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Dynamics of Cry1Ac protein and soil enzyme activity in the rhizosphere of transgenic *Bt* oilseed rape

Zhengjun Guan[1], Wei Wei[2], Yanlin Huo[3], C. Neal Stewart Jr.[4], Zhixi Tang[2]*

[1]*Department of Life Sciences, Yuncheng University, Yuncheng, Shanxi, P.R. China*
[2]*State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing, P.R. China*
[3]*Science Experiment Center, Yuncheng University, Yuncheng, Shanxi, P.R. China*
[4]*Department of Plant Sciences, University of Tennessee, Knoxville, USA*
**Corresponding author: zhengjunguan@126.com*

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Abstract: In this study, three insect-resistant transgenic *Bacillus thuringiensis* (*Bt*) oilseed rape events (GT1, GT5 and GT9) under field conditions were utilised to analyse the dynamics of Cry1Ac protein and the changes in soil enzyme activities in the rhizosphere soil of transgenic *Bt* plants during different growth stages over two successive cultivation years. The results indicated that compared to the non-transgenic control plant cv. Westar, the amount of Cry1Ac protein in the rhizosphere soil of the three transgenic oilseed rape events was significantly higher during the flowering and podding stages in the first cultivation year. Additionally, in the second cultivation year, transgenic GT1 and GT9 had significantly higher amounts of Cry1Ac protein in the rhizosphere soil during the flowering stage, and all three transgenic oilseed rape events had significantly higher amounts of Cry1Ac protein in the rhizosphere soil during the podding stage. Over the two successive cultivation years, the sucrase activity in the rhizosphere soil of transgenic events showed significant changes during bolting, flowering and podding stages, while all three transgenic events exhibited significant changes in phosphatase activity during the four different stages. Furthermore, different transgenic events showed varying significant changes in urease and protease activities during the bolting, flowering and podding stages of the first year, and all three transgenic events had significant changes in dehydrogenase activities during the four different stages of the second cultivation year. PCA and correlation analysis clearly demonstrated a strong correlation between the Cry1Ac protein and five soil enzyme activities, as well as a close interconnectedness among those five soil enzyme activities. These findings suggest that the amount of insecticidal crystal proteins in the rhizosphere soil of transgenic *Bt* (Cry1Ac) oilseed rape varies with different growth periods, and the enzyme activities in the rhizosphere soil of transgenic *Bt* oilseed rape plants undergo significant changes over two successive planting years.

Keywords: *Brassica napus* L.; transgenic plant; ecological risk assessment; soil ecosystem; toxin accumulation

The potential impacts of the global adoption of transgenic plants on the soil ecosystem have concerned scientists and the public. Foreign proteins and other expression products from transgenic plants can enter the soil ecosystem through root exudation, crop residue, and other pathways (Krogh et al. 2020). The expression of insecticidal crystal proteins, such as *Bacillus thuringiensis* (*Bt*) protein, in plants, represents a potential case of accumulation and persistence of transgenic products in the soil (Veettil et

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al. 2017, Ortiz and Sansinenea 2023). Studies have shown that *Bt* protein can quickly bind to soil clay and humic acid upon entering the soil, retaining its insecticidal activity and resisting breakdown by soil microorganisms (Li et al. 2022). Low levels of *Bt* toxin produced by transgenic plants may persist in the soil for weeks or months (Strain and Lydy 2015), potentially affecting the soil environment and leading to toxin accumulation with repeated use of *Bt*-producing plants (Li et al. 2022). Therefore, the effects of transgenic *Bt* plants on the soil ecological environment have become a continuing focus in research on the biosafety of transgenic *Bt* plants.

Soil plays a crucial role in material cycling and energy conversion within ecosystems (Vezzani et al. 2018). The activities of soil enzymes, important biological indicators of soil health, can reflect the various biochemical processes occurring in the soil (Ibrahim et al. 2020, Lebedev et al. 2022). While some studies have suggested that the cultivation of transgenic *Bt* plants could alter soil enzyme activities, other studies show negligible impact (Singh and Dubey 2016). Experimental evidence is still needed to address these controversial views. Transgenic maize had minimal effects on urease activity (Bai et al. 2019). A global meta-analysis by Li et al. (2019) highlighted the responses of soil enzymatic activities to transgenic *Bt* crops, emphasising the need for more systematic research in this area. Research on the effects of transgenic *Bt* plants on soil enzymes, which are crucial for ecosystem health, has been limited following field release. However, recent studies have shed light on this important topic. For instance, Zheng et al. (2022) observed significant differences in some soil enzyme activities between transgenic and wild-type poplar (*Populus alba* × *Populus berlinensis*) at different growth stages. Conversely, Arshad et al. (2022) found no significant changes in enzyme activities in the soil solution of AVP1-transgenic wheat rhizospheres. Additionally, a comprehensive three-year study on salt-tolerant transgenic maize conducted by Zeng et al. (2022) revealed no discernible effects on soil enzyme activities. These findings underscore the complex and context-dependent nature of the interactions between transgenic plants and soil enzymes, highlighting the need for further research in this area better to understand the ecological implications of transgenic plant cultivation.

