Effects of Bacillus thuringiensis (Bt) gene on potassium efficiency of cotton (Gossypium hirsutum L.)

Wang Qianqian
China Agricultural University
Yan Wei
China Agricultural University
Zhang Yichi
China Agricultural University
Zhan Manman
China Agricultural University
Luo Xiaoli
Shanxi Agricultural University
Eneji A. Egrinya
University of Calabar
Zhang Anhong
Shanxi Agricultural University
Xiao Juanli
Shanxi Agricultural University
Li Fangjun
China Agricultural University
Xiaoli Tian (tianxl@cau.edu.cn)
China Agricultural University

Research Article

Keywords: Biomass, Bt (Bacillus thuringiensis) gene, Cotton (Gossypium hirsutum L.), K uptake, K utilization index, Yield

Posted Date: July 26th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1849752/v1

Abstract

Potassium (K) deficiency became a common field production problem following the widespread adoption of *Bacillus thuringiensis* (*Bt*) transgenic cotton (*Gossypium hirsutum* L.) worldwide. The purpose of this study was to clarify whether the introduction of *Bt* gene directly reduces the K-use efficiency of cotton to cause K deficiency. The cotton variety, Jihe 321 (wild type, WT) and its two *Bt* (*Cry1Ac*)-transgenic lines (*OE*-29317, *OE*-29312) were studied in growth chamber and field with low soil-test K^+ (47.8 mg kg^{-1}). When silencing of *Bt* gene (~ 70%) in *OE*-29317 and *OE*-29312 plants by virus-induced gene silencing (VIGS), VIGS-*Bt* plants did not produce more biomass than VIGS-Ctrl plants. In the field with low soil-test K^+, *OE*-29317 but not *OE*-29312 had less biomass and K^+ accumulation than those of WT at some growth stages. Moreover, both *Bt* lines produced similar or even greater seed cotton yield than WT in the field. Taken together, the introduction of *Bt* gene did not necessarily hinder the K use efficiency of the cotton lines under study.

1 Introduction

Potassium (K) is one of the essential macronutrients required for plant growth and development **(Sale et al., 2003)**. It plays crucial roles in many physiological and developmental processes of plants, such as osmotic adjustment, phloem loading and sugar transport to heterotrophic organs, water relations, stomatal regulation, enzyme activation and resistance to biotic and abiotic stresses **(Urrego et al., 2014; Wang et al., 2013).** However, with the increases in nitrogen (N) and phosphorus (P) fertilizer applications and the release of higher-yielding crop varieties **(Dong et al., 2010; Hu et al., 2016)**, a negative K^+ balance in soil (around − 60 kg ha^{-1} every year) occurred and is becoming worse **(Balik et al., 2020; Steiner et al., 2012)**.

Cotton needs K as much as N or even more **(Rochester, 2007)**, and it is more sensitive to K deficiencies than most other field crops due to its sparse root system **(Cassman et al., 1989; Mullins & Burmester, 2010)**. Since the 1990s, the premature senescence of cotton caused by K deficiencies has occurred frequently and with greater intensity worldwide **(Wright et al., 1999)**, which coincided with the commercialization and popularization of transgenic *Bacillus thuringiensis* (*Bt*) cotton cultivars that was developed to produce proteins toxic to lepidopterous insects and thus reduce their damage to cotton yield **(Perlak et al., 1990)**.

Genetic engineering and plant transformation have played a pivotal role in crop improvement by introducing beneficial foreign gene(s) into crop plants **(Kumar et al., 2020)**. However, the improvement of a plant variety by inserting one or two qualitative genes may lead to unintended effects (i.e. going beyond that of the original genetic modification) **(Ladics et al., 2015; Verhalen et al., 2003)** because of random gene insertion (that could disrupt the function of native gene of the host genome) **(Marrelli et al., 2006)**, random mutation, somaclonal variation, pleiotropy, position effect, the tissue culture process during the development of genetically modified plants **(Ladics et al., 2015; Miki et al., 2009; Schnell et al., 2015)**, and the added burden by the constitutive over-expression of the alien transgene **(Gurr & Rushton, 2005)**.

In order to reveal whether *Bt* cotton was responsible for the K deficiency of cotton production due to its lower K efficiency (one of unintended effects), we previously compared K efficiency between 33 *Bt*- and 15 conventional cotton cultivars/lines, and found that *Bt* cotton showed more severe K deficiency symptoms than conventional cotton at the seedling stage, and yielded less than the latter in the field **(Tian et al., 2009)**. However, we are not sure yet if the *Bt* gene transformation directly decreased cotton K use efficiency since the genetic background of tested *Bt*- and conventional cotton cultivars/lines was different.

Therefore, we generated two independent *Bt* cotton lines by introducing *Bt* gene into a wild type (WT) of cotton variety. In this study, we used virus-induced gene silencing (VIGS) method to knock down the *Bt* gene of transgenic lines and compared their K efficiency with VIGS-Ctrl (control) plants. Also, the K efficiency of *Bt* lines was compared with WT via field experiments. The results will provide direct evidences as to whether the introduction of *Bt* gene influences the K use efficiency of cotton, and could be helpful in K management of *Bt* cotton production.

2 Materials And Methods

2.1 Generation of transgenic *Bacillus thuringiensis* (*Bt*) lines

Cotton (*Gossypium hirsutum*) variety of Jihe 321 was used as wild type (WT). Seeds were sterilized with 70% alcohol and 10% (w/v) hydrogen peroxide (H_2O_2). After germination, they were cultured on half-strength Murashige and Skoog (MS) medium. Five days later, the middle part of the hypocotyl of sterile seedlings was cut into 5 ~ 7 mm segments as the transformation recipients.

A synthetic *Bacillus thuringiensis* (*Bt*) gene (*Cry1Ac*) with signal peptide (*Bt*S29K) was inserted into the pBin438 vector to generate the plant expression vector, pBin438-*Bt*S29K. *Agrobacterium* with the vector was inoculated into LB liquid medium. The cut hypocotyl segments were inoculated with the *Agrobacterium* suspension for 5 ~ 10 min, and cultured on the co-culture medium at 22 ~ 24℃ for 2 d in the dark. Then, they were placed into callus induction medium under a 12 h/12 h (day/night) photoperiod, 2000 lx light intensity at 25°C for 2 months.

