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Spatial and temporal distribution of Bt proteins in Bt maize leaves

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ABSTRACT

To learn the spatial and temporal distribution of Bt proteins in Bt maize leaves, Bt protein levels of Bt maize leaves and their corresponding non-transgenic lines were detected and analysed by the ELISA method. The results showed that Bt proteins were expressed in the leaves of Bt maize varieties during all periods of their growth, while no such Bt proteins were detected in the leaves of non-transgenic maize varieties. The range of leaf Bt proteins was 1.08~5.81 $\mu\text{g}\cdot\text{g}^{-1}$ FW, which could effectively protect Bt maizes from the corn borer pest during their growth periods. Furthermore, the younger leaves of Bt maizes had significantly lower levels of Bt proteins than their older leaves. In most periods, Bt protein content of Bt maize leaves under drought stress was lower than that under normal growth conditions, but Bt protein content under nutrition stress was higher than that under normal growth conditions. The results provided a full pattern of the spatial and temporal distribution of Bt proteins in Bt maize leaves.

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1. Introduction

Corn (*Zea mays* L.(Paniagua-Zambrana et al., 2020)) is becoming a major food material in China. It is also an important feed material in the industry (Montes et al., 2009; Mostafa et al., 2020). However, corn production and quality were often affected by Asian corn borer, Lepidoptera and other insects (Chunruan et al., 2011; Chvátalová, 2020). Farmers were not able to effectively manage by insecticides due to environmental concerns and other multiple uncertainties. The most effective strategy is still to engineer Bt genes into the crops to generate insecticidal proteins. The control of some insect pests can be managed by the commercialization of genetically modified Bt crops. For example, the evolution of resistance has been reported in field populations of *Busseola fusca*

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targeted by Bt corn expressing Cry1Ab (Kotey et al., 2017). Most commercialized Bt crops exhibited enhanced agronomic traits, such as insect resistance and/or herbicide tolerance (Tian et al., 2015), and many new Bt crops with enhanced nutritional traits are still under development. The examination of Bt proteins in Bt crops became important for the understanding of those traits.

Bt crops and the corresponding Bt proteins are under intense investigation in China, Zhang and Zhao (2000) developed the immunological method of determining Bt protein level in Bt cotton in different growth periods and cotton components. Xing et al. (2001) studied the temporal and spatial distribution of insecticidal protein content in transgenic Bt cotton and its effect on resistance to cotton bollworm. Wang et al. (2003) investigated the content and expression of toxic protein in different organs of transgenic Bt cotton. Few studies have been conducted for Bt proteins in Bt maize cultivated in China.

Fearing et al. (1997) reported the expression of Bt protein in Bt maize plants; Liu and Wang (2003) compared the expression dynamics of Bt genes in four Bt maize strains with the same plasmid (pMG6); Wang et al. (2003) used freeze-dried root, stem and leaf organs as materials and studied the dynamics of Bt protein content at different growth . Tian Xiaoyan et al. (2012) used the enzyme-linked immunosorbent assay (ELISA) to quantitatively detect Bt protein expression and leaf surface Bt protein secretion in Mon810 corn leaves, indicating that Bt protein can be detected in leaf tissues and leaf surfaces. Yang Yingli (2013) used the ELISA to determine the temporal and spatial dynamics of the expression of two proteins: Cry1Ab and IAc in transgenic Bf maize. Those studies of Bt proteins in Bt maize were still fragmentary. There are no full reports on the spatial and temporal distribution of Bt proteins in fresh leaves of maize plants under stress conditions.

To understand the distribution pattern and dynamics of Bt protein in Bt maize leaves, in this paper, Bt protein contents in different leaves of different Bt maize and its near-isogenic lines, as well as non-GM maize currently widely grown in northern China's maize production areas, were studied by using the ELISA with standard kits from the US EPA (Castillo et al., 1998). The leaf Bt protein expressions of Bt maize under nutrient stress and water drought stress were also measured to provide a theoretical basis for a better understanding of trans-Bt gene-resistant maize to control the damage of pests.

2. Materials and methods

2.1. Maize varieties for tests

The Bt maize varieties offered for testing and their corresponding near-isogenic lines are shown in Table 1. Since we did not have access to near-isogenic lines corresponding to the Bt maize varieties 1426 × 1482 and Nongda 61 for comparison, we selected the non-GM maize variety Nongda 3138, which is currently widely grown in the maize

Table 1. Bt maize varieties tested and their corresponding near-isogenic lines.