Transgenic oilseed rape (*Brassica napus* L.) is one of the earliest developed transgenic crops with widespread application and has undergone research in multiple environmental releases and field trials (Tang et al. 2019). Although transgenic *Bt* oilseed rape is not yet commercialised, this plant and its relatives have become a model system for evaluating the ecological impacts of transgenic *Bt* plants (Cao et al. 2014). While some studies have focused on changes in soil microorganisms or for herbicide-resistant or insect-resistant transgenic oilseed rape plants (Guan et al. 2021), the impact of insect-resistant transgenic oilseed rape containing Cry1Ac on rhizosphere soil enzyme activities remains unexplored. Nevertheless, the effect of insect-resistant transgenic oilseed rape with Cry1Ac on the activities of enzymes in rhizosphere soil has not been reported. This study aims to evaluate the effects of insect-resistant transgenic *Bt* oilseed rape on rhizosphere soil enzyme activities to provide a theoretical basis for the ecological risk assessment of transgenic plants.

MATERIAL AND METHODS

Plant materials. *B. napus* cv. Westar, a spring-type oilseed rape transformed with genetically linked GFP and *Bt* Cry1Ac controlled by separate CaMV 35S promoters within the pSAM12 plasmid (Halfhill et al. 2001) were used. Three transformed events were also utilised, namely GT1, GT5, and GT9.

Field trials and soil sampling. The field trials were conducted at the experimental field of the Institute of Botany, Chinese Academy of Sciences in Beijing, China (116°12'E, 39°59'N) for two consecutive growing seasons – seeds from three transgenic events (GT1, GT5, and GT9) and the non-transgenic cv. Westar was planted in a randomised block design with three replicates for each variant. Each plot measured 2 × 2 m, with a 45 cm spacing between them. Standard irrigation and fertiliser management were applied to the experimental area. This experimental setup was replicated in the same plots over the two growing seasons, maintaining consistent material varieties and plot locations. Soil samples were collected at four key growth stages: seedling, bolting, flowering, and podding. Rhizosphere soils were sieved (< 2 mm), placed in sterile plastic bags, promptly transported to the laboratory, and stored at 4 °C for subsequent analysis of *Bt* toxin and enzyme activity.

Analysis of Cry1Ac protein concentrations in rhizosphere soil. The concentration of Cry1Ac protein was quantified using the Qualitative double antibody sandwich *Bt* Cry1Ab/1Ac enzyme-linked immunosorbent assay (ELISA) kit (Envirologix Inc.,

https://doi.org/10.17221/330/2024-PSE

Portland, USA). 0.5 mL of extraction buffer was added to 0.5 g of soil sample. The mixture was then shaken for 10 min, incubated at 4 °C for 2–4 h, and centrifuged at 6 000 rpm for 10 min at 4 °C. The Cry1Ac protein in the supernatant was analysed using the ELISA kit, with 1.0 ppm CryAb/CryAc protein serving as the positive control. The absorbency of samples was measured at 620 nm.

Measurement of soil enzyme activities. The activities of sucrase, urease, phosphatase, dehydrogenase, and protease were determined following the manufacturer's instructions (Komin, Biotechnology Co., Ltd. Suzhou, China), and the specified protocols were followed. All enzymatic activity measurements were conducted in triplicate.

Statistical analysis. The presented data represents the arithmetic means (± standard error) of three replicates of each treatment. Statistical analysis was conducted using one-way ANOVA in SPSS Statistics 18.0 (SPSS Inc., Chicago, USA). Least significant difference (*LSD*) values were calculated for parameters that exhibited significant changes ($P < 0.05$). The relationships between the various indexes were evaluated using Pearson correlations. Principal component analysis (PCA) was performed using SPSS 18.0 software (Chicago, USA).

RESULTS

Dynamics of Cry1Ac protein in rhizosphere soil of transgenic oilseed rape

The presence of Cry1Ac protein was detected not only in soil samples from the three transgenic oilseed rape events (GT1, GT5, and GT9) but also in the control plant Westar, indicating a basal level of Cry1Ac protein in the typical soil (Figure 1A). One-way ANOVA analysis revealed significant differences in Cry1Ab/1Ac protein levels among plant types across different developmental stages. In the first-year field trial, the levels of Cry1Ac protein in the rhizosphere soil of transgenic oilseed rape events GT1, GT5, and GT9 were significantly higher than those in the control plant Westar during the flowering ($F_{3,8} = 7.721$, $P < 0.05$) and podding ($F_{3,8} = 19.459$, $P < 0.05$) stages.

