PHYSICAL PROGRESS REPORT
OF THE PARB PROJECT NO. 193
(01-06-2010 TO 31-05-2017)

GENETIC IMPROVEMENT OF SUGARCANE FOR HERBICIDE AND BORER RESISTANCE

Project Manager

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LAHORE.

PHYSICAL PROGRESS REPORT FOR PARB CGS PROJECT

(01 June 2010 to 31 May 2017)

A. Basic Information

1.	**Name of the project**	Genetic Improvement of Sugarcane for Herbicide and Borer Resistance.
2.	**Project No.**	PARB Project No 193
3.	**Total Project cost**	Rs. 22.350 million
4.	**Total project duration**	Five Years
5.	**Funds released so far**	
6.	**Project commencement Date**	1-06-2010
7.	**Name of the Project Manager or Team Leader with designation**	Dr, Idrees Ahmad Nasir

B. Physical Research Achievements

Output/Activity	Description	Planned Start date	Planned Completion date	Achievement Indicator as planned	**Achievements** (Please attach data in brief as annexure if activity completed)	Reasons for deviation if any
Component objective	**Development of transgenic Sugarcane tolerant to Borers and Glyphosate herbicide through incorporation of CEMB-Bt and GTGene via gene gun in four elite sugarcane cultivars and Pilot scale propagation of two of the transgenic sugarcane cultivars i.e. HSF 240 and CPF-246.**	**01.06. 10**	**30.5. 17**	**HSF 240, SPF 234, SPF 213 and CPF 246 sugarcane cultivars containing herbicide tolerant gene and additional Bt gene in CPF 246 will be available.**	**Completed**	**Annexure-I**
Output-1	**Bt and GTGene transformation in sugarcane cultivars HSF 240, SPF 234, SPF 213 and CPF 246.**	**01.02. 15**	**30.11.15**	**HSF 240, SPF 234, SPF 213 and CPF 246 sugarcane cultivars containing herbicide tolerant gene and Bt gene (in CPF 246) will be available.**	**Completed**	**Annexure-I**

Activity-1	Transformation of sugarcane cultivars by biolistic gun with CEMB-Bt and GTGene (at least 10 events) in appx.100 calli per cultivar.	15.02.15	10.05. 15	10 transformed calli plates per variety will be available.	**Completed**	**Annexure-I**
Activity-2	Regeneration of Putative transgenic sugarcane plants.	15.3.15	30.07.15	100 regenerated sugarcane plants from transformed calli of each variety will be available.	**Completed**	**Annexure-I**
Activity-3	Delivery of Sugarcane to SRI containing CEMB-Bt & GTGene and maintenance for micro propagation at CEMB.	1.10.15	30.11.15	100 plantlets of each cultivar with multiple shoots containing CEMB-Bt & GTGene will be delivered to SRI.	**Completed**	**Annexure-I**
Output-2	**Confirmation of transgenic sugarcane lines via molecular analyses and bioassays.**	**15.5.15**	**15.05. 17**	**Positive transgenic sugarcane plants of cultivars, HSF-240, SPF 234, SPF 213 and CPF 246.**	**Completed** PCR for GTG is done detail is attached as Annex-01)	**Annexure-I**
Activity-1	Bioassay, ELISA and PCR analysis of transgenic plants during regeneration, in-vitro multiplication, green house evaluation, field trials.	5.3.16	15.3.17	Confirmation of Integration of transgenes in plant genome, their multiplication and efficacy of Bt and GTGene in transgenic plants.	**Completed** Bioassay for GTG of 10 Transgenic plant of CPF-246 and ELISA of the positive plants is performed (Annex-01). Multiplication of Transgenic Plants is in progress.	**Annexure-I**
Activity-2	Southern analyses of transgenic plants during in-vitro multiplication, green house evaluation, field trials.	1.1.17	15.3.17	Copy number of gene/ genome and multiplication of transgenic plants	**Completed**	**Annexure-I**
Output 3	**Bio-safety Assessments of CEMB-Bt and GTGene transformed cultivars HSF240, CPF 246, SPF-213 and SPF-234**	**1/4/2016**	**1/5/2017**	**Data for bio-safety study will be available**	**Completed**	**Annexure-I**

Activity 1	Study of Horizontal Gene flow to evaluate the presence of CEMB-Bt and GTGene protein in soil, its kinetics and effect on soil organisms.	1/4/2016	1/4/2017	Data for Horizontal gene flow will be available	**Completed** Horizontal gene flow was studied for **transformed cultivars HSF240, CPF 246, SPF-213 and SPF-234 in field for presence of CEMB Bt and GTGenes.(Attach as Annex-01**)	**Annexure-I**
Activity 2	Study of Effect of CEMB-Bt and GTGene on non target insects	1/4/2016	1/5/2017	Data for effect of CEMB-Bt and GTGene on non target insects will be available.	**Completed** No non target insects were found dead in sugarcane field.	**Annexure-I**
Activity 3	Study of effect of CEMB-Bt and GTGene protein on Animals (Mice)	1/4/2016	1/5/2017	Data for effect of CEMB-Bt and GTGene on Animals (Mice) insects will be available.	**Completed** +ive Transgenic Plants were fed to animals and found no significant effect of CEMB Bt and GTGene on Target animals	**Annexure-I**

Signature of Project Manager/Team Leader

Annexure-1

Selected Sugarcane Cultivars

Variety	Year of Release	Maturity	Cane Yield (t/ha)	Sugar Recovery(%)
SPF-213	2000	Mid	100	11.0
SPF-234	2002	Early	100	11.6
HSF-240	2002	Early	95	11.7
CPF-246	2011	Mid	105	12.0

Varietal tissue culture response after optimized tissue culture conditions

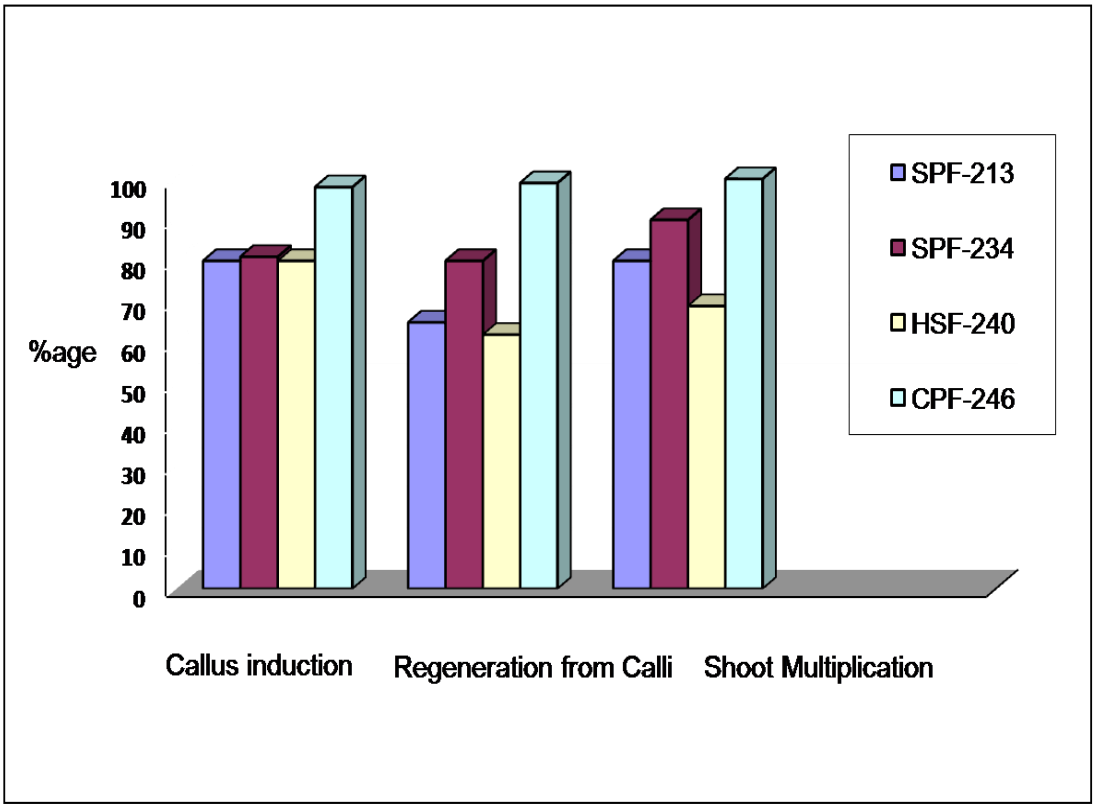


Figure: 1 Comparison b/w four sugarcane varieties (SPF-213, SPF-234, HSF-240, and CPF-246) on the basis of their growth responses at three different stages during tissue culturing.

Fig2: Physical Mapping of CEMB-Cry1Ac, Cry2A and CEMB-GTG genes cloned in plant expression vectors for transformation in sugarcane (in this study)

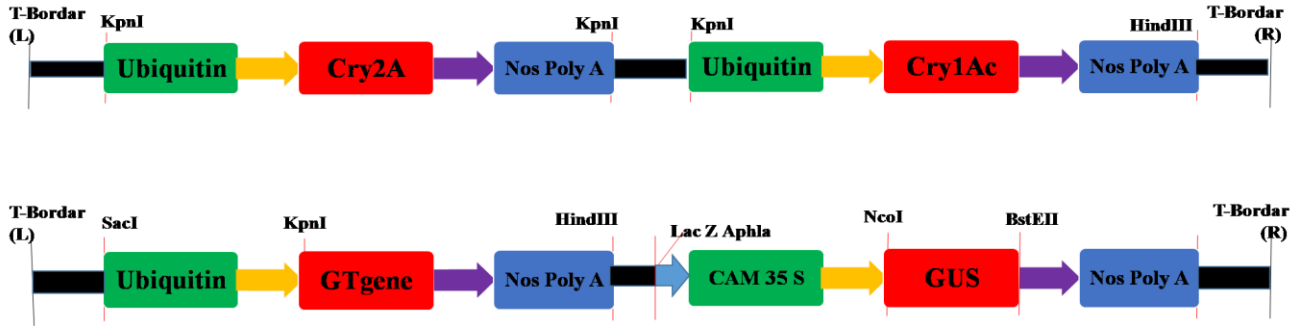
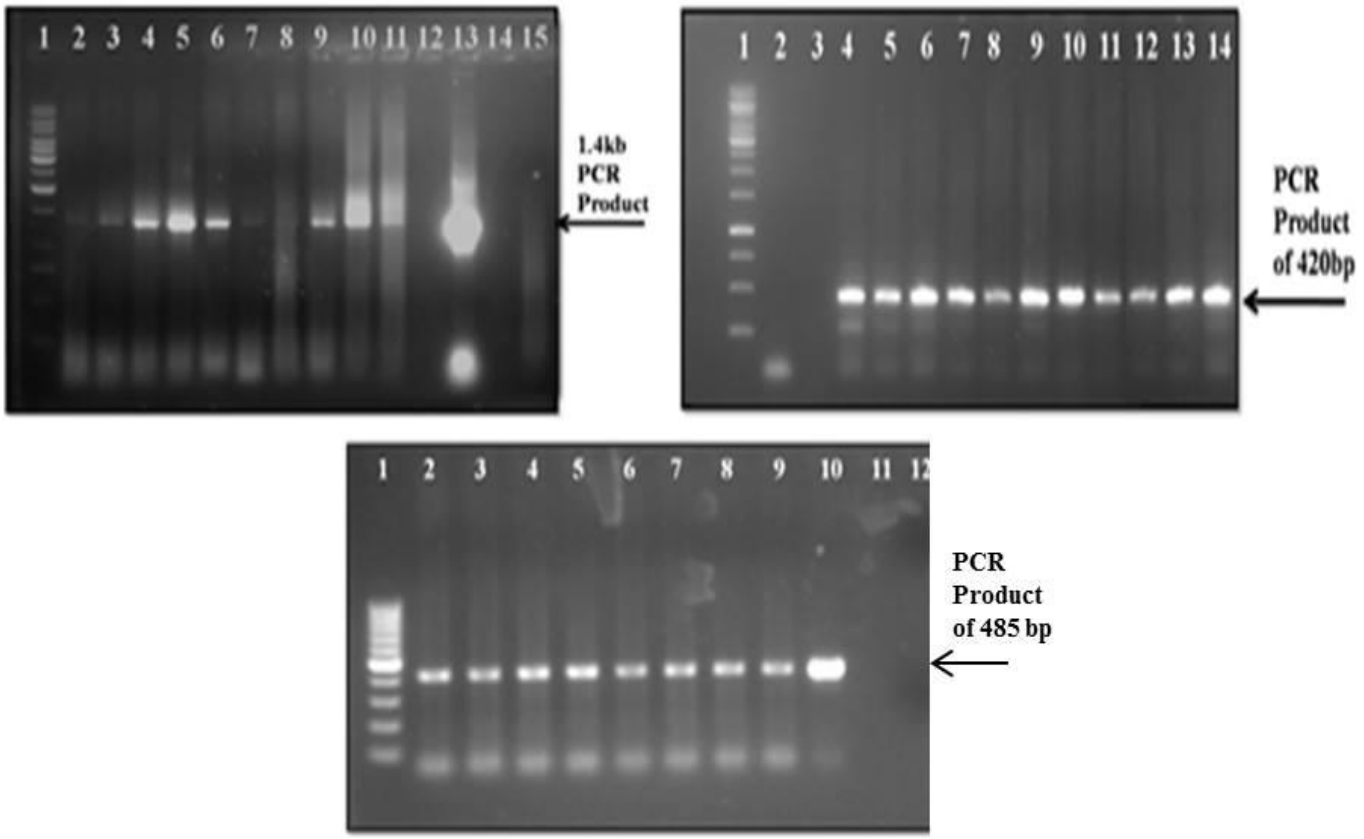


