



केन्द्रीय कपास अनुसंधान संस्थान

(भारतीय कृषि अनुसंधान परिषद)

पोस्ट बैग नं. 02, शंकर नगर, पो. ऑ. नागपुर-440 010 (महाराष्ट्र), भारत

CENTRAL INSTITUTE FOR COTTON RESEARCH

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No.F.CPt.Bt/2017/
26666

By Speed post

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. Krantibhi

(S. Krantibhi)

Head I/c

Encl : a/a

Dhawle

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 – Karmbad Taluka- Parshivani, Dist.Nagpur.
2	ATM	Village- Karmbad 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

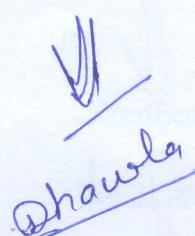
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Sr.No	Sample name	Cry2Ab test	Cry1Ac test
178	7 ₃	Positive	Negative
179	7 ₄	Positive	Positive
180	7 ₅	Positive	Positive
181	7 ₆	Negative	Positive
182	7 ₇	Positive	Positive
183	7 ₈	Positive	Positive
184	7 ₉	Positive	Negative
185	7 ₁₀	Positive	Negative
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


D. Shweta

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
Manollya Kranth