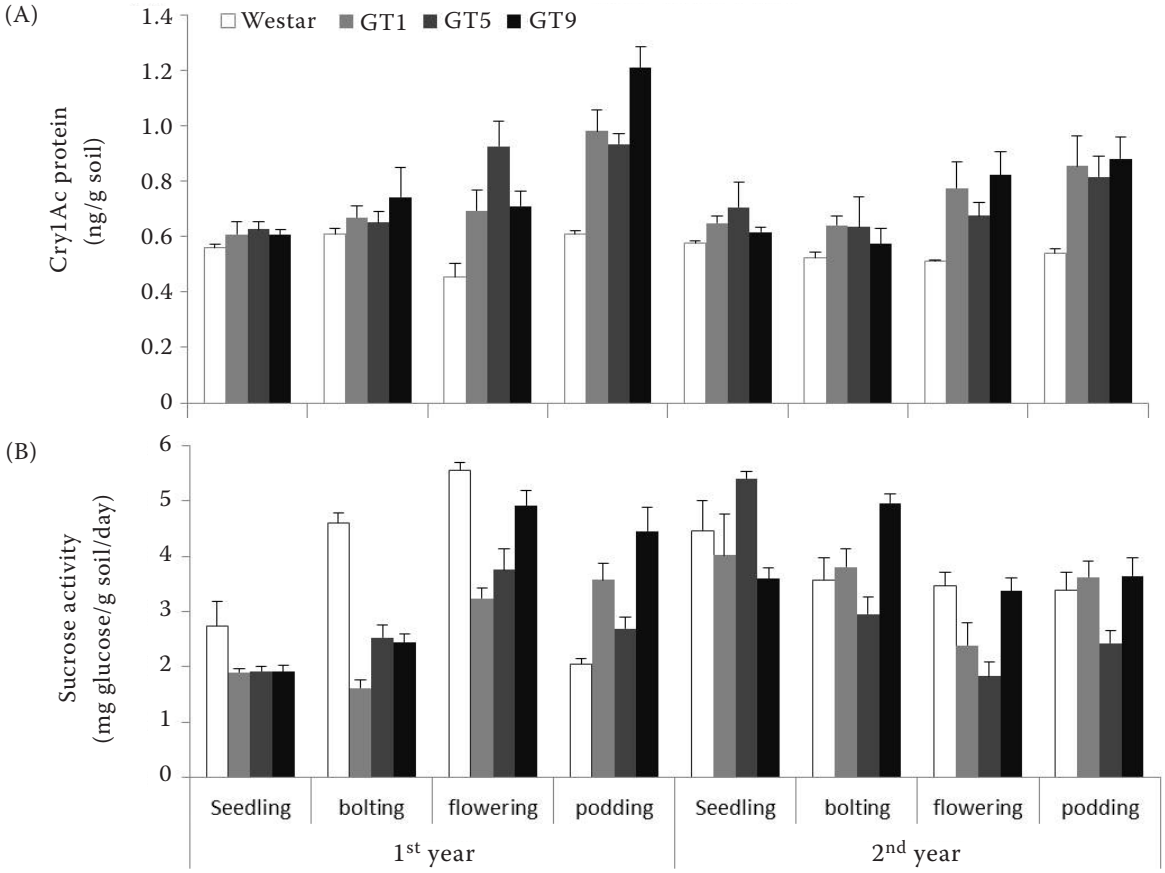

Figure 1. Dynamics of (A) Cry1Ac protein and (B) the changes of sucrose activity in rhizosphere soil of transgenic *Bt* oilseed rape (GT1, GT5, and GT9) and the control (Westar) over two consecutive growing seasons

https://doi.org/10.17221/330/2024-PSE

In the second-year field trial, transgenic oilseed rape plants GT1 and GT9 exhibited significantly higher levels of Cry1Ac protein in the rhizosphere soil compared to the control plant Westar during the flowering stage ($F_{3,8}$ = 4.233, P < 0.05). Moreover, during the podding stage, the Cry1Ac protein levels in the rhizosphere soil of the three transgenic oilseed rape events were significantly higher than those in the control plant Westar ($F_{3,8}$ = 4.252, P < 0.05). No significant differences were observed in Cry1Ac protein levels in the rhizosphere soil between the four oilseed rape plant types during the other two stages of the two successive planting years.

Soil enzyme activities change in rhizosphere soil of transgenic oilseed rape.

Sucrase activity. In the first year's rhizosphere soil, the sucrase activities of the three transgenic plant events (GT1, GT5, and GT9) were significantly lower ($F_{3,8}$ = 48.769, P < 0.05) than that of the control plant Westar during the bolting stage. Additionally, the sucrase activities of transgenic plant events GT1 and GT5 were significantly lower ($F_{3,8}$ = 16.526, P < 0.05) than Westar during the flowering stage (Figure 1B). During the podding stage, while transgenic plant events GT1 and GT9 exhibited significantly higher sucrase activities ($F_{3,8}$ = 13.559, P < 0.05) than Westar, transgenic plant events GT1 and GT9 exhibited significantly higher sucrase activities ($F_{3,8}$ = 13.559, P < 0.05) than Westar. In the second year's rhizosphere soil, compared to the control plant Westar, transgenic plant GT9 displayed significantly higher sucrase activity ($F_{3,8}$ = 7.06, P < 0.05) during the bolting stage. Conversely, transgenic plant events GT1 and GT5 showed significantly lower sucrase activities ($F_{3,8}$ = 6.926, P < 0.05) during the flowering stage (Figure 1B).