Fig3: Transformation of two Bt and GTG genes in sugarcane varieties (SPF-213, SPF-234, HSF-240, and CPF-246)



A. Amplification of GTgene in Transgenic sugarcane Lane 1: 1kb DNA Ladder, Lane 2-11: GTG Positive Lines, Lane 13: Positive Control and Lane 15: Non-transgenic Negative Control. **B.** Amplification of Cry1Ac gene in transgenic sugarcane Lane 1:1kb DNA Ladder, Lane 2: Non-transgenic negative control Lane, 4-13: Cry1Ac positive lines and Lane 14: Positive control. **C.** Amplification of Cry2Ac gene in transgenic sugarcane Lane 1: 100bp DNA Ladder Lane, 2-9: Cry2Ac positive lines, Lane 10: Positive control and Lane 12: Non-transgenic negative Control.

Table 1: Total distribution of sugarcane plants at different stages of Research

Variety	Total No of Calli bombarded	No of calli survived on Kanamycine Selection (%)	GUS positive callus	No of calli survived on kanamycine + glyphosate selection (%)	No of calli regenerated on selection medium (%)	No. of plantlets after multiplication	In process of acclimatization	In tubes on rooting media	Shifted to green house In pots	Survived In pots After Spray	In field	In field after glyphosate spray	Total tested	Positive for Cry1Ac	Positive for Cry2A	Positive for GTG	positive plants for three genes	Transgenic plants survived In field after Glyphosate Spray
SPF-213	100	70	70	34	21	1000	800	752	656	200	122	54	53	18	10	12	15	11
SPF-234	100	74	74	40	32	1500	1000	780	725	250	141	90	86	15	17	16	27	14
HSF-240	100	45	45	29	13	300	220	205	125	75	60	40	37	8	9	5	11	10
CPF-246	100	91	90	81	48	2500	1800	1724	1630	900	751	300	160	30	20	15	21	11
										Total			**336**	**71**	**56**	**48**	**74**	**46**

Table 2: Transformation efficiency on the basis of calli survived on selected media

Variety	No. of calli bombarded	Control	No. of calli survived on selection media	transformation efficiency
HSF-240	100	10	29	29%
CPF-246	100	10	81	81%

Transformation efficiency in HSF-240 = 29%
Transformation efficiency in CPF-246 = 81%

Table 3: Transformation efficiency on the basis of calli regenerated on selected media

Variety	No. of calli bombarded	Control	No. of calli regenerated on selection media	transformation efficiency
HSF-240	100	10	13	13%
CPF-246	100	10	48	48%

Transformation efficiency in HSF-246 = 13%
Transformation efficiency in CPF-246 = 48%

Fig4: Field Evaluation and confirmation of transgenic cotton plants containing Cry1Ac+Cry2A and GTG in local sugarcane at V0 generation through PCR.

Table 4: Determination of Purity ratio of double Bt genes in Transgenic Sugarcane Variety HSF-240 through PCR

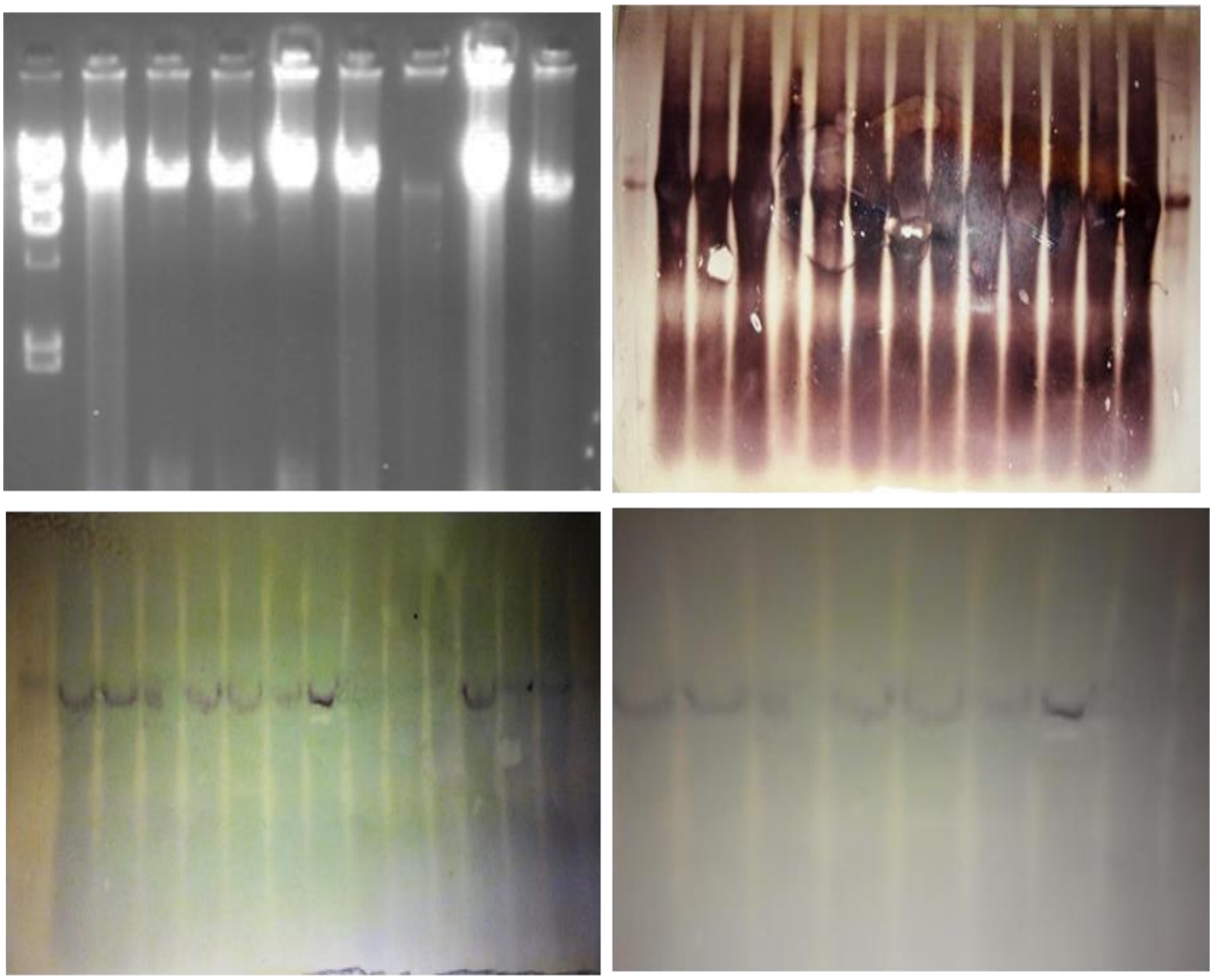
Plants Name	GTG PCR amplification	Cry1Ac PCR amplification	Cry2A PCR amplification
HSF-L (1)	Positive	Positive	Positive
HSF-L(2)	Positive	Positive	Positive
HSF-L (3)	Positive	Positive	Positive
HSF-L (4)	Positive	Positive	Positive
HSF-L (5)	Positive	Positive	Positive
HSF-L (6)	Positive	Positive	Positive
HSF-L (7)	Positive	Positive	Positive
HSF-L (8)	Positive	Positive	Positive
HSF-L (9)	Positive	Positive	Positive
HSF-L (10)	Positive	Positive	Positive
Negative control	Negative	Negative	Negative
Positive control	Positive	Positive	Positive

Table5: Determination of Purity ratio of double Bt genes in Transgenic Sugarcane Variety CSF-246 through PCR

Plants Name	**GTG PCR amplification**	**Cry1Ac PCR amplification**	**Cry2A PCR amplification**
CPF-L (1)	Positive	Positive	Positive
CPF -L(2)	Positive	Positive	Positive

CPF -L (3)	Positive	Positive	Positive
CPF -L (4)	Positive	Positive	Positive
CPF -L (5)	Positive	Positive	Positive
CPF -L (6)	Positive	Positive	Positive
CPF -L (7)	Positive	Positive	Positive
CPF -L (8)	Positive	Positive	Positive
CPF -L (9)	Positive	Positive	Positive
CPF -L (10)	Positive	Positive	Positive
CPF -L (11)	Positive	Positive	Positive
Negative control	Negative	Negative	Negative
Positive control	Positive	Positive	Positive

Fig6: Southern Blot Analysis For CEMB-GTG, CEMB-Cry1Ac,CEMB-Cry2A in transgenic sugarcane varieties



A. Genomic DNA isolaton B. GTG-Southern C.Cry1Ac-Southern D. Cry2A-Southern

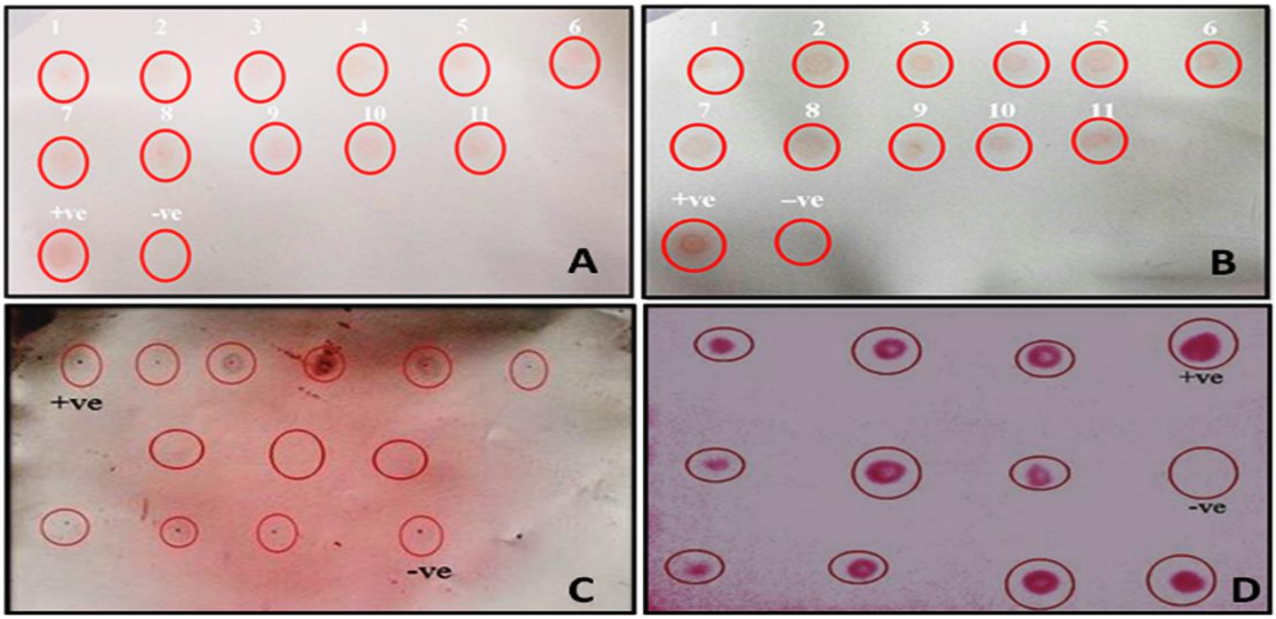
Fig7: Determination of Crude protein of Bt and GTGene through Bradford assay

