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Introduction of *Bacillus thuringiensis* (*Bt*) gene does not reduce potassium use efficiency of *Bt* transgenic cotton (*Gossypium hirsutum* L.)

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Abstract

Background: Potassium (K) deficiency has become 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 induce K deficiency.

Results: The cotton variety, Jihe 321 (wild type, WT) and its two *Bt* (*Cry1Ac*)-transgenic overexpression lines (OE-29317, OE-29312) were studied in field with low soil-test K^+ (47.8 mg·kg^{-1}). In the field with low soil-test K^+, only OE-29317 had less biomass and K^+ accumulation than the WT at some growth stages. Both *Bt* lines produced similar or even greater seed cotton yield than WT in the field. When the *Bt* gene (~ 70%) in OE-29317 and OE-29312 plants was silenced by virus-induced gene silencing (VIGS), the VIGS-*Bt* plants did not produce more biomass than VIGS-green fluorescent protein (control) plants.

Conclusions: The introduction of *Bt* gene did not necessarily hinder the K use efficiency of the cotton lines under this study.

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

Introduction

Potassium (K) is one of the essential macronutrients for plant growth and development (Sale 2003). It plays a crucial role 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, a negative K^+ balance in the soil (around − 60 kg·hm^{-2} annually) was reported (Dong et al. 2010; Hu et al. 2016) and has continued to exacerbate (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 deficiency than most other field crops due to its sparse root system (Cassman et al. 1989; Mullins et al. 2010). Since the 1990s, the premature senescence of cotton caused by K deficiencies has occurred frequently and with greater intensity worldwide (Wright 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 the insects damage to cotton yield (Perlak et al. 1990).

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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 (Ladics et al. 2015; Verhalen et al. 2003) because of random gene insertion (that could disrupt the function of the native gene of the host genome) (Marrelli et al. 2006), random mutation, somaclonal variation, pleiotropy, position effect, the tissue culture process during the construction 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 transgenes (Gurr et al. 2005).

To determine whether the common K deficiency under *Bt* cotton production was due to its lower K use efficiency (one of the unintended effects), we previously compared K use 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 2009). We have also carried out more recent studies on the question of K use in cotton (Wang et al. 2019; Zhang et al. 2021; Yang et al. 2021). However, we are not sure yet whether the *Bt* gene transformation directly decreased the K use efficiency since the genetic background of the tested *Bt*- and conventional cotton cultivars/lines was different.

Therefore, we generated two independent *Bt* overexpression cotton lines by introducing the *Bt* gene into a wild type (WT) cotton variety. Here, the K use efficiency of *Bt* lines was compared with WT via field experiments. Also, virus-induced gene silencing (VIGS) technique was used to knock down the *Bt* gene of transgenic lines and compared their K use efficiency with VIGS-green fluorescent protein (GFP) (control) plants. 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 under *Bt* cotton production.

Materials and methods

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

The cotton variety, Jihe 321, was used as the 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 a callus induction medium under a 12 h/12 h (day/night) photoperiod and 2 000 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. The embryoids were then transferred to a differentiation medium to grow into plantlets that were sequentially moved into a seedling growth medium. When the regenerated plants grew to 5 ~ 8 cm in height, 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 generated; the plant size of OE-29312 was similar to WT, and that of OE-29317 was smaller than WT under normal growth conditions.

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 the early squaring—(54 days after sowing, DAS), squaring—(63 DAS), and early bloom (78 DAS) stages to determine the content of Bt protein, using ELISA kit (CryIAb/Cry1Ac Plate kit AP003, EnviroLogix, Portland, ME, USA).

