

## Identification of Superior Transgressive Segregants with Cry1Ac Gene for Yield and Yield Related Traits in UASD Bt Cotton EVENT No. 78 Based F<sub>2</sub> Intra *Gossypium hirsutum* L. Populations

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### ABSTRACT

Three F<sub>2</sub> populations derived from three non Bt *hirsutum* female lines (DHS-114, DHS-121, DHS-108) and male Bt parent UASD Event No. 78, independently, were evaluated for yield and yield related traits. F<sub>2</sub> generation of three crosses recorded transgressive segregants for number of bolls per plant (45), number of monopodia per plant (38), average boll weight (36) and seed cotton yield per plant (18). Cross DHS-114 × Event No.78 recorded 15 transgressive segregants, with 0.7% to 104.6% higher seed cotton yield than check ARBH-813 (56.2 g/plant). In crosses DHS-121

× Event No.78 and DHS-108 × Event No.78, 15 and five segregants showed higher performance with yield advantage of 0.4 to 71.7% and 14.4 to 56.6%, respectively. These selected transgressive segregant plants can be advanced to further generations to help in isolating and stabilizing superior genotypes with Cry1Ac gene.

**Keywords** Bt cotton, UASD event no. 78, Cry1Ac gene, Transgressive segregants, Seed cotton yield.

### INTRODUCTION

Cotton, the ‘King of Fiber’ known for its desirable properties is called ‘White Gold’ due to its global importance in agriculture and industrial economy. The word “cotton” has Arabic origin, derived from the Arabic word qutun. In India, cotton has 75 % share in total fiber consumption and contributes 58 % in fiber consumption in textile industry and provides direct livelihood to 6 million farmers and about 40-50 million people are employed in cotton trade and processing thus making it an important ‘cash’ crop.

Indian cotton ecosystem is prone to 162 insect pest species, causing monetary yield loss of Rs 2, 87,000 million annually (Dhawan *et al.* 2008). Among which *H. armigera* is the most dominant and difficult

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to control chiefly due to its wide spread insecticide resistance, multivoltine nature and prolific feeding pattern and polyphagous nature. As genetic resistance, one of the important pest management strategies is not available in cotton gene pool against the bollworms, farmers are mainly dependent on chemical insecticides for control of bollworms which has led to increase in cost of cultivation. Therefore, an alternate strategy to control insect pest in cotton was developed through genetic modification technique commonly known as Bt cotton.

The Bt gene Cry1Ac was used to develop the first Bt-cotton hybrid and approved for field trials in 1993 and for commercial use in the United States in 1995. In India, on March 25, 2002 approval was given for commercial cultivation of Bt cotton hybrids with Cry1Ac (Mon 531) event of M/s. MAHYCO by GEAC. At present there are several private and public sector Bt cotton hybrids developed using Monsanto 531 event.

The recent reports of breakdown of resistance to pink bollworms emphasize the need for development of diverse events to overcome the present monopoly of single commercial event. The development of cotton transgenic events with diverse genes and with higher concentration of cry toxins is necessary for the sustainability of the transgenic technology. In this direction a new public sector event of UAS, Dharwad, UASD Bt cotton Event No.78 has been developed with Cry1Ac gene from ICGEB source in the genetic background of cultivar RAH-100, a released variety belonging to *Gossypium hirsutum* L. Simultaneously there is an urgent need to develop Bt varieties and hybrids using this potential event by creating variability through hybridization with superior breeding lines and isolation of superior Bt transgressive segregants in F<sub>2</sub> generations.

Many plant breeders have reported transgressive segregants in hybrid progenies and suggested that transgressive segregation may be used as a positive tool in plant breeding. Transgressive breeding aims at improving yield or its contributing characters through transgressive segregation. Such plants are produced by an accumulation of the positive or favorable genes from both the parents as a consequence of recombina-

tion. Obviously, the parents involved in hybridization must combine well with each other and preferably be genetically diverse that is quite different.

In such a situation, each parent is expected to contribute different positive genes, which when brought together by recombination gives rise to transgressive segregation. As a result, the intensity of character in the transgressive segregants will be greater than that of either of the parents. Genetic studies indicates that transgressive segregation mostly results from the combination of alleles from both the parents that show effects in the same direction (complementary gene action) that is, hybrid individuals those combine 'positive' alleles from both parents or 'negative' alleles from both the parents are likely to have the extreme phenotypes. Hence, present study was conducted to select transgressive segregants for seed cotton yield and its component traits in three F<sub>2</sub> segregating populations derived from crossing three non Bt lines DHS-114, DHS-121 and DHS-108 with Event No.78, independently.