Table 6:: Concentration of Crude Protein in Transgenic plants (HSF-240) by Bradfrd Assay			
Serial No.	**Plant Name**	**Protein Extraction**	**Concentration of Protein (µg/mL)**
1	HSF240-L (3)	Extracted	270
2	HSF240-L(16)	Extracted	180
3	HSF240-L (22)	Extracted	230
4	HSF240-L (54)	Extracted	210
5	HSF240-L (55)	Extracted	270
6	HSF240-L (61)	Extracted	150
7	HSF240-L (67)	Extracted	180
8	HSF240-L (72)	Extracted	230
9	HSF240-L (74)	Extracted	190
10	HSF240-L (82)	Extracted	140

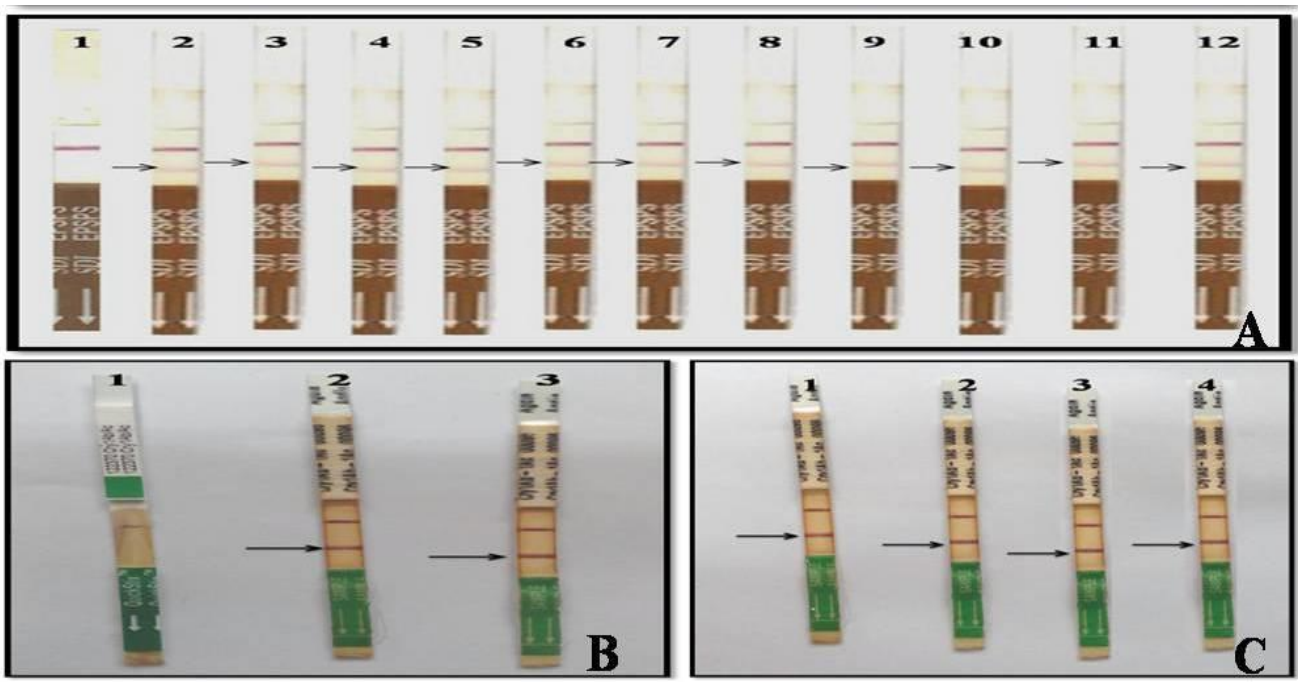
Table: Concentration of Crude Protein in Transgenic plants (HSF-240) by Bradford Assay			
Serial No.	**Plant Name**	**Protein Extraction**	**Concentration of Protein (µg/mL)**
1	CPF246-L (2)	Extracted	220
2	CPF 246-L(18)	Extracted	280
3	CPF 246-L (24)	Extracted	190
4	CPF 246-L (51)	Extracted	160
5	CPF 246-L (53)	Extracted	230
6	CPF 246-L (55)	Extracted	180

7	CPF 246-L (67)	Extracted	160
8	CPF 246-L (69)	Extracted	190
9	CPF 246-L (71)	Extracted	220
10	CPF 246-L (76)	Extracted	170
11	CPF 246-L (81)	Extracted	178

Fig8: Protein Dot Blot of sugarcane transgenic plants



Protein Dot Blot of A, B) CEMB-*GTG, C) CEMB-Cry1Ac* D) CEMB-Cry2A



A.*GTG* qualitative protein expression through Dipsticks under Ubiquitin promoter, 1: Negative control, 2-12: Positive assay of *GTG* Dipstick. **B. :** *GTG* qualitative protein expression through Dipsticks under with CaMV 35S promoter, Negative Control, 2-12: Positive assay of *GTG* Dipstick. **C.** *Cry1Ac* qualitative protein expression through Dipsticks with Ubiquitin Promoter, 1: Negative control, 2-3: Positive assay of *Cry1Ac*. **D.** *Cry1Ac* protein qualitative expression through Dipsticks with CaMV 35S promoter, 1-4: Positive assay of *Cry1Ac* Dipstick.

Fig.10: Bio Toxicity Leaf assay after 80-days

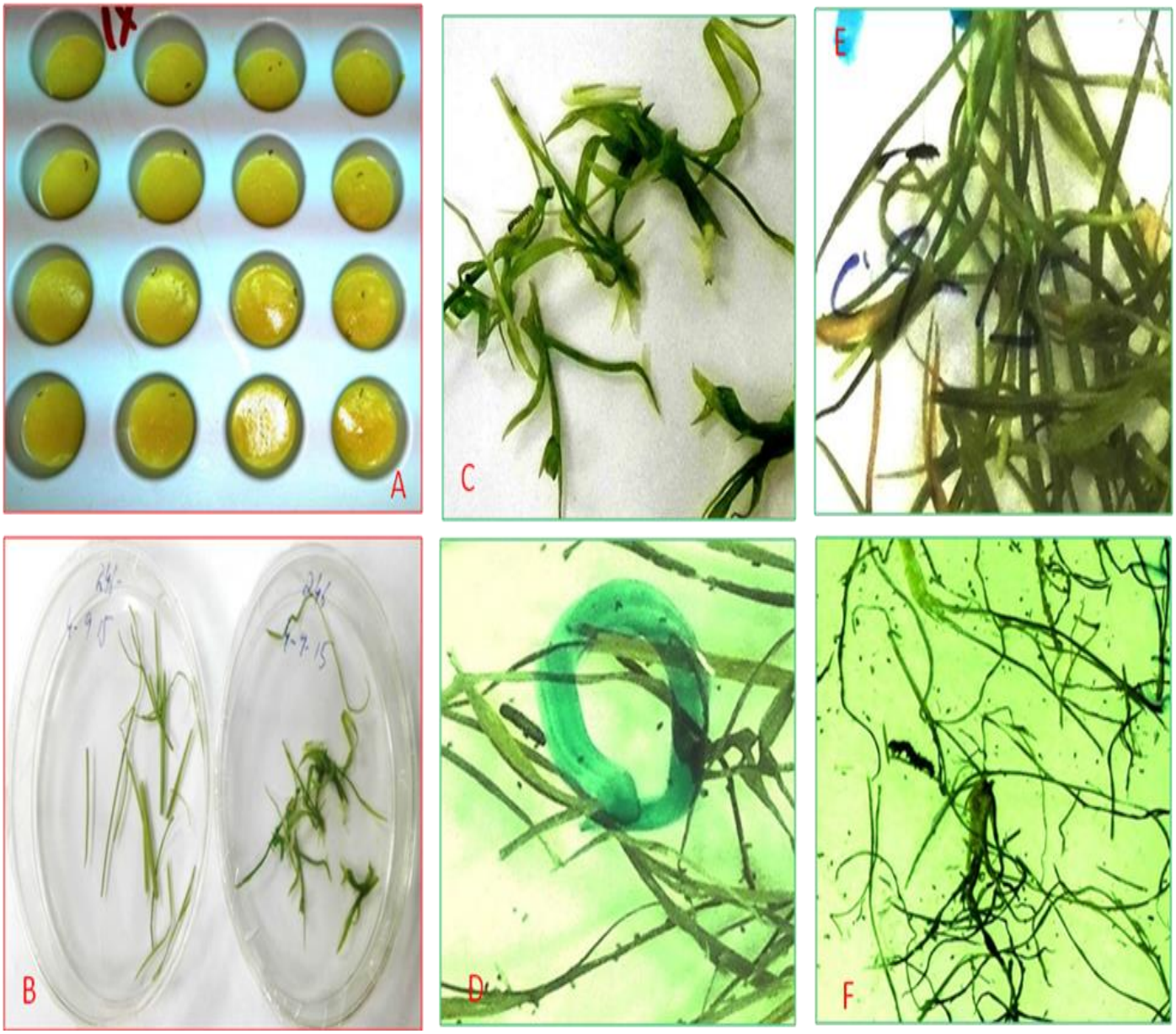


Fig: Insect Bio-Assay of transgenic sugarcane plants

A: Insect ***Chilo infuscatellus*** from CEMB-insectory Lab

B: Transgenic sugarcane plant leaf after 80 days

C: *Chilo infuscatellus* insect with transgenic leaf

D: Cane Borer dead, 4th day of infestation with single gene Cry1Ac

E: Cane Shoot Borer Died on 2nd day with infestation of double BT genes leaf

F. Insect is healthy with non transgenic Control sugarcane plant Leaf

Fig.11: Quantification of Cry1A protein in transgenic sugarcane plants of varieties during field evaluation

Fig: A1: Non transgenic plant Control, with Buffer
B1: Positive controls given in kit

Fig.12: Quantification of Cry2A protein in transgenic sugarcane plants of varieties during field evaluation.