Bt corn varieties	Corresponding near-isogenic lines	Logarithm of different genes	Source
B24	B23	1 (Bt gene)	American pioneer seed company
G30	G26	2 (Bt gene、Yellow Maize gene)	American pioneer seed company

production areas of northern China. Nongda 3138, 1426 × 1482 and Nongda 61 were provided by the academician Jingrui Dai of China Agricultural University, and the rest of the varieties were given by Dr. Cindy Nakatus of the Department of Agronomy, Purdue University, USA.

2.2. Design of experiments

- (1) Pot experiment: The above maize varieties were used as materials and each variety was planted in 4 pots (i.e. 4 replications) with 2 plants in each pot; each pot was randomly arranged. One whole seedling was taken from each pot 20 d after sowing when it had 6~7 leaves, and Bt protein content of the inverted 1, inverted 3 and inverted 5 leaves was measured separately. The soil was collected from 0 to 20 cm layer of the field in the internship farm of South China Agricultural University, with a clay loam texture, pH 6.1% and 1.81% organic matter content.
- (2) Greenhouse plot experiment: Bt maize varieties B24 and G30 and the corresponding non-transgenic near-isogenic lines B24 and G26 were used as materials for three-factor experiments of maize variety, water stress and nutrient stress in a glass greenhouse, with two levels of each factor. For example, two levels were set for the Bt maize variety and non-GM maize variety; two levels were set for normal water stress and drought (i.e. no water at all, but a little water when the leaves wilted); and two levels were set for nutrient stress: normal fertilization (20 g of compound fertilizer per plot every two weeks) and no fertilization. A total of eight treatment combinations were used to match the three factors. Each treatment combination was planted in one plot and randomly arranged in the field. Each plot was 3.4 m × 0.75 m and 10 plants were planted in each. Each plot was randomly sampled five times (three replicates each) in the first and middle of the entire reproductive period, at 18, 25, 32, 39 and 46 d after sowing. 1, 2 and 3 leaves of a single plant were taken as material in the first two periods, and 1, 3 and 5 leaves of a single plant were taken as material in the last three periods, and Bt protein content was measured to understand its spatial and temporal distribution pattern. The number of leaves damaged by corn borer in each plot was observed at the same time of each sampling.

2.3. Determination of Bt protein content

Nowadays, proteins are commonly detected by the ELISA (Ahmad & Cheema, 2019; Chen et al., 2020; Seo & Joo, 2014; Walschus et al., 2002). Bt protein content was determined by the ELISA method, using the AP003 ELISA quantitative kit from EnviroLogix, Inc. This is the standard kit used by the US EPA for the detection of Bt protein. The kit includes one 96-well (12×8) plate with embedded antibodies and supporting reagents, four Cry1A(b) specimens at concentrations of 0, 0.5, 2.5 and 5.0 ng.g⁻¹. The extraction of Bt protein from maize leaves and the ELISA determination procedure for its content were referred to the kit instructions. Briefly, 20–30 mg of fresh leaves was added to a 1.5 ml centrifuge tube, mashed and 0.5 ml of 1×Cry1A(b) extraction/dilution buffer

was added, shaken for 2 min at 200 rpm and centrifuged for 2 min at 12,000 r/min to mix thoroughly, 10 μ l of the supernatant night was taken and diluted 101 times with extraction/dilution buffer to be measured. 100 μ l of sample extract was added to each well, covered with a plastic wrap and shaken at 200 rpm for 15 min; 100 μ l of Cry1A(b)-enzyme conjugate was added to each well, covered a with plastic wrap and shaken at 200 rpm for 1 h; the membrane was removed and Wash Buffer was rinsed 3 times; 100 μ l of reaction substrate was added to each well. It was covered with a plastic wrap, and shaken at 200 rpm for 30 min; finally, 100 μ l of the termination solution was added and mixed thoroughly, and the OD value was measured at 450 nm for 30 min using an enzyme standard (Sunrise Remote). Protein content per gram of fresh leaf is (unit: μ g·g⁻¹FW).

2.4 Analysis of data

All data from this experiment were compiled in Excel and statistically analysed with the statistical software SAS 6.12. The *t*-test was performed according to the method of the Statistical Group of the Institute of Mathematics, Chinese Academy of Sciences (1973).