Virus-induced gene silencing (VIGS) and phenotypic identification

Vector construction, transformation and infection

VIGS assay was performed as described previously (Li et al. 2017). Briefly, 161 bp fragments of the *Bt* gene (*Cry1Ac*) were amplified and cloned into a binary tobacco rattle virus (TRV) vector. The forward primer sequence (5′-3′) was GGGGTACCTGTGTCTCTCTTCCCG AAC, and reverse primer sequence was CGGAATTCT GCTGGTTGTTGATACCG. Plasmids of TRV vectors pTRV-RNA1 or pTRV-RNA2 [pYL156-GFP (green fluorescent protein, negative control), pYL156-*Cry1Ac*, pYL156-*GhCLA1* (positive control)] were transformed into the *Agrobacterium tumefaciens* strain, GV3101 by electroporation, respectively. 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 infiltrated into just fully expanded cotyledons of cotton plants, using a needle-less syringe in a growth chamber.

Growth conditions

The growth chamber was set with photosynthetic photon flux density of 600 μmol·cm^{-2}·s^{-1}, mean humidity of 70%∼90%, and light/dark regime of 14 h/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 (in mmol·L^{-1}) 1.25 KNO_3, 2.5 $Ca(NO_3)_2$, 1.0 $MgSO_4$, 0.5 $NH_4H_2PO_4$, 2×10^{-4} $CuSO_4$, 1×10^{-3} $ZnSO_4$, 0.1 EDTA Fe-Na, 2×10^{-2} H_3BO_3, 5×10^{-6} $(NH_4)_6Mo_7O_{24}$, and 1×10^{-3} $MnSO_4$. The solution was changed every 3∼4 d and continuously aerated with an air pump.

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

When the leaves of pYL156-*GhCLA1* plants showed an albino phenotype, the VIGS-GFP and VIGS-*Bt* plants were transferred to 0.1 mmol·L^{-1} K^+ (KNO_3) solutions. After 40 days, all leaves of the seedlings were photographed to observe the symptoms of K deficiency, then leaves were inactivated at 105 °C for 30 min and dried at 80 °C to a constant weight. After grinding the dried sample, K was extracted by wet digestion with 1 mol·L^{-1} HCl solution, and the K content was determined using an atomic absorption spectrometer (SpectAA-50/55, Varian, Australia).

Quantitative real time polymerase chain reaction (qRT-PCR) analysis of *Bt* gene expression

When positive control plants of pYL156-*GhCLA1* showed an albino phenotype, total RNA was extracted from the young leaves of VIGS-GFP and VIGS-*Bt* seedlings using an RNAprep Pure Plant Kit and purified with RNase-free DNase I (both from Tiangen). cDNA was synthesized with 2 μg RNA using Oligo (d T) primer and M-MLV reverse transcriptase (TaKaRa). qRT-PCR was conducted in an Applied Biosystems 7500 Fast Real-Time PCR System using SYBR® Premix Ex Taq™ (TaKaRa) under the following procedures: 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 relative expression level of *Bt* gene was determined relative to the reference gene, *GhACTIN9* and was calculated using the $2^{-\triangle\triangle CT}$ method (Livak et al. 2001). The forward and reverse primers sequence for qRT-PCR of *Bt* gene were CGTGGTTCTGCCCAAGGTAT, and CGATACGTT GTTGTGGAGCG, respectively. Three biological replicates were performed.

Field experiments

Experimental design

Field experiments were conducted at Shangzhuang experimental station (40° 08′ 12.15″ N; 116° 10′ 44.83″ E) of China Agricultural University, in Beijing during 2010–2011. The soil was sandy loam with pH of 7.8 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 cotton growing seasons are shown in Table 1.

The experiment was arranged into a split-plot design with four replications. The levels of K supply (0 and 225 kg·hm^{-2} K_2O) were assigned to main plots, and WT and transgenic *Bt* lines (OE-29312 and OE-29317) were assigned to the subplots. The pre-plant fertilizer treatment included 120 kg·hm^{-2} N (in the form of diammonium phosphate and urea), 186 kg·hm^{-2} P_2O_5 (in the form of diammonium phosphate), and 135 kg·hm^{-2} K_2O (in the form of potassium sulphate, only for K-supplied plots). At squaring and peak bloom stages,

Table 1 Monthly mean temperature and precipitation during cotton growing seasons

Month	Mean temperature /℃			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

69 kg·hm^{-2} N (in the form of urea) were top dressed. For K treated plots, 90 kg·hm^{-2} K_2O (in the form of potassium sulphate) was top dressed at the 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·hm^{-2}. Seeds were planted on May 8, 2010, and April 26, 2011. Insect pests were chemically controlled to eliminate bollworm infestation.

