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Effect of Potash Application on Gene Expression of Cry1Ac and Cry2Ab2 in Bt Cotton Hybrid

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ABSTRACT: This study investigates the impact of different levels of potash (K_2O) application on the gene expression of Cry1Ac and Cry2Ab2 endotoxins in Bt cotton hybrid G. Cot. Hy. 8 BG II. A field experiment was conducted at the Main Cotton Research Station, Surat, with 12 treatments, including varying potash levels (0, 40, and 80 kg ha^{-1}), the application of potash mobilizing bacteria (KMB), and foliar sprays of potassium nitrate (KNO_3). Potash application significantly influenced the incidence of pink bollworm (PBW), reducing larval populations and flower damage in Bt cotton. Cry1Ac and Cry2Ab2 expression levels in different plant parts remained above critical thresholds for bollworm management, with higher expressions in treatments with potash application. The study indicates that potash supplementation can enhance bollworm resistance by stabilizing Bt gene expression in cotton hybrids.

Keywords: cotton, potash, bollworm, Cry1Ac and Cry2Ac.

INTRODUCTION

Cotton (*Gossypium* spp.) is a major economic crop with high demand in textile industries worldwide. Bt cotton hybrids expressing Cry1Ac and Cry2Ab2 have proven to be effective against lepidopteran pests, particularly bollworms. However, various environmental factors, including nutrient availability, influence the efficacy of gene expression. Potassium (K), essential for plant growth and fiber quality, has been shown to affect transgene expression and pest resistance in cotton. This study explores the effect of K_2O application on Cry1Ac and Cry2Ab2 gene expression in Bt cotton hybrid G. Cot. Hy. 8 BG II and its impact on bollworm incidence. Cotton is cultivated in varied climate and soil type with mono-cropping approach in Gujarat state due to economic cash crop. As per the soil type, texture and structure, nutrient up-take by plant is differed in rain fed and irrigated cotton. Based on regional suitability balanced fertilizer use is recommended in cotton. Though, Gujarat soil is rich in K content (Bhambhaneeya *et al.*, 2017), the availability may be increased through the use of such organic amendments, foliar applications or potash mobilizing bacteria with supplementation of the K in deficient soils. The balanced use of fertilizers does not affect much the gene expression but the lower dose of nitrogen reduced the expression of gene in Bt cotton. Further, cotton appears to be more sensitive to K deficiencies than other crops, as root system of cotton is less dense than that of other crops (Mithaiwala *et al.*, 1981). It has been found that potash application enhanced protein synthesis resulting in reduced amino acid content of the plant sap, may stabilize expression of genes in transgenic crop and reduced the development and multiplication of insect pests especially of bollworms.

MATERIALS AND METHODS

A split-plot field experiment was conducted using Bt cotton hybrid G. Cot. Hy. 8 BG II, with 12 treatments including three K_2O application rates (0, 40, and 80 kg ha^{-1}), two levels of KMB, and two foliar spray treatments of KNO_3. The study was designed to measure bollworm incidence, larval populations, flower damage, and gene expression levels of Cry1Ac and Cry2Ab2 across different plant parts at various growth stages. The incidence of bollworms was monitored, and Cry1Ac and Cry2Ab2 gene expression in different plant tissues were quantified using ELISA.

Experimental Details:

SI	Particulars		Detail
(i)	Location	:	Main Cotton Research Station, NAU, Surat
(ii)	Season and Year	:	*Kharif* 2018
(iii)	Crop and Variety	:	Cotton, G. Cot. Hy. 8 BG II
(iv)	Layout/Design	:	Split Plot Design (SPD)
(v)	Replications	:	3
(vi)	Spacing	:	1.20m × 0.45 m
(vii)	Plot Size	:	Gross: 6.00m × 5.40m Net: 3.60m × 4.50m
(viii)	Sowing date	:	Sowing: 27/06/2018 Re-sowing:17/07/2018
	Date of Gap filling	:	06/07/2018
	Date of 1st Picking	:	28/11/2018
	Date of Ring line Picking	:	01/12/2018
	Date of 2nd Picking	:	28/12/2018
(ix)	Manures and Fertilizers	:	FYM 10 t ha^{-1} & NPK:240:40:00 kg ha^{-1} (Recommended dose of nutrition) common to all the experimental unit
(x)	Cultural operations & Plant protection	:	Interculturing and weeding as and when required and ETL based sprays for sucking pests (as per Table 1)
(xi)	No. of Irrigations	:	One irrigation was given one month after cessation of rain (October)
(xii)	**Treatment Details**	:	
	Main Treatment	:	K_2O application as Murate of Potash (00:00:60), By Indian Potash Ltd. (Plate I)
	First level	:	K_0=0 kg ha^{-1}
	Second level	:	K_{40}= 40 kg ha^{-1}
	Third level	:	K_{80}= 80 kg ha^{-1}
	Sub Treatment 1	:	Potash Mobilizing Bacteria (Plate II)
	First level	:	B_0=No application of KMB
	Second level	:	B_1=KMB @2.5 lit ha^{-1}
	Sub Treatment 2	:	Foliar sprays of KNO_3(13:00:45)
	First level	:	NFS=No foliar spray of KNO_3
	Second level	:	FS=Foliar sprays of KNO_3 @3 % (Plate III)

