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Validation of Bt-gene and study of packaging materials on seed longevity in Bt-cotton hybrids

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A laboratory experiment was carried out to validate Bt gene and to study the storage potential of Bt-cotton hybrids. Results revealed that, all the six Bt-cotton hybrid seed lots selected for the study were positive for Bt-gene (*Cry 1 Ac*). In the storage study, the seed lot L_1 recorded highest germination (71.55%) and lowest in L_3 (68.11%) at the end of ten months of storage. Among the packaging materials, seeds stored in P_1 and P_2 recorded highest germination (71.50 and 71.50%, respectively) compared to P_3 (68.11 %). Seedling vigour index was highest (2007) in L_1 and lowest (1792) in L_6. Irrespective of seed lots, P_1 recorded highest vigour (1981) followed by P_2 (1963) and lowest in P_3 (1759) at the end of ten months of storage. Germination per cent in polythene bag and polylined cloth was above the minimum seed certification standards (70.00%) upto ten months of storage. However, genotypic differences were observed with respect to seed quality.

Key words : Cottton, Validation, Bt gene, Storability, Seed quality

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INTRODUCTION

Cotton is a king of fibre crop belongs to family Malvaceae and the genus *Gossypium* which includes 20 wild as well as cultivated species. It is one of the most important fibre crops playing a key role in textile industry, economic and social affairs of the world. It is the oldest among the commercial crops of the world. Cotton is used as a fabric in India from time immemorial. Globally, China stands first in cotton production (32 million bales) followed by India (25.81 million bales). India has the world's largest acreage of 9.43 million hectares representing about one quarter of global area (35 million ha) under cotton cultivation. The average yield of cotton in India is 466 kg/ha, which is far below the world average of 677 kg/ha and in India the overall production accounts for 25.81 million bales (Anonymous, 2008). The main cause for reduced yield is due to bollworm attack and depending on rainfed cultivation. To overcome bollworm infestation Bt-cotton was developed by insertion of *Cry 1AC* gene which confers resistance against bollworms.

Seed purity and germination are two important factors, which determine seed quality. The rapid loss of seed viability and vigour in storage leads to poor stand establishment of the crop and low productivity. Hence, storage of seeds after harvest till next sowing season is of prime importance for better seed yields. Many a times the seed is to be stored for many years as buffer stock. The seed deterioration starts right at the field levels immediately after the physiological maturity. The seed has to be stored safely so that the viability and vigour is maintained intact, as cotton seeds deteriorates at faster rate being an important oilseed crop. Therefore, the present study was carried out to study the effect of packaging materials on longevity of cotton hybrids.

RESEARCH METHODOLOGY

The experiment was carried out in the Department of Seed Science and Technology, University of Agricultural Sciences, Bangalore during 2010-11. The Bt-cotton hybrid seed lots treated with imidacloprid @10g/kg and thiram 1g/kg of seeds were obtained from seed companies located at Rannebennur, Karnataka *viz.*, L_1: Jai-Bt (a), L_2: Jai-Bt (b), L_3: Bunny-Bt, L_4: Cheeranjeevi-Bt (a), L_5: Cheeranjeevi-Bt (b), L_6: Mallika-Bt seeds were cleaned, graded, subjected for validation of Bt-gene, analyzed for initial seed quality parameters (Table 1) and stored different packaging materials *viz.*, P_1: Polythene bag (700 guage), P_2: Polylined cloth bag and P_3: Cloth bag for a period of ten months under ambient conditions of Bangalore.

Validation of *Cry 1Ac* gene was carried out by using Bt-kit (Plate 1) as per the protocol provided by Central Institute for Cotton Research, Nagpur (CICR), Maharashtra. A single Bt-cotton hybrid seed was taken randomly from each hybrid seed lots and seed coat was removed. The white colored internal embryo was transferred into vials provided along with kit. After placing embryo into vial, 0.5 ml of sample extraction buffer which was also provided along with kit was added. Subsequently, embryo was crushed with pestle provided with kit. Then, *Cry 1Ac* Bt instant-check strip was dipped into vial and left for 15-20 minutes for the sample solution to travel till top end of the strip and the filter pad at top end is almost completely wet. One band developed halfway through the strip along with another at the top (two bands) indicating the presence of *Cry 1Ac* gene and it is positive for Bt-cotton sample.

