भा कृ अनु प
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केन्द्रीय कपास अनुसंधान संस्थान
(भारतीय कृषि अनुसंधान परिषद)
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By speed post

No.F.CPt.Bt/2017/ 26666

February 7, 2017

To
Shri Amol Pusadkar
52, Shivshakti Nagar-1
Behind Tapasya Vidyalaya
Ring Road, Nagpur – 440 034

Sub : Test Report Bollgard II and EPSPS, ROUND-UP- READY- FLEX reg.

Dear Sir,

Please find enclosed herewith the test report pertaining to Bollgard II and EPSPS, ROUND-UP- READY for further necessary action at your end.

Thanking you,

Yours faithfully,

(S.Kranthi)
Head I/c

Encl : a/a

: पांजरी एल. पी.जी. बॉटलिंग प्लॉन्ट के पास वर्धा रोड, नागपुर – 441108 Office : Panjari, Near LPG Bottling Plant, Wardha Road, Nagpur-441108

CENTRAL INSTITUTE FOR COTTON RESEARCH

PB No, 2, Shankarnagar PO, Nagpur 440 010

BT REFERRAL LABORATORY

REPORT

SUMMARY:

ELISA (Enzyme linked immunosorbent assay) and PCR (Polymerase chain reaction) tests confirmed the presence of *Cry1Ac + Cry2Ab + epsps* genes in six field samples of seed-cotton collected from nine fields of seven villages in Nagpur district. The results confirm that bolls collected from six fields were positive for Bollgard-II and EPSPS, ROUND-UP-READY-FLEX.

Results of tests conducted on seed-cotton boll samples obtained from farmer fields to determine the presence of Cry1Ac + Cry2Ab (Bollgard-II BG-II) and *epsps* gene (Round-up Ready Flex):

Table 1. Details of hybrids and locations of samples

Code No.	BG-II Hybrid	Location
1	Jadoo	Village – Karmbhad Taluka- Parshivani, Dist.Nagpur.
2	ATM	Village- Karmbhad Taluka -.Parshivani, Dist. Nagpur.
3	Balbhadra	Taluka – Savner, Dist- Nagpur.
4	Krishna gold	Village – Issapur, katol.
5	Arjun-151	Village- Nimba Taluka- Parshivani, Dist-Nagpur.
6	Jambo	Village – Savner, Dist- Nagpur.
7	Krishna gold	Village – Malapur, Narkhed.
8	Supershakti	Village – Yerla, Narkhed
9	Balbhadra	Village – Savner.

Table 2. Results of Cry1Ac and Cry2Ab tests conducted with the seeds from samples collected from the nine fields

Sr.No	Sample name	Cry2Ab test	Cry1Ac test
1	1_1	Positive	Positive
2	1_2	Positive	Negative
3	1_3	Positive	Positive
4	1_4	Positive	Positive
5	1_5	Positive	Negative
6	1_6	Positive	Positive
7	1_7	Positive	Negative
8	1_8	Positive	Positive
9	1_9	Positive	Positive
10	1_{10}	Positive	Positive
11	1_{11}	Positive	Negative
12	1_{12}	Positive	Positive
13	1_{13}	Positive	Positive
14	1_{14}	Positive	Positive
15	1_{15}	Positive	Positive
16	1_{16}	Positive	Positive
17	1_{17}	Positive	Negative