Determination of biomass accumulation, K concentration, K accumulation and K utilization index

Three uniform plants per plot were harvested at squaring (63 DAS), early bloom (78 DAS), peak bloom (92 DAS), boll-filling (111 DAS), and boll-opening (143 DAS) stages and separated into roots, stem, leaves and reproductive organs including squares, flowers, and bolls. Dry weights of each part were recorded after oven-drying at 105 °C for 30 min, then 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 the product of K concentration and dry weight; K utilization index was estimated by dividing dry matter by K concentration at the plant level (Siddiqi et al. 1981).

Determination of yield and its components

At the boll-opening stage, ten plants were randomly selected per plot to count boll numbers and measure the 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.

Data analysis

Analysis of variance (ANOVA) was performed using SAS V8 (SAS Institute, 2000), and the data 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).

Results

The transgenic *Bt* lines had much more Bt protein than WT

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), respectively; and OE-29317 had more Bt protein than OE-29312 (Additional file 1: Fig. S1).

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

Symptoms of K deficiency

In plots without application of K fertilizers, we observed obvious symptoms of K deficiency in the youngest mature leaf (the fourth leaf from apex) at 61 DAS. Unexpectedly, WT showed the most severe symptoms (interveinal chlorosis) relative to *Bt* lines (Fig. 1A).

Biomass yield and K concentration, accumulation and utilization index

The plants showed a logistic growth in the field (Fig. 1B, C). There were almost no differences in dry matter accumulation among WT and *Bt* lines at 63 DAS (the squaring stage). Thereafter, OE-29317 showed the lowest dry matter accumulation 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 among WT and the *Bt* lines (Fig. 1B, C). In addition, the dry matter accumulation of OE-29317 decreased after 111 DAS (the boll-filling stage) (Fig. 1B, C), probably due to its leaf loss.

From 63 to 143 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 concentrations in roots of OE-29317 at 92, 111 and 143 DAS] (Fig. 2A–C). The K application (225 kg·hm^{-2} K_2O) increased K concentrations in roots, stem, and leaves to some extent. Under this situation, there were no consistent differences in K concentrations in roots of WT and *Bt* lines (Fig. 2A), and the K concentrations in stem and leaves of *Bt* lines were statistically similar or even significantly higher than those of WT from 63 to 111 DAS (except OE-29317 at 111 DAS); the K concentration in the stem of *Bt* lines was significantly lower than that in WT at 143 DAS (Fig. 2B, C). In reproductive organs, the K concentrations declined slightly at 111 and 143 DAS compared with previous growth stages, and there were almost no differences in K concentrations among K supply levels as well as among WT and *Bt* lines (Fig. 2D).

The K accumulations in roots, stem and leaves of OE-29317 were less than those of WT from 63 to 143 DAS, whereas those of OE-29312 were similar to WT in most situations when no K fertilizers were applied (Fig. 2E–G). No consistent differences among *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 and 143 DAS (Fig. 2H). The application of K fertilizers enhanced K accumulation in most organs (Fig. 2E–H) mainly owing to greater biomass (Fig. 1B, C). From 78 (the early bloom stage) to 143 DAS, OE-29317 had less