3. Results and analysis

3.1. Bt protein content of leaves of maize varieties

(1) Comparison of Bt protein content in leaves of various maize varieties

Bt protein content in the penultimate 1st, 3rd and 5th expanded leaves of each maize variety was measured at 20 d after sowing (Table 2). The results showed that the levels of Bt protein in different parts of the leaves of each Bt maize variety ranged from 1.83 to 4.08 μ g·g⁻¹ FW at 20 d after sowing, whereas in all non-transgenic maize varieties, no Bt protein content could be detected.

Among the four Bt maize varieties measured, Bt protein content of the inverted one leaf was highest in variety 1426 × 1482 (Table 2), significantly higher than B24 and Nongda 61, and not significantly different from G30; Nongda 61 had lowest Bt protein content, not significantly different from B24 and G30, and significantly lower than 1426 × 1482. Bt protein content of the inverted 3 leaves was highest in variety B24 and G30, which were significantly higher than other varieties; lowest in variety was 1426 ×

Table 2. The level of Bt protein (Mean $\pm$ S.E.M.) in different location leaves of 20-d old corn seedlings (μ g·g⁻¹ fresh wt.).

Leaf location	B24	B23	G30	G26	Nongda 61	1426×1482	Nongda 3138
The first leaf from the top	2.70 $\pm$ 0.31b	ND	3.05 $\pm$ 0.12b	ND	2.30 $\pm$ 0.05b	3.50 $\pm$ 0.30a	ND
The third leaf from the top	4.08 $\pm$ 0.06a	ND	4.01 $\pm$ 0.39a	ND	2.82 $\pm$ 0.11a	2.44 $\pm$ 0.21b	ND
The fifth leaf from the top	3.13 $\pm$ 0.15b	ND	3.30 $\pm$ 0.09b	ND	1.84 $\pm$ 0.14c	1.83 $\pm$ 0.20b	ND

Notes: Means with the same letter within the column were not significantly different ($P > 0.05$) using Duncan's multiple test (Dafaallah, 2019). ND indicated not detected. The same as below.

1482. Bt protein content of the inverted 5 leaves was also highest in varieties B24 and G30, significantly higher than 1426 × 1482 and Nongda 61.

(2) Spatial distribution of Bt protein in leaves of different Bt maize varieties

The distribution pattern of Bt protein in leaves of different Bt maizes varied. The varieties with the highest Bt protein content in the first leaf from the top were 1426 × 1482 (Table 2), and the remaining three varieties all had the highest Bt protein content in the third leaf from the top. Among the four observed trans-Bt maize varieties, Nongda 61, 1426 × 1482 and other varieties had the lowest Bt protein content in the fifth leaf from the top, while B24 and G30 had the lowest Bt protein content in the inverted 1 leaf.

3.2. Distribution dynamics of Bt protein content in Bt maize leaves

(1) Comparison of leaf Bt protein content among different varieties

To understand the distribution dynamics of Bt protein in Bt maize, two Bt maize varieties, B24 and G30, were selected as materials from the above four varieties. A three-factor test, variety, water stress and nutrient stress, was designed to determine the Bt protein content of different parts of leaves at different periods. The results showed (Table 3) that Bt protein was detected in leaves of different parts of Bt maize under different growth conditions at all growth periods; the levels ranged from 1.08 to 5.81 $\mu\text{g}\cdot\text{g}^{-1}$ FW. The *t*-test showed ($P < 0.01$) that Bt protein content was generally higher in B24 than in G30.

This difference in Bt protein content among different Bt maize varieties may be related to different structures and promoters of Bt genes. It may also be related to the way the gene was introduced, the way it was transformed and the genetic background of the recipient (Wang et al., 2003). However, what they have in common is that the content of Bt protein has reached the level of resistance to maize borer damage.

(2) Changes of Bt protein content in different parts of leaves

The content of Bt protein in the first leaf from the top of both Bt maize varieties was significantly lower than in the second leaf from the top, third leaf from the top and fifth leaf from the top at all measurement periods. The test results showed that the expression of Bt protein in young leaves of Bt maize was significantly lower than that in mature leaves.

(3) Changes in Bt protein content of leaves at different growth periods

Using the Bt protein content of maize leaves measured at the first time (18 d after planting) as a standard for comparison, the average Bt protein content increased by 14.47% and 17.25% for each treatment of varieties B24 and G30, respectively, at 25 d after planting; at 32 d after planting, it increased by 34.21% and 29.15%, respectively. Combining the treatments, the Bt protein content increased with time of maize growth until 32 d

Table 3. The dynamic of leaf Bt protein in different locations of Bt corns under different growth conditions.