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Sr.No	Sample name	Cry2Ab test	Cry1Ac test
18	1_{18}	Positive	Positive
19	1_{19}	Positive	Negative
20	1_{20}	Positive	Positive
21	1_{21}	Positive	Positive
22	1_{22}	Positive	Positive
23	1_{23}	Positive	Positive
24	1_{24}	Positive	Positive
25	1_{25}	Positive	Positive
26	2_{1}	Positive	Positive
27	2_{2}	Positive	Positive
28	2_{3}	Positive	Positive
29	2_{4}	Positive	Positive
30	2_{5}	Positive	Positive
31	2_{6}	Positive	Positive
32	2_{7}	Positive	Positive
33	2_{8}	Positive	Positive
34	2_{9}	Positive	Positive
35	2_{10}	Positive	Positive
36	2_{11}	Positive	Positive
37	2_{12}	Positive	Positive
38	2_{13}	Positive	Positive
39	2_{14}	Positive	Positive
40	2_{15}	Positive	Positive
41	2_{16}	Positive	Positive
42	2_{17}	Positive	Positive
43	2_{18}	Positive	Positive
44	2_{19}	Positive	Positive
45	2_{20}	Positive	Positive
46	2_{21}	Positive	Positive
47	2_{22}	Positive	Positive
48	2_{23}	Positive	Positive
49	2_{24}	Positive	Positive
50	2_{25}	Positive	Positive
51	2_{26}	Positive	Positive
52	2_{27}	Positive	Positive
53	2_{28}	Positive	Positive
54	2_{29}	Positive	Positive
55	2_{30}	Positive	Positive
56	2_{31}	Positive	Positive
57	2_{32}	Positive	Positive
58	2_{33}	Positive	Positive
59	2_{34}	Positive	Positive
60	3_{1}	Negative	Negative
61	3_{2}	Positive	Positive
62	3_{3}	Positive	Negative
63	3_{4}	Negative	Negative
64	3_{5}	Positive	Positive
65	3_{6}	Positive	Positive
66	3_{7}	Positive	Positive
67	3_{8}	Negative	Positive
68	3_{9}	Positive	Positive
69	3_{10}	Negative	Positive
70	3_{11}	Negative	Positive
71	3_{12}	Positive	Positive

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Sr.No	Sample name	Cry2Ab test	Cry1Ac test
72	3_{13}	Positive	Negative
73	3_{14}	Positive	Positive
74	3_{15}	Negative	Positive
75	3_{16}	Positive	Positive
76	3_{17}	Positive	Negative
77	3_{18}	Positive	Positive
78	3_{19}	Negative	Positive
79	3_{20}	Negative	Positive
80	3_{21}	Negative	Positive
81	3_{22}	Positive	Positive
82	3_{23}	Positive	Positive
83	3_{24}	Positive	Positive
84	3_{25}	Negative	Negative
85	3_{26}	Negative	Positive
86	3_{27}	Positive	Positive
87	3_{28}	Positive	Positive
88	3_{29}	Positive	Positive
89	3_{30}	Positive	Positive
90	3_{31}	Positive	Positive
91	4_{1}	Positive	Positive
92	4_{2}	Positive	Positive
93	4_{3}	Positive	Positive
94	4_{4}	Negative	Positive
95	4_{5}	Positive	Positive
96	4_{6}	Positive	Positive
97	4_{7}	Positive	Positive
98	4_{8}	Negative	Positive
99	4_{9}	Negative	Positive
100	4_{10}	Positive	Positive
101	4_{11}	Positive	Positive
102	4_{12}	Positive	Positive
103	4_{13}	Negative	Positive
104	4_{14}	Positive	Positive
105	4_{15}	Positive	Positive
106	4_{16}	Positive	Positive
107	4_{17}	Positive	Positive
108	4_{18}	Positive	Positive
109	4_{19}	Positive	Positive
110	4_{20}	Positive	Positive
111	4_{21}	Positive	Positive
112	4_{22}	Positive	Positive
113	4_{23}	Positive	Positive
114	4_{24}	Positive	Positive
115	5_{1}	Positive	Positive
116	5_{2}	Positive	Positive
117	5_{3}	Positive	Positive
118	5_{4}	Negative	Positive
119	5_{5}	Negative	Positive
120	5_{6}	Negative	Positive
121	5_{7}	Positive	Positive
122	5_{8}	Positive	Positive
123	5_{9}	Negative	Negative
124	5_{10}	Negative	Negative
125	5_{11}	Positive	Positive