The calluses with 1 ~ 2 cm diameter were successively transferred to the selective medium, proliferation medium, and differentiation medium until embryoids were formed. Then, the embryoids were transferred to differentiation medium to grow into plantlets that were sequentially moved into seedling growth medium. When the regenerated plants grew to 5 ~ 8 cm, they were grafted onto the rootstock with 1 ~ 3 true leaves **(Wang et al., 2016; Wu et al., 2008)**

In this study, two independent homozygous transgenic *Bt* lines (*OE*-29312 and *OE*-29317) were used, and the plant size of *OE*-29312 was similar to WT, and that of *OE*-29317 was smaller than WT under normal growth condition.

2.2 Identification of transgenic Bt cotton lines with Enzyme-linked immunosorbent assays (ELISA)

In the field, the youngest mature leaves (the fourth leaf from apex) were sampled at early squaring − (54 days after sowing, DAS), squaring − (63 DAS), and early bloom stages (78 DAS) to determine the content of Bt protein, using ELISA kit (CryIAb/Cry1Ac Plate kit AP003, EnviroLogix, Portland, ME, USA).

2.3 Agrobacterium-mediated virus-induced gene silencing (VIGS)

2.3.1 Vector construction

VIGS assay was performed as **(Li et al., 2017)** described. 161 bp fragments of *Bt* gene (*Cry1Ac*) were amplified and cloned into a binary tobacco rattle virus (TRV) vector. The forward primer (5'-3') is GGGGTACCTGTGTCTCTCTTCCCGAAC, and reverse primer is CGGAATTCTGCTGGTTGTTGATACCG. Plasmids of TRV vectors pTRV-*RNA1* or pTRV-*RNA2* [pYL156-Ctrl, pYL156-*Cry1Ac*, pYL156-*GhCLA1* (positive control)] were transformed into *Agrobacterium tumefaciens* strain, GV3101 by electroporation. The Agrobacterial culture carrying the above *pTRV-RNA2* constructs (OD_{600} = 1.5) was mixed with that carrying the *pTRV-RNA1* construct in a 1:1 ratio, and then was infiltrated into just fully expanded cotyledons of cotton plants using a needle-less syringe at a growth chamber.

2.3.1 Growth conditions

The growth chamber was set with photosynthetic photon flux density 600 μM cm^{-2} s^{-1}, mean humidity 70–90%, and light/dark regime of 14/10 h at 22 ± 2 ℃/24 ± 2 ℃. After being surface-sterilized by soaking in 9% H_2O_2 for 20–30 min, seeds were rinsed with tap water and then germinated in a sand medium with only distilled water present for four days in the dark. After emergence, uniform seedlings were transplanted into half-strength modified Hoagland's solution containing 1.25 mM KNO_3,2.5 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 0.5 mM $NH_4H_2PO_4$, 2×10^{-4} mM $CuSO_4$, 1×10^{-3} mM $ZnSO_4$, 0.1 mM EDTA Fe-Na, 2×10^{-2} mM H_3BO_3, 5×10^{-6} mM $(NH_4)_6Mo_7O_{24}$ and 1×10^{-3} mM $MnSO_4$. The solution was changed every 3–4 d and continuously aerated with an air pump.

2.3.3 Symptom of K deficiency, biomass yield, K concentration and accumulation

When the leaves infiltrated with pYL156-*GhCLA1* showed an albino phenotype, the VIGS-Ctrl and VIGS-*Bt* plants were transferred to 0.1 mM K^+ solutions. After 40 days, all leaves of seedlings were photographed to show symptoms of K deficiency, then leaves were inactivated at 105°C for 30 min and dried at 80°C to a constant weight. The dried samples were milled for the determination of K (SpectAA-50/55, Varian, Australia).

2.4 Quantitative RT-PCR analysis of *Bt* gene expression

When positive control plants of pYL156-*GhCLA1* showed an albino phenotype, total RNA was extracted from the primary roots and young leaves of VIGS-Ctrl and VIGS-*Bt* seedlings using an RNAprep Pure Plant Kit and purified with RNase-free DNase I (both Tiangen). cDNA was synthesized with 2 μg RNA using Oligo (d T) primer and M-MLV reverse transcriptase (TaKaRa). Quantitative real-time (qRT-) PCR was conducted in an Applied Biosystems 7500 Fast Real-Time PCR System using SYBR® Premix Ex Taq™ (TaKaRa) under the following conditions: 95°C for 30 s, 40 cycles of 95°C for 5 s, 60°C for 34 s, and 95°C for 15 s, 60°C for 60 s, then 95°C for 30 s, and finally 60°C for 10 s. A melting-curve was performed from 60 to 95°C to check the specificity of the amplified products. The expression level of each gene was determined relative to the references, *GhACTIN9* and was calculated using the $2^{-\triangle\triangle CT}$ method **(Livak & Schmittgen, 2001)**. The forward and reverse primer for qRT-PCR is CGTGGTTCTGCCCAAGGTAT, and CGATACGTTGTTGTGGAGCG, respectively. Three biological replicates were performed.

2.5 Field experiments

Field experiments were conducted at Shangzhuang experimental station (N 40° 08’ 12.15”; E 116° 10’ 44.83”) of China Agricultural University during 2010–2011. The soil was sandy loam with 7.8 pH and contained 6 g kg^{-1} organic matter, 37.8 mg kg^{-1} alkaline-hydrolyzable N, 19.5 mg kg^{-1} available P (Olsen P), and 47.8 mg kg^{-1} available K. The monthly average temperature and cumulative precipitation during the growing seasons are shown in Table 1.

Table 1
Monthly mean temperature and precipitation during cotton growing seasons

Month	Mean temperature (°C)			Precipitation (mm)		
	2010	2011	Perennial average	2010	2011	Perennial average
April	11.2	15.2	14.2	17.5	15.3	21.2
May	21.7	21.3	19.9	29.5	21.8	34.2
June	24.7	26.4	24.4	88.7	117.4	78.1
July	28.6	27.5	26.2	34.0	265.7	185.2
August	26.5	26.4	24.8	177.8	171.3	159.7
September	21.3	20.2	20.0	80.8	64.0	45.5
October	13.6	14.2	13.1	59.0	28.7	21.8
The data were provided by Meteorological Bureau of Haidian District, Beijing.						