The required quantity of seeds were drawn at monthly intervals from each bag and subjected to seed quality as per ISTA (2007). Germination test was conducted by placing 100 seeds in four replicates in paper towels and incubated at 25 ± 1^0C for 14 days. The seedling growth parameters were recorded, seedling vigour index was calculated and expressed as a whole number as suggested by Abdul-Baki and Anderson (1973). The data obtained from other experiments was statistically analyzed by using suitable ANOVA and critical differences between the treatments at five per cent significance as per Snedecor and Cochran (1967).

RESEARCH FINDINGS AND ANALYSIS

The seeds of six cotton were procured from the seed companies and kept in different packaging materials upto ten months. Presence of *Cry 1 Ac* gene was confirmed by Bt-strip test developed specifically for the purpose. Presence of *Cry 1Ac* gene in all the six hybrids was revealed by manifestation of two distinct bands from the samples of Bt-cotton hybrid seeds unlike single band on non-Bt samples (Plate 1). All the six Bt-cotton hybrids under study were positive for *Cry 1Ac* gene under strip test. Development of two distinct band on test strips was due to interaction between antigen and an antibody, where *Cry 1Ac* gene acts as antigen (foreign body) and binds to specific antibody (*Cry 1Ac* antibody) coating on the test strips, first band indicates the control and below that second band indicating the presence of *Cry 1Ac* gene. Two distinct bands develop only if samples contain *Cry 1Ac* gene unlike single band an indicative of non-Bt sample (Randhawa,

Plate 1 : Validation of Bt-gene (Cry 1Ac) using Bt detection kit A and B- Bt cotton kit and its components 1 to 6- Bt cotton hybrids 1-Jai-Bt (a), 2-Jai-Bt (b), 3: Bunny-Bt, 4-Cheeranjeevi-Bt (a), 5:Cheeranjeevi-Bt (b), 6-Mallika-Bt

Table 1: Initial seed quality parameters of cotton hybrids

Seed lots (L)	Seed quality parameters				
	Seed moisture content (%)	Germination (%)	Field emergence (%)	Mean seedling length (cm)	Vigour index
L_1 - Jai-Bt (a)	7.00	85.33	82.66	36.67	3132
L_2 –J ai-Bt (b)	7.75	84.66	81.33	36.80	3109
L_3 - Bunny-Bt	7.00	84.00	81.33	37.46	3143
L_4 - Cheeranjeevi-Bt (a)	7.00	86.00	82.66	34.80	2992
L_5 - Cheeranjeevi-Bt (b)	7.50	84.00	84.00	37.33	3136
L_6 - Mallika-Bt	7.00	82.66	82.00	35.91	2968
Mean	7.21	84.44	82.33	36.50	3080
S.E. ±	0.19	1.28	1.15	0.80	55
C.D. (P=0.05)	0.60	3.19	3.14	2.40	145

2005).

Storage potential of seeds is basically under genetic control and it differs with the species and genotypes (Delouche *et al.*, 1973). It is known that vapour proof packing is better to preserve the seed in storage and seed has to be dried to a lower moisture level compared to packing in vapour pervious package. Packaging materials also acts as a barrier between seeds and the external environment and protects the seeds from high relative humidity and prevents entry of moisture and pathogens (Doijode, 2002). In the present study, seeds stored in polythene bag (700 guage) recorded lowest (7.08%) moisture content at the end of the storage period compared to cloth bag (8.44%). There was more moisture fluctuation was recorded between the hybrids (Table 2). Increase in moisture content in cloth might be due to the higher vapour pressure gradient between the seeds and external environment and also due to the fluctuations in the atmospheric relative humidity and temperature.

