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SOIL PROPERTIES ASSESSMENT IN LONG-TERM CULTIVATED Bt COTTON FARMLAND IN SOUTHERN SHANXI, CHINA

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Abstract

This study assessed the impact of over eight years of continuous cultivation of transgenic Bt cotton on the soil ecological environment by examining rhizosphere and non-rhizosphere soils from five regions in southern Shanxi, China. Changes in enzyme activities, Bt protein residues, and physicochemical characteristics of soils in the rhizosphere and non-rhizosphere environment during the harvest period were investigated. Results showed that rhizosphere soil had significantly higher organic matter and water content, but significantly lower alkali-hydrolyzable nitrogen, available phosphorus, and available potassium. Rhizosphere soil contained more Bt protein residues with regional variations. Enzyme activities differed significantly; rhizosphere soil had higher polyphenol oxidase, alkaline phosphatase, sucrase, and peroxidase activities, but lower urease. Correlation analysis revealed relationships between soil physicochemical properties, Bt protein content, and soil enzyme activities. Principal component analysis identified soil available potassium and urease activity as key factors influencing the rhizosphere environment. These findings highlight the needs for long-term monitoring to support rational cultivation practices and ecological safety assessment of Bt crops.

Introduction

With the rapid advancement of transgenic technology, the promotion and cultivation of transgenic crops worldwide have emerged as a significant trend in modern agriculture. Transgenic Bt cotton has achieved considerable economic and ecological benefits in production due to its remarkable insect-resistant properties (Liu *et al.* 2019). However, as transgenic Bt cotton becomes more prevalent in the fields, concerns about the ecological implications of its exogenous genes have come to the fore. Once released into the environment, these exogenous genes could disrupt the delicate balance of the soil ecosystem, making this area a prime focus of current research (Krogh *et al.* 2020, Guan *et al.* 2021). Therefore, exploring the long-term influence of transgenic Bt cotton on the field environment is crucial for ensuring the safe, healthy, and sustainable development of transgenic Bt cotton breeding.

Soil enzyme activity, a key indicator of soil biological activity, is closely linked to nutrient cycling, energy transfer, and soil clay and humus content (Vezzani *et al.* 2018). Enzymes like urease, phosphatase, and invertase affect the cycling of N, P, S, C, and plant nutrition (Zhou and Staver 2019, Wulandari *et al.* 2021, Guan *et al.* 2024). Studies on transgenic plants show various effects: BADH transgenic maize had minimal impact on urease (Bai *et al.* 2019); while AVP1-transgenic wheat showed no significant changes in rhizosphere enzyme activities (Arshad *et al.* 2022). Thus, soil enzyme activity is crucial for assessing transgenic plants' long-term impact on soil ecosystems. Rhizosphere soil, an active zone for soil-plant root interactions (Lebedev *et al.* 2022, Alam *et al.* 2024), differs from non-rhizosphere soil in nutrients, enzyme activities, and microbial traits, but research on such differences in transgenic crops remains relatively limited.

Bt protein from transgenic insect-resistant crops can enter the soil and affect nutrient transformation (Li *et al.* 2022, Xu *et al.* 2023). Most studies on Bt cotton soil enzyme activities are

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short-term pot/greenhouse experiments with inconsistent conclusions (Xie *et al.* 2017), and long-term impacts of soil Bt protein remain unclear. Research on transgenic cotton's field effects focuses on artificial cultivation, with scarce studies on farmland rhizosphere soil. This study thus examines natural southern Shanxi Bt cotton fields with over 8-year cultivation, exploring rhizosphere/non-rhizosphere differences in soil physicochemical properties and enzyme activities during harvest. It aids understanding of nutrient cycling and ecological adaptation mechanisms of transgenic cotton, providing a theoretical basis for risk assessment of transgenic crop cultivation.

Materials and Methods

The study was conducted in natural cotton farmlands with over 8 years of continuous Bt cotton cultivation (≥2 acres) in southern Shanxi, China. Five sampling sites were selected from five regions such as Longju, Jinjing, Yongji, Xiandi, and Daning. Three samplings per region were carried out during late harvest. The exact geographical location of five sampling sites is shown in Table 1. Rhizosphere and non-rhizosphere soils were collected, sieved (<2 mm) and stored at 4°C for subsequent laboratory analysis.

Table 1. Sample information of Bt transgenic cotton farmlands.