The experiment was arranged into a split-plot design with four replications. The levels of K supply (0, 225 kg K_2O ha^{-1}) were assigned to main plots, and WT and transgenic *Bt* lines (*OE*-29312 and *OE*-29317) were assigned as subplots. The pre-plant fertilizer treatment included 120 kg N ha^{-1} (in the form of diammonium phosphate and urea), 186 kg P_2O_5 ha^{-1} (in the form of diammonium phosphate), and 135

kg K_2O ha^{-1} (in the form of potassium sulphate, only for K-supplied plots). At squaring and peak bloom stage, 69 kg N ha^{-1} (in the form of urea) were top dressed. For K treated plots, 90 kg K_2O ha^{-1} (in the form of potassium sulphate) was top dressed at peak bloom stage.

The treatment plots were 10 m long and comprised six rows, spaced 90 + 50 cm apart. The inter-plant spacing was 25 cm, and the planting density was 57 000 plants ha^{-1}. Seeds were planted on May 08, 2010, and April 26, 2011. Insects were reinforced chemically controlled to eliminate bollworm infestation.

2.5.1 Biomass, K concentration and accumulation and K utilization index

Three uniform plants per plot were harvested at squaring (63 DAS), early bloom (78 DAS), peak bloom (92 DAS), and boll-filling (111 DAS) stages and separated into roots, stem, leaves and reproductive organs including squares, flowers and bolls. Dry weights of each part were recorded after being fixed at 105°C for 30 min, then dried at 80°C to a constant weight. The K content was determined with an atomic absorption spectrophotometer (SpectAA-50/55, Varian, Australia); K accumulation was calculated as K concentration times dry weight, and K utilization index was estimated by dividing dry matter by K concentration at the plant level **(Siddiqi & Glass, 1981)**.

2.5.2 Yield and its components

At boll-opening stage, ten plants were randomly selected per plot to count boll numbers and obtain boll weight. Plants of the inner 4 rows per plot were manually picked twice to determine seed cotton yield. Thirty mature bolls were harvested per plot to determine lint percentage.

2.6 Data analysis

Analysis of variance (ANOVA) was performed using SAS V8 (SAS Institute, 2000), and treatment means were compared using Duncan's multiple range tests at $P \leq 0.05$. The figures were plotted using OriginLab 2018 software (OriginLab Corp., Northampton, MA, USA).

3 Results

3.1 The transgenic *Bt* lines had more Bt protein

While grown in the field, the content of Bt protein in the youngest mature leaves of *OE*-29317 and *OE*-29312 was significantly higher than that of WT during squaring and flowering period (54–78 DAS); and *OE*-29317 produced more Bt protein than *OE*-29312 (Fig. S1).

3.2 Effect of *Bt* gene silencing on the K efficiency of cotton seedlings

As shown in Fig. 1B, the *Bt* gene was effectively silenced in transgenic lines, and its relative expression in VIGS-*Bt* plants was only equivalent to 30–36% of VIGS-Ctrl. Grown in low K^+ solutions (0.1 mM K^+) for 40 days, the VIGS-*Bt* plants showed similar interveinal chlorosis in the 3rd -5th leaves compared with VIGS-Ctrl plants (Fig. 1A). Moreover, there were no significant differences in dry matter production (Fig. 1C), K concentration (Fig. 1D) and accumulation (Fig. 1E) in leaves between VIGS-*Bt* and VIGS-Ctrl plants.

3.3 Effect of *Bt* gene on the K efficiency of cotton in the field

3.3.1 Symptoms of K deficiency

In plots without K fertilizers, we observed obvious symptoms of K deficiency in the youngest mature leaf (the fourth leaf from apex) 61 days after sowing (DAS). Unexpectedly, WT showed the most severe symptoms (interveinal chlorosis) relative to *Bt* lines (Fig. 2A).

3.3.2 Biomass yield and K concentration, accumulation and utilization index

Cotton plants showed a logistic growth in the field (Fig. 2B, C). There were no differences in dry matter production between WT and *Bt* lines at 63 DAS (the squaring stage). Thereafter, *OE*-29317 showed the least dry matter per plant, whereas *OE*-29312 had similar value with WT. The application of K fertilizer increased the dry weight of plants, but did not affect the differences between WT and the *Bt* lines (Fig. 2B, C). In addition, the dry matter production of *OE*-29317 decreased after 111 DAS (the boll-filling stage) (Fig. 2B, C), probably due to its severe premature senescence and excessive leaf loss.

From 63 to 111 DAS, the K concentrations in roots, stem and leaves of *OE*-29317 and *OE*-29312 grown in plots without K supply were not significantly lower than those of WT [except the K concentrations in roots of *OE*-29317 at 92 (the peak bloom stage) and 111 DAS] (Fig. 3A, B, C). The K application (225 kg K_2O ha^{-1}) increased K concentrations in roots, stem and leaves to some extent. Under this situation, there were no consistent differences in K concentrations in roots between WT and *Bt* lines (Fig. 3A), and K concentrations in stem and leaves of *Bt* lines were not lower and even significantly higher than those of WT (except *OE*-29317 at 111 DAS) (Fig. 3B, C). The K concentrations in reproductive organs slightly declined at 111 DAS compared with previous growth stages, and there were almost no differences between K supply levels as well as between WT and *Bt* lines (Fig. 3D).

The K accumulations in roots, stem and leaves of *OE*-29317 were less than those of WT from 63 to 111 DAS, whereas those of *OE*-29312 were similar to WT in most situations when no K fertilizers were applied (Fig. 3E, F, G). No consistent differences between *Bt* lines and WT were found in K accumulation in reproductive organs before 111 DAS. However, the *Bt* lines accumulated more K^+ in their reproductive organs than WT at 111 DAS (Fig. 3H). The application of K fertilizers enhanced K accumulation in most organs (Fig. 3E, F, G, H) mainly owing to greater biomass (Fig. 2B, C). From 78 (the early bloom stage) to 111 DAS, *OE*-29317 had less or significantly less K accumulations in roots, stem and leaves than WT; and

OE-29312 accumulated similar K^+ as WT (Fig. 3E, F, G). The *Bt* lines accumulated same or even greater levels of K^+ as WT in the reproductive organs (Fig. 3H).