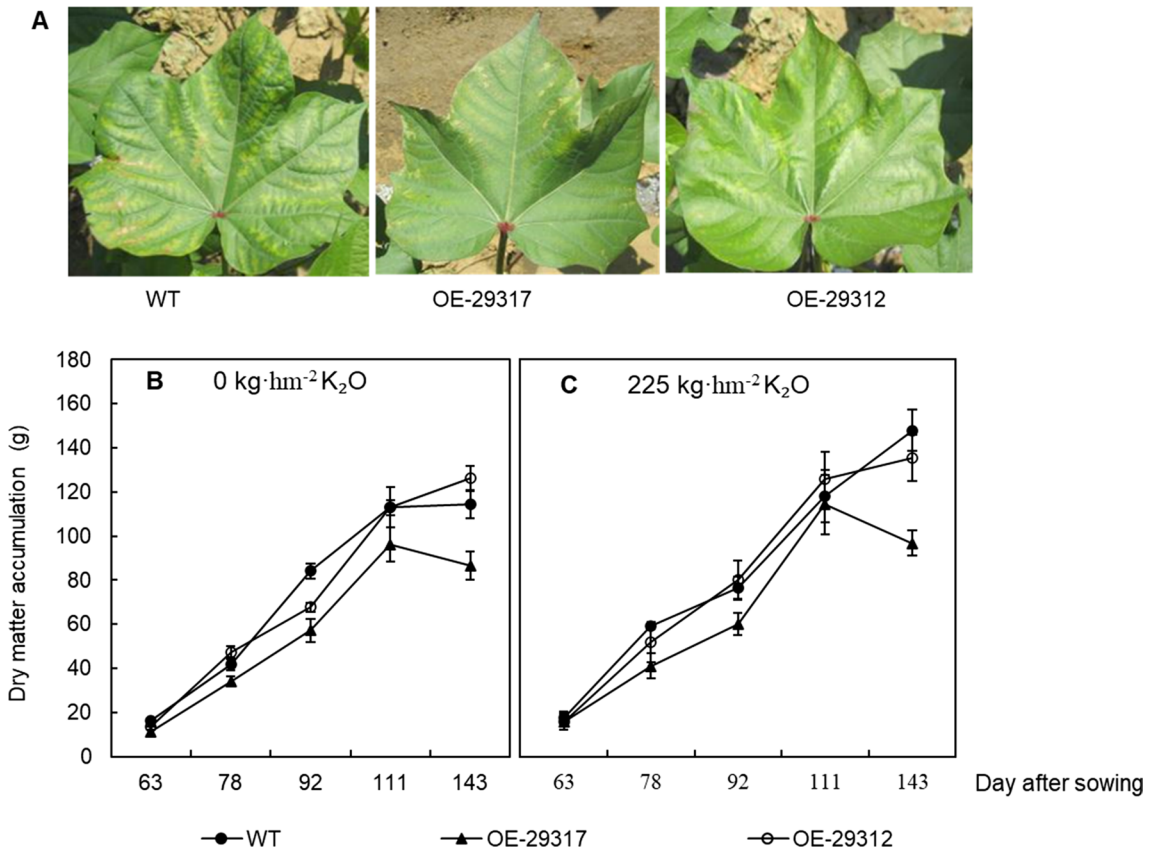

Fig. 1 Effects of *Bacillus thuringiensis* (*Bt*) gene on the symptoms of potassium (K) deficiency in the youngest fully expanded leaf (the fourth leaf from apex) 61 days after sowing in the field (**A**), dry matter production of cotton plants in plots without (**B**) or with (**C**) K fertilizer (225 kg·hm^{-2} K_2O). 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

or significantly less K accumulations in roots, stem, and leaves than WT; the K accumulation in roots, stem, and leaves of OE-29312 was not significantly lower than that of WT from 78 to 111 DAS under the application of K fertilizers, but the situations in roots and stem was opposite at 143 DAS (Fig. 2E–G). The *Bt* lines accumulated same or even greater levels of K^+ as WT in the reproductive organs in most situations (Fig. 2H).

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. 2I). There were little differences in this trait between K supply levels. Both *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 the K utilization index of *Bt* lines was significantly higher than that of WT with K supply at 111 DAS (Fig. 2I).