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Sr.No	Sample name	Cry2Ab test	Cry1Ac test
126	5_{12}	Positive	Positive
127	5_{13}	Positive	Negative
128	5_{14}	Positive	Positive
129	5_{15}	Positive	Positive
130	5_{16}	Negative	Negative
131	5_{17}	Negative	Negative
132	5_{18}	Positive	Positive
133	5_{19}	Positive	Positive
134	5_{20}	Positive	Positive
135	5_{21}	Negative	Positive
136	5_{22}	Positive	Positive
137	5_{23}	Negative	Positive
138	5_{24}	Positive	Positive
139	5_{25}	Positive	Positive
140	5_{26}	Positive	Positive
141	5_{27}	Positive	Positive
142	5_{28}	Positive	Positive
143	6_{1}	Positive	Positive
144	6_{2}	Positive	Positive
145	6_{3}	Positive	Positive
146	6_{4}	Positive	Positive
147	6_{5}	Positive	Positive
148	6_{6}	Positive	Positive
149	6_{7}	Positive	Positive
150	6_{8}	Negative	Positive
151	6_{9}	Positive	Positive
152	6_{10}	Positive	Positive
153	6_{11}	Positive	Positive
154	6_{12}	Negative	Positive
155	6_{13}	Positive	Positive
156	6_{14}	Positive	Positive
157	6_{15}	Positive	Positive
158	6_{16}	Negative	Positive
159	6_{17}	Positive	Positive
160	6_{18}	Positive	Positive
161	6_{19}	Positive	Positive
162	6_{20}	Positive	Positive
163	6_{21}	Positive	Positive
164	6_{22}	Positive	Positive
165	6_{23}	Negative	Positive
166	6_{24}	Negative	Positive
167	6_{25}	Positive	Positive
168	6_{26}	Positive	Positive
169	6_{27}	Negative	Positive
170	6_{28}	Negative	Positive
171	6_{29}	Positive	Positive
172	6_{30}	Positive	Positive
173	6_{31}	Negative	Positive
174	6_{32}	Positive	Positive
175	6_{33}	Positive	Positive
176	7_{1}	Negative	Positive
177	7_{2}	Positive	Positive

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Sr.No	Sample name	Cry2Ab test	Cry1Ac test
178	7_3	Positive	Negative
179	7_4	Positive	Positive
180	7_5	Positive	Positive
181	7_6	Negative	Positive
182	7_7	Positive	Positive
183	7_8	Positive	Positive
184	7_9	Positive	Negative
185	7_{10}	Positive	Negative
186	7_{11}	Negative	Positive
187	7_{12}	Positive	Negative
188	7_{13}	Positive	Negative
189	7_{14}	Positive	Positive
190	7_{15}	Positive	Positive
191	7_{16}	Positive	Negative
192	7_{17}	Positive	Positive
193	7_{18}	Positive	Positive
194	7_{19}	Negative	Negative
195	7_{20}	Negative	Positive
196	7_{21}	Positive	Negative
197	7_{22}	Positive	Negative
198	7_{23}	Positive	Positive
199	7_{24}	Positive	Positive
200	7_{25}	Positive	Positive
201	7_{26}	Positive	Positive
202	7_{27}	Negative	Positive
203	7_{28}	Positive	Positive
204	7_{29}	Positive	Positive
205	8_1	Negative	Positive
206	8_2	Negative	Positive
207	8_3	Positive	Positive
208	8_4	Positive	Positive
209	8_5	Negative	Positive
210	8_6	Positive	Negative
211	8_7	Positive	Positive
212	8_8	Positive	Positive
213	8_9	Positive	Negative
214	8_{10}	Positive	Positive
215	8_{11}	Positive	Positive
216	8_{12}	Positive	Positive
217	8_{13}	Positive	Positive
218	8_{14}	Positive	Positive
219	8_{15}	Positive	Positive
220	8_{16}	Positive	Positive
221	8_{17}	Positive	Positive
222	8_{18}	Positive	Positive
223	8_{19}	Positive	Positive
224	8_{20}	Positive	Positive
225	8_{21}	Positive	Negative
226	8_{22}	Positive	Positive
227	8_{23}	Positive	Negative
228	8_{24}	Positive	Positive
229	8_{25}	Positive	Positive