The K utilization index increased from around 1.5-2.0 $g^2\ mg^{-1}$ at 63 DAS to around 15.0–20.0 $g^2\ mg^{-1}$ at 111 DAS (Fig. 3I). There were little differences in this trait between K supply levels. The *Bt* lines showed a lower K utilization index than WT at 63, 78 (except *OE*-29312 without K supply) and 92 DAS (except *OE*-29312 with K supply) but not at 111 DAS (Fig. 3I).

3.3.3 Yield and yield components

Both *Bt* lines had more bolls than WT (Fig. 4A), and the boll weight of *OE*-29312 was similar to that of WT while *OE*-29317 produced smaller bolls (Fig. 4B). The introduction of *Bt* gene did not significantly decrease the seed cotton yield; it even increased yield in some situations (Fig. 4C). The lint percentage of *Bt* lines was not lower than that of WT (Fig. 4D).

4 Discussion

Definitions of plant nutrient efficiency vary greatly. Blair (1993) defined nutrient efficiency of a genotype/cultivar as the ability to acquire nutrients from a growth medium and/or to incorporate or utilize them in the production of biomass. The internal nutrient requirement of the plant was generally defined as total plant biomass produced per unit nutrient absorbed **(Gourley et al., 1994)**. However, Siddiqi and Glass (1981) suggested a more appropriate measure of nutrient efficiency, utilization index, which is the product of yield and the reciprocal of nutrient concentration (i.e. biomass produced by per unit nutrient concentration). We found that in the field, *OE*-29312 acquired similar or more K^+ compared with WT from squaring stage (63 DAS) to boll-filling stage (111 DAS), and *OE*-29317 accumulated less or similar K^+ as WT (Fig. 3E, F, G, H). In addition, there was a tendency that the two *Bt* lines had lower K utilization index than WT during squaring and flowering period (63–92 DAS). However, the *Bt* lines showed similar K utilization index as WT (no fertilizer application) or greater K utilization index than WT at 225 kg $K_2O\ ha^{-1}$ supply at 111 DAS (Fig. 3I). These results suggest that the ability of *Bt* lines to acquire and utilize K was not always inferior to WT.

Sometimes the efficient plants are defined as those with fewer deficiency symptoms **(Clark, 1990; Tian et al., 2008)**. **Cakmak (2005)** considered that leaf chlorosis caused by K deficiency was related to oxidative degradation of chlorophyll by excess production of reactive oxygen species (ROS). In this study, *OE*-29312 and *OE*-29317 showed less interveinal chlorosis in mature leaves than WT in the field with low available K (Fig. 2A), which does not support the view that *Bt* gene introduction reduced the K efficiency of Bt cotton.

Relative to the ability to acquire and utilize nutrients as well as deficiency symptoms, nutrient efficiency was more broadly considered as the ability of a genotype to grow well in a soil deficient in that nutrient (very low bioavailability) and produce higher biomass, especially in the harvestable component, than other standard genotypes **(Blair, 1993; Buso & Bliss, 1988; Clark, 1990; Fageria et al., 2008; Gourley et al.,**

1994; Zhu et al., 2002). In this study, the knockdown of *Bt* gene in *OE*-29317 and *OE*-29312 by using virus-induced gene silencing (VIGS) did not result in more biomass production and greater K concentration and accumulation relative to VIGS-Ctrl plants (Fig. 1). Moreover, the seed cotton yield of *OE*-29317 and *OE*-29312 in the field with low soil-test K (47.8 mg kg^{-1}) was not lower than that of WT under the reinforced chemical control of insects (Fig. 4), indicating that the *Bt* gene introduction does not affect K efficiency of cotton concerning the harvestable product. Also, **Wilson et al. (1994)** reported that the expression of *Bt* insecticidal gene caused no general reduction in lint yield compared with their parental cultivar. **Verhalen et al. (2003)** found that the Bollgard (BG) genes (*Cry1Ac* and *Cry2Ab*) were stable for lint yield across genetic backgrounds.

Therefore, we think that the *Bt* gene introduction did not necessarily decrease the K use efficiency of cotton. Cotton growers can improve the K efficiency of *Bt* cotton by careful varietal selection.

5 Conclusions

Because of the concurrence of widespread potassium (K) deficiency and popularization of transgenic *Bacillus thuringiensis* (*Bt*) cotton, the introduction of *Bt* genes was speculated to be a main factor that impairs the K efficiency of cotton. However, the results of our study indicated that *Bt* gene did not necessarily reduce the K efficiency of cotton at least under the conditions of this study. When the *Bt* gene in two transgenic lines was subjected to virus-induced gene silencing (VIGS), no additional dry biomass was produced. In addition, the seed cotton yield and lint percent of *Bt* lines were not lower than those of wild type in the field with low soil-test K^+.

Declarations

Author Contributions

F. L., and X. T., designed the research; Q. W., M. Z. and W. Y. performed the experiments with the assistance of Y. Z.; X. L., A. Z. and J. X. generated the transgenic *Bt* lines; Q. W., M. Z., W. Y., F. L., and X. T. analyzed the data; Q. W., M. Z., and X. T. wrote the draft manuscript; A. E. gave important technical suggestions and improved the writing. All authors reviewed the manuscript.

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study, data collection, analyses, or interpretation, writing of the manuscript, or the decision to publish the results.

Acknowledgements

This study was supported by the Genetically Modified Organisms Breeding Major Projects of China Grant (2016ZX08005–004). We appreciate Professor Yuejin Shi of Shanxi Agricultural University/Institute of

Cotton Research, Shanxi Academy of Agriculture Science for the help in generation of cotton transgenic *Bt* lines, and thank graduate student Zhixin Tan for the help in processing data.