Sampling regions	Geographic location information of every sample site		
	Sample site I	Sample site II	Sample site III
Yongji	E110.552517; N34.954093	E110.551654; N34.954492	E110.551743; N34.954625
Jinjing	E110.788538; N35.007212	E110.785663; N35.008866	E110.785745; N35.008971
Longju	E110.885775; N35.018827	E110.885653; N35.018759	E110.885543; N35.018632
Daning	E110.564722; N36.442777	E110.576687; N35.448351	E110.552163; N35.444170
Xiandi	E111.604891; N36.032764	E111.599404; N36.039892	E111.608342; N36.063369

Soil water content (SWC, oven-drying method), pH value (electrode method), organic matter (OM, potassium dichromate volumetric method), alkali-hydrolyzable nitrogen (AN, alkaline hydrolysis diffusion method), available phosphorus (AP, sodium bicarbonate extraction-molybdenum antimony anti-colorimetric method), and available potassium (AK, neutral ammonium acetate extraction-flame photometry method) were determined.

Bt Cry1Ac protein content was quantified using ELISA assay kit (Envirologix Inc., USA). Soil enzyme activities were assayed using kits (Suzhou Keming Biotechnology Co., Ltd., China), including sucrase (SUC), alkaline phosphatase (ALP), urease (URE), polyphenol oxidase (PPO), and peroxidase (POD).

Data (mean ± SE) were analyzed via one-way ANOVA (SPSS 27.0) with LSD test ($P < 0.05$). Pearson correlations and principal component analysis (PCA) were performed using SPSS 27.0.

Results and Discussion

The results indicated that soil water content was generally higher in rhizosphere than non-rhizosphere soils except in Jinjing, with regional variations (Table 2). All soils were alkaline, with rhizosphere pH consistently higher ($p < 0.05$) than non-rhizosphere; rhizosphere also had higher organic matter. For nutrients, rhizosphere alkali-hydrolyzable nitrogen was lower in most regions except Longju and Xiandi. Available phosphorus was generally lower in rhizosphere with regional variations, and both rhizosphere and non-rhizosphere showed significant regional differences. Available potassium was consistently lower in rhizosphere across most regions, with significant regional variations in both rhizosphere and non-rhizosphere.

Table 2. Physicochemical properties and soil nutrient content of rhizosphere and non-rhizosphere soils in transgenic Bt cotton farmlands across different regions.

Sampling regions	Index	Rhizosphere	Non-rhizosphere
Yongji	SWC	43.62 ± 0.99Aa	40.41 ± 1.15Ba
	pH	8.57 ± 0.04Aa	8.43 ± 0.06Ba
	OM	20.96 ± 0.57Ab	19.39 ± 0.78Ba
	AN	0.86 ± 0.09Bb	1.13 ± 0.13Ab
	AP	12.72 ± 1.14Bbc	15.73 ± 1.36Abc
	AK	130.67 ± 6.26Bd	153.57 ± 6.48Ac
Jinjing	SWC	44.26 ± 1.21Aa	41.20 ± 1.77Aa
	pH	8.47 ± 0.11Aa	8.25 ± 0.04Bb
	OM	21.49 ± 0.92Aab	19.48 ± 0.71Ba
	AN	0.86 ± 0.09Bb	1.27 ± 0.09Aab
	AP	14.94 ± 1.50Ba	18.46 ± 1.23Aa
	AK	155.56 ± 6.52Abc	169.73 ± 7.05Ab
Longju	SWC	43.02 ± 1.53Aab	39.75 ± 0.74Ba
	pH	8.44 ± 0.09Aa	8.22 ± 0.04Bb
	OM	22.45 ± 0.65Aa	20.23 ± 0.90Ba
	AN	1.15 ± 0.13Aa	1.38 ± 0.08Aa
	AP	14.47 ± 0.64Bab	16.80 ± 0.81Aab
	AK	145.94 ± 9.99Bc	168.58 ± 7.82Ab
Daning	SWC	39.16 ± 0.47Ab	35.63 ± 1.04Bb
	pH	8.24 ± 0.08Ab	8.02 ± 0.04Bc
	OM	21.79 ± 0.69Aab	19.85 ± 0.25Ba
	AN	1.00 ± 0.09Bab	1.30 ± 0.06Aab
	AP	11.27 ± 0.62Ac	12.42 ± 1.16Ad
	AK	167.22 ± 6.13Aab	175.66 ± 4.26Ab
Xiandi	SWC	41.53 ± 0.82Ab	37.39 ± 1.07Bb
	pH	8.60 ± 0.12Aa	8.42 ± 0.05Aa
	OM	21.74 ± 0.81Aab	19.34 ± 0.88Ba
	AN	1.01 ± 0.16Aab	1.24 ± 0.15Aab
	AP	12.57 ± 1.01Abc	14.63 ± 0.91Ac
	AK	176.34 ± 3.81Ba	192.59 ± 4.96Aa

Different uppercase and lowercase letters indicate significant differences between rhizosphere and non-rhizosphere soils at $P < 0.05$.