Bt corn	Drought stress	Nutrition stress	18-d after sowing					25-d after sowing					32-d after sowing					39-d after sowing					46-d after sowing				
			1st leaf	2nd leaf	3rd leaf	1st r leaf	2nd leaf	3rd leaf	1st leaf	2nd leaf	3rd leaf	1st leaf	3rd leaf	5th leaf	1st leaf	3rd leaf	5th leaf	1st leaf	3rd leaf	5th leaf	1st leaf	3rd leaf	5th leaf	1st leaf	3rd leaf	5th leaf	
B24	Normal	Normal	4.19 ± 0.34a	4.78 ± 0.09a	5.03 ± 0.61a	3.81 ± 0.26a	3.54 ± 0.12b	4.98 ± 0.29a	2.83 ± 0.40d	3.95 ± 0.35c	5.23 ± 0.11a	2.79 ± 0.35a	3.52 ± 0.15a	3.65 ± 0.19a	1.73 ± 0.24d	3.92 ± 0.27a	4.30 ± 0.06a	1.73 ± 0.24d	3.92 ± 0.27a	4.30 ± 0.06a	1.73 ± 0.24d	3.92 ± 0.27a	4.30 ± 0.06a	1.73 ± 0.24d	3.92 ± 0.27a	4.30 ± 0.06a	
B24	Normal	Nitrogen deficiency	3.42 ± 0.56a	4.09 ± 0.22b	3.22 ± 0.39c	3.37 ± 0.40ab	4.11 ± 0.23a	4.01 ± 0.25b	4.54 ± 0.16a	5.39 ± 0.41a	5.73 ± 0.07a	3.15 ± 0.19a	3.26 ± 0.19a	3.86 ± 0.19a	3.50 ± 0.07a	3.36 ± 0.20b	4.00 ± 0.27ab	3.50 ± 0.07a	3.36 ± 0.20b	4.00 ± 0.27ab	3.50 ± 0.07a	3.36 ± 0.20b	4.00 ± 0.27ab	3.50 ± 0.07a	3.36 ± 0.20b	4.00 ± 0.27ab	
B24	Drought	Normal	2.46 ± 0.12ab	3.31 ± 0.27b	3.02 ± 0.51c	3.58 ± 0.15a	4.02 ± 0.06a	4.46 ± 0.20b	4.04 ± 0.12b	5.14 ± 0.11a	5.37 ± 0.52a	2.71 ± 0.05a	2.74 ± 0.11b	3.00 ± 0.14b	2.71 ± 0.06b	2.74 ± 0.06c	3.00 ± 0.10c	2.71 ± 0.06b	2.74 ± 0.06c	3.00 ± 0.10c	2.71 ± 0.06b	2.74 ± 0.06c	3.00 ± 0.10c	2.71 ± 0.06b	2.74 ± 0.06c	3.00 ± 0.10c	
B24	Drought	Nitrogen deficiency	2.72 ± 0.31b	3.76 ± 0.26b	4.03 ± 0.28b	3.25 ± 0.22ab	4.22 ± 0.38a	4.38 ± 0.04b	4.52 ± 0.06a	5.81 ± 1.00a	4.88 ± 0.34ab	2.02 ± 0.32b	2.28 ± 0.12c	3.04 ± 0.25b	2.63 ± 0.24b	3.31 ± 0.21b	4.55 ± 0.03a	2.63 ± 0.24b	3.31 ± 0.21b	4.55 ± 0.03a	2.63 ± 0.24b	3.31 ± 0.21b	4.55 ± 0.03a	2.63 ± 0.24b	3.31 ± 0.21b	4.55 ± 0.03a	
G30	Normal	Normal	2.62 ± 0.25b	3.85 ± 0.35b	5.15 ± 0.57a	3.46 ± 0.33ab	3.91 ± 0.19a	4.47 ± 0.22b	2.34 ± 0.09c	3.91 ± 0.29c	4.47 ± 0.17b	1.61 ± 0.18c	2.80 ± 0.07b	3.08 ± 0.14b	2.64 ± 0.08b	3.63 ± 0.32ab	4.30 ± 0.14a	2.64 ± 0.08b	3.63 ± 0.32ab	4.30 ± 0.14a	2.64 ± 0.08b	3.63 ± 0.32ab	4.30 ± 0.14a	2.64 ± 0.08b	3.63 ± 0.32ab	4.30 ± 0.14a	
G30	Normal	Nitrogen deficiency	2.56 ± 0.36b	3.23 ± 0.41b	2.90 ± 0.40c	3.23 ± 0.21ab	4.07 ± 0.37a	3.40 ± 0.26c	4.24 ± 0.27ab	4.60 ± 0.30b	4.33 ± 0.42b	2.00 ± 0.08b	2.31 ± 0.10c	2.65 ± 0.29b	2.76 ± 0.27b	3.52 ± 0.17ab	3.70 ± 0.22b	2.76 ± 0.27b	3.52 ± 0.17ab	3.70 ± 0.22b	2.76 ± 0.27b	3.52 ± 0.17ab	3.70 ± 0.22b	2.76 ± 0.27b	3.52 ± 0.17ab	3.70 ± 0.22b	
G30	Drought	Normal	2.81 ± 0.75b	3.65 ± 0.30b	3.35 ± 0.28c	3.03 ± 0.25b	3.67 ± 0.16b	4.08 ± 0.10b	3.29 ± 0.56c	4.69 ± 0.29b	4.50 ± 0.32b	1.08 ± 0.04d	2.47 ± 0.13bc	2.21 ± 0.10c	2.25 ± 0.11c	3.10 ± 0.10bc	3.67 ± 0.05b	2.25 ± 0.11c	3.10 ± 0.10bc	3.67 ± 0.05b	2.25 ± 0.11c	3.10 ± 0.10bc	3.67 ± 0.05b	2.25 ± 0.11c	3.10 ± 0.10bc	3.67 ± 0.05b	
G30	Drought	Nitrogen deficiency	1.45 ± 0.47c	3.57 ± 0.14b	3.04 ± 0.18c	3.21 ± 0.09ab	3.44 ± 0.12b	4.72 ± 0.28ab	3.71 ± 0.28bc	4.76 ± 0.08b	4.49 ± 0.21b	1.94 ± 0.07b	2.15 ± 0.09c	2.70 ± 0.06b	2.16 ± 0.11cd	3.14 ± 0.10bc	3.71 ± 0.09b	2.16 ± 0.11cd	3.14 ± 0.10bc	3.71 ± 0.09b	2.16 ± 0.11cd	3.14 ± 0.10bc	3.71 ± 0.09b	2.16 ± 0.11cd	3.14 ± 0.10bc	3.71 ± 0.09b	