References

1. Balik, J., Kulhanek, M., Cerny, J., Sedlar, O., & Suran, P. (2020). Potassium fractions in soil and simple K balance in long-term fertilising experiments. *Soil and Water Research, 15*(4), 211–219.
2. Blair, G. (1993). Nutrient efficiency — what do we really mean? *Springer Netherlands*. In: Randall P.J., Delhaize E., Richards R.A., Munns R. (eds) Genetic Aspects of Plant Mineral Nutrition. *Developments in Plant and Soil Sciences*, vol 50. Springer, Dordrecht.
3. Buso, G. S. C., & Bliss, F. A. (1988). Variability among lettuce cultivars grown at two levels of available phosphorus. *Plant and Soil, 111*(1), 67–73.
4. Cakmak, I. (2005). The role of potassium in alleviating detrimental effects of abiotic stresses in plants. *Journal of Plant Nutrition and Soil Science, 168*(4), 521–530.
5. Cassman, K. G., Roberts, B. A., Kerby, T. A., Bryant, D. C., & Higashi, S. L. (1989). Soil Potassium-Balance and Cumulative Cotton Response To Annual Potassium Additions on a Vermicultitic Soil. *Soil Science Society of America Journal, 53*(3), 805–812.
6. Clark, R. B. (1990). Physiology of cereals for mineral nutrient uptake,use,and efficiency. *Crops As Enhancers of Nutrient Use*.
7. Dong, H. Z., Kong, X. Q., Li, W. J., Tang, W., & Zhang, D. M. (2010). Effects of plant density and nitrogen and potassium fertilization on cotton yield and uptake of major nutrients in two fields with varying fertility. *Field Crops Research, 119*(1), 106–113.
8. Fageria, N. K., Baligar, V. C., & Li, Y. C. (2008). The role of nutrient efficient plants in improving crop yields in the twenty first century. *Journal of Plant Nutrition, 31*(6), 1121–1157.
9. Gourley, C. J. P., Allan, D. L., & Russelle, M. P. (1994). Plant Nutrient Efficiency - a Comparison of Definitions and Suggested Improvement. *Plant and Soil, 158*(1), 29–37.
10. Gurr, S. J., & Rushton, P. J. (2005). Engineering plants with increased disease resistance: how are we going to express it? *Trends in Biotechnology, 23*(6), 283–290.
11. Hu, W., Lv, X. B., Yang, J. S., Chen, B. L., Zhao, W. Q., Meng, Y. L., Oosterhuis, D. M. (2016). Effects of potassium deficiency on antioxidant metabolism related to leaf senescence in cotton *(Gossypium hirsutum L.)*. *Field Crops Research, 191*, 139–149.
12. Kumar, K., Gambhir, G., Dass, A., Tripathi, A. K., Singh, A., Jha, A. K., Rakshit, S. (2020). Genetically modified crops: current status and future prospects. *Planta, 251*(4).
13. Ladics, G. S., Bartholomaeus, A., Bregitzer, P., Doerrer, N. G., Gray, A., Holzhauser, T., Glenn, K. (2015). Genetic basis and detection of unintended effects in genetically modified crop plants. *Transgenic Research, 24*(4), 587–603.
14. Li, F. J., Li, M. Y., Wang, P., Cox, K. L., Duan, L. S., Dever, J. K., He, P. (2017). Regulation of cotton *(Gossypium hirsutum)* drought responses by mitogen-activated protein (MAP) kinase cascade-

mediated phosphorylation of GhWRKY59. *New Phytologist, 215*(4), 1462–1475.

15. Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method. *Methods, 25*(4), 402–408.
16. Marrelli, M. T., Moreira, C. K., Kelly, D., Alphey, L., & Jacobs-Lorena, M. (2006). Mosquito transgenesis: what is the fitness cost? *Trends in Parasitology, 22*(5), 197–202.
17. Miki, B., Abdeen, A., Manabe, Y., & MacDonald, P. (2009). Selectable marker genes and unintended changes to the plant transcriptome. *Plant Biotechnology Journal, 7*(3), 211–218.
18. Mullins, G. L., & Burmester, C. H. (2010). Relation of Growth and Development To Mineral Nutrition. *Physiology of Cotton*, 97–105.
19. Perlak, F. J., Deaton, R. W., Armstrong, T. A., Fuchs, R. L., Sims, S. R., Greenplate, J. T., & Fischhoff, D. A. (1990). Insect Resistant Cotton Plants. *Bio-Technology, 8*(10), 939–943.
20. Rochester, I. J. (2007). Nutrient uptake and export from an Australian cotton field. *Nutrient Cycling in Agroecosystems, 77*(3), 213–223.
21. Ryan, J. G., Estefan, G., & Rashid, A. (2002). Soil–Plant-AnalysisSoil and Plant Analysis Laboratory Manual.
22. Sale, P. (2003). Principles of Plant Nutrition: K. Mengel, E.A. Kirkby; Kluwer Academic Publishers, Dordrecht, The Netherlands, July 2001, ISBN No.: 079237150X (USD 320; EUR 350; GBP 220). *35*(8), 1159–1160.
23. Schnell, J., Steele, M., Bean, J., Neuspiel, M., Girard, C., Dormann, N., & Macdonald, P. (2015). A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments. *Transgenic Research, 24*(1), 1–17.
24. Siddiqi, M. Y., & Glass, A. D. M. (1981). Utilization Index - a Modified Approach To the Estimation and Comparison of Nutrient Utilization Efficiency in Plants. *Journal of Plant Nutrition, 4*(3), 289–302.
25. Steiner, F., Pivetta, L. A., Castoldi, G., Costa, M. S. S. D., & Costa, L. A. D. (2012). Phosphorus and potassium balance in soil under crop rotation and fertilization. *Semina-Ciencias Agrarias, 33*(6), 2173–2186.
26. Tian, X. L., Wang, G. W., Zhu, R., Yang, P. Z., Duan, L. S., & Li, Z. H. (2008). Conditions and Indicators for Screening Cotton (Gossypium hirsutum L.) Varieties Tolerant to Low Potassium. *ACTA AGRONOMICA SINICA*.
27. Tian, X. L. (2009). Differences in Tolerance to Low-Potassium Supply among Different Types of Cultivars in Cotton *(Gossypium hirsutum L.)*. *ACTA AGRONOMICA SINICA, 34*(10), 1770–1780.
28. Urrego, D., Tomczak, A. P., Zahed, F., Stuhmer, W., & Pardo, L. A. (2014). Potassium channels in cell cycle and cell proliferation. *Philos Trans R Soc Lond B Biol Sci, 369*(1638), 20130094.
29. Verhalen, L. M., Greenhagen, B. E., & Thacker, R. W. (2003). Lint yield, lint percentage, and fiber quality response in Bollgard, Roundup Ready, and Bollgard/Roundup Ready cotton. *Journal of Cotton Science*(2).

