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A-ONE: HIGH YIELDING, CLCV TOLERANT AND TRANSGENIC BT. COTTON VARIETY FOR IRRIGATED AREAS OF PAKISTAN

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ABSTRACT

Allah Din One (A-One) has been bred through hybridization of a local commercial cotton variety FVH-53 and an exotic variety SL-223 containing Cry1 Ac Gene MON-531 event. Cross was attempted during 2001 under green house conditions at Allah Din Research Centre, Bahaudin Zakariya University, Multan. The strain was bulked during early 2005 in F7th generation. All developmental work of A-One had been completed before promulgation of National Bio-Safety rules, 2005. After completion of all Micro, Varietals and Zonal Varietals trials, A-One was tested for two consecutive years 2010-12 for various preliminary yield, disease, lint characters and confirmation of Bt Gene by Distinctness, Uniformity and Stability test, Punjab Seed Corporation yield test and National Coordinated Bt. Trial (NCBT). On the bases of over all good performance, A-One was approved by Provincial Seed Council, Punjab and registered by FSC & RD, Islamabad for general cultivation in 2011-12. The optimum sowing time of A-One is 1st May to 15th May and 1st week of March for early sowing. The development of A-One strengthened the much needed gene pool for Bt Cotton in the country.

Key words: Bt cotton, Variety development, high yielding

INTRODUCTION

Cotton is a pivotal ingredient of the economic growth of Pakistan contributing 11% of the GDP, having 9.3 billion US dollar shares in export earnings contributing towards two third of country's export earnings (Anonymous, 2012). Though cotton is mainly grown for its fiber (80%), nonetheless, cotton seed is also an important food source for livestock and human beings. It's through cotton culture that is sustaining lives of millions of people, daily wage people and numerous businessmen running small and medium based industries, who are greatly influenced by fluctuations in yield and growth of this crop that in turn affects country's exports, (Ilyas *et al*. 2010).

Cotton is the leading fiber crop not only in Pakistan but also in the world. It is threatened by various *biotic* stresses during its life cycle. Bollworms like *Helicoverpa armigera, Earias insulana* and *Pectinophora gossypiella*, are the pests causing huge damages to this crops causing almost 30 to 40 percent reduction in yield (Haque, 1991) as well as shrinkage of 20-60 percent in market value of yarn (Verma, 1999). Each year a substantial amount of foreign exchange of Rs.10 billion incurred to import chemicals to control different pests in Pakistan and of that 80% is used for cotton (Mohyuddin *et al.* 1997). The unchecked use of pesticides for increased yield and cotton quality negatively influenced farmers not only by developing pest resistance against most of pesticides but also posed greater health hazards to rural community and the environment.

Although, Pakistan's textile sector is enjoying the production of fine quality cotton for various value added products, but, this segment is not free from administrative and environmental challenges. Use of excessive amount of chemicals for protecting cotton from various insect pests and diseases, earmarked greater hazards to environment and human health directly exposed to these chemicals. The production and promotion of GM cotton has been the added advantage of maneuvering the use of excess chemicals having insect resistant qualities besides fine quality cotton production (Ilyas *et al*. 2010).

Bt cotton truly backed by the modern biotechnology wherein one or more foreign genes extracted from the soil borne bacterium *Bacillus thuringiensis* (Bt). Bt introduced by having a mechanism of built-in toxins to kill some of the most injurious pests of cotton. Only few chemical sprays are also needed to check population of crops's secondary pests. To be honest, Bt is not the total cure from problems being faced by the current cotton germplasm, but to satisfied extent, it serves the farmers to reduce certain insect pests. Bt was developed by the US renowned company, Monsanto, to made available the first GM crop technologies for commercial use in the mid 1990's (Gandhi and Namboodiri, 2006), after that a significant increase in the area of transgenic crops has been witnessed. Bt is currently grown in a large number of countries, including US (80%), India (70%), Argentina (40%), Indonesia and

Pakistan (70%) **(**Reddy, *et.al*, 2006). The added advantages of GM cotton to resist target organism having no adverse effects on human health and environment as well as assuring handsome premiums to farmers. A study of Bt Cotton with three levels of Bt toxin in different morphological traits with lint yield revealed significant differences in bolls per plant and lint percentage (Feiyu *et al*, 2012). Arshad *et al*, 2009, showed the significant results of Bt over non Bt cotton.

The comparative advantage of Bt cotton containing Cry 1Ac gene for increased income, better product and assuring safety to environment, increased area under this crop. There is need to increase gene pool of this technology for earning substantial foreign exchange and to compete the world in fineness of the produce and value added advantage. The objective of the present study was to create useful genetic variability for developing Bt variety that promises to help enhance productivity of cotton across the country. A-One is a Bollgard I variety of Bt seed which has been approved for general cultivation.