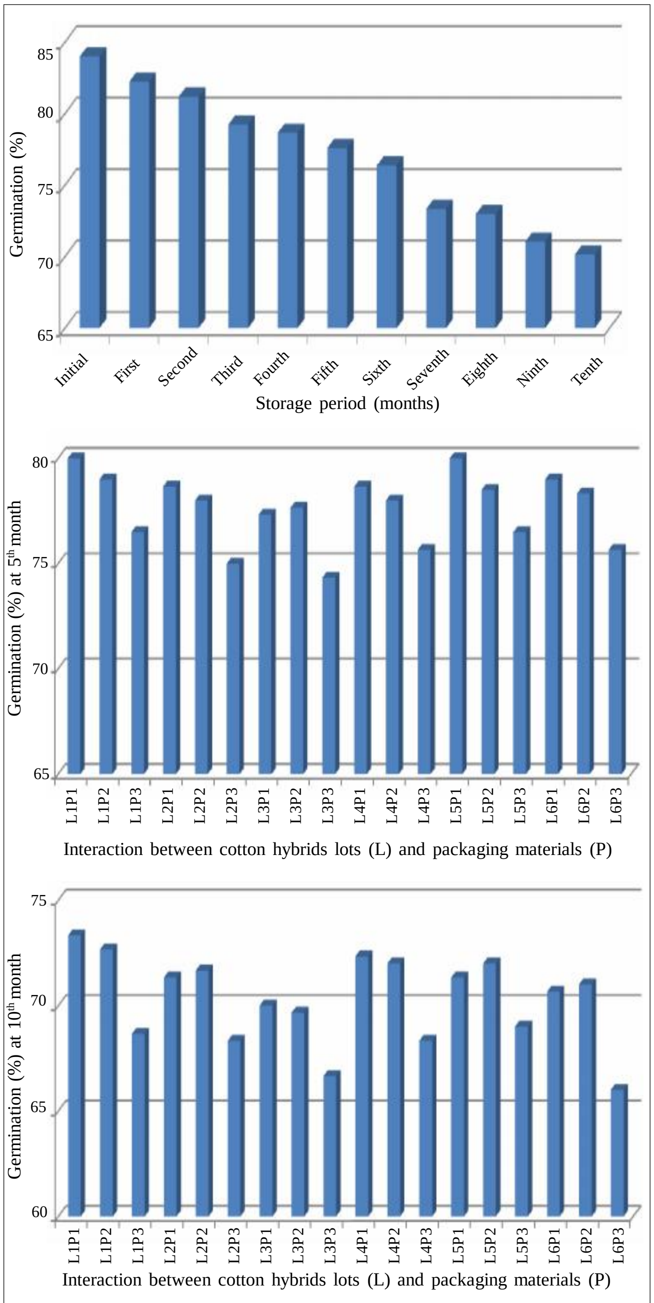

Fig. 1: Seed germination as influenced by cotton hybrids and packaging materials during storage

Germination declined progressively over a period of storage (Fig. 1). Among the seed lots L_1 recorded significantly highest germination (71.55%) and lowest in L_3 (68.11%). Among packaging materials, seeds stored in P_1 and P_2 recorded highest germination (71.50 and 71.50%, respectively) compared to P_3 (68.11%). Decline in germination percentage might be due to decrease in protein content (Abdelmagid and Osman (1973) also attributed to genotypic differences, leakage of electrolytes, depletion of food reserve, increased accumulation of total peroxide and malondialdehyde content and decreased activities of antioxidant enzymes, due to ageing, higher respiration rate (Goel *et al.*, 2003). Field emergence also decreased progressively with advancement of storage period (Fig. 2). As field emergence and germination are positively correlated, the decline in field emergence may attributed to decrease in germination per cent, seedling vigour, due to seed ageing and seed deterioration and loss of seed viability over a period of storage. These results are similar to the findings of Selvaraj and Ramaswamy (1977), Wood *et al*. (1977), Bishnoi and Delouche (1980) in cotton.

Seedling length declined with advancement of storage period at end of the storage period. Among the cotton hybrids, L_1 significantly recorded highest seedling length (28.29 cm) and L_6 recorded lowest (25.85 cm). The highest seedling length was recorded in seeds stored in P_1 (27.63 cm) followed by P_2 (27.39 cm) compared to P_3 (26.04 cm). The decrease in seedling dry weight may be attributed to decrease in germination and due to ageing which resulted in deterioration of seed. These results are in agreement with the findings of Shivayogi