30. Wang, M., Zheng, Q. S., Shen, Q. R., & Guo, S. W. (2013). The Critical Role of Potassium in Plant Stress Response. *International Journal of Molecular Sciences, 14*(4), 7370–7390.
31. Wang, Y. Q., Liang, C. Z., Wu, S. J., Zhang, X. Y., Tang, J. Y., Jian, G. L., & Chu, C. C. (2016). Significant Improvement of Cotton Verticillium Wilt Resistance by Manipulating the Expression of Gastrodia Antifungal Proteins. *Molecular Plant, 9*(10), 1436–1439.
32. Wilson, F. D., Flint, H. M., Deaton, W. R., & Buehler, R. E. (1994). Yield, Yield Components, and Fiber Properties of Insect-Resistant Cotton Lines Containing a Bacillus-Thuringiensis Toxin Gene. *Crop Science, 34*(1), 38–41.
33. Wright, P. R. (1999). Premature senescence of cotton *(Gossypium hirsutum L.)* - Predominantly a potassium disorder caused by an imbalance of source and sink. *Plant and Soil, 211*(2), 231–239.
34. Wu, S. J., Wang, H. H., Li, F. F., Chen, T. Z., Zhang, J., Jiang, Y. J.,Zhang, T. Z. (2008). Enhanced Agrobacterium-mediated Transformation of Embryogenic Calli of Upland Cotton via Efficient Selection and Timely Subculture of Somatic Embryos. *Plant Molecular Biology Reporter, 26*(3), 174–185.
35. Zhu, Y. G., Huang, C., Howes, N. K., & Smith, S. E. (2002). Breeding for low-nutrient environments. *Plant & Soil, 245*(1), 47–57.

Figures

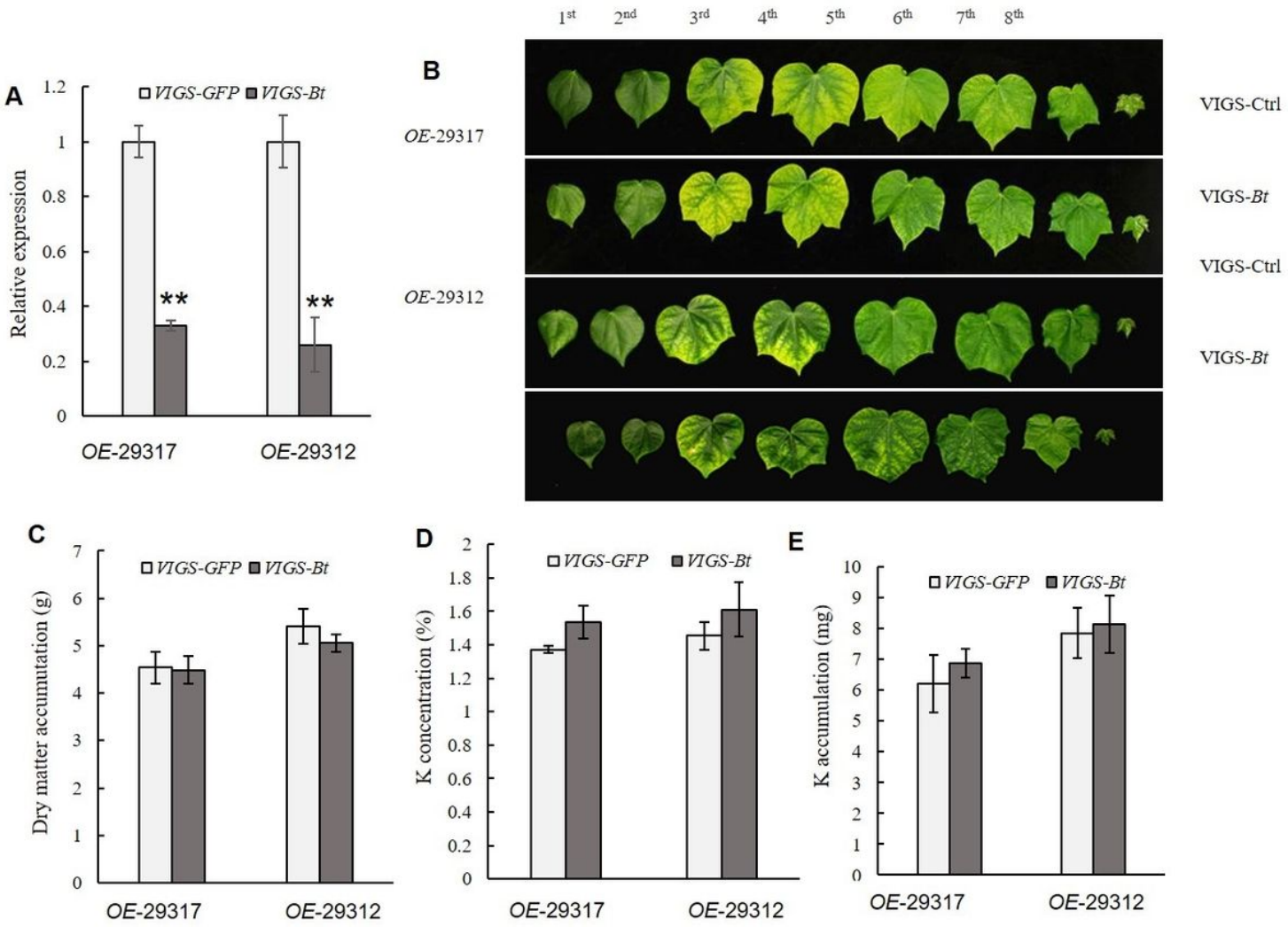

Figure 1

The symptoms of K deficiency (A), relative expression of *Bacillus thuringiensis* (*Bt*) gene (B), dry weight (C), K concentration (D) and accumulation (E) in leaves of transgenic *Bt* lines whose *Bt* gene was knocked down by virus-induced gene silencing (VIGS) method. The VIGS was performed on fully expanded cotyledons. When leaves of plants infiltrated with pTRV:*GhCLA1* (positive control) showed an albino phenotype, part of the VIGS-Ctrl and VIGS-*Bt* plants were sampled to perform the quantitative RT-PCR analysis of *Bt* gene, and the rest of seedlings were transferred to 0.1 mM K^+ solutions. After 40 days, VIGS-Ctrl and VIGS-*Bt* plants were photographed and harvested. ** represents the significant differences ($P < 0.01$).