Phosphatase activity. In the first year of cultivation, the phosphatase activity in the rhizosphere soil of transgenic plant event GT1 was significantly higher ($F_{3,8}$ = 3.278, P < 0.05) than that in the control plant Westar during the seedling stage (Figure 2A). However, the soil phosphatase activities of three transgenic plant events (GT1, GT5 and GT9) were significantly lower ($F_{3,8}$ = 13.778, P < 0.05) than that

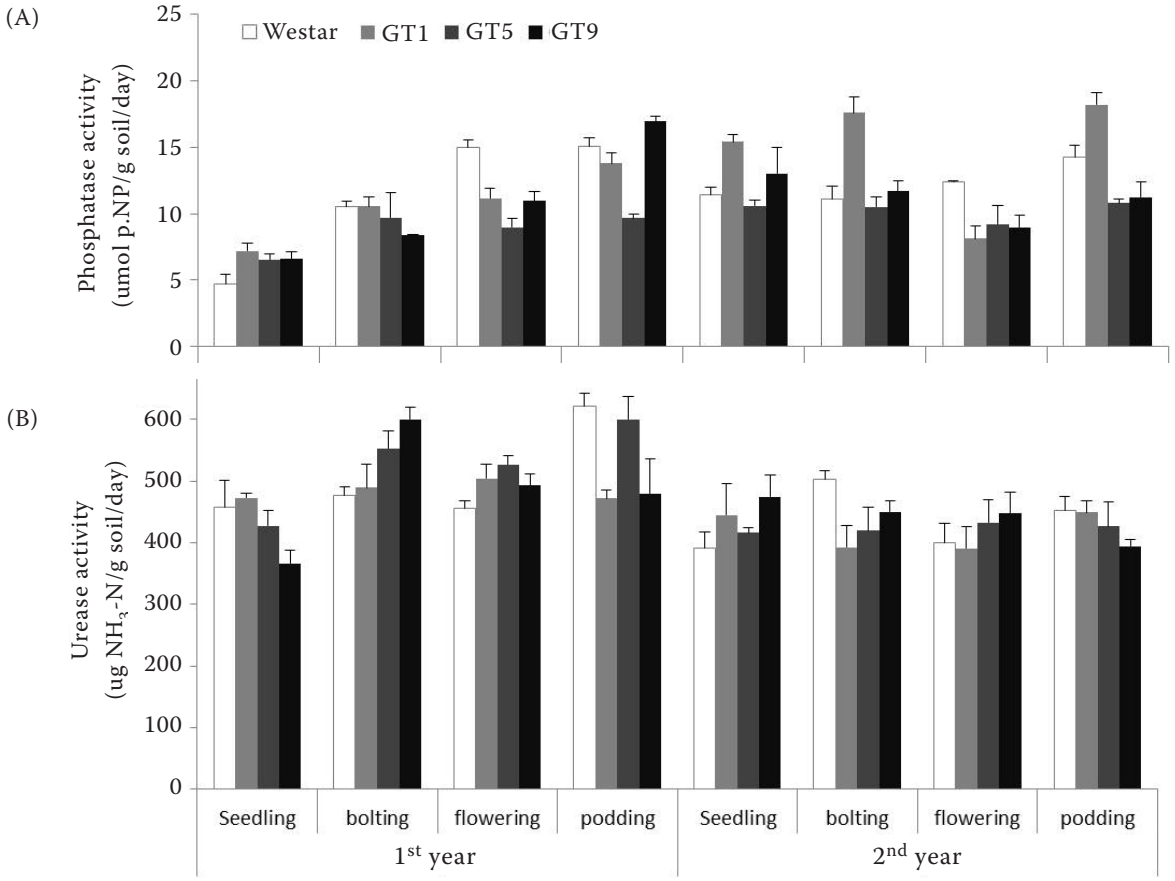

Figure 2. The changes of (A) phosphatase and (B) urease activities in rhizosphere soil of transgenic *Bt* oilseed rape (GT1, GT5, and GT9) and the control (Westar) over two consecutive growing seasons

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of the control plant Westar during the flowering stage. Specifically, transgenic GT5 had significantly lower soil phosphatase activity ($F_{3,8}$ = 32.628, P < 0.05) than the control Westar during the podding stage. In the second year, compared to the control plant Westar, transgenic plant event GT1 exhibited significantly higher soil phosphatase activities during the seedling ($F_{3,8}$ = 3.772, P < 0.05), bolting ($F_{3,8}$ = 12.085, P < 0.05), and podding ($F_{3,8}$ = 15.986, P < 0.05) stages. Furthermore, all three transgenic plant events (GT1, GT5 and GT9) had significantly lower ($F_{3,8}$ = 4.03, P < 0.05) soil phosphatase activities during the flowering stage (Figure 2A).