Fig: A1: Non transgenic plant Control, with Buffer
B1: Positive controls given in kit

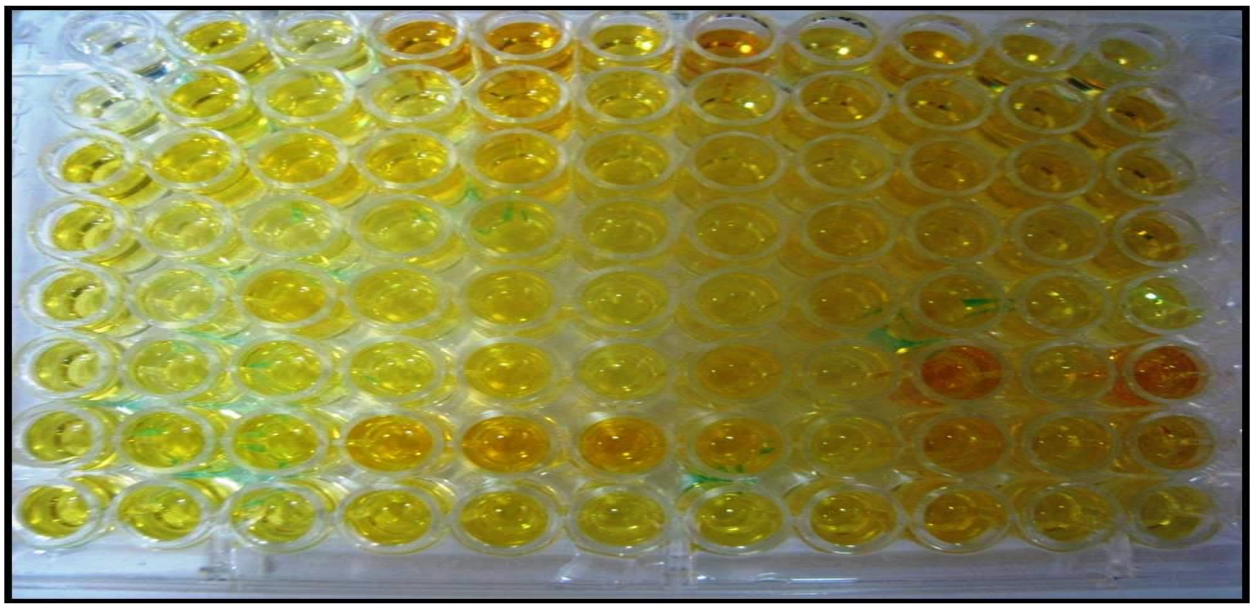


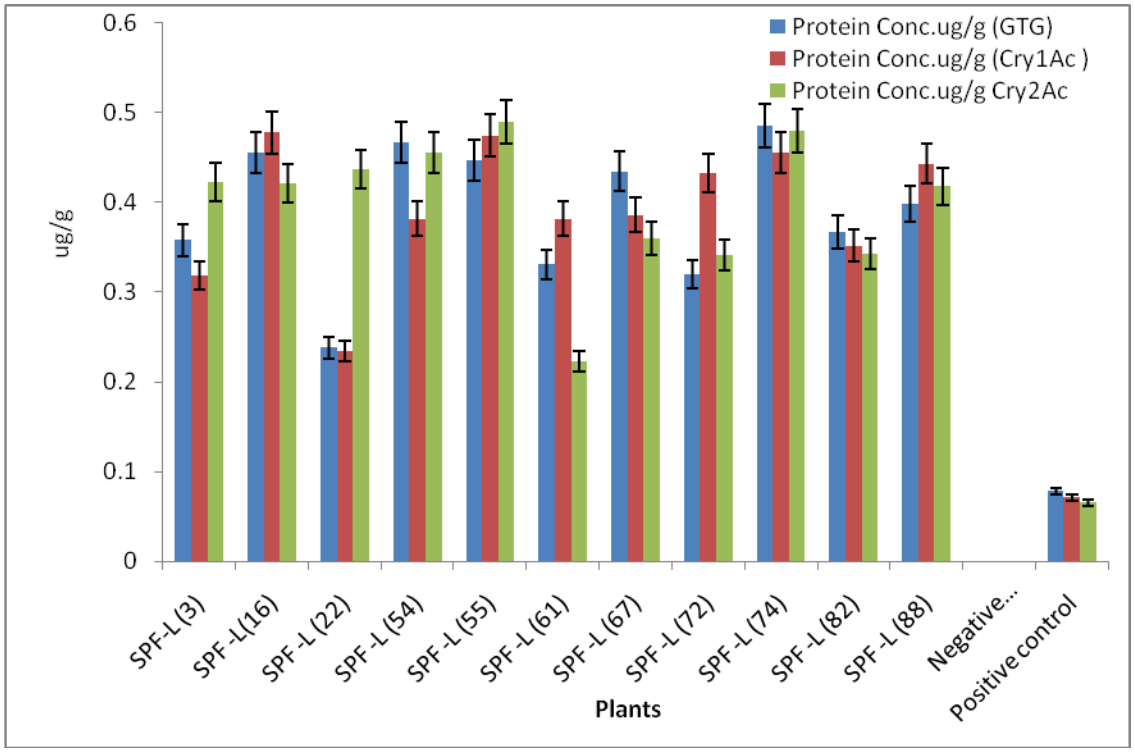
Fig.13: Quantification of GTG protein in transgenic sugarcane plants of varieties during field evaluation

Fig: A1: Non transgenic plant Control, with Buffer
B1: Positive controls given in kit

Table :Quantification of Cry1Ac, Cry2A and GTG protein in putative transgenic sugarcane plants of HSF-240 through ELISA at T0 generation

Plants Name	Protein Conc.ug/g (GTG)	Protein Conc.ug/g (Cry1Ac)	Protein Conc.ug/g Cry2Ac	Result
HSF-L (3)	0.358	0.319	0.423	Plant have two Bt and GTG protein
HSF -L(16)	0.456	0.478	0.421	Plant have two Bt and GTG protein
HSF -L (22)	0.238	0.234	0.437	Plant have two Bt and GTG protein

HSF -L (54)	0.467	0.382	0.456	Plant have two Bt and GTG protein
HSF -L (55)	**0.447**	**0.475**	**0.490**	Plant have two Bt and GTG protein
HSF -L (61)	0.331	0.382	0.223	Plant have two Bt and GTG protein
HSF -L (67)	0.435	0.386	0.360	Plant have two Bt and GTG protein
HSF -L (72)	0.320	0.4327	0.341	Plant have two Bt and GTG protein
HSF -L (74)	**0.486**	**0.456**	**0.480**	Plant have two Bt and GTG protein
HSF -L (82)	0.367	0.352	0.343	Plant have two Bt and GTG protein
HSF -L (88)	0.398	0.443	0.418	Plant have two Bt and GTG protein
Negative control	0.00	0.00	0.00	-
Positive control	0.078	0.071	0.065	-



Quantification of Cry1Ac, Cry2A and GTG protein in putative transgenic sugarcane plants of CPF-246 through ELISA at T0 generation

Plants Name	Protein Conc.ug/g (GTG)	Protein Conc.ug/g (Cry1Ac)	Protein Conc.ug/g Cry2Ac	Result
CPF-L (2)	0.252	0.415	0.314	Plant have two Bt and GTG protein
CPF -L(18)	0.320	0.360	0.278	Plant have two Bt and GTG protein
CPF -L (24)	0.322	0.431	0.345	Plant have two Bt and GTG protein
CPF -L (51)	0.425	0.356	0.401	Plant have two Bt and GTG protein
CPF -L (53)	**0.436**	**0.460**	**0.421**	Plant have two Bt and GTG protein
CPF -L (55)	0.334	0.241	0.241	Plant have two Bt and GTG protein
CPF -L (67)	0.227	0.426	0.430	Plant have two Bt and GTG protein
CPF -L (69)	**0.480**	**0.447**	**0.567**	Plant have two Bt and GTG protein
CPF -L (71)	0.348	0.252	0.322	Plant have two Bt and GTG protein
CPF -L (76)	0.386	0.351	0.400	Plant have two Bt and GTG protein
CPF -L (81)	0.378	0.451	0.511	Plant have two Bt and GTG protein
Negative control	0.00	0.00	0.00	-
Positive control	0.058	0.032	0.056	-

Fig.14: A: Weeds growing along with transgenic sugarcane in the field

B: Glyphosate spray assay of transgenic sugarcane plants (GTGene) at the rate of 1300ml/acre at T0 generation. Weeds dead.

Fig.15: Estimation of Crude protein of Bt and GTGene through Bradford assay in transgenic sugarcane V1 gertation

Table: **Concentration of Crude Protein having CaMV 35S Promoter by Bradford Assay**

Serial No.	**Variety**	**Plant Name**	**Concentration of Protein (µg/mL)**
1	234	234-1	270
2	234	234-2	180
3	234	234-3	230
4	234	234-4	210
5	234	234-5	270
6	240	240-6	150
7	240	240-7	180
8	240	240-8	230
9	213	213-9	190
10	213	213-10	140
11	213	213-11	110

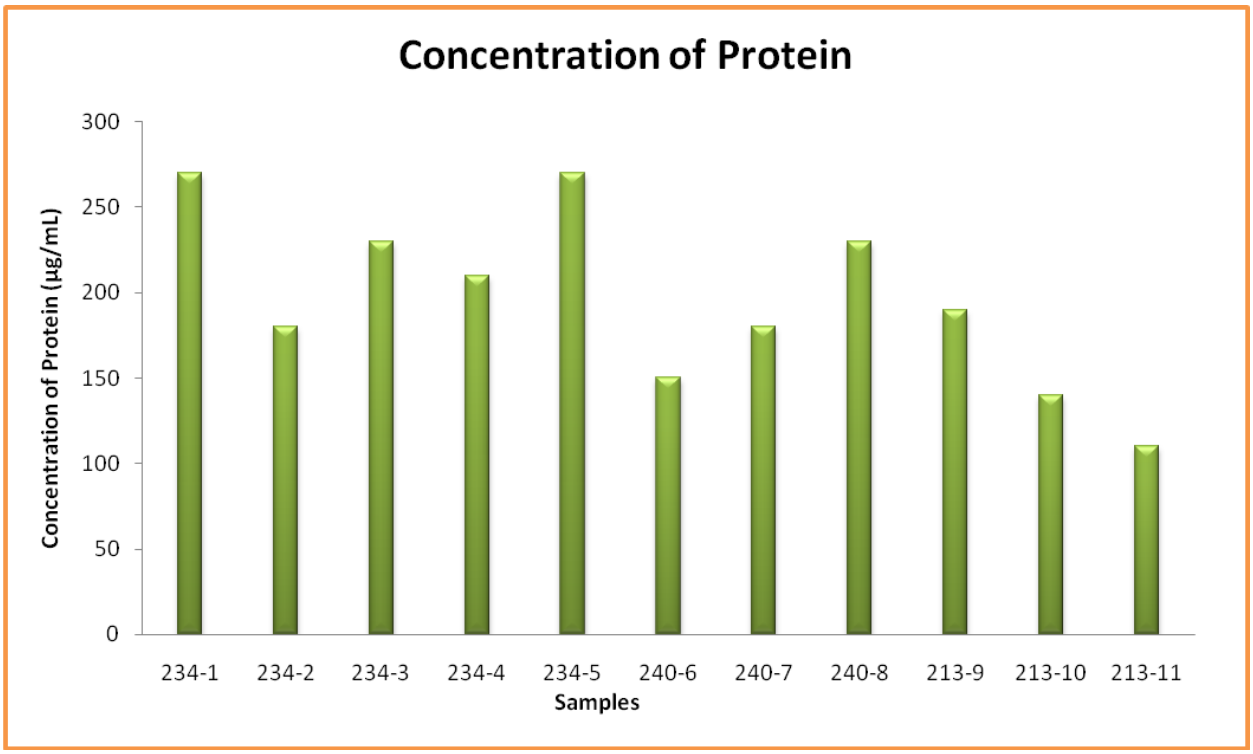


Fig.16: Graph for Concentration of Crude Protein in Transgenic Plant by Bradford Assay

Table 4.2: Concentration of Crude Protein having Ubiquitin Promoter by Bradford Assay			
Serial No.	**Variety**	**Plant Name**	**Concentration of Protein (µg/mL)**
1	234	234-1	50
2	234	234-2	70
3	234	234-3	190
4	234	234-4	160
5	234	234-5	130
6	240	240-6	110
7	240	240-7	130
8	240	240-8	90
9	213	213-9	70
10	213	213-10	160
11	213	213-11	90