Bt protein residues were detected in rhizosphere soils across all regions with variations (Fig. 1A). Longju had the highest residual level of Bt protein (0.1672 ng/g), Daning following closely (0.1541 ng/g), and Yongji the lowest (0.1166 ng/g, $p < 0.05$). Non-rhizosphere residues ranged from 0.0559 to 0.0664 ng/g, with no significant regional differences.

In each tested region, rhizosphere soil of transgenic Bt cotton had significantly higher polyphenol oxidase activity than non-rhizosphere soil, with no significant differences between regions (Fig. 1B). Alkaline phosphatase activity in rhizosphere soil of all five regions was significantly higher than in non-rhizosphere soil, with Yongji's rhizosphere soil having the highest (24.46 U phenol/g soil) (Fig. 2A). Urease activity in rhizosphere soil was generally lower than in non-rhizosphere soil, with significant difference only in Daning; Jinjing's rhizosphere and non-rhizosphere soils had relatively high urease activity (Fig. 2B). Sucrase and peroxidase activities in rhizosphere soil of all tested regions were significantly higher than in non-rhizosphere soil, with no significant differences between regions (Fig. 3A-B).

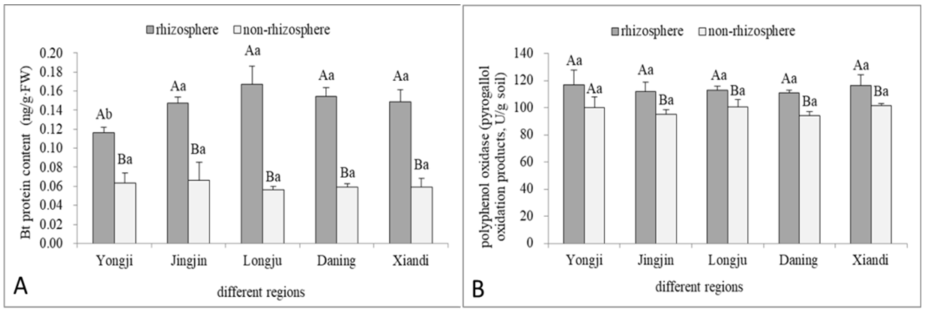

Fig.1. Dynamics of Cry1Ac protein (A) and the changes of polyphenol oxidase (B) in soil of transgenic Bt cotton in different regions. Different uppercase and lowercase letters indicate significant differences at (P <0.05).

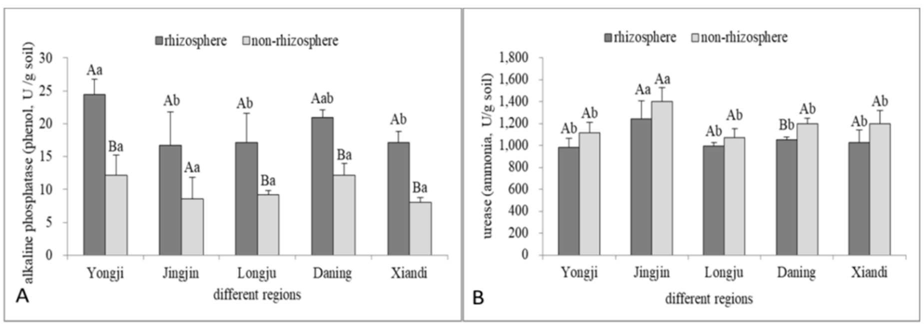

Fig. 2. The changes of alkaline phosphatase (A) and urease activities (B) in soil of transgenic Bt cotton in different regions. Different uppercase and lowercase letters indicate significant differences at (P <0.05).