SI			
(xiii)	**Treatment combinations : 12 (Twelve)**		
	TC1	:	No K_2O , No KMB, No foliar sprays of KNO_3
	TC2	:	No K_2O , No KMB, Foliar sprays of KNO_3
	TC3	:	No K_2O , KMB @2.5 l ha^{-1} , No foliar sprays of KNO_3
	TC4	:	No K_2O , KMB @2.5 l ha^{-1}, Foliar sprays of KNO_3
	TC5	:	K_2O @40 kg ha^{-1}, No KMB, No foliar sprays of KNO_3
	TC6	:	K_2O @40 kg ha^{-1}, No KMB, Foliar sprays of KNO_3
	TC7	:	K_2O @40 kg ha^{-1}, KMB @2.5 l ha^{-1} , No foliar sprays of KNO_3
	TC8	:	K_2O @40 kg ha^{-1}, KMB@2.5 l ha^{-1}, Foliar sprays of KNO_3
	TC9	:	K_2O @80 kg ha^{-1} , No KMB, No foliar sprays of KNO_3
	TC10	:	K_2O @80 kg ha^{-1}, No KMB, Foliar sprays of KNO_3
	TC11	:	K_2O @80 kg ha^{-1} , KMB @2.5 l ha^{-1}, No foliar sprays of KNO_3
	TC12	:	K_2O @80 kg ha^{-1} , KMB @2.5 l ha^{-1}, Foliar sprays of KNO_3
(xiv)	**Application of treatments:**		
	The main and sub-treatment-1 were applied as basal application 15 days after germination of the crop in respective treatments. The sub-treatment-1 was prepared by thoroughly mixing the liquid potash Mobilizer after proper shaking in the compost and mixed well @2.5 lit 20 kg^{-1} of compost (Plate IV) and applied near the root zone at 30 DAS (Plate V). Whereas sub-treatment-2 was applied at squaring (60 DAS), flowering (75 DAS) and boll development (90 DAS) stages of the crop (Plate VI).		

Table 1: Incidence of pink bollworm larvae (big) on G. Cot. Hy. 8 BG II during 2018-19.

