality of *Helicoverpa armigera* (Lepidoptera: Noctuidae). J Econ Entomol. 2000;93:1293–9.
Pommerrenig B, Papini-Terzi FS, Sauer N. Differential regulation of sorbitol and sucrose loading into the phloem of plantago major in response to salt stress. Plant Physiol. 2007;144:1029–38.
Reddy AR, Chaitanya KV, Vivekanandan M. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J Plant Physiol. 2004;161:1189–202.
Roslin T, Gripenberg S, Salminen JP, et al. Seeing the trees for the leaves: oaks as mosaics for a host-specific moth. Oikos. 2006;113:106–20.
Saleh B. Effect of salt stress (NaCl) on biomass and K^+/Na^+ ratio in cotton. J Stress Physiol Biochem. 2011;7:5–14.
Saleh B. Water status and protein pattern changes towards salt stress in cotton. J Stress Physiol Biochem. 2013;9:113–23.
Ullah I, Asif M, Arslan M, Ashfaq M. Temporal expression of Cry1Ab/c protein in Bt-cotton varieties, their efficacy against *Helicoverpa armigera* (Lepidoptera: Noctuidae) and population dynamics of sucking arthropods on them. Int J Agric Biol. 2014;16:879–85.
Wang Q, Eneji AE, Kong X, et al. Salt stress effects on secondary metabolites of cotton in relation to gene expression responsible for aphid development. PLoS One. 2015;10:e0129541.
Wang T, Kong X, Song Y, Dong H. The effects of NaCl stress on cotton leaf senescence characteristics and physiological mechanisms. Cotton Sci. 2014;26:66–72.
Wu CA. The cotton GhNHX1 gene encoding a novel putative tonoplast Na+/H+ antiporter plays an important role in salt stress. Plant and Cell Physiology. 2004;45:600–7.
Wu KJ, Gong PY. A new and practical artificial diet for the cotton bollworm. Entomologia Sinica. 1997;4:277–82.
Xie ZX, Duan LS, Li ZH, et al. Dose-dependent effects of coronatine on cotton seedling growth under salt stress. Journal of Plant Growth Regulation. 2015;34:651–64.
Yao D, Zhang X, Zhao X, et al. Transcriptome analysis reveals salt-stress-regulated biological processes and key pathways in roots of cotton (*Gossypium hirsutum* L.). Genomics. 2011;98:47–55.
Yao L, Wu Z, Zheng YY, et al. Growth promotion and protection against salt stress by Pseudomonas putida, Rs-198 on cotton. European Journal of Soil Biology. 2010; 46:49–54.
Zhang F, Zhu G, Du L, et al. Genetic regulation of salt stress tolerance revealed by RNA-Seq in cotton diploid wild species, *Gossypium davidsonii*. Sci Rep. 2016;6:20582.