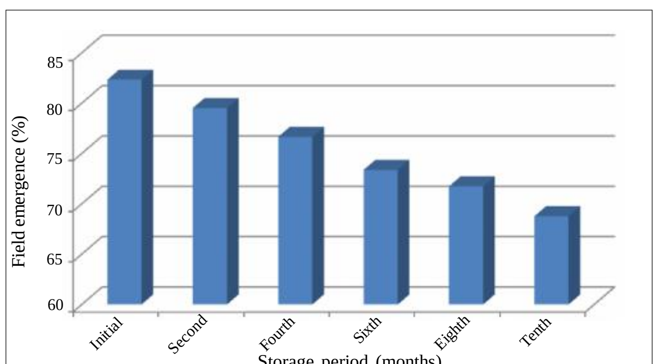

Fig. 2: Field emergence as influenced seed storage in cotton hybrids

Table 2: Influence of packaging materials on seed moisture content in Bt-cotton hybrids during storage

Seed lots (L)	Storage period (months)				
	2	4	6	8	10
L_1	7.32(15.70)	7.56(15.95)	7.51(15.90)	7.67(16.07)	7.66(16.05)
L_2	7.55(15.94)	7.76(16.16)	7.85(16.26)	8.00(16.41)	7.68(16.08)
L_3	7.48(15.86)	7.70(16.09)	7.76(16.16)	8.10(16.51)	7.93(16.34)
L_4	7.50(15.88)	7.72(16.11)	7.80(16.20)	7.94(16.35)	7.70(16.10)
L_5	7.77(16.18)	7.80(16.21)	7.99(16.40)	7.98(16.39)	7.64(16.04)
L_6	7.54(15.92)	7.65(16.03)	7.92(16.32)	8.06(16.47)	7.93(16.35)
Mean	7.52(15.91)	7.70(16.09)	7.80(16.21)	7.96(16.37)	7.76(16.16)
S.E. ±	0.06	0.11	0.12	0.13	0.09
C.D. (P=0.05)	0.17	0.21	0.10	0.25	0.10
Packaging materials (P)					
P_1	7.16(15.51)	7.13(15.49)	7.09(15.45)	7.28(15.65)	7.08(15.43)
P_2	7.26(15.64)	7.29(15.66)	7.81(16.23)	7.86(16.27)	7.75(16.16)
P_3	8.15(16.58)	8.68(17.13)	8.51(16.95)	8.73(17.19)	8.44(16.89)
Mean	7.52(15.91)	7.70(16.09)	7.80(16.21)	7.96(16.37)	7.76(16.16)
S.E. ±	0.04	0.08	0.08	0.09	0.06
C.D. (P=0.05)	0.12	0.24	0.24	0.27	0.19

Note: Figures in parentheses are *arc sine* values.

Table 3: Influence of packaging materials on total seedling length and vigour index in Bt-cotton hybrids during storage

Seed lots	Storage period (months)											
	1	3	5	7	9	10	1	3	5	7	9	10
	Seedling length (cm)						Seedling vigour index					
L_1	36.32	35.18	32.18	29.93	28.41	28.29	2986	2776	2527	2242	2069	2007
L_2	36.33	35.18	31.20	29.57	27.78	26.97	3020	2803	2401	2163	1985	1901
L_3	36.77	35.28	30.82	28.85	27.82	26.22	3048	2779	2365	2066	1924	1822
L_4	34.58	33.98	31.27	29.73	28.33	27.60	2827	2703	2422	2196	2036	1958
L_5	37.01	35.64	31.15	28.91	28.02	27.20	3006	2820	2441	2145	1993	1926
L_6	35.68	34.52	31.91	29.35	27.53	25.85	2921	2727	2475	2131	1928	1792
Mean	36.11	34.96	31.42	29.39	27.98	27.02	2968	2768	2438	2157	1989	1901
S.E. ±	0.32	0.23	0.17	0.23	0.26	0.42	32.39	20.47	17.38	19.73	20.06	35.26
C.D. (P=0.05)	0.92	0.65	0.50	0.66	NS	1.20	92.64	58.57	49.71	56.45	57.38	100.9
Packaging materials												
P_1	36.06	35.00	31.34	30.02	28.57	27.63	3030	2846	2552	2263	2076	1981
P_2	35.81	34.58	30.58	29.50	28.26	27.39	2950	2788	2452	2185	2023	1963
P_3	36.11	34.96	31.42	28.66	27.11	26.04	2924	2670	2312	2024	1868	1759
Mean	0.22	0.16	0.12	29.39	27.98	27.02	2968	2768	2438	2157	1989	24.93
S.E. ±	NS	0.46	0.35	0.16	0.18	0.29	22.90	14.47	12.29	13.95	14.18	71.31
C.D. (P=0.05)	36.11	34.96	31.42	0.47	0.53	0.85	65.60	41.41	35.15	39.92	30.57	150.12