## MATERIALS AND METHODS

Three F<sub>2</sub> populations were generated by crossing potential transgenic UASD Cry1Ac Bt cotton Event No. 78 as male parent to three non Bt hirsutum female lines DHS-114 (compact type), DHS-121 (high yielding) and DHS-108 (high yielding). UASD Bt cotton Event No.78 is a public sector event of UAS, Dharwad, developed with Cry1Ac gene from ICGEB, New Delhi source and has been confirmed to be significantly superior to Mon BG-II for cry toxin expression. The event is in the genetic background of cultivar RAH-100, a released variety belonging to *Gossypium hirsutum* L. The investigation was conducted at Agricultural Research Station, Hebballi, University of Agricultural Sciences, Dharwad, India, during 2019-20, situated in the northern transitional zone (Zone No. 8) of Karnataka with latitude of 15°17' N, latitude of 75° 2' E and altitude of 678 meters above mean sea level (MSL). Seeds were hand dibbled in rows of 8 m length with spacing of 90 cm between rows and 40 cm between plants in an unreplicated design. Two rows were assigned to each of P<sub>1</sub>, P<sub>2</sub>, F<sub>1</sub>s and checks, while 24 rows to F<sub>2</sub> generation for each cross. Two to three protective irrigations were given at critical stages to

get good crop stand. All the agronomic management practices were followed according to recommended packages of practices. The biometrical observations were recorded on randomly selected five plants of parents, checks,  $F_1$  and on all the individual plant in each  $F_2$  generation of three crosses. Observations were recorded on different characters viz., days to 50% flowering, plant height, number of monopodia per plant, number of sympodia per plant, number of bolls per plant, average boll weight and seed cotton yield per plant. Desirable segregants in the three populations were identified based on their superior performance over the corresponding better parent in the desirable direction for each of the traits under consideration. The identified transgressive segregants were tested for the presence of Bt gene using ELISA and lateral flow strips (Envirologix Cat No. AP 003 CRBS). The three  $F_2$  populations and their population size is mentioned below.

Sl. No.	Cross	$F_2$ population size
1	DHS – 114 × UASD Cry1Ac Bt cotton Event No.78	227
2	DHS – 121 × UASD Cry1Ac Bt cotton Event No.78	135
3	DHS – 108 × UASD Cry1Ac Bt cotton Event No.78	127
Checks	Sahana and ARBH-813 (non Bt checks)	

In genetics, transgressive segregation is the formation of extreme (transgressive) phenotypes, observed in segregating populations as compared to parents. There are many causes for transgressive segregation that are recombination of additive alleles, high mutation rate, reduced developmental stability and epistasis. Recombination results in new pairs of alleles at two or more loci and this new alleles pair can give rise to new/extreme phenotypes. When mutation rates are high, it is more probable that a mutation will occur and cause an extreme phenotypic change. These causes lead to the appearance of these extreme phenotypes and create a hybrid species that will deviate from the parent species niche. Frequency of transgressive segregants for yield and its component traits is presented in Table 1. In  $F_2$  generation of three crosses highest proportion of transgressive segregants were reported for days to 50 % flowering (88) followed by number of sympodia per plant (81), plant height (65), number of bolls per plant (45), number of monopodia per plant (38), average boll weight (36) and seed cotton yield per plant (18). Similar results were reported by Anusha *et al.* (2018) where they observed higher proportion of transgressive segregants for seed cotton yield per plant followed by number of sympodia per plant, number of bolls per plant, average boll weight, plant height, ginning percentage and days to flowering. Basamma (2007) recorded different frequency of transgressive segregants for yield attributing characters in five  $F_2$  populations of desi cotton.

## RESULTS AND DISCUSSION

In case of seed cotton yield per plant 1.6 to 7.5 %

**Table 1.** Number of superior Bt transgressive segregants for morphological and yield traits in three  $F_2$  populations.