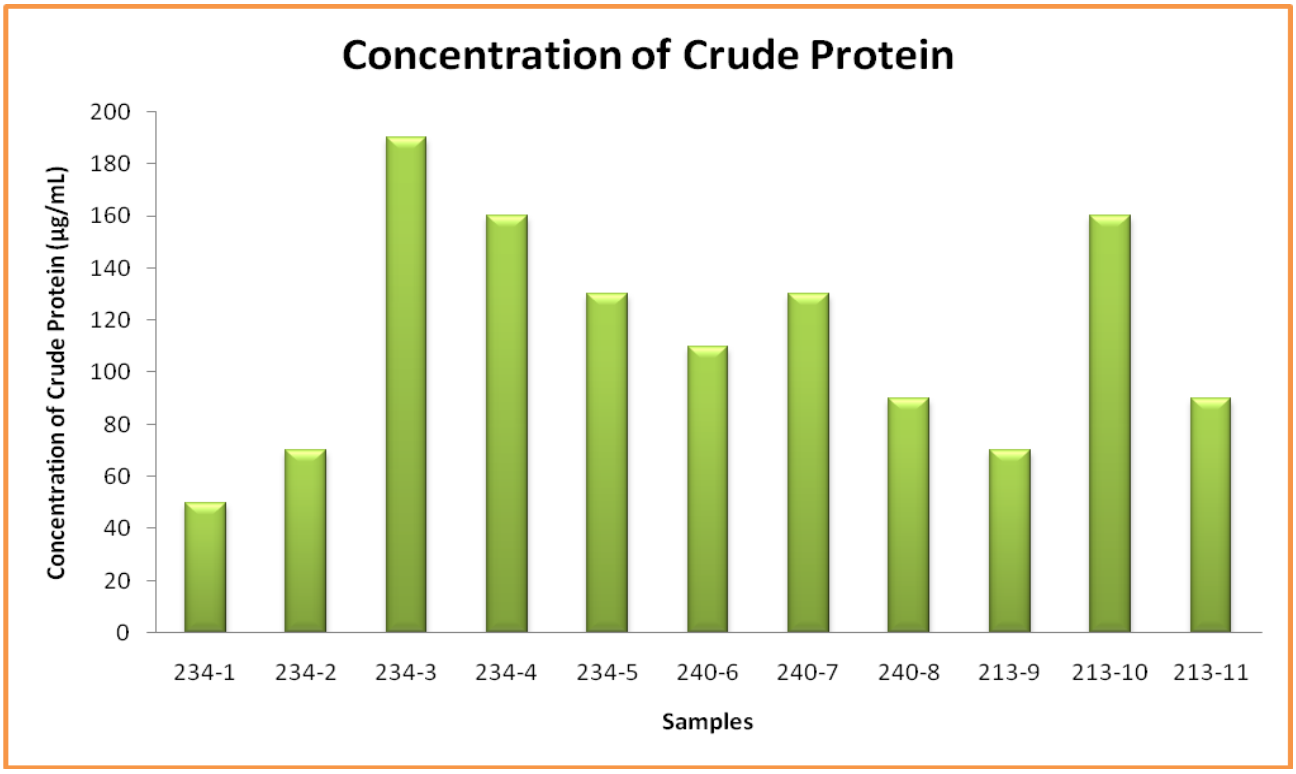


Fig.17: Graph for Concentration of Crude Protein in Transgenic Plants by Bradford Assay

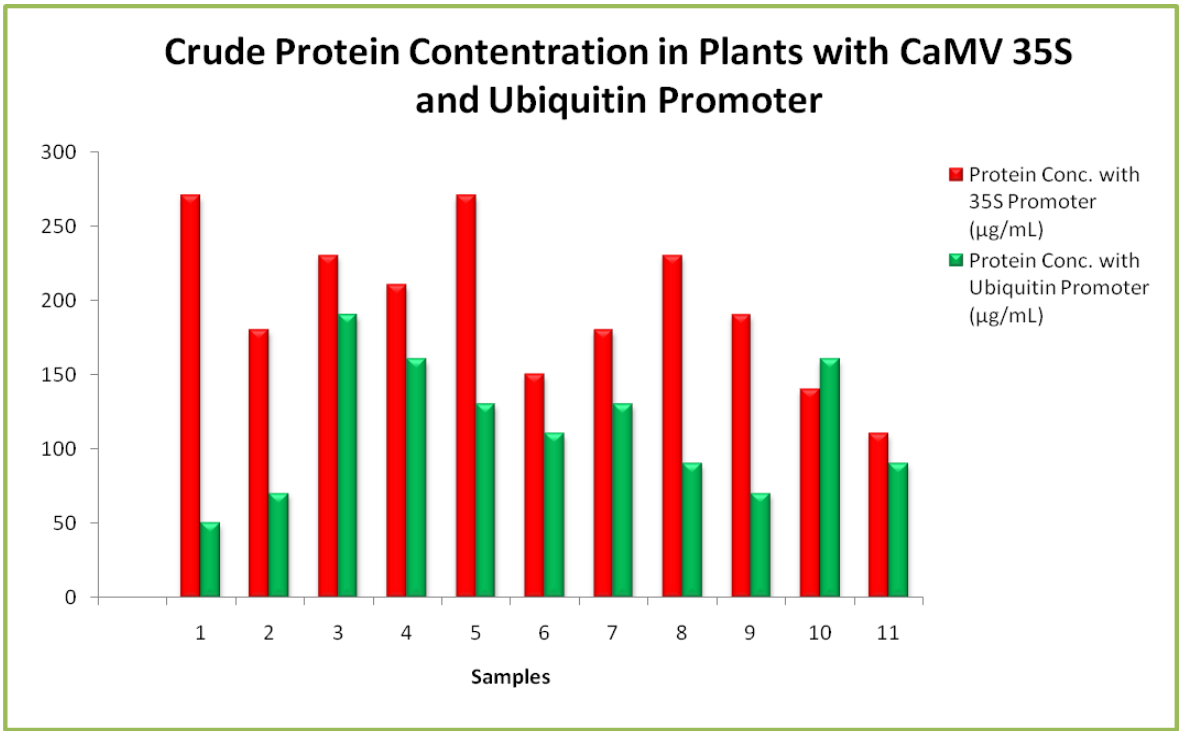


Table: ELISA for GTG Protein with CaMV 35S Promoter in Transgenic Sugarcane Plants			
Well No.	**Plant Name**	**Optical Density (OD) at 450 nm**	**ELISA GTG**
1	Negative Control	0.072	-
2	Positive Control	2.179	-
3	234-1	1.802	Positive
4	234-2	2.042	Positive
5	234-3	2.906	Positive
6	234-4	3.674	Positive
7	234-5	2.284	Positive
8	240-6	1.831	Positive
9	240-7	1.655	Positive
10	240-8	2.657	Positive
11	213-9	3.732	Positive
12	213-10	3.534	Positive
13	213-11	2.418	Positive

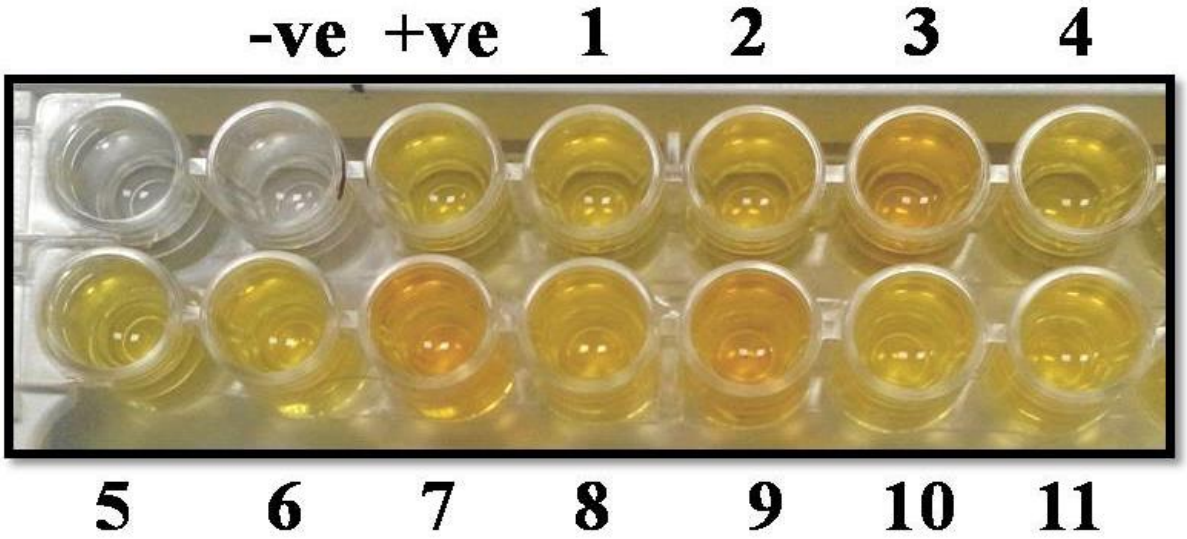


Fig 18: DAS-ELISA of GTG Protein with 35S Promoter in Transgenic Sugarcane Plants

Well 1: Negative Control

Well 2: Positive Control

Well 4-14: GTG ELISA Positive Plants

Table: ELISA for GTG Protein with Ubiquitin Promoter in Transgenic Sugarcane Plants			
Well No.	**Plant Name**	**Optical Density (OD) at 450 nm**	**ELISA GTG**
1	Negative Control	0.072	-
2	Positive Control	2.179	-
3	234-1	0.944	Positive
4	234-2	0.333	Positive
5	234-3	0.336	Positive
6	234-4	0.442	Positive
7	234-5	0.658	Positive
8	240-6	1.884	Positive
9	240-7	0.543	Positive
10	240-8	0.783	Positive
11	213-9	0.664	Positive
12	213-10	0.492	Positive
13	213-11	0.325	Positive

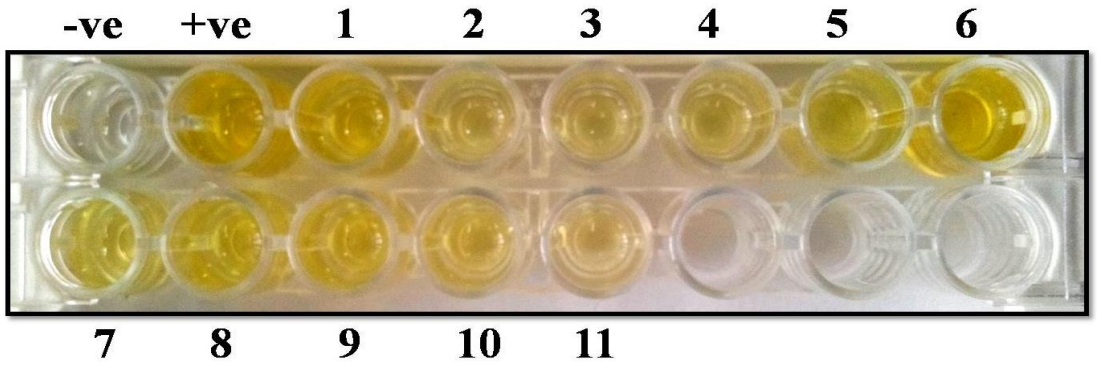
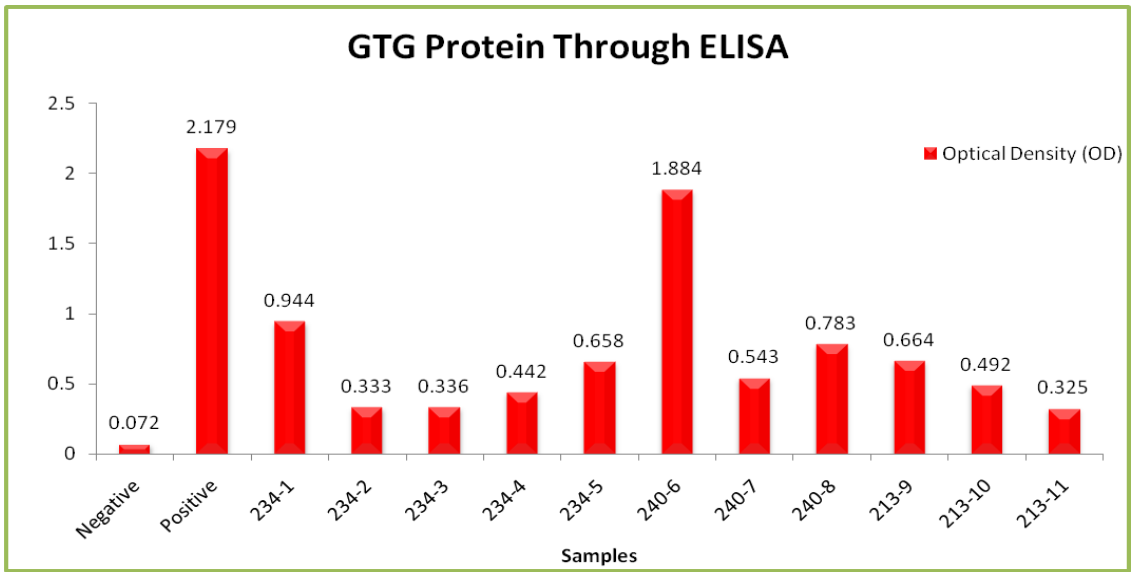