Yield and its components

Both *Bt* lines had more bolls than WT (Fig. 3A), and the boll weight of OE-29312 was similar to that of WT, while OE-29317 produced smaller bolls (Fig. 3B). The introduction of *Bt* gene did not significantly decrease the seed cotton yield; it even increased seed cotton yield in some situations (Fig. 3C). The lint percentage of *Bt* lines was comparable to that of WT in most situations (Fig. 3D).

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

Grown in low K^+ solutions (0.1 mmol·L^{-1}) for 40 days, the VIGS-*Bt* plants showed similar interveinal chlorosis in the 3rd∼5th leaves as VIGS-GFP plants (Fig. 4A). As shown in Fig. 4B, the *Bt* gene was effectively silenced in transgenic lines, and its relative expression levels in VIGS-*Bt* plants were only equivalent to 30%∼36% of VIGS-GFP. Moreover, there were little differences in dry matter accumulation (Fig. 4C), K concentration (Fig. 4D), and K accumulation (Fig. 4E) in leaves of VIGS-*Bt* and VIGS-GFP plants.

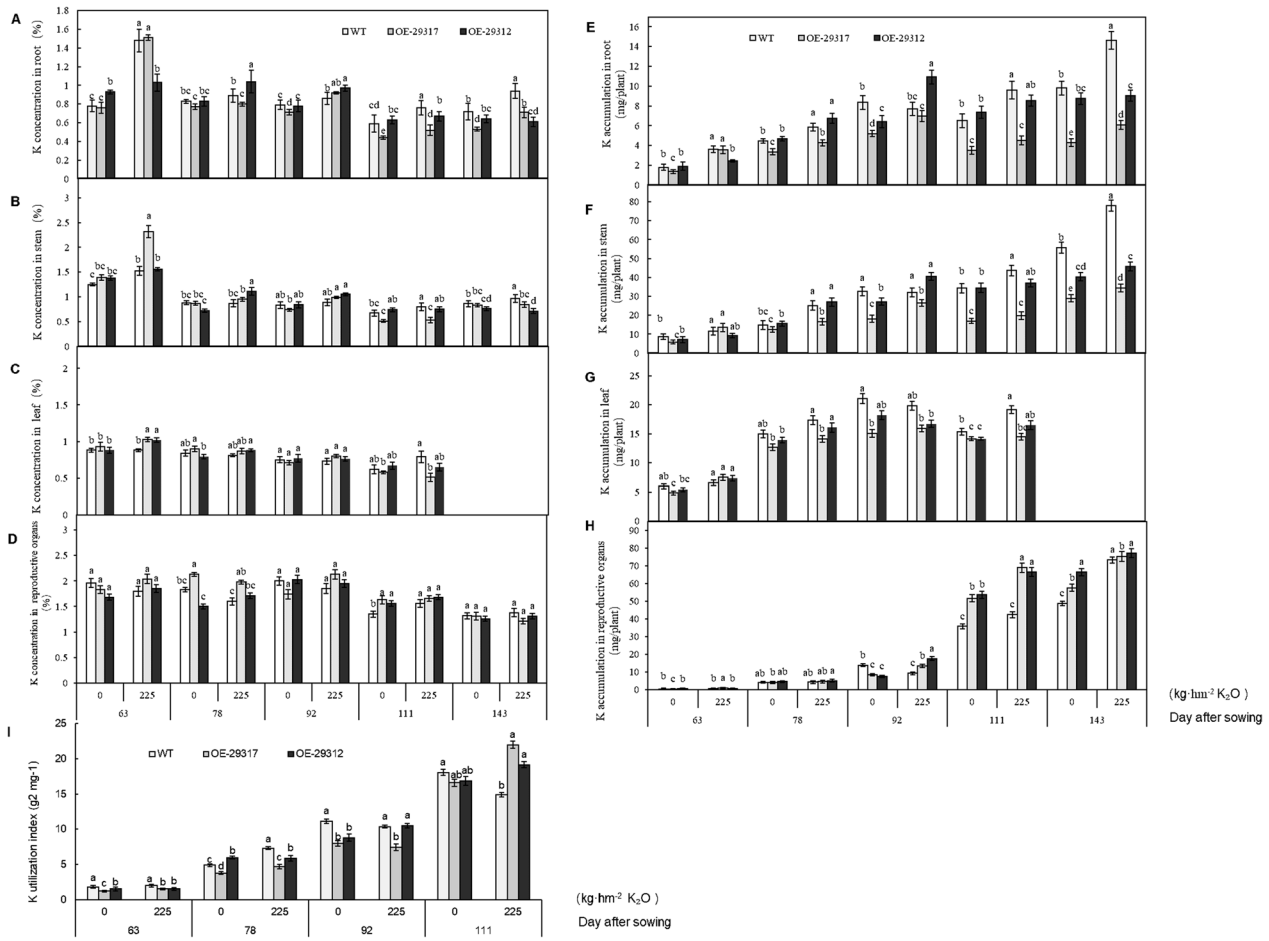

Fig. 2 Effects of *Bacillus thuringiensis* (*Bt*) genes on potassium (K) concentration (**A**–**D**), accumulation (**E**–**G**) and utilization index (I, the dry matter produced per unit K concentration at the plant level) in roots (**A**, **E**), stems (**B**, **F**), leaves (**C**, **G**) and reproductive parts (**D**, **H**, 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

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 et al. (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 per unit nutrient concentration). In this study, it was found that in the field, OE-29312 acquired similar or more K^+ than WT in most situations in reproduction organs from squaring stage (63 DAS) to boll-filling stage (111 DAS), and OE-29317 accumulated less or similar K^+ as WT in roots, stem, and leaves from 78 to 111 DAS (Fig. 2E–H). In addition, the two *Bt* lines tended to have a 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 (without K fertilizer application) or significantly higher K utilization index than WT (with 225 kg·hm^{-2} K_2O supply) at 111 DAS (Fig. 2I). These results suggested that the ability of *Bt* lines to acquire and utilize K was not always inferior to that of WT.

Sometimes the efficient plants are defined as those with fewer deficiency symptoms (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