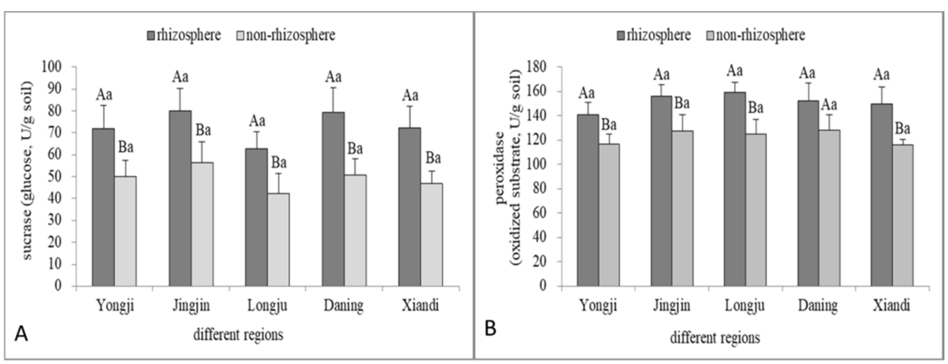

Fig. 3. The changes of sucrase (A) and peroxidase activities (B) in soil of transgenic Bt cotton in different regions. Different uppercase and lowercase letters indicate significant differences at (P < 0.05).

Rhizosphere correlations analysis (Table 3) showed that Polyphenol oxidase (PPO) correlated positively with soil water content (SWC) and pH, negatively with organic matter (OM). The alkaline phosphatase (ALP) correlated negatively with OM, available phosphorus (AP), and significantly with Bt protein (p <0.05). The Bt protein was correlated negatively with pH, positively with OM and available potassium (AK) (p <0.05). The SWC showed positive correlation with pH, and negative correlation with AK. The OM had positive correlation with alkali-hydrolyzable nitrogen (AN) and AK. Non-rhizosphere correlations (Table 4) showed that PPO correlated positively with SWC and pH ($p < 0.05$); urease with sucrose (SUC) ($p < 0.05$); SWC significantly with AP ($p < 0.01$); SUC with peroxidase (POD). PCA (Fig. 4) showed rhizosphere PC1 (96.51%) driven by AK, PC2 (1.80%) by urease; non-rhizosphere PC1 (23.14%) by pH, PC2 (19.33%) by AP and urease.

Table 3. Correlation analysis of physicochemical properties and soil enzyme activities of rhizosphere soil from transgenic Bt cotton in different regions.

	PPO	ALP	URE	SUC	POD	SWC	pH	OM	AN	AP	AK	Bt protein
PPO	1	0.044	-0.193	-0.241	-0.161	0.146	0.297	-0.102	0.096	0.186	0.028	-0.224
ALP		1	-0.129	0.168	-0.13	0.059	0.237	-0.23	-0.081	-0.453	-0.399	-0.622*
URE			1	0.325	0.036	0.205	-0.114	-0.145	-0.213	0.283	0.136	0.097
SUC				1	0.387	-0.245	-0.115	-0.319	-0.286	0.007	0.085	-0.314
POD					1	-0.042	0.01	0.289	0.219	0.324	0.156	0.267
SWC						1	0.546*	-0.166	-0.208	0.578*	-0.524*	-0.33
pH							1	-0.101	-0.039	0.215	-0.199	-0.492*
OM								1	0.201	-0.067	0.333	0.59*
AN									1	0.17	0.081	0.42
AP										1	-0.32	0.052
AK											1	0.478*
Bt protein												1

* indicates significance at the 0.05 level.

Table 4. Correlation analysis of physicochemical properties and soil enzyme activities of non-rhizosphere soil from transgenic Bt cotton in different regions.

	PPO	ALP	URE	SUC	POD	SWC	pH	OM	AN	AP	AK	Bt protein
PPO	1	-0.105	-0.106	-0.175	-0.333	0.307	0.54*	-0.025	0.132	0.22	0.176	0.08
ALP		1	-0.115	0.217	0.307	-0.137	-0.091	-0.142	0.005	-0.38	-0.304	-0.364
URE			1	0.516*	0.202	0.112	-0.09	-0.321	0.081	0.344	0.183	0.428
SUC				1	0.521*	0.015	0.008	-0.304	-0.075	0.114	-0.132	0.186
POD					1	-0.124	-0.339	0.208	0.235	0.038	-0.044	-0.332
SWC						1	0.462	0.124	-0.171	0.721**	-0.364	-0.009
pH							0.54*	-0.282	-0.38	0.324	-0.006	-0.033
OM							-0.091	1	-0.217	-0.178	0.139	-0.428
AN									1	0.168	0.139	0.052
AP										1	-0.339	0.403
AK											1	-0.314
Bt protein												1