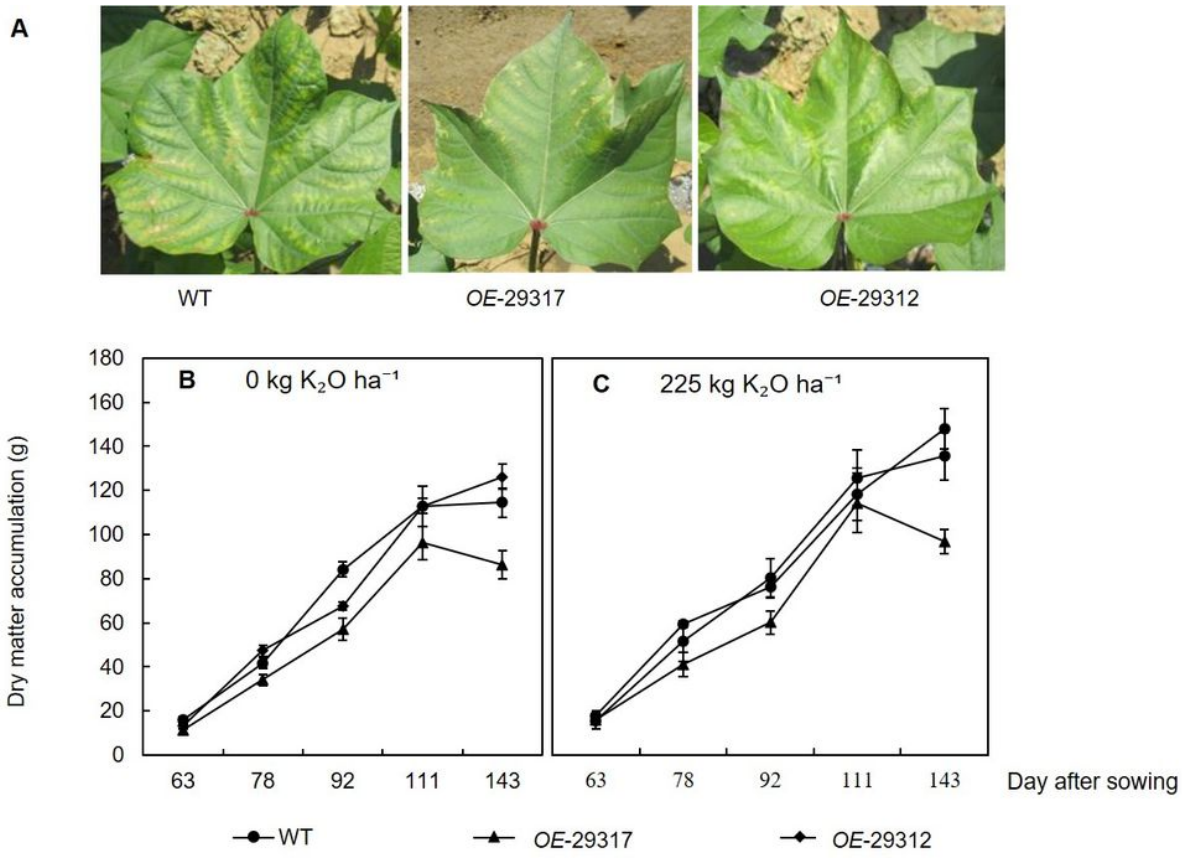

Figure 2

Effects of *Bacillus thuringiensis* (*Bt*) gene on the symptoms of potassium (K) deficiency in the youngest fully expanded leaf (the fourth leaf from apex) 61 days after sowing in the field (A), dry matter production of cotton plants in plots without (B) or with (C) K fertilizer (225 kg K_2O ha^{-1}). The available K^+ in soil was 47.8 mg kg^{-1}. DAS: days after sowing. WT: wild type (Jihe 321); *OE*-29317 and *OE*-29312: transgenic *Bt* lines.