Factors	Number of pink bollworm larvae (big)/ 10 green bolls recorded at 15 days interval									
	90 DAS		105 DAS		120 DAS		135 DAS		Pooled	
	OV	TV	OV	TV	OV	TV	OV	TV	OV	TV
A. Main Treatment (Potash Fertilizer) K										
K_0	1.16	1.27	1.50	1.40	1.33	1.34	1.25	1.30	1.31	1.32
K_{40}	0.91	1.15	1.41	1.37	1.08	1.24	1.16	1.25	1.14	1.25
K_{80}	0.83	1.11	1.16	1.25	1.08	1.22	1.00	1.18	1.02	1.19
GM		**1.17**		**1.34**		**1.27**		**1.24**		**1.26**
SEm ±		0.05		0.03		0.07		0.09		**0.03**
CD (5%)		NS		NS		NS		NS		**0.08**
CV %		16.17		9.99		20.19		26.94		**19.16**
B. Sub Treatments										
Potash Mobilizng Bacteria (B)										
B_0	1.05	1.21	1.44	1.38	1.33	1.33	1.38	1.36	1.30	1.32
B_1	0.88	1.14	1.27	1.30	1.00	1.20	0.88	1.13	1.01	1.19
GM		**1.18**		**1.34**		**1.27**		**1.25**		**1.26**
SEm ±		0.07		0.05		0.05		0.06		**0.03**
CD (5%)		NS		NS		NS		0.18		**0.09**
Foliar sprays of Potassium Nitrate (F)										
NFS	1.05	1.21	1.38	1.35	1.22	1.29	1.27	1.30	1.23	1.29
FS	0.88	1.14	1.33	1.33	1.11	1.24	1.00	1.18	1.08	1.22
GM		**1.17**		**1.34**		**1.26**		**1.24**		**1.25**
SEm ±		0.07		0.05		0.05		0.06		**0.03**
CD (5%)		NS		NS		NS		NS		**NS**
Interactions	**SEm ±**	**CD (5%)**	**SEm ±**	**CD (5%)**	**SEm ±**	**CD (5%)**	**SEm ±**	**CD (5%)**	**SEm ±**	**CD (5%)**
KB	0.12	NS	0.10	NS	0.10	NS	0.11	NS	0.05	**NS**
KF	0.12	NS	0.10	NS	0.10	NS	0.11	NS	0.05	**NS**
BF	0.10	NS	0.08	NS	0.08	NS	0.09	NS	0.04	**NS**
KBF	0.18	NS	0.14	NS	0.14	NS	0.15	NS	0.07	**NS**
PK									0.06	**NS**
PB									0.06	**NS**
PF									0.06	**NS**
PKBF									0.15	**NS**
CV%		26.75		18.51		19.99		21.65		**21.69**

Note: P=Period, TV= Square root + 0.5 whereas, OV= Original Values, NS= Non-Significant, GM= General Mean

RESULTS AND DISCUSSION

The application of K_2O significantly reduced PBW damage, with the highest potash application (80 kg ha^{-1}) showing the lowest flower and boll damage. Cry1Ac and Cry2Ab2 expression levels were well above critical thresholds in all treatments but showed increased levels in treatments with potash application. Potassium mobilizing bacteria and KNO_3 sprays further enhanced gene expression, though differences between treatments were marginal. In the present study, the pink bollworm infestation and damage was found above ETL on G. Cot. Hy. 8 BG II as the technology of Bt cotton lost its effectiveness against pink bollworm. Similar results were aslo found in potash application populations of pink bollworm to Cry1Ac (Dhurua and Gujar 2011) and Cry2Ab2 (Fabrick *et al*., 2015; Malthankar and Gujar 2016; Gao *et al*., 2018) or both the genes (Fabrick *et al*., 2014).

Gene Expression: The expression of Cry1Ac and Cry2Ab2 in leaves, squares, and boll rinds was significantly higher in potash-treated plots, particularly at 80 kg/ha. Potash mobilizing bacteria did not have a significant impact on gene expression, while foliar sprays of KNO_3 further reduced bollworm incidence.

- Seed Cotton Yield: The highest yield (2692.90 kg/ha) was recorded in plots treated with 80 kg/ha of potash, followed by the 40 kg/ha treatment. KMB application marginally improved yields, but the interaction between treatments was not significant. Bhambhaneeya *et al.* (2017) studied the soil available nutrient status and their indexing in cotton growing areas of south Gujarat and reported high status of K(>280 kg ha^{-1}) both in irrigated and rain fed regions in selected samples.

The findings suggest that potash enhances the expression of Cry genes in Bt cotton, leading to reduced bollworm damage and improved yields. This aligns with previous studies showing the role of potassium in plant resistance and gene expression stability. While KMB application did not significantly impact bollworm incidence, foliar sprays of KNO_3 were effective in reducing larval populations, especially of pink bollworm. Application of hormone and micro-nutrient did not affect the population bollworms (Abro *et al.*, 2004). With respect to synthetic and organic source of nutrition, some workers did not find any marked effect on whitefly, jassid, thrips and spotted bollworms (Ahmed *et al.*, 2003) whereas less infestation of all three bollworms and sucking pests was noticed in organic source of nutrition (Kedar *et al.*, 2010).

Table 2: Open bolls and locule damage by pink bollworm at harvest.