** indicates significance at the 0.01 level. * indicates significance at the 0.05 level.

One ecological risk of transgenic Bt crops is their impact on soil ecology and functionality. Transgenic crops may affect soil nutrient transformation via root exudate changes (Song *et al.* 2020), and exogenous Bt protein entering soil could alter soil fertility (Tang *et al.* 2014). While Liu *et al.* (2012) found higher available phosphorus in transgenic phyA cotton rhizosphere, other studies showed no obvious effects of Bt plant return on soil enzymes or nutrients (Zhou *et al.* 2016). This study shows long-term Bt cotton rhizosphere has higher organic matter but lower alkali-hydrolyzable nitrogen, available phosphorus, and potassium, with rhizosphere fertility changes linked to root metabolism, suggesting long-term Bt cultivation may negatively affect soil fertility factors.

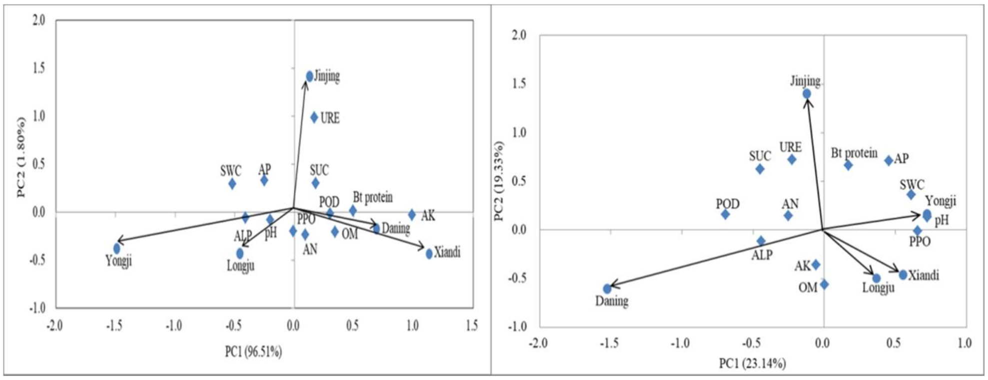

Fig. 4. PCA analysis on correlation of soil enzyme activities and physicochemical properties of rhizosphere (A) and non-rhizosphere (B) soils in different regions.

Soil enzymes play key roles in humus synthesis/decomposition, organic matter conversion, and inorganic oxidation-reduction (Kotroczó *et al.* 2014, Schwarz *et al.* 2015, Propa *et al.* 2021). While some studies note no significant changes in rhizosphere enzyme activities during Bt cotton growth (Xie *et al.* 2017), others find transgenic plants, especially Bt varieties, impact enzyme activities (Mina *et al.* 2012). This study shows long-term Bt cotton cultivation leads to regional differences in rhizosphere enzyme activities, linked to nutrient release, humus formation, and soil structure (Lebedev *et al.* 2022), requiring long-term monitoring to clarify insecticidal crystal proteins' effects on enzymes and nutrient conversion.

In this study, we found that soil organic matter content in the rhizosphere of Bt cotton was significantly higher than that in the non-rhizosphere soil, but the contents of alkali-hydrolyzable nitrogen, available phosphorus, and available potassium were significantly lower. There were significant differences in the Bt protein content between rhizosphere and non-rhizosphere soils, and there might be a low dose of Bt protein residue in the rhizosphere soil of cotton fields. Significant differences in enzyme activity were also observed between the rhizosphere and non-rhizosphere soils of transgenic cotton. The activities of polyphenol oxidase, alkaline phosphatase, sucrase, and peroxidase were generally higher in the rhizosphere soil, while the urease activity in the rhizosphere soil was generally lower than that in the non-rhizosphere soil. Correlation and principal component analyses revealed complex interactions among soil physicochemical properties, Bt protein content, and soil enzyme activities. This study concludes that long-term cultivation of transgenic Bt cotton induces significant differences in physicochemical properties, Bt protein residues, and enzyme activities between rhizosphere and non-rhizosphere soils with complex interactions; while limited to five regions in southern Shanxi, it highlights the need for expanded regional and indicator monitoring to support ecological risk assessment and sustainable cultivation of Bt crops.

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