Urease activity. In the first planting year, transgenic plant events exhibited higher urease activities in the rhizosphere soil during bolting (GT5 and GT9, $F_{3,8}$ = 4.649, P < 0.05) and flowering stages (GT5, $F_{3,8}$ = 2.814, P < 0.05), and lower urease activities during the podding stage (GT1 and GT9, $F_{3,8}$ = 4.695, P < 0.05) compared to the control plant Westar (Figure 2B). In the second planting year, the urease activity in the rhizosphere soil of transgenic plant event GT1 was significantly lower ($F_{3,8}$ = 2.701, P < 0.05) than in the control plant Westar during the bolting stage. There were no significant differences in urease activities of the rhizosphere soil between all transgenic events and the control plant during the other three stages (Figure 2B).

Protease activity. During the first planting year, the transgenic plant event GT9 had lower protease activity in the rhizosphere soil during the flowering stage ($F_{3,8}$ = 5.76, P < 0.05) and higher protease activity during the podding stage ($F_{3,8}$ = 2.536, P < 0.05) than the control plant Westar (Figure 3A). In the successive planting year, the protease activity in the rhizosphere soil of transgenic plant GT9 was significantly higher ($F_{3,8}$ = 4.431, P < 0.05) than in the control plant Westar during the bolting stage. The enzyme activities in the rhizosphere soil of transgenic plant events GT1 and GT5 were significantly lower ($F_{3,8}$ = 7.501, P < 0.05) than in the control during the flowering stage.

Dehydrogenase activity. In the first year, the dehydrogenase activity in the rhizosphere soil of the

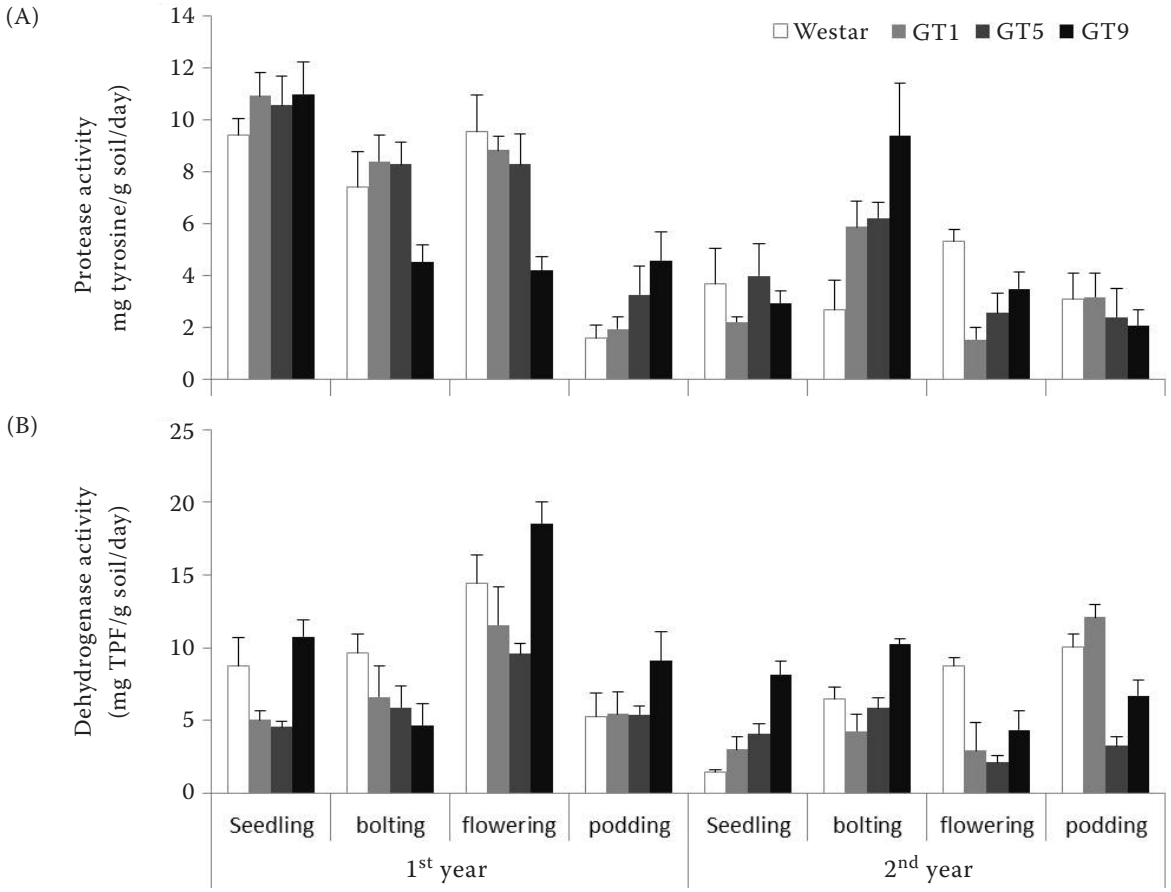

Figure 3. The changes of (A) protease and (B) dehydrogenase activities in rhizosphere soil of transgenic *Bt* oilseed rape (GT1, GT5, and GT9) and the control (Westar) over two consecutive growing seasons