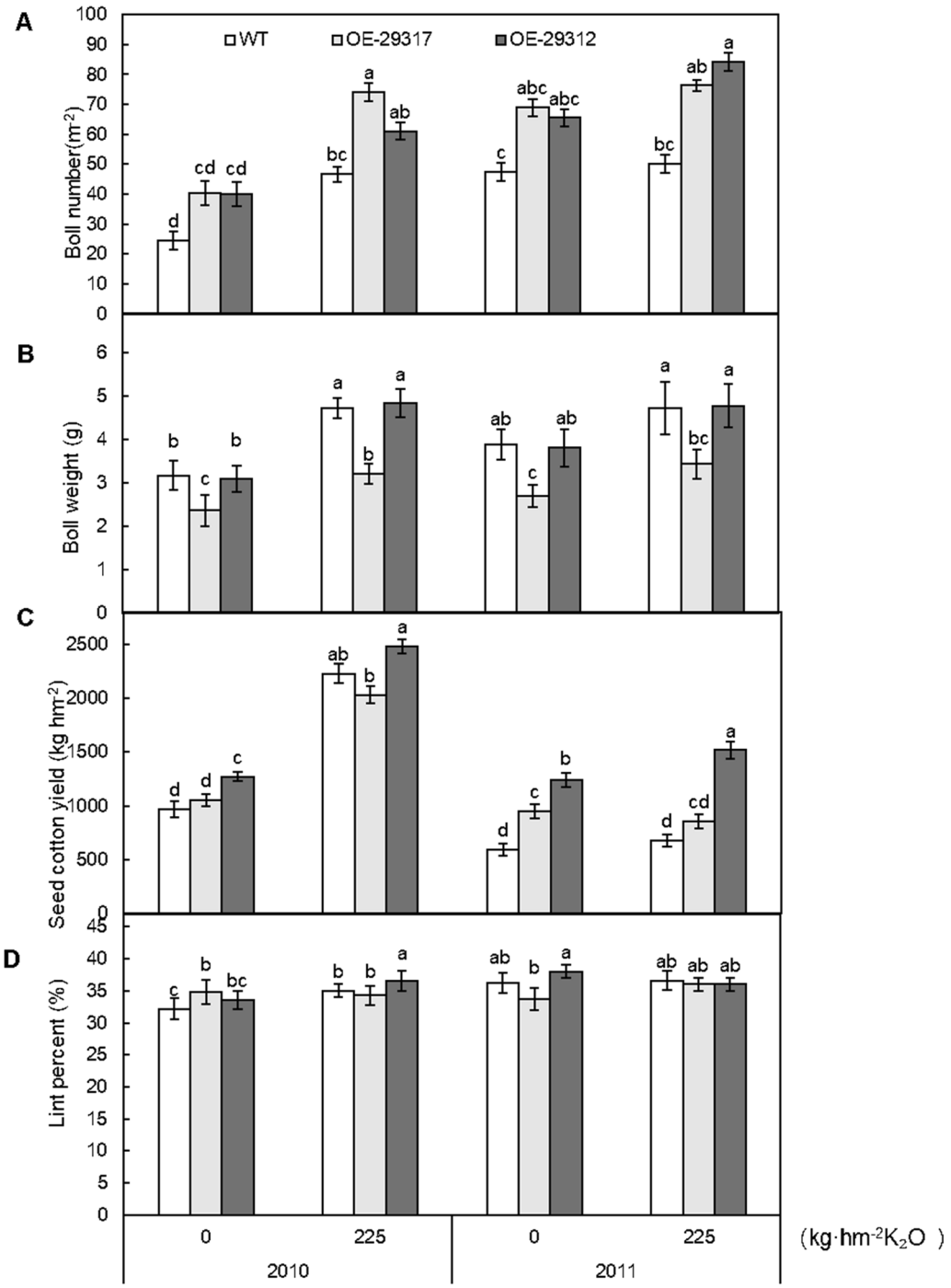

Fig. 3 Effects of *Bacillus thuringiensis* (*Bt*) gene on the boll number (**A**), boll weight (**B**), seed cotton yield (**C**), and lint percentage (**D**) in the field without or with potassium fertilizer (225 kg·hm^{-2} K_2O). The available K^+ in soil was 47.8 mg·kg^{-1}. WT: wild type (Jihe 321); 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

the field with low available K (Fig. 1A), which does not support the view that *Bt* gene introduction reduced the K use 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