Note: All Bt protein contents in the Table are $\mu\text{g}\cdot\text{g}^{-1}$ FW

after planting. From 32 d to 46 d after planting, there was a decline in Bt expression as the number of days of maize growth increased, and the amount of Bt protein decreased, but the decrease was not significant.

3.3 Effect of drought stress on leaf Bt protein content

Leaf Bt protein content was lower in both Bt maize varieties under drought stress than in normal growth conditions during most of the observation periods (Table 3). The results of the sign test ($P < 0.10$) indicated that the expression of Bt protein was decreased in maize leaves under drought stress.

3.4 Effect of nitrogen nutrient stress on leaf Bt protein content

From 32 d after planting, the Bt protein content in the first leaf from the top of both Bt maize varieties under nitrogen nutrient stress was consistently higher than under normal growth conditions (Table 3). The results indicated that nitrogen nutrient stress favoured the expression of Bt protein in the inverted 1 leaf of maize plants.

3.5 Protective effect of Bt maize on leaves

Throughout the experimental observation period, leaves of Bt maize plants were free of corn borer damage, whereas leaves with corn borer damage were found in all non-transgenic maize plants corresponding to them, with a significant level of difference between the two (Table 4). The results showed that the level of Bt protein content expressed by the leaves of Bt maize plants during all growth periods in the test materials used has been effective in controlling the damage of corn borer. Since the Bt maize plants continued to express Bt protein efficiently throughout the growth period, they were able to effectively control pests such as corn borer throughout the growth period.

4. Discussion

In this experiment, all non-transgenic maize failed to detect Bt protein, while all Bt maize consistently expressed Bt protein throughout the growth period; the levels of Bt protein in leaves of each Bt maize variety ranged from 1.08 to 5.81 $\mu\text{g}\cdot\text{g}^{-1}$ FW under different growth conditions during the observation period. This indicates that all the Bt maize varieties selected for this experiment have indeed integrated the exogenous Bt gene and that the gene can be expressed efficiently and consistently throughout the growth period.