Ryavalad *et al.* (2009) in cotton. Seedling vigour index was assessed based on seedling length (Table 3). Seedling vigour index was highest in L_1 (2007) and lowest in L_6 (1792). Irrespective of seed lots, P_1 recorded highest vigour (1981) followed by P_2 (1901) and lowest in (1759). The decline seedling vigour may be attributed to decrease in germination per cent and seedling length. These findings are in agreement with results obtained by Meena *et al.* (1998), Freitas *et al.* (2002) and Shivayogi Ryavalad *et al.* (2009) in cotton.

Among seed lots, L_1 - Jai-Bt performed better compared to other Bt-cotton hybrid seed lots. Among the packaging materials the seed quality parameter values were higher in polythene bag (700 guage) and polylined cloth bag as compared to cloth bag. Germination (%) in polythene bag and polylined cloth bag was above the minimum seed certification standards (70%). Thus, the study can be concluded that Bt-cotton hybrid seeds treated with imidacloprid @ 10g/kg and thiram 1g/kg of seeds could be stored well in polythene bag (700 guage) and polylined cloth bag for the period of ten months without considerable loss in seed viability as compared to cloth bag.

LITERATURE CITED

Abdelmagid, A.S. and Osman, A.M. (1973). Influence of storage period and temperature on viability and chemical composition of cotton seeds. *Ann. Bot.,* **39**: 237-248.

Abdul-Baki, A.A. and Anderson, J.D. (1973). Vigour determination in soybean seeds by multiple criteria. *Crop Sci*., **13**: 639-633.

Bishnoi, U.R. and Delouche, J.C. (1980). Relationship of vigour tests and seed lots to cotton seedling establishment. *Seed Sci. & Technol*., **8**: 341-346.

Delouche, J.C. (1973). Seed maturation. In: *Seed production manual*. NSC and Rockfeller Foundation. pp. 162-165.

Doijode, S.D. (2002). *Seed storage of horticultural crops*. CBS publishers and distributors, pp. 269-273.

Freitas, R.A., Dias D.C.F.S., Cecon, P.R., Reis, M.S. and Lias, Dias (2002). Storability of cotton seeds predicted by vigour tests, *Seed Sci. & Technol*., **30**: 403-410.

Goel, Anuradha, Goel, Ajay Kumar and Sheoran, Inder Singh (2003). Changes in oxidative stress enzymes during artificial ageing in cotton (*Gossypium hirsutum* L.) seeds. *J. Pl. Physiol.,* **160**: 1093-1100.

ISTA (2007). International rules for seed testing, Ed. 2007, ZURICH (SWITZERLAND).

Meena, R.A., Rathinavel, R.K., Deshmukh, R.K. and Tuteja, O.P. (1998). Storage potential of tetraploid and diploid cottons under ambient conditions, *Seed Res.,* **27**(1): 125-127.

Randhawa, G.J. (2005). Detection methods for GMO's/LMO's, *NRC on DNA fingerprinting,* NBPGR, NEW DELHI (INDIA).

Selvaraj, J.A. and Ramaswamy, K.R. (1977). Studies on seed quality as influenced by picking in cotton cv. MCU, *Madras Agric. J*., **65**: 658-663.

Shivayogi Ryavalad, Biradar Patil, N.K., Girradi, R.S. and Katageri, I.S. (2009). Effect of acid delinting seed treatment and containers on storability of cotton hybrid. *Karnataka J. agric. Sci*., **22**(1): 56-60.

Snedecor, G.W. and Cochran, W.G. (1967). *Statistical methods*, The IOWA State University Press, USA, pp.593.

Wood, D.W., Longeden, P.C. and Scoot, R.K. (1977). Seed size variation, its extent, source and significance in field crops. *Seed Sci. & Technol*., **5**: 337-352.

■ WEBLIOGRAPHY

Anonymous (2008). *http//www.agricoop.nic.in.*

(*www. nbpgr.com*)