Characters	DHS-114 × Event No.78				DHS-121 × Event No.78				DHS-108 × Event No.78			
	$F_2$	P1	P2	Percent	$F_2$	P1	P2	Percent	$F_2$	P1	P2	Percent
Days to 50% flowering	31 (106-116)	117.4	124.8	18.7	31 (103-123)	126.4	124	23.1	26 (106-115)	116	123	20.4
Plant height (cm)	24 (20-53)	54.4	84.8	14.5	16 (39-71)	65.4	85	11.9	35 (31-82)	82.6	83	27.5
Number of monopodia per plant	22 (0-1)	0.6	1.2	13.3	13 (0-1)	0.8	1.6	9.7	3 (0-1)	1.2	1	2.3
Number of sympodia per plant	45 (20-28)	12.8	19.2	27.2	13 (20-25)	13.4	19	9.7	23 (20-28)	17.8	18	18.1
Number of bolls per plant	22 (18-30)	11	17	13.3	15 (18-28)	12	17.6	11.1	8 (19-26)	11.3	18	6.2
Average boll weight (g)	16 (4.5-6.0)	3.6	4.53	9.6	15 (4.6-5.8)	3.4	4.51	11.1	5 (4.7-6.6)	3.5	4.5	3.9
Seed cotton yield per plant (g/plant)	6 (80-115)	33.5	73.5	3.6	10 (76-96.5)	39.3	74.2	7.4	2 (79-88)	36.8	75.5	1.5





**Table 4.** Performance of Bt transgressive segregants superior to better check ARBH-813 for yield and fiber related traits in the F<sub>2</sub> population of cross. DHS – 108 × Event No.78.

Sl. No.	Bt segregant	Number of bolls per plant	Average boll weight (g)	Seed cotton yield (g/plant)	GOT (%)	Lint index (g)	Seed index (g)	UHML (mm)
1	F <sub>2</sub> -III-8	26	3.6	88	40.1	5.7	8.6	26.6
2	F <sub>2</sub> -III-89	21	4	79	40	5.8	8.7	26.3
3	F <sub>2</sub> -III-82	11	6.6	69	39.8	5.6	8.5	27.6
4	F <sub>2</sub> -III-19	18	4	66	37.8	5.6	9.3	26.3
5	F <sub>2</sub> -III-62	19	3.9	64.3	39	5.4	8.6	27.1
6	ARBH-813	14	4.4	56.2	38.3	5.2	9	28.9
	(C)(Mean)							
7	F <sub>1</sub>	19	4.25	65.8	-	-	-	-
8	P1	11.3	3.5	36.8	37.1	4.1	7.8	-
9	P2	18	4.5	75.5	38.2	5.9	9.3	-

**Table 4.** Continued.

Sl. No.	Bt segregant	Strength (g/tex)	Mic (µg/inch)	Uniformity index (%)	Elongation (%)	S/L	% over c for SY	% over F <sub>1</sub> for SY	% over F1 for SY
1	F <sub>2</sub> -III-8	25.9	3.2	83.8	5.5	0.9	56.6	33.738602	16.556
2	F <sub>2</sub> -III-89	25.6	3.4	84.5	5.5	0.9	40.6	20.06079	4.6358
3	F <sub>2</sub> -III-82	27.3	4.1	87.6	6.2	0.9	22.8	4.8632219	-8.6093
4	F <sub>2</sub> -III-19	25.6	3.4	84.5	5.5	0.9	17.4	0.3039514	-12.583
5	F <sub>2</sub> -III-62	27.1	3.9	85.2	5.5	1	14.4	-2.279635	-14.834
6	ARBH-813	29.7	4.5	83	6.9	1			
	(C)(Mean)								
7	F <sub>1</sub>	-	-	-	-	-	-	-	-
8	P1	-	-	-	-	-	-	-	-
9	P2	-	-	-	-	-	-	-	-

71.7% and 14.40 to 56.60%, respectively. When compared to Bt parent, UASD Cry1Ac Bt cotton Event No.78, Population-I recorded three segregants that recorded yield advantage ranging from 8.80 to 56.4 % followed by crosses DHS-121 × Event No.78 and DHS-108 × Event No.78 which recorded six and two transgressive segregants with yield advantage ranging from 2.4 to 30.0 % and 4.60 to 16.5 %, respectively (Tables 2-4). Similar results were reported by Kumar *et al.* (2018) where they observed top 20 seed cotton yield transgressive segregants from all three segregation generations (F<sub>2</sub>, B1 and B2), recorded yield advantage ranging from 104.02 to 169.46% over BCS 23-18-7 (116.9 g). Similarly Ramesh *et al.* (2017) recovered 21.72 to 44.72% yield advantage and 44.18% GOT advantage from RILs derived from DCH 32, interspecific hybrid.

In the three F<sub>2</sub> populations, 15 (37.9 % - 40 %), 15 (38.1 % - 40 %) and 5 (38.2 % - 40.1 %) plants

recorded high ginning outturn over its corresponding female parents DHS-114 (36.2 %), DHS-121 (37.1 %) and DHS-108 (36.5 %) and its male parent Event No. 78 (37.5 %), respectively.