There are differences in Bt protein content among different Bt maize varieties. This may be related to the differences in the structure of the exogenous gene, the mode of

Table 4. The number of leaves infested by corn borer in different corns during growth stages.

Varieties	18 d	25 d	32 d	39 d	46 d
B23	1.75 $\pm$ 0.48a	2.25 $\pm$ 0.25a	2.50 $\pm$ 0.48a	2.75 $\pm$ 1.03a	2.75 $\pm$ 1.03a
B24 (Bt)	ND	ND	ND	ND	ND
G26	2.25 $\pm$ 0.36a	2.25 $\pm$ 0.50a	2.75 $\pm$ 0.75a	2.75 $\pm$ 0.75a	3.00 $\pm$ 0.86a
G30 (Bt)	ND	ND	ND	ND	ND

introduction, the mode of transformation, the insertion site, the genetic background of the recipient, the promoter and the copy number, and many other factors, or it may be the result of the combination of these factors and environmental conditions. Wang et al. (2003) suggested that the spatial and temporal dynamics of Bt gene expression in different varieties of Bt maize may be due to the interaction of gene expression with factors, such as maize growth and developmental characteristics, and the external environment to be further explored. When comparing the expression dynamics of Bt genes in four Bt maize strains with the same plasmids, Liu and Wang (2003) also found significant differences in Bt protein expression between different tissues of the same plant and between different strains and speculated that this might be the effect of different insertion sites and copy numbers. The results of Miluse T et al. (2015) showed that Bt content depends not only on transgene expression but also on the genetic background of the corn variety. Under stress conditions, the concentration of Bt protein is more difficult to predict. Therefore, the reasons for the differences in Bt protein content among different Bt maize varieties remain to be investigated in depth.

In this experiment, three Bt maize varieties had lower levels of Bt protein in young leaves (the first leaf from the top) than in mature leaves. This result is similar to the condition of Bt protein distribution measured by Wang Baomin et al. (2002) in trans-Bt gene cotton. The reason may be that the nitrogen metabolism of young maize leaves is more vigorous and requires more nitrogen nutrients to synthesize the related amino acids and proteins; therefore, the fraction of nitrogen nutrients allocated for the synthesis of Bt protein is less.

Under nutrient stress conditions, the content of Bt protein in the first leaf from the top of Bt maize was significantly increased. It seems that certain nitrogen stress favours benefit the synthesis and accumulation of Bt protein in Bt maize. The relationship between Bt protein content and nitrogen metabolism in Bt maize deserves further study.

Drought stress affects plant growth and also the expression of Bt genes. The content of Bt protein in leaves of Bt maize varieties B24 and G30 under drought stress was significantly lower than normal growth conditions during most of the observed periods, indicating that drought stress inhibited the expression of Bt protein in maize leaves. Why is this the case? Any organism in nature must pay the price to survive and reproduce. Organisms have always evolved to develop a mechanism to obtain their survival and development at minimal cost (Bazzaz et al., 1987). Under drought stress, the main threat to the survival of the plant is drought, so the plant always has to mobilize all factors to resist drought. In the results of the present group, it was found that the protective enzyme activity of Bt maize leaves increased and the root callus content increased under PEG drought osmotic stress (published separately); these results suggest that under drought stress, the Bt gene could promote the expression of resistance genes in the maize plant itself, increase callus content, improve protective enzyme activity, as well as synthesize and produce a number of other substances that contribute to the resistance of (published in a separate paper). These physiological processes require a certain material and energy base, and the material and energy resources available to the plant are relatively limited; therefore, under stressful conditions such as drought, the material and energy available to the plant for synthesizing Bt protein is relatively reduced due to the promotion of Bt gene on the expression of the resistance gene in the maize plant itself, and thus Bt protein content decreases. This is because the ability to synthesize Bt proteins is relatively less important for the plant under drought stress; in this case, the entire

metabolic focus of the plant will be more centred on drought resistance, and the synthesis of Bt proteins will be relatively inhibited. Therefore, although Bt genes are available to express Bt protein, the expression of Bt protein decreases because the plant has to invest more material and energy for drought resistance. The exact biochemical mechanism needs further studies.

Disclosure statement

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