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Sr.No	Sample name	Cry2Ab test	Cry1Ac Test
230	8_{26}	Positive	Positive
231	8_{27}	Positive	Positive
232	8_{28}	Positive	Positive
233	8_{29}	Positive	Positive
234	8_{30}	Positive	Positive
235	8_{31}	Positive	Positive
236	8_{32}	Positive	Negative
237	8_{33}	Positive	Negative
238	8_{34}	Positive	Negative
239	9_1	Positive	Positive
240	9_2	Positive	Positive
241	9_3	Negative	Positive
242	9_4	Negative	Positive
243	9_5	Positive	Positive
244	9_6	Negative	Positive
245	9_7	Positive	Positive
246	9_8	Positive	Positive
247	9_9	Negative	Positive
248	9_{10}	Positive	Negative
249	9_{11}	Positive	Positive
250	9_{12}	Positive	Negative
251	9_{13}	Positive	Positive
252	9_{14}	Positive	Positive
253	9_{15}	Positive	Negative
254	9_{16}	Negative	Positive
255	9_{17}	Positive	Positive
256	9_{18}	Positive	Positive
257	9_{19}	Positive	Positive
258	9_{20}	Positive	Positive
259	9_{21}	Positive	Negative
260	9_{22}	Positive	Positive
261	9_{23}	Positive	Positive
262	9_{24}	Positive	Positive
263	9_{25}	Positive	Positive
264	9_{26}	Negative	Positive
265	9_{27}	Positive	Negative
266	9_{28}	Positive	Positive
267	9_{29}	Positive	Negative
268	9_{30}	Negative	Positive
269	9_{31}	Negative	Negative
270	9_{32}	Positive	Positive

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CONFIRMATORY TEST FOR BOLLGARD-II & RRF (ROUND UP READY FLEX)

Seeds that were positive for both Cry1Ac + Cry2Ab were subjected to PCR (polymerase chain reaction) test for tested for the presence of 5-enolpyruvylshikimate-3-phosphate synthase (*epsps*) gene expressed in Roundup Ready Flex.

S.No.	Hybrid	Code no	Cry2Ab test	Cry1Ac test	RRF test
1	Jadoo (Karmbadh)	1_{14}	Positive	Positive	Positive
		1_{22}	Positive	Positive	Positive
		1_{24}	Positive	Positive	Positive
2	ATM (Karmbadh)	2_{3}	Positive	Positive	Positive
		2_{4}	Positive	Positive	Positive
		2_{13}	Positive	Positive	Positive
3	Balbhadra (Saoner)	3_{5}	Positive	Positive	Positive
		3_{7}	Positive	Positive	Positive
		3_{18}	Positive	Positive	Positive
4	Krishna Gold (Esapur)	4_{5}	Positive	Positive	Negative
		4_{18}	Positive	Positive	Negative
		4_{24}	Positive	Positive	Negative
5	Arjun (Nimba)	5_{11}	Positive	Positive	Positive
		5_{19}	Positive	Positive	Positive
		5_{26}	Positive	Positive	Positive
6	Jambo (Saoner)	6_{7}	Positive	Positive	Negative
		6_{19}	Positive	Positive	Negative
		6_{26}	Positive	Positive	Negative
7	Krishna Gold (Malapur)	7_{15}	Positive	Positive	Positive
		7_{24}	Positive	Positive	Positive
		7_{26}	Positive	Positive	Negative
8	Supershakti (Yerla)	8_{13}	Positive	Positive	Negative
		8_{19}	Positive	Positive	Negative
		8_{34}	Positive	Positive	Negative
9	Balbhadra (Saoner)	9_{18}	Positive	Positive	Positive
		9_{25}	Positive	Positive	Positive
		9_{32}	Positive	Positive	Positive

RESULT AND CONCLUSION

All the nine samples tested positive for Bollgard-II (BG-II). Six out of the nine samples that were positive for BG-II were also found to contain the EPSPS gene. **The results confirm that bolls collected from six fields were positive for Bollgard-II and EPSPS, ROUND-UP-READY-FLEX.**

Tested by
Shilpa Chawla

6/2/17.
Verified by
Vandhya Kranth