Fig. 19: DAS-ELISA of GTG Protein with Ubiquitin Promoter in Transgenic Sugarcane Plants

Well 1: Negative Control

Well 2: Positive Control

Well 3-13: GTG ELISA Positive Plants



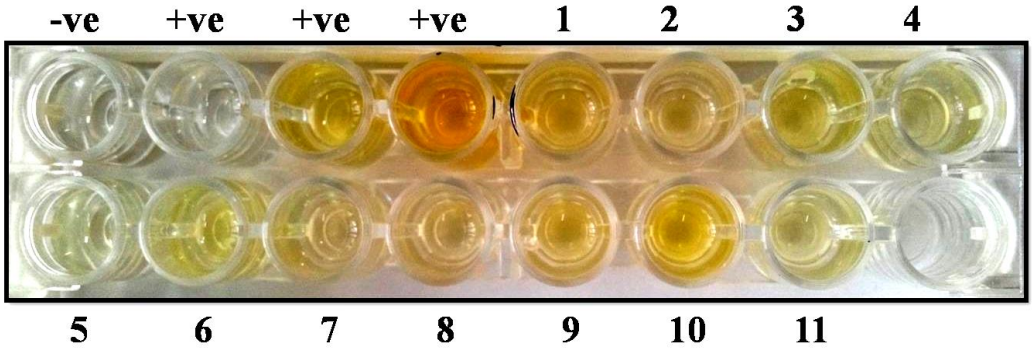


Fig.21: DAS-ELISA of Cry1Ac Protein under CaMV 35S Promoter in Transgenic Sugarcane Plants

Well 1: Negative Control

Well 2-4: Positive Control of different Concentrations

Well 5-15: Cry1Ac ELISA Positive Plants

Table: ELISA for Cry1Ac under CaMV 35S Promoter of Transgenic Sugarcane Plants

Well No.	**Plant Name**	**Optical Density (OD) at 450 nm**	**Concentration of Cry1Ac Protein (µg/gm)**	**ELISA Cry1Ac**
1	Negative Control	0.068	0	-
2	Positive Control	0.071	0	-
3	Positive Control	0.796	0.314	-
4	Positive Control	1.459	0.602	-
5	234-1	3.43	0.656	Positive
6	234-2	1.48	0.572	Positive
7	234-3	0.88	0.352	Positive
8	234-4	1.42	0.567	Positive
9	234-5	3.412	0.627	Positive
10	240-6	0.826	0.327	Positive
11	240-7	0.817	0.323	Positive
12	240-8	1.173	0.477	Positive
13	213-9	3.42	0.615	Positive
14	213-10	0.873	0.348	Positive
15	213-11	0.893	0.356	Positive

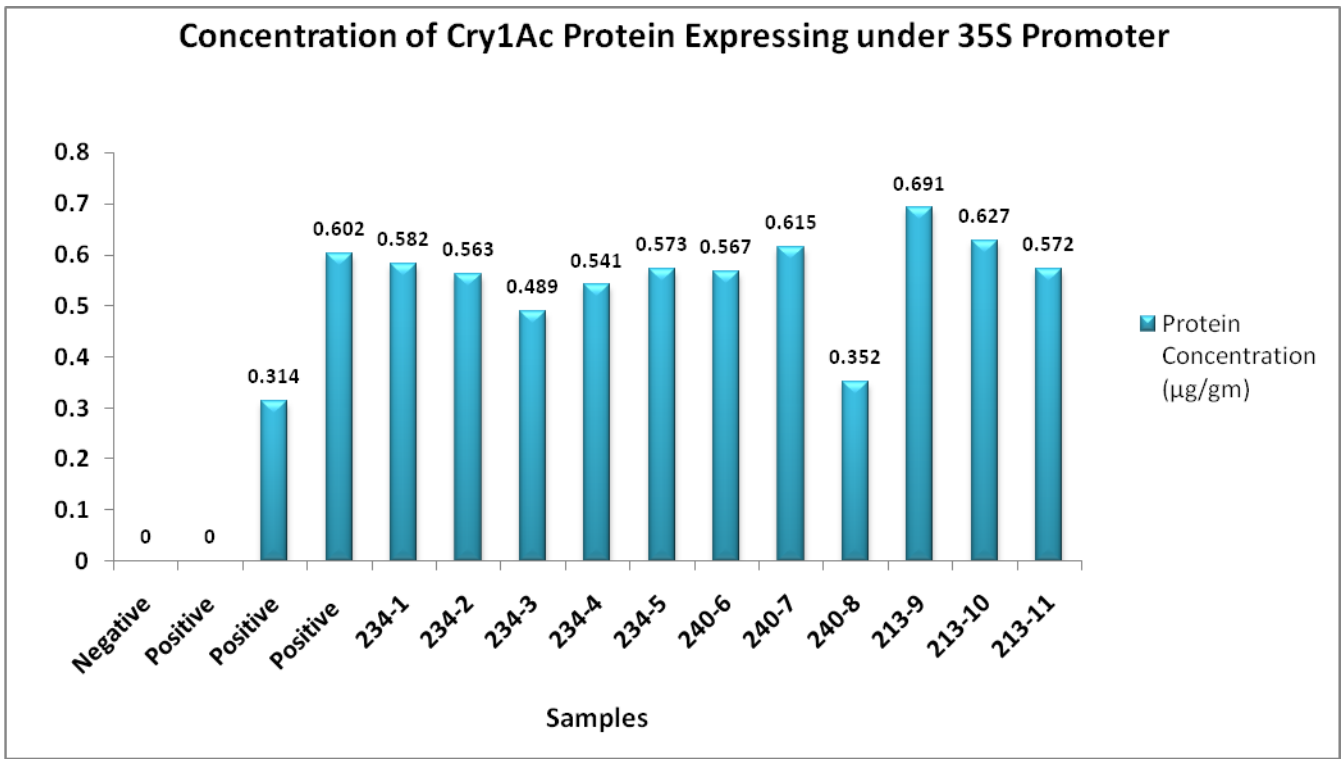


Fig.22: Graph Showing Concentration of Cry1Ac Protein under CaMV 35S Promoter in Transgenic Sugarcane Plants

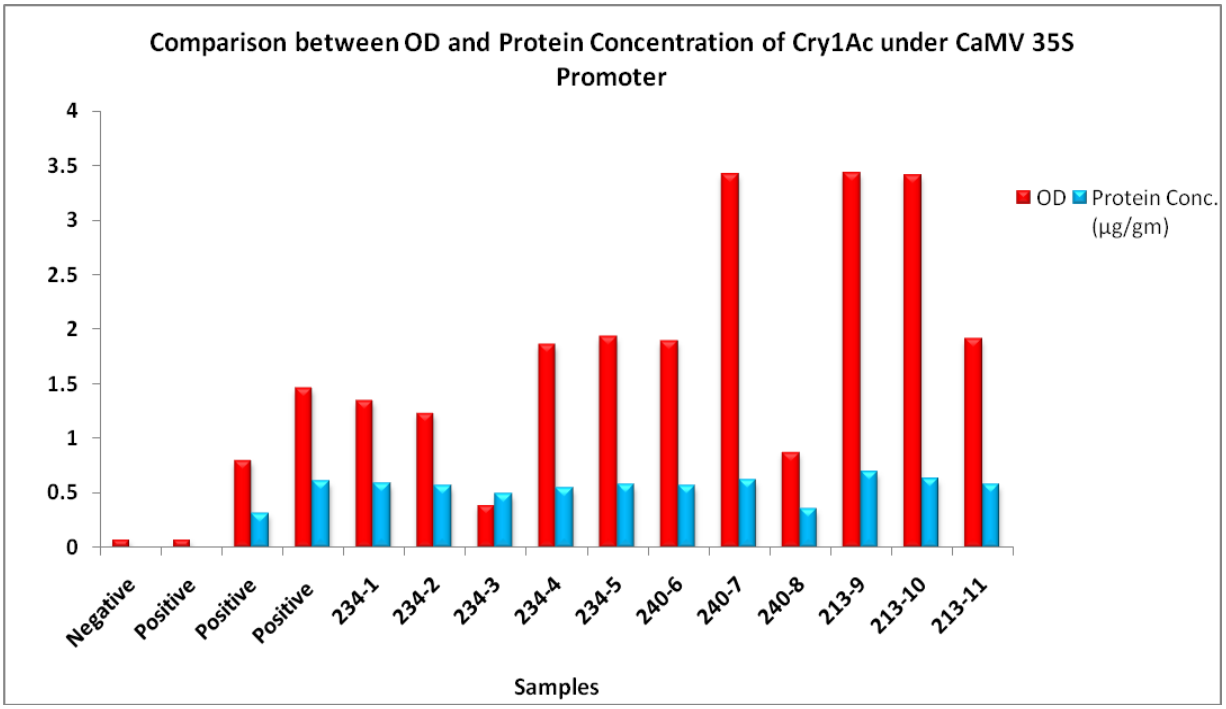


Fig.23: Graph for Showing Co-relation between OD and Concentration of Cry1Ac Protein Expressing under CaMV 35S Promoter

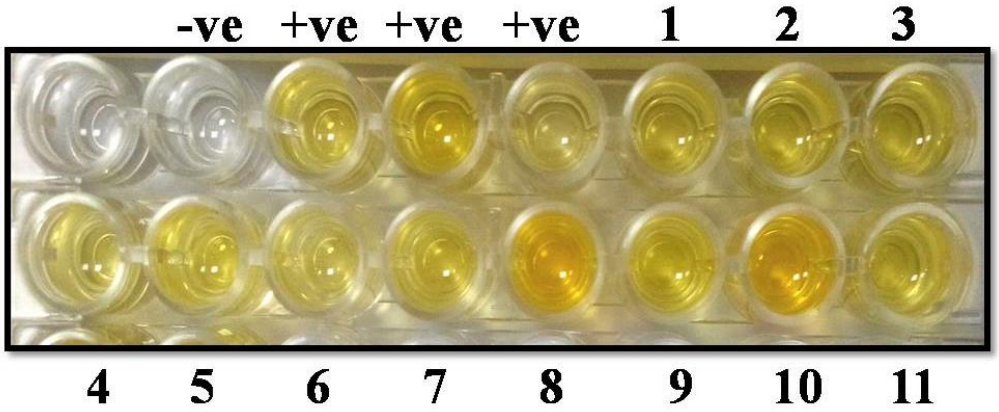


Fig.24: DAS-ELISA of Cry1Ac Protein with Ubiquitin Promoter in Transgenic Sugarcane Plants