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transgenic plant event GT5 was lower ($F_{3,8}$ = 6.181, P < 0.05) than the control plant Westar during the seedling stage. There were no significant differences in dehydrogenase activities of the rhizosphere soil between all transgenic events and the control plant during the other three stages (Figure 3B). In the second year, compared with the control, transgenic plant events had higher dehydrogenase activities during the seedling (GT5 and GT9, $F_{3,8}$ = 15.912, P < 0.05) and bolting (GT9, $F_{3,8}$ = 10.163, P < 0.05) stages, and lower ones during the flowering (GT1, GT5 and GT9, $F_{3,8}$ = 5.732, P < 0.05) and podding (GT5 and GT9, $F_{3,8}$ = 19.143, P < 0.05) stages (Figure 3B).

PCA analysis of enzyme activity changes in rhizosphere soil of transgenic oilseed rape

Principal components analysis (PCA) was conducted to explore the relationships between soil enzyme activities in rhizosphere soil of transgenic oilseed rape. In the first year, the Eigenvalues from PCA of the four plant types revealed that the first principal component explained 45.55% of the total sample variation. In contrast, the second principal component explained 28.23% (Figure 4A). Sucrase and dehydrogenase activities significantly positively impacted PC1 (> 0.800), indicating that these two enzyme activities were strongly correlated and might play a crucial role in the overall enzymatic activity pattern in the rhizosphere. Urease activity had a major positive effect on PC2 (> 0.800), suggesting that it contributed significantly to the variation explained by the second principal component. The soil enzyme activities of transgenic event GT9 and the control Westar in the first quadrant of the PCA ordination diagram differed from those of the other two transgenic plant events, GT1 and GT5. This suggests that the enzyme activity profiles of GT9 and Westar might be more similar to each other, while GT1 and GT5 have distinct patterns. In the second year, the Eigenvalues from PCA indicated that the first principal component accounted for 52.34% of the total sample variation, and the second principal component accounted for 20.02% (Figure 4B). Sucrase had a major positive effect on PC1 (> 0.800), highlighting its importance in driving the variation along the first principal component. Phosphatase had a major positive effect on PC2 (> 0.900). The soil enzyme activities of all three transgenic events, GT1, GT5 and GT9, differed from those of the control Westar, suggesting that the transgenic plants have a distinct impact on the soil enzyme activity profile compared to the non-transgenic control. These findings can be combined with the other results of the study to

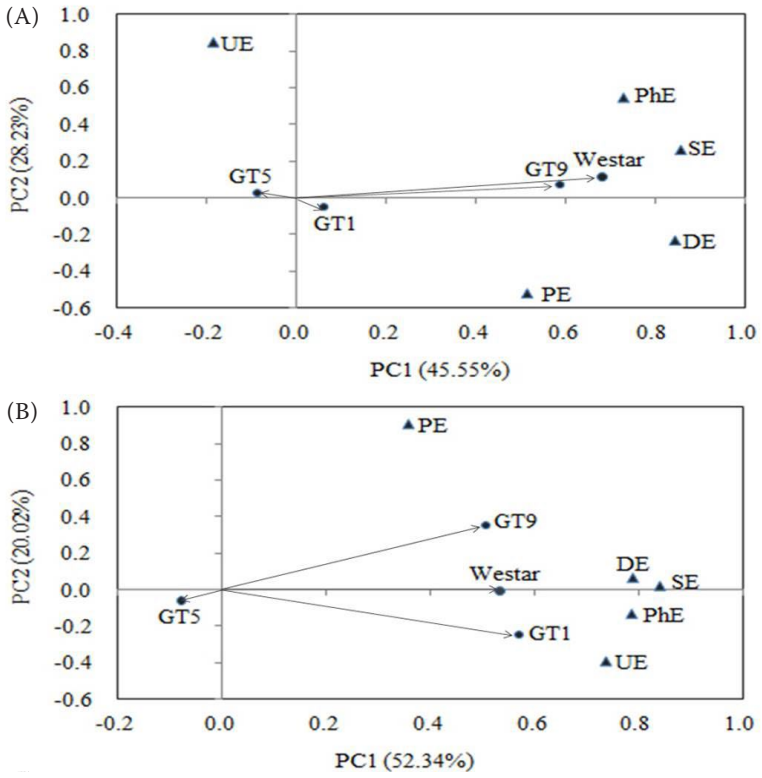

Figure 4. Principal components analysis (PCA) analysis of enzyme activities in rhizosphere soil of transgenic *Bt* oilseed rape (GT1, GT5, and GT9) and the control (Westar) in (A) the first cultivation year and (B) the second cultivation year. SE – sucrose; DE – dehydrogenase; PhE – phosphatase; UE – urease; PE – protease

https://doi.org/10.17221/330/2024-PSE

draw more comprehensive conclusions about the dynamics of Cry1Ac protein and the changes in soil enzyme activities in the rhizosphere of transgenic *Bt* oilseed rape.