Factors	Open bolls damage (%)		Locules damage (%)	
	OV	TV	OV	TV
A. Main Treatment (Potash Fertilizer) (K)				
K_0	15.01	22.73	9.93	18.33
K_{40}	12.77	20.83	8.15	16.51
K_{80}	9.01	17.22	5.09	12.93
GM		**20.26**		**15.92**
SEm ±		**1.02**		**0.68**
CD (5%)		**4.02**		**2.69**
CV %		**17.50**		**14.91**
B. Sub Treatment				
Potash Mobilizing Bacteria				
B_0	12.20	20.28	7.91	16.14
B_1	12.33	20.24	7.54	15.71
GM		**20.26**		**15.93**
SEm ±		**0.54**		**0.27**
CD (5%)		**NS**		**NS**
Foliar sprays of Potassium Nitrate (F)				
NFS	12.09	20.06	7.51	15.65
FS	12.43	20.46	7.94	16.20
GM		**20.26**		**15.93**
SEm ±		**0.54**		**0.27**
CD (5%)		**NS**		**NS**
Interactions				
	SEm ±	**CD (5%)**	**SEm ±**	**CD (5%)**
KB	0.94	NS	0.47	NS
KF	0.94	NS	0.47	NS
BF	0.77	NS	0.39	NS
KBF	1.33	NS	0.67	NS
CV%		**11.44**		**7.35**

Note: TV= Transformed mean (Arc sine) whereas, OV= Original Values,
NS= Non- Significant, GM= General Mean

Table 3: Effect of potash application on gene expression of square tissue in Bt cotton hybrid.

Factors	*Cry2Ab* Square (μg g^{-1} fresh weight of sample)									
	75		**90**		**105**		**120**		**Pooled**	
	OV		OV		OV		OV		**OV**	
A. Main Treatment (Potash Ferilizer) K										
K_0	72.91		74.43		69.26		68.92		71.38	
K_{40}	74.56		76.22		76.62		71.51		74.56	
K_{80}	74.34		76.62		75.62		76.54		76.54	
GM	**73.94**		**75.42**		**73.83**		**72.32**		**73.88**	
SEm ±	2.34		2.73		1.57		2.03		**1.09**	
CD (5%)	NS		NS		NS		NS		**3.20**	
CV %	10.99		12.54		7.41		9.76		**10.38**	
B. Sub Treatment										
Potash Mobilizing Bacteria (B)										
B_0	74.09		75.03		71.64		70.38		72.78	
B_1	73.58		75.80		76.02		74.27		74.97	
GM	**73.84**		**75.42**		**73.83**		**72.33**		**73.88**	
SEm ±	1.50		1.45		1.38		1.59		**0.76**	
CD (5%)	NS		NS		4.12		NS		**2.10**	
Foliar sprays of Potassium Nitrate (F)										
NFS	74.16		74.77		72.98		70.45		73.09	
FS	73.72		76.07		74.69		74.20		74.67	
GM	**73.94**		**75.42**		**73.83**		**72.33**		**73.88**	
SEm ±	1.50		1.45		1.38		1.59		**0.73**	
CD (5%)	NS		NS		NS		NS		**NS**	
Interactions	**SEm ±**	**CD(5%)**	**SEm ±**	**CD(5%)**	**SEm ±**	**CD(5%)**	**SEm ±**	**CD(5%)**	**SEm ±**	**CD(5%)**
KB	2.59	NS	2.52	NS	2.40	NS	3.00	NS	1.26	**NS**
KF	2.59	NS	2.52	NS	2.40	NS	3.00	NS	1.27	**NS**
BF	2.12	NS	2.05	NS	1.96	NS	2.44	NS	1.03	**NS**
KBF	3.67	NS	3.56	NS	3.39	NS	4.24	NS	1.78	**NS**
PK									2.21	**NS**
PB									1.48	**NS**
PF									1.48	**NS**
PKBF									3.64	**NS**
CV%	8.61		8.19		7.97		9.35		**8.53**	
Note: OV= Original Values, NS= Non-Significant, GM= General Mean, P= Period										

Note: OV= Original Values, NS= Non-Significant, GM= General Mean, P= Period

CONCLUSION

This study demonstrates that potash application can significantly influence the efficacy of Bt genes in controlling bollworm populations by enhancing Cry1Ac and Cry2Ab2 expression in Bt cotton. These findings suggest that optimal K_2O application can improve Bt cotton's resistance to bollworm pests and should be considered in pest management strategies.

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