Well 2: Negative Control

Well 3-5: Positive Control of different Concentrations

Well 6-16: Cry1Ac ELISA Positive Plants

Table : ELISA for Cry1Ac with Ubiquitin Promoter of Transgenic Sugarcane Plants

Well No.	**Plant Name**	**Optical Density (OD) at 450 nm**	**Concentration of Cry1Ac Protein (µg/gm)**	**ELISA Cry1Ac**
1	Negative Control	0.068	0	-
2	Positive Control	0.073	0	-
3	Positive Control	0.796	0.314	-
4	Positive Control	1.459	0.602	-
5	234-1	1.52	0.582	Positive
6	234-2	3.42	0.656	Positive
7	234-3	1.167	0.457	Positive
8	234-4	3.44	0.656	Positive
9	234-5	1.33	0.546	Positive
10	240-6	0.817	0.336	Positive
11	240-7	1.18	0.489	Positive
12	240-8	1.32	0.541	Positive
13	213-9	1.401	0.563	Positive

14	213-10	1.93	0.573	Positive
15	213-11	0.904	0.361	Positive

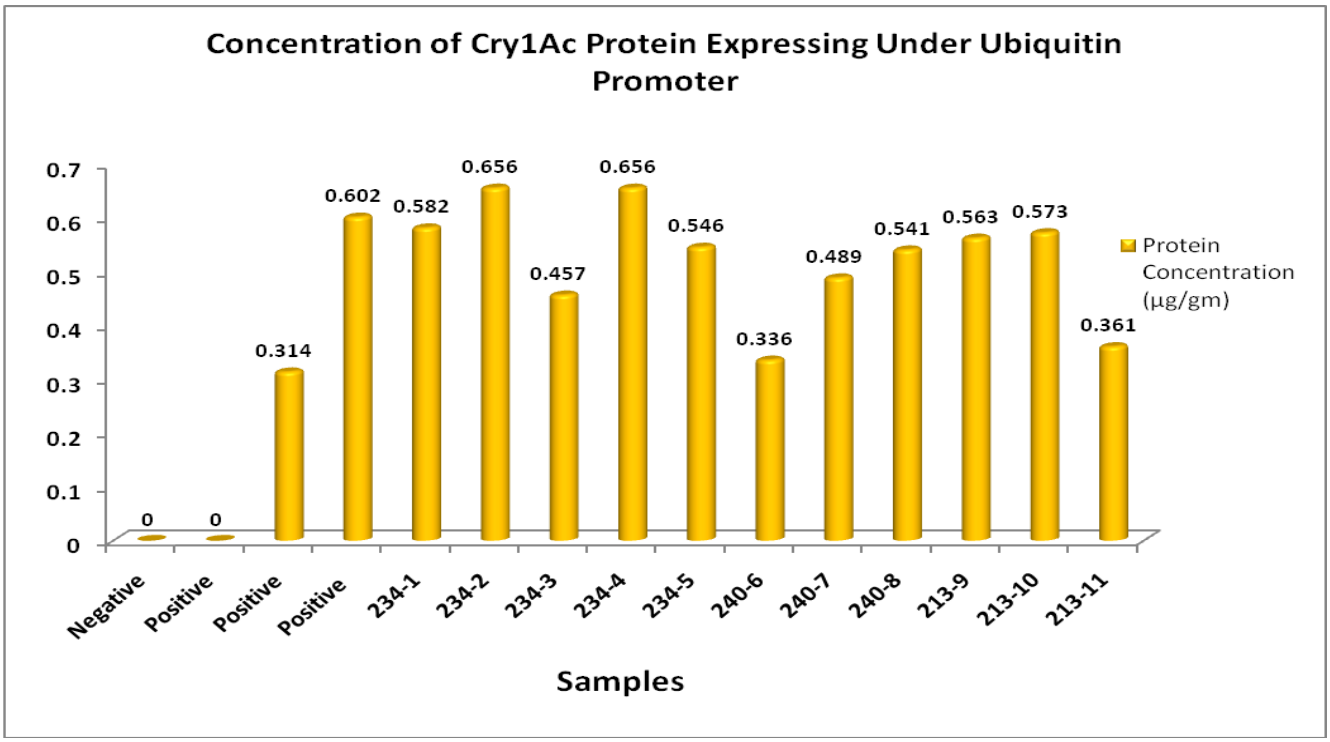


Fig.25: Graph Showing Concentration of Cry1Ac Protein with Ubiquitin Promoter in Transgenic plants

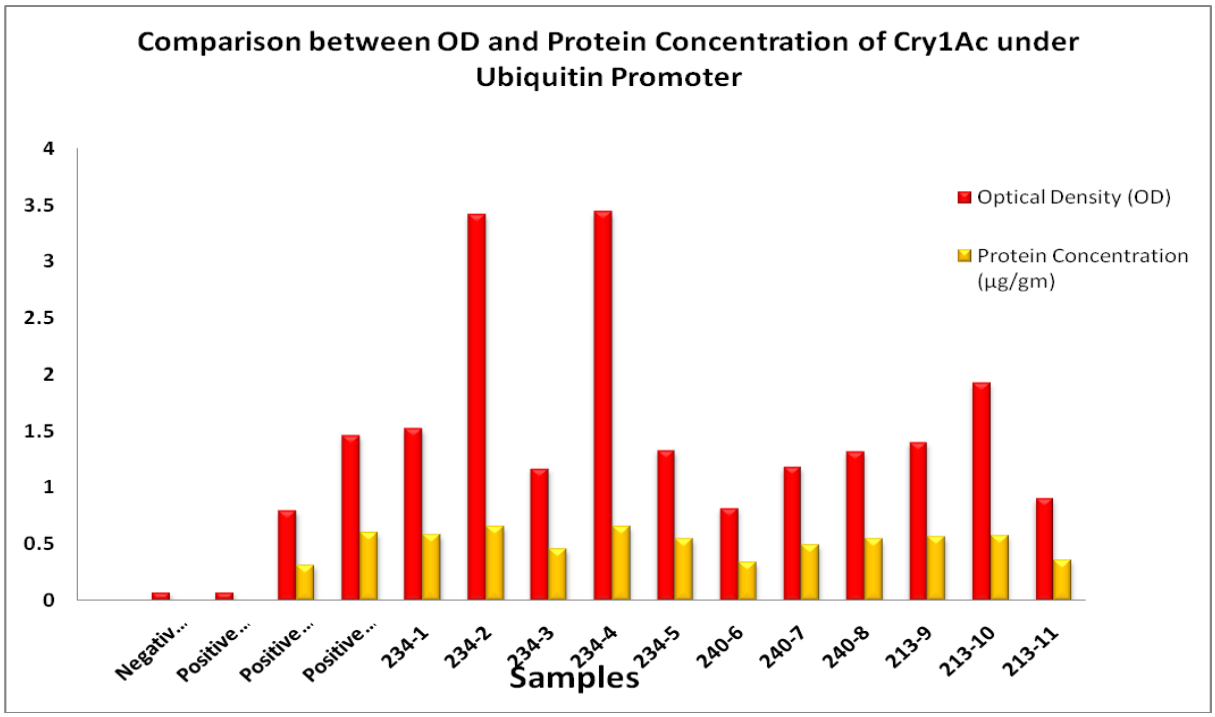


Fig.26: Graph Showing Co-relation between OD and Concentration of Cry1Ac Protein Expressing under Ubiquitin Promoter

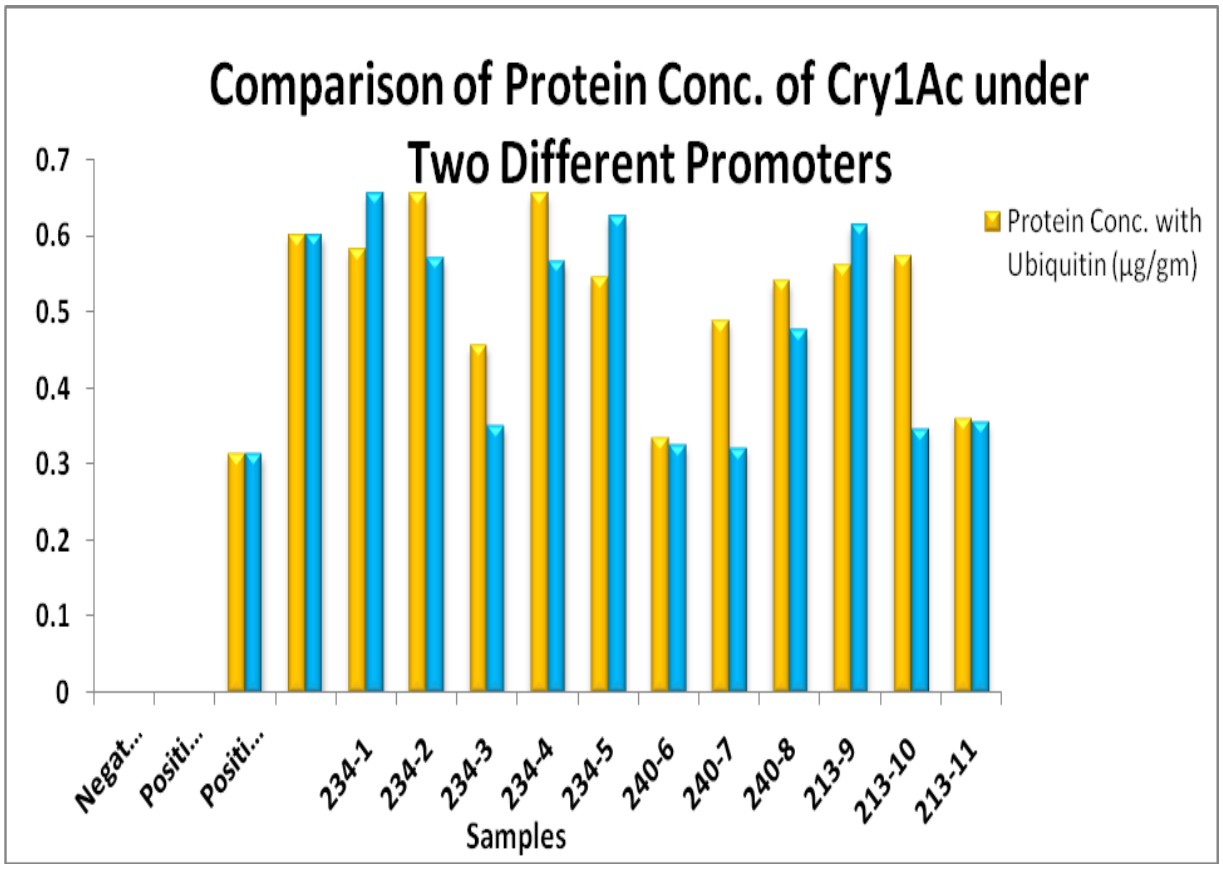


Fig.27: Graph Showing Comparison of Cry1Ac Protein Concentration Expressing under Ubiquitin and CaMV 35S Promoter

A: Weeds growing along with transgenic sugarcane in the field

B: Glyphosate spray assay of transgenic sugarcane plants (GTGene) at the rate of 1300ml/acre at T0 generation. Weeds dead.

Five Lines with three genes (CEMB-Cry1Ac,CEMB- Cry2A, and CEMBGTGene) of transgenic sugarcane free of borers selected from the field trials.




Five lines from 21 are free of any kind insect attack (Stem, Root and Top borers) 4L/2, 5L/4, 8L/4, 6L/5

Outcomes of Project:

Development of Product

Sugarcane sets of HSF 240, SPF 234, SPF 213 and CPF 246 sugarcane cultivars containing herbicide tolerant gene and additional Bt gene in CPF 246 were handed over to SRI for multiplication.