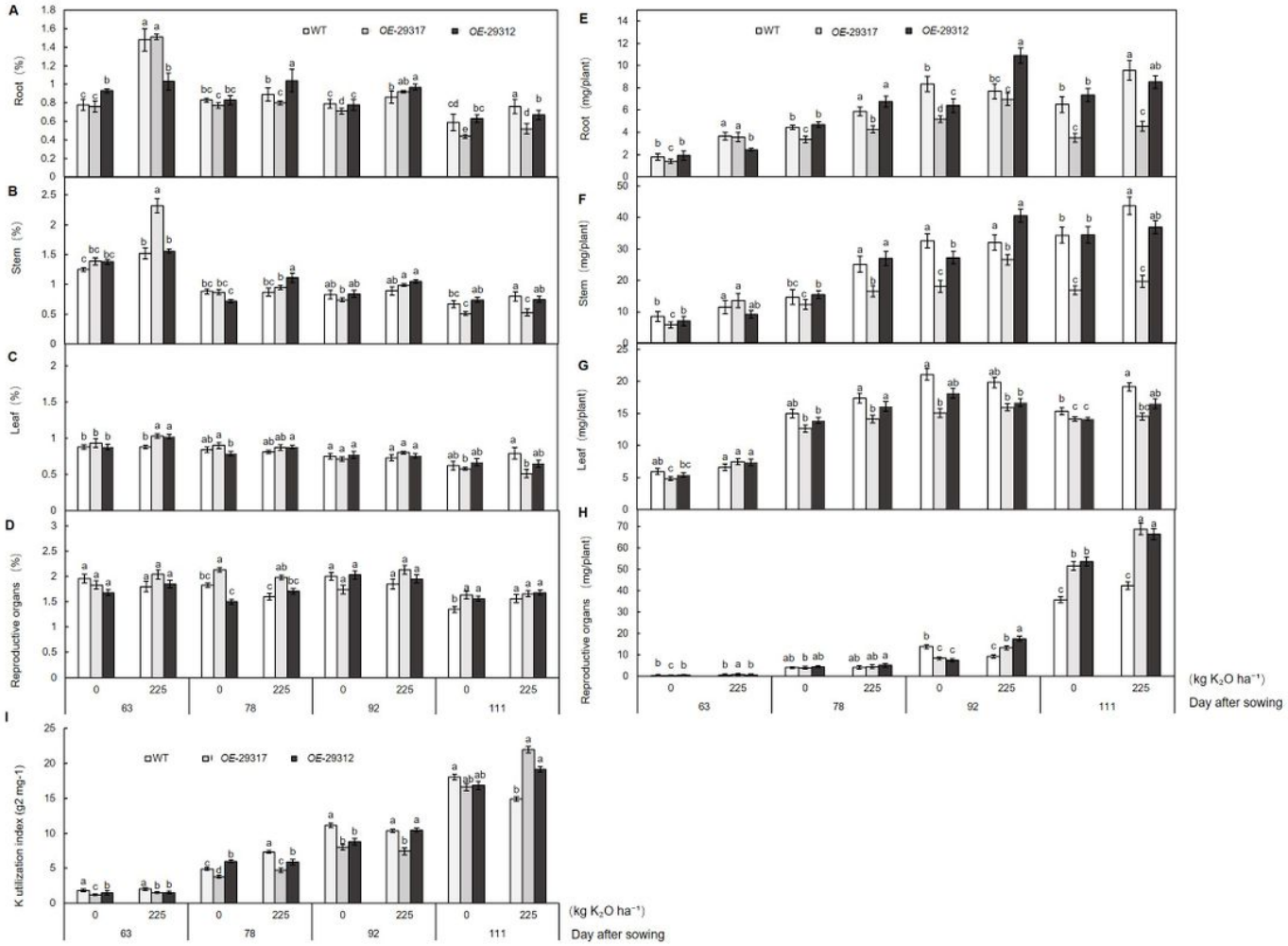

Figure 3

Effects of *Bacillus thuringiensis* (*Bt*) genes on potassium (K) concentration (A-D), accumulation (E-G) and utilization index (I, the dry matter produced per unit K concentration at the plant level) in roots (A, E), stems (B, F), leaves (C, G) and reproductive parts (D, F, including squares, flowers and bolls) of cotton plants in the field. The available K^+ in soil was 47.8 mg kg^{-1}. DAS: days after sowing. WT: wild type (Jihe 321); *OE*-29317 and *OE*-29312: transgenic *Bt* lines.. Bars with different letters indicate significant differences within the same day ($P < 0.05$). Error bars depict standard errors of the mean.

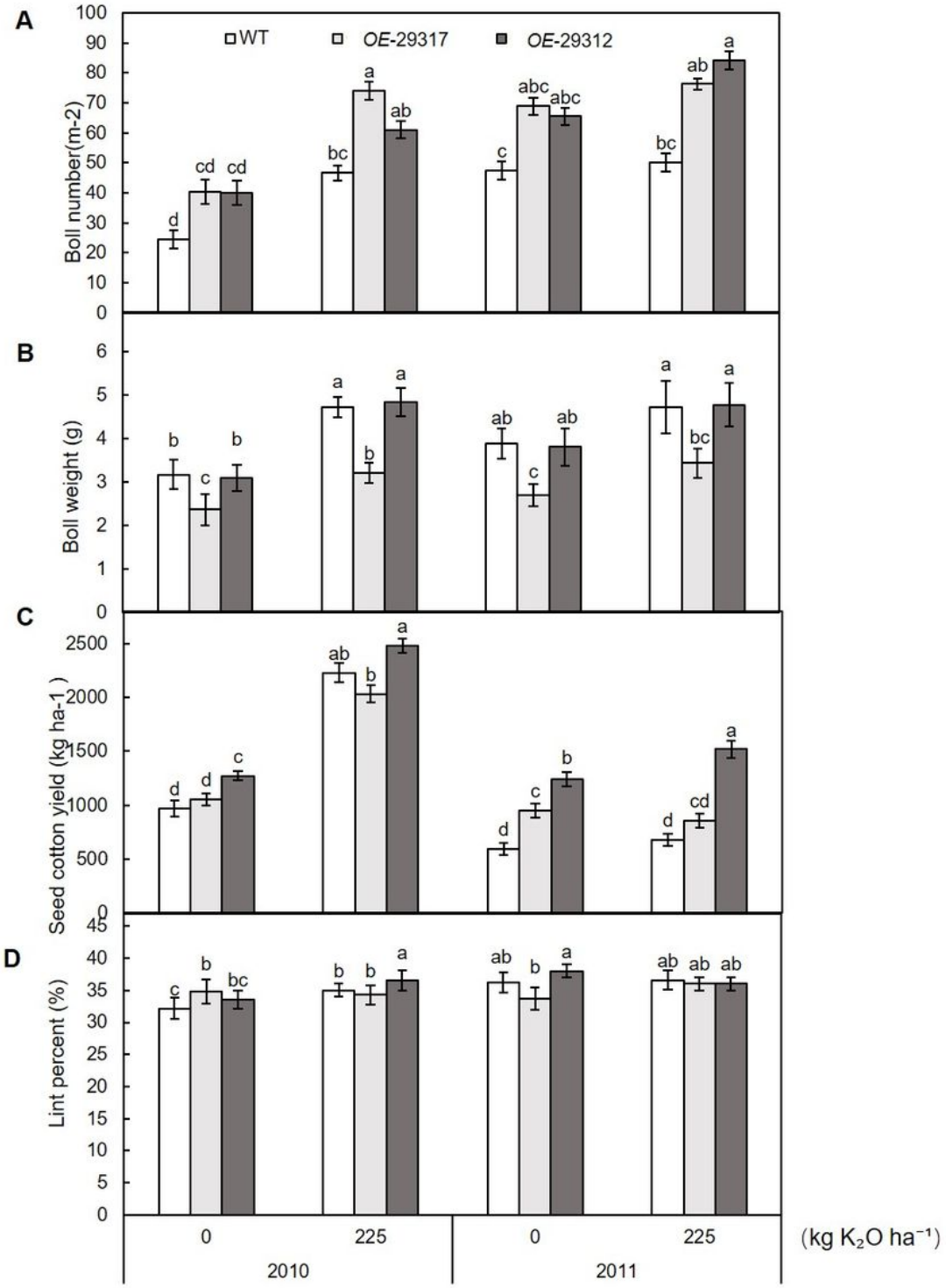

Figure 4

Effects of *Bacillus thuringiensis* (Bt) gene on the boll number (A), boll weight (B), seed cotton yield (C) and lint percent (D) in the field without or with potassium fertilizer (225 kg K_2O ha^{-1}). The available K^+ in soil was 47.8 mg kg^{-1}. WT: wild type (Jihe 321); *OE*-29317 and *OE*-29312: transgenic *Bt* lines. Bars with different letters indicate significant differences within the same year ($P < 0.05$). Error bars depict standard errors of the mean.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FIGS1.docx