Relationships between soil enzyme activities and Cry1Ac protein

Correlation analysis among the five soil enzyme activities revealed an extremely significant and positive correlation between soil dehydrogenase activity and the other four enzymes ($P < 0.01$) (Table 1). Soil sucrase activity showed extremely significant positive correlations with phosphatase (0.590, $P < 0.01$), urease (0.311, $P < 0.01$), and dehydrogenase (0.538, $P < 0.01$). Additionally, soil phosphatase activity had extremely significant positive correlations with urease (0.270, $P < 0.01$) and dehydrogenase (0.382, $P < 0.01$). However, soil protease activity was negatively correlated with phosphatase (–0.213, $P < 0.01$).

The relationships between soil enzyme activities and Cry1Ac protein were examined in this study (Table 1). The results demonstrated that Cry1Ac protein had an extremely significant positive correlation with phosphatase (0.242, $P < 0.01$) and a significant positive correlation with urease (0.185, $P < 0.05$). However, Cry1Ac protein was significantly and negatively correlated with soil protease activity (0.248, $P < 0.01$).

DISCUSSION

The long-term effects of transgenic *Bt* plants on the soil ecosystem are indeed a matter of concern. Previous studies have shown that the expression of insecticidal crystal proteins in plants can have varying effects on the soil ecosystem. For example, some research has indicated that *Bt* proteins can quickly bind to soil clay and humic acid, retaining their insecticidal activity and potentially persisting in the soil for extended periods (Li et al. 2022). This highlights the need to further explore the fate and stability of Cry1Ac protein in the soil environment. *Bt* crops produce insecticidal crystal proteins, which endow specific insect resistance to the plant tissues expressing the transgene. Radioactivity detection in watermelon and wheat plant tissues confirmed the adsorption, uptake, and translocation of the *Bt* Cry1Ac protein peptide into the seedlings of these plants (Zhang et al. 2020). Researchers have analysed the expression patterns of toxic proteins in various organs of insect-resistant crops (Chen et al. 2022). The spatial and temporal expression of insecticidal crystal proteins in different transgenic *Bt* crops during various growth stages is influenced by external factors such as crop regulation, growing environment, and management conditions. A 3-year study indicated that *Bt* rice can release detectable amounts of Cry1Ab/1Ac protein into the soil and field water during the growth period (Wang et al. 2013). Low levels of *Bt* toxin produced in transgenic plants, similar to commercial microbial *Bt* formulations, may persist in the soil for weeks or months (Strain and Lydy 2015). *Bt* proteins from transgenic crops may enter into soil ecosystems primarily through root exudates and aquatic ecosystems through plant residues (Liu et al. 2016). *Bt* proteins were detected at low levels in the rhizosphere soils of transgenic 2A-7 maize plants (Xu et al. 2023). This study also revealed that transgenic *Bt* (Cry1Ac) oilseed rape plants could introduce insecticidal crystal proteins into the soil through root exudates, and the protein levels varied across different growth periods. Given that soil is a complex ecological system, the accumulation of toxic proteins in the soil can be influenced by factors such as the types, forms, and concentrations of insecticidal crystal proteins, soil types, moisture levels, microbial compositions, farming depth, continuous cropping

Table 1. Correlation coefficients between soil enzyme activities and Cry1Ac protein (n = 120)

	Sucrase	Phosphatase	Urease	Protease	Dehydrogenase	Cry1Ac protein
Sucrase	1.000	0.590**	0.311**	0.053	0.538**	0.119
Phosphatase		1.000	0.270**	–0.213**	0.382**	0.242**
Urease			1.000	0.082	0.335**	0.185*
Protease				1.000	0.329**	–0.248**
Dehydrogenase					1.000	–0.161
Cry1Ac protein						1.000

*$P < 0.05$; **$P < 0.01$

https://doi.org/10.17221/330/2024-PSE

practices, and climate conditions (Xue et al. 2014, Guan et al. 2016). Therefore, assessing the biological activity of insecticidal crystal proteins released from transgenic *Bt* plants into the soil is crucial for evaluating their potential impacts on non-target species. Regular and comprehensive monitoring of the levels of Cry1Ac protein and soil enzyme activities in the rhizosphere soil should be established to track any changes over an extended period closely. This could involve using advanced analytical techniques and frequent sampling to ensure accurate and timely detection of potential issues. For example, adjusting the cultivation methods, such as optimising irrigation and fertilisation regimes, could help maintain a balanced soil environment. Furthermore, using soil amendments, like organic matter or beneficial microorganisms, might enhance the soil's resilience and ability to cope with any disturbances caused by transgenic *Bt* plants.