Published 4 articles in world reputed journal

a. Nasir, I. A., Tabassum, B., Qamar, Z., Javed, M. A., Tariq, M., Farooq, A. M., Butt, S. J., Qayyum, A., Husnain, T., (2014). Herbicide-tolerant sugarcane (Saccharum officinarum L.) plants: an unconventional method of weed removal. Turk J Biol 38: 439-449.

b. Qamar, Z., Riaz, S., Nasir, I. A., Ali, Q. and Husnain, T. (2015). Transformation and Transgenic Expression studies of Glyphosate tolerant and Cane Borer Resistance Genes in Sugarcane (Sccharum officinarum L.), Molecular Plant Breeding, 6(12) 1- 17.

c. Qamar, Z., Nasir, I. A., Riaz, S., Ali, Q. and Husnain, T. (2016). Molecular cloning, transformation and evaluation of different transgenic sugarcane lines for GTGene and Cry1Ac gene. 51(1): Cytology and Genetics.

d. Hameed, A., Nasir, I. A., Tabassum, B., Qamar, Z., Younus, M., Rao, A. Q., Rashid, B., Tariq, M., Zameer, M., Khan, G. A., Ali, M., Anjum, M. S., Ahmed, S., Bhatti, J. A., Samiullah, T. R. and Husnain, T. (2016). Biosafety Assessment of 20 Locally Developed Transgenic Sugarcane. J. Anim. Plant Sci. 26(4)

PHYSICAL PROGRESS REPORT
OF THE PARB PROJECT NO. 193
(01-06-2010 TO 31-05-2017)

GENETIC IMPROVEMENT OF SUGARCANE FOR HERBICIDE AND BORER RESISTANCE

TEAM LEADER

DR. MUHAMMAD ZAFFAR IQBAL

AGRICULTURAL BIOTECHNOLOGY RESEARCH INSTITUTE, FAISALABAD

FINAL PHYSICAL PROGRESS REPORT FOR PARB CGS PROJECT # 193
(Physical progress from 01-06-2013 to 31-05-2017)

A. Basic Information

1.	**Name of the project**	Genetic Improvement of Sugarcane for Herbicide and Borer Resistance
2.	**Project No.**	193
3.	**Total Project cost**	22.350 Million Rupees
4.	**Total project duration**	Five years (June 1st, 2010 to May 31st, 2015)
5.	**Funds released so far**	
6.	**Project commencement Date**	June 1st, 2010
7.	**Name of the Team Leader with designation**	Dr. Muhammad Zaffar Iqbal Director Agricultural Biotechnology Research Institute, Faisalabad.

Output activity	**Description**	**Planned Completion date**	**Achievement indicator as planned**	**Achievements**	**Reason for deviation if any**
Output-1	Pilot scale multiplication/propagation of herbicide tolerant Sugarcane CVs SPF 213 and SPF-234.	31-05-2013	One lac seed sets of two Herbicide tolerant sugarcane lines for field trials will be available	More than one Lac seed sets of sugarcane containing CEMB-GT gene were produced from 2 Kanals of SPF-213 and 1 Kanal 9 marlah of SPF-234 planted at ABRI, AARI, Faisalabad.	Out of 1 lac 72 thousand sets, 24352 sets were transferred to SRI for field trial. However, after these field trials SRI did not to take the remaining sets from ABRI. The reason for not taking the remaining

					sets was the unavailability of Biosafety permission by NBC.
Activity-1	Sugarcane Plantlets of cultivars SPF 213 and SPF-234 delivery from CEMB containing CEMB-GT Gene	15-07-2011	1000 calli of each of the two sugarcane cultivars SPF-213 and SPF-234 containing GT Gene received from CEMB	1000 calli of sugarcane cultivars SPF-234 received and 34 test tube containing callus of sugarcane variety SPF-213 received on 27-09-2011.	
Activity-2	*In-vitro* multiplication and acclimatization of Sugarcane plants containing CEMB-GT Gene	31-05-2013	10000 Plantlets of cultivars SPF 213 and CPF 246 will be available for green house/tunnel evaluation.	1. 20000 plantlets of sugarcane variety SPF-213 and SPF-234 available in test tubes. 2. 10000 plantlets of sugarcane cultivars SPF-213 and SPF-240 shifted in the field.	
Activity-3	Sugarcane Sets delivery to SRI containing CEMB-GT Gene for field trial.	31-05-2013	One Lac Seed sets of sugarcane containing CEMB-GT Gene will be delivered to SRI for Field trial.	1- 30000 plantlets of sugarcane cultivars SPE-213 and SPF-234 available in the test tubes**.** 2- 1949 plantlets of sugarcane cultivars of SPF-213 in polythene bags and 20 plants SPF-234 in pots available and ready to transplants in field. 3- 120 fully grown plants of SPF-234 are	Out of 1 lac 72 thousand sets, 24352 sets were transferred to SRI for field trial. However, after these field trials SRI did not to take the remaining sets from

				available in the field. Each plants have average has six tillers and about six sets. Total numbers of sets of SPF-234 are 120x6x6=4320. 4- More than one Lac 72 thousand seed sets of sugarcane containing CEMB-GT gene were produced from 2 Kanals of SPF-213 and 1 Kanal 9 marlah of SPF-234 planted at ABRI, AARI, Faisalabad. Almost 24352 sets were delivered to SRI for field trial.	ABRI. The reason for not taking the remaining sets was the unavailability of Biosafety permission by NBC.

DR. M. ZAFFAR IQBAL
TEAM LEADER/PROJECT NO. 193
DIRECTOR AGRICULTURAL BIOTECHNOLOGY
RESEARCH INSTITUTE, FAISALABAD

PHYSICAL PROGRESS REPORT
OF THE PARB PROJECT NO. 193
(01-06-2013 TO 31-05-2017)

GENETIC IMPROVEMENT OF SUGARCANE FOR HERBICIDE AND BORER RESISTANCE

TEAM LEADER

MR. MUHAMMAD MUNIR

SUGARCANE RESEARCH INSTITUTE, FAISALABAD

FINAL REPORT OF THE PARB CGS PROJECT 193

(From 10.10.2013 to 31.05.2017)

A. Basic Information

1.	**Name of the project**	***Genetic Improvement of Sugarcane for Herbicide and Borer Resistance***
2.	**Project No.**	**193**
3.	**Total Project cost**	**Rs. 22.350 million**
4.	**Total project duration**	60 months
5.	**Funds released so far to SRI, Faisalabad**	2.220 million
6.	**Project commencement Date**	01-06-2010
7	**Project extension period**	01.06.2015 to 31.05.2017
8	**Name of the Team Leader with designation**	**Mr. Muhammad Munir,** Assistant Entomologist/Team Leader, SRI, Faisalabad

B. Physical Research Achievements

Output/ Activity	**Description**	**Planned Completion date**	**Achievement Indicator as planned**	**Achievements** (Please attach data in brief as annexure if activity completed)	**Reasons for deviation if any**
Output-1	Sugarcane lines HSF-240, SPF-234, SPF-213 and CPF-246 with CEMB BT and GT gene will be	31.05.2015	Confirmed transgenic sugarcane lines HSF-240, SPF-234, SPF-213 and CPF-246	- Entries of transgenic sugarcane variety HSF-240 and CPF-246 cane setts were received from CEMB against herbicide and borer tolerance, respectively in month of Oct 2013. - Entries of two transgenic varieties HSF-240 and SPF-213 cane setts were received from ABRI,	

	ready for approval as variety		with tolerance to borer/herbicide	Faisalabad in the month of Oct 2013, tolerant against herbicide (glyphosate) - Transgenic varieties i.e., HSF-240, SPF-213, and CPF-246 were sown in three separate non-replicated experiments against their tolerance character (BT/GT gene) at SRI Faisalabad in the months of Oct 2013 - Seed of 23 transgenic clones of HSF-240 & SPF-213 having GT gene were received from ABRI on March 17, 2014 and had been sown in field - Glyphosate **@ 1900 ml/acre** was applied to herbicide tolerant HSF-240 dated April 22, 2014. **All the transgenic events showed poor performance compared to non-transgenic plants**. The same outcomes/findings were endorsed by PARB monitoring team comprising of Dr. Hafeez Ahmad Sadaqat and Abdul Rehman in their visit on May 10, 2014	

<table>
<tr>
<td>Activity-2</td>
<td>Testing of CEMB BT and GT gene against borer/herbicide tolerance in sugarcane lines in different sugarcane growing areas at 8 locations in autumn and spring seasons in south, central and northern Punjab in addition to SRI, Faisalabad and SRS, Khanpur</td>
<td>31.05.2017</td>
<td>Data on the performance of transgenic versions of selected varieties in comparison with non-transgenic versions of these varieties</td>
<td>- Sixty one (61) entries of transgenic CPF-246 were received from CEMB Lahore on 10.10.2013. These were sown in the research area of Sugarcane Research Institute, Faisalabad on 11.10.2013. All the recommended inputs were given to the crop throughout the season except insecticide application. The data on dead heart % age was recorded twicely during February & March 2014 with one month interval from all entries sown in the area. At harvest time during December, 2014, internode damage % age was recorded by collecting a sample of ten randomly selected canes. These canes were splitted longitudinally and borar damage was observed and is being presented in the following table.
<table>
<tr><th>S. #</th><th>Entry Name</th><th>Dead-heart %age</th><th>commulative internode damage</th><th></th></tr>
<tr><td>1</td><td>2L / 3</td><td>2.10</td><td>2.23</td><td></td></tr>
<tr><td>2</td><td>2L / 6</td><td>2.85</td><td>6.18</td><td></td></tr>
<tr><td>3</td><td>2L / 8</td><td>5.27</td><td>6.25</td><td></td></tr>
<tr><td>4</td><td>3L / 3</td><td>9.28</td><td>2.09</td><td></td></tr>
<tr><td>5</td><td>3L / 4</td><td>6.25</td><td>5.69</td><td></td></tr>
<tr><td>6</td><td>4L / 2</td><td>2.68</td><td>2.60</td><td></td></tr>
<tr><td>7</td><td>4L / 7</td><td>3.22</td><td>2.44</td><td></td></tr>
<tr><td>8</td><td>4L / 8</td><td>9.73</td><td>4.45</td><td></td></tr>
<tr><td>9</td><td>5L / 1</td><td>14.08</td><td>4.82</td><td></td></tr>
<tr><td>10</td><td>5L / 5</td><td>9.20</td><td>1.45</td><td></td></tr>
<tr><td>11</td><td>6L / 2</td><td>4.96</td><td>4.76</td><td></td></tr>
<tr><td>12</td><td>6L / 3</td><td>3.10</td><td>5.17</td><td></td></tr>
<tr><td>13</td><td>6L / 5</td><td>6.57</td><td>2.18</td><td></td></tr>
<tr><td>14</td><td>7L / 2</td><td>16.23</td><td>6.85</td><td></td></tr>
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15	7L / 7	15.99	6.58
16	8L / 4	7.76	2.4
17	9L / 6	7.25	5.00
18	CPF 246 (non-transgenic)	2.46	7.78

It is clear from the table that no entry was found free of dead heart and internode damage. These seventeen entries were re-sown in research area of Sugarcane Research Institute, Faisalabad during spring 2015 in varying lines. Dead heart % age data was recorded in May, 2015 and is being presented in the following table:

Sr.#	Dead heart (%)	Internode damage (%)		Cumulative internode damage (%)
		Stem borer	Root borer	
1	9.13	8.57	3.67	**12.24**
2	10.73	9.79	4.49	**14.29**
3	8.61	6.95	3.82	**10.77**
4	8.42	8.02	3.78	**13.06**
5	9.42	9.92	5.41	**15.39**
6	10.12	10.88	4.55	**15.42**
7	10.73	8.36	7.36	**15.73**
8	10.59	10.84	3.68	**14.53**
9* **(Control)**	9.89	9.35	4.11	**13.45**

*= Non transgenic

- All entries showed dead hearts at early stage and internode damage at crop maturity.

- Conclusively it is reported that no resistant against herbicide & borers were found in transgenic sugarcane entries.

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PARB Project No. 193
SRI, Faisalabad.