In the present study highest number of Bt transgressive segregants for yield related traits were observed in the F<sub>2</sub> populations derived from DHS-114 and DHS-121 as female parents indicating that these two inbred lines combine well with male parent Event No. 78 and potential Bt genotypes were recovered when crosses are made using these two lines with Event No. 78. Out of these segregants, F<sub>2</sub>-I-55 of the cross recorded highest seed cotton yield of 115 g/plant. Out of the how many identified Bt segregants, 25 segregants recorded S/L Ratio of 1.0 and the segregant F<sub>2</sub>-I-55 recorded highest UHML value of 28.9 mm, uniformity ratio of 88.8%, S/L ratio of 1.0 and seed cotton yield of 115 g/plant.

Thirty six Bt transgressive segregants were observed across three F<sub>2</sub> populations for seed cotton yield ranging from 56 g to 115 g reporting superiority over both commercial checks Sahana (51 g) and ARBH 813 (56.2 g). The segregants also exhibited superiority for number of bolls per plant and boll weight with the range of from 11 to 29 and 2.88 to 6.6 g, respectively. A similar work by Nandhini *et al.* (2019) revealed 175 transgressive segregants in RILs of cross MCU 5 × TCH 1218 for seed cotton yield ranging from 85.5 g to 149.36 g, compared to its superior check MCU 5 (80.59 g).

### Transgressive segregants for group of important traits

The transgressive segregants common for important traits like plant height, number of bolls per plant, average boll weight and seed cotton yield in the three segregating populations were identified and are listed in the Table 5. The segregant No. 58 and 3 were found to be the most promising transgressive segregant in the cross DHS – 114 × Event No.78 for seed cotton yield giving 16.6 and 15.4% higher seed cotton yield, respectively. In addition to this it had higher intensity of expression than the increasing parent for plant height, number of sympodia per plant, number bolls per plant and average boll weight. The transgressive segregant No. 6 and 97 were found to be most promising in Cross DHS – 121 × Event No.78. They out yielded the increasing Bt parent by 28.8 and 8.7 %, respectively. In addition higher intensity of expression than the better parent was noticed for plant height, number of sympodia per plant, number of bolls per

plant and average boll weight.

In Cross DHS-108 × Event No.78, the transgressive segregant was No. 89, which out yielded the increasing parent by 3.8%. In addition to this, it expressed higher intensity for number of sympodia per plant, number of bolls per plant and halo length than the increasing parent. Similar work by Anusha *et al.* (2018) who reported the transgressive segregants which out yielded the superior parent by 82.2, 79.7 and 82.8 % in three different F<sub>2</sub> populations of desi cotton, respectively.

From the study it was evident that when the desired intensity of a character is not available in the parents, transgressive segregation concept can be utilized to extend the limit of character expression. Genetic recombination between both linked and unlinked alleles decides the success in obtaining the desired transgressive segregants. It is therefore, concluded that the concept, transgressive segregation, is effective for extending the limit of character expression when the plant breeder is interested in isolating the rare genotypes. The study on identification of transgressive segregants revealed that in early segregating F<sub>2</sub> population, the phenotypic expression for the trait of interest goes beyond one or both the parents. Hence it was concluded that, transgressive breeding can be successfully used to extend the intensity of expression of character by accumulation of favorable alleles, when the desired intensity of a character is not available in the parents by hybridization. The potential Bt transgressive segregants identified for yield and yield attributing traits from this study need to be evaluated

**Table 5.** Promising transgressive segregants (TS) having combinations of desirable attributes. \*Intensity of expression of the character higher than the Bt parent.

Sl. No.	Days to 50% flowering	Plant height (cm)	Number of monopodia per plant	Number of Sympodia per plant	Number of bolls per plant	Average boll weight (g)	Seed cotton yield (g/plant)	% over P2 for SY
DHS – 114 × UASD Cry1Ac Bt cotton Event No.78								
F <sub>2</sub> - I- 58	133	44*	0	13	18	5.2*	86.9*	16.6
F <sub>2</sub> - I- 3	120	36*	4	8	19	5.5*	86*	15.4
DHS – 121 × UASD Cry1Ac Bt cotton Event No.78								
F <sub>2</sub> - II- 6	107	64*	3	16	25*	4.1	96*	28.8
F <sub>2</sub> - II- 97	110	66*	1	15	20*	4.6	81*	8.7
DHS – 108 × UASD Cry1Ac Bt cotton Event No.78								
F <sub>2</sub> -III- 89	127	45*	1	15	21*	4.0	73.2*	3.8



further, for consistency in their performance for further generations to develop Bt varieties.

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