The long-term effects of transgenic *Bt* plants on the soil ecosystem are indeed a matter of concern. The insecticidal crystal proteins produced by these plants can likely interact with soil particles, affecting the availability of nutrients and the activity of soil microorganisms. This, in turn, can lead to changes in soil enzyme activity. For example, the binding of Cry1Ac protein to soil minerals and humic acids may alter the structure and function of soil enzymes, potentially inhibiting their activity. Additionally, the changes in microbial communities due to the presence of transgenic *Bt* plants may also influence soil enzyme production and activity. While this study has revealed changes in soil enzyme activities over two consecutive planting years, the long-term implications of these changes require further investigation. Compared with other related studies, our findings are consistent with those of Zheng et al. (2022), who observed significant differences in soil enzyme activities between transgenic and wild-type plants at different growth stages. However, in contrast to the study by Arshad et al. (2022), which found no significant changes in enzyme activities in the rhizosphere of AVP1-transgenic wheat, our research shows clear alterations in the activities of several soil enzymes in the rhizosphere of transgenic *Bt* oilseed rape. These comparisons demonstrate the complex and context-dependent nature of the interactions between transgenic plants and the soil ecosystem. To monitor and mitigate any potential adverse effects, it is essential to establish long-term monitoring programs that track the dynamics of soil enzyme activities, soil microbial communities, and other relevant parameters over an extended period.

The implications of the changes in soil enzyme activities for soil fertility, nutrient cycling, and overall ecosystem functioning are highly valuable. Soil enzyme activities are closely linked to these processes. For instance, alterations in sucrase activity can influence the breakdown of carbohydrates, affecting the availability of energy sources for soil microorganisms. Changes in urease activity can impact nitrogen cycling, as it plays a key role in converting urea to ammonia. Phosphatase activity is crucial for releasing phosphorus from organic compounds, which are essential for plant growth. Dehydrogenase activity is an indicator of overall microbial activity and can reflect the health of the soil ecosystem. Under ambient temperature, pressure, and suitable pH conditions, soil enzymes play a vital role in accelerating essential biochemical processes such as the synthesis and decomposition of humic substances, hydrolysis and transformation of organic matter, and oxidation and reduction of inorganic materials (Schwarz et al. 2015). These processes are closely linked to soil nutrient element release and storage, soil humus formation and development, and soil structure and physical conditions (Lebedev et al. 2022). Studies have indicated that soil enzyme activity correlates with the content of soil clay particles and humus (Tabatabai et al. 2002). Upon entering the soil, insecticidal crystal proteins can adsorb and bind to the soil's minerals, humic acids, and organo-mineral complexes (Crecchio and Stotzky 2001). They may compete for active binding sites on soil particles with soil enzymes, potentially affecting soil enzyme activities. Research has shown that certain soil enzyme activities significantly decreased in the soil after 4 years of consecutive cultivation of transgenic cotton (Chen et al. 2011). The dehydrogenase, alkaline phosphatase and urease activities were higher in the rhizosphere of *Bt* cotton isolines (Mina and Chaudhary 2012), while the changes varied in our study depending on growth stages, events and years. In contrast, studies showed that the activities of soil enzymes, such as dehydrogenase, urease, and phosphatase activities, were not impacted by transgenic *Bt* rice or cotton (Wei et al. 2012, Xie et al. 2017). These changes in soil enzyme activities can have far-reaching implications for soil fertility. For example, if enzyme activities are disrupted, it may lead to imbalances in nutrient availability, affecting plant nutrition and productivity. Nutrient cycling can

https://doi.org/10.17221/330/2024-PSE

also be affected, as the efficient cycling of nutrients like nitrogen, phosphorus, and carbon relies on the proper functioning of soil enzymes. Overall, the functioning of the ecosystem may be compromised if the soil ecosystem is not stable and healthy. Further research is essential to develop effective monitoring strategies and mitigation measures that are based on a thorough understanding of the interactions between transgenic *Bt* plants and the soil ecosystem. This will enable us to ensure the sustainable use of these plants while minimising any potential risks to the soil ecosystem. Additionally, a comprehensive understanding of the detailed implications of soil enzyme activity changes on soil fertility and ecosystem functioning is imperative for making informed decisions regarding the management of transgenic crops and for maintaining the long-term health and stability of our agricultural systems.

In this study, we found that the amount of insecticidal crystal proteins in the rhizosphere soil of transgenic *Bt* (Cry1Ac) oilseed rape changed with different growth periods, and the enzyme activities in the rhizosphere soil of transgenic *Bt* oilseed rape plants underwent significant changes over two successive planting years. Specifically, the levels of Cry1Ac protein in the rhizosphere soil of transgenic oilseed rape events were significantly higher than those in the control during certain growth stages. Additionally, the activities of sucrase, urease, phosphatase, dehydrogenase, and protease in the rhizosphere soil of transgenic oilseed rape showed varying significant changes across different growth stages and years. Further research is needed to elucidate the long-term effects of insecticidal proteins on soil enzyme activities and the transformation of soil nutrient elements through extended field experiments.

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