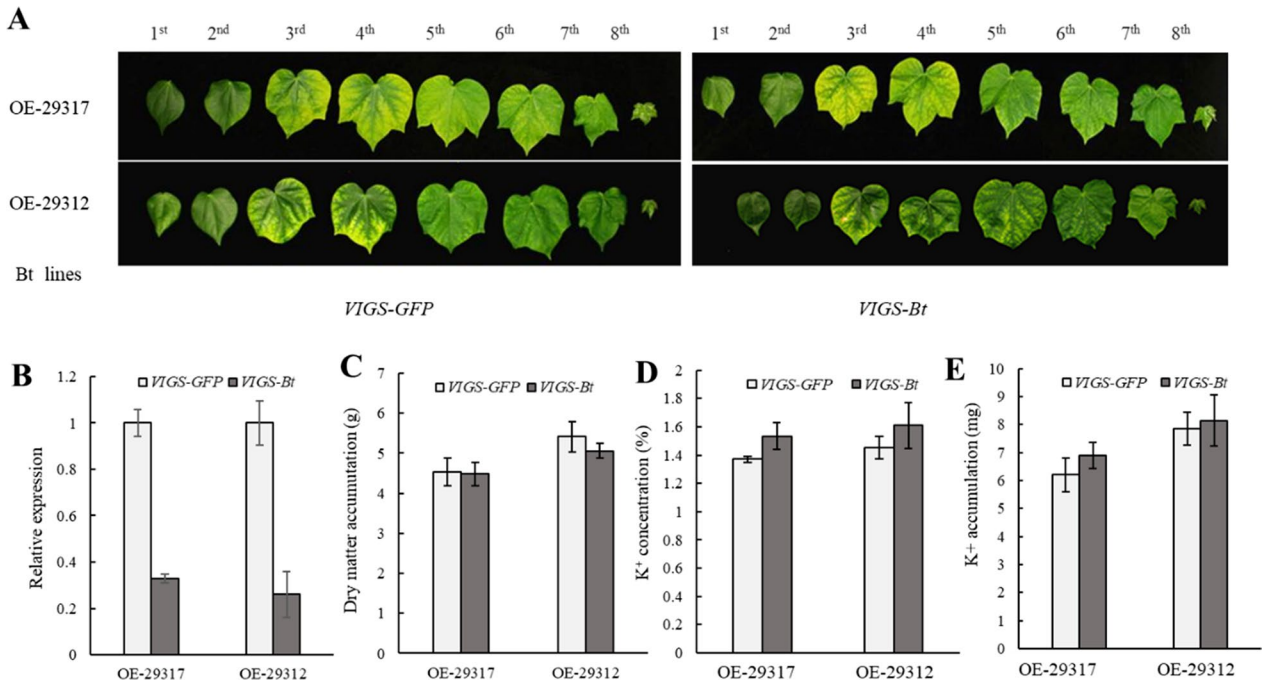

Fig. 4 The symptoms of K use deficiency (**A**), relative expression of *Bacillus thuringiensis* (*Bt*) gene (**B**), dry weight (**C**), K concentration (**D**), and accumulation (**E**) in leaves of transgenic *Bt* lines whose *Bt* gene was knocked down by virus-induced gene silencing (VIGS) method

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 et al. 1988; 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 VIGS did not result in more biomass production and greater K concentration and accumulation relative to VIGS-GFP plants (Fig. 4). 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. 3), indicating that the *Bt* gene introduction does not affect the K use 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 the 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 different genetic backgrounds.

It is well known that K deficiency is most likely to cause premature senescence, which means that leaves turn chlorotic during boll filling stages and shed much earlier than normal conditions (Wright 1999). In this study, the dry matter accumulation of OE-29317 plants declined at the last sampling time (143 DAS), possibly due to leaf abscission. However, since we did not investigate the maturity process during the study, it can not be accurately estimated whether earliness or premature senescence occurred in OE-29317.

Overall, our data indicated that the *Bt* gene introduction did not necessarily decrease the K use efficiency of cotton. Cotton growers can improve the K use efficiency of *Bt* cotton by careful varietal selection.

Conclusions

Form this study, it was determined that the seed cotton yield and lint percentage of two independent *Bacillus thuringiensis* (*Bt*) overexpression cotton lines were comparable to those of the wild type in the field with low soil-test K^+. In addition, when the *Bt* gene in the two transgenic lines was knocked down by VIGS, no additional dry biomass was produced. These results indicated that the *Bt* gene did not necessarily reduce the K use efficiency of cotton at least under the conditions of this study.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42397-022-00132-9.

Additional file 1: Fig. S1. The content of Bt protein in the youngest mature leaves of wild type (WT, Jihe 321) and *Bacillus thuringiensis (Bt)* gene transgenic lines (OE-29317 and OE-29312). Each value is the mean of three replications. Bars with different letters differ significantly ($P < 0.05$) on the same day. Error bars depict standard errors of the mean.

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Author contributions
Li FL and Tian XL designed the research; Wang QQ, Zhan MM, and Yan W performed the experiments with the assistance of Zhang YC; Luo XL, Zhang AH, and Xiao JL generated the transgenic *Bt* lines; Wang QQ, Zhan MM, Yan W, Li FL, and Tian XL analyzed the data; Wang QQ, Zhan MM, and Tian XL wrote the draft manuscript; Eneji AE gave important technical suggestions and improved the writing. All authors read and approved the final manuscript.

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Availability of data and materials
No other data related to this study is available at this time.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no conflict of interest.

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