MATERIALS AND METHODS

A-One has been bred through the hybridization of a local commercial cotton variety FVH-53 and an exotic variety SL-223 during 2001 at Allah Din Research Centre, BZU, Multan. Selection and screening procedure continued from F1 to F6 generation. Additional generation obtained every year using green house. This variety developed through pedigree method of breeding involving diversified parents having contrasting genetic make up for various traits. The strain was bulked during early 2005 in F7 generation. The chronological development of A-One is explained in Table. 1.

Table. 1. Chronological development of A-One.

Years	Parentage/Pedigree	Generations	Remarks
2001-02	Cross attempted (FVH-53 × SL-223)	-	Green house/Field
2002-05	Single plant selections from different Families	F1 to F6	Green house/Field
2005-06	AGC-331	F7	Bulk & MVT
2006-07	-do-	-	VT
2007-08	-do-	-	VT & Zonal VT
2008-09	-do-	-	VT & Zonal VT
2009-10	-do-	-	1.25 Acre Trial at PSC, Khanewal
2010-11	-do-	-	NCBT (1st year)
2011-12	-do-	-	NCBT (2nd year)
Feb, 2012	The Punjab Seed Council (PSC) approved A-one for cultivation in Punjab province of Pakistan.		

A-One was extensively evaluated in Micro Varietal Trials (MVT) & Varietal Trials (VT) conducted at Allah Din Research Centre, BZU, Multan and farmers fields as compared with standard cotton varieties, MNH-786 and CIM-496. The trials were laid out in a randomized complete block design having plot size 3.5m X 10m with three replications. At growers fields the variety was tested in zonal varietal trials at 27 different locations in the districts of Multan, Khanewal, Vehari, D.G. Khan, Bahawalpur, Khanpur and Rahimyar Khan. The standard plant protection measures to control the attack of sucking pests and bollworms were adopted throughout the cotton crop season in all experiments. Its performance was tested on PSC farm, Khanewal and in NCBT for two years. The data for various morphological characteristics and fibre properties were recorded and means were compared using Fisher's protected least significant difference (LSD) procedure.

RESULTS AND DISCUSSION

Yield: Various yield trials viz-a-viz varietal trials (micro, variety, zonal) were conducted to evaluate performance of A-One in comparison with standard check varieties of cotton. In MVT and VT with the standard cotton varieties on the basis of four years average, A-One gave higher yield of seed cotton (3028 kg/ha) compared with 2026 kg/ha of MNH-786 and 1730 kg/ha of CIM-496 (Table-2). Regarding Zonal Varietal Trials (ZVT) for two years (2007-09) having 43 un-replicated trials conducted at Allah Din Research Centre and progressive growers in different ecological zones. The averaged data presented in Table-3 showed that A-One yielded the highest (2950 kg/ha) followed by MNH-786 (1783 kg/ha).

Ginning Out Turn (GOT %): The data of ginning out turn (%) of MVT and VT of A-One on the basis of four years testing (Table 4) showed that A-One had 38.5% ginning out turn compared with 37.5% of MNH-786. Similarly, in ZVT A-One had higher GOT % (38.41) followed by 36.92% of MNH-786 (Table 5).

Staple Length (mm): Measurement of staple length during MVT and VT conducted for four years (Table 6) showed that A-One (27.6 mm) was better than one of the standard MNH-786 (26.3 mm). A perusal of summarized data of forty three ZVT conducted during two years

presented in Table 7, again confirmed that A-One had better staple length (27.4 mm) than MNH-786 (26.3 mm).

Micronaire Value and Fiber Strength: The data of Micronaire value and Fiber strength (Average of four years) presented in Table 8 showed that micronaire of A-One had the best value i.e., 4.55 followed by CIM-496 (4.79) & MNH-786 (5.02). Fiber strength showed that A-One (96.5 tppsi) is better than MNH-786 (94.7 tppsi).

Plant Characteristics: The data of plant characters i.e., plant height, number of monopodial branches per plant, number of sympodial branches per plant and maximum boll weight (Table 9) showed two year average wherein A-One had plant height (159.4 cm), number of monopodial branches (1.65), number of sympodial branches (22) and maximum boll weight (4.4 g).

Insect Pests and Disease Infestation: The data (Table 10) showed that A-One had low number of insects per leaf for sucking insects. Moreover, it also showed least percentage damage by bollworms. Pathological studies on A-One relating to cotton leaf curl virus were conducted at Allah Din Research Centre, BZU, Multan. The data of virus incidence (Table 11) showed that A-One proved to be highly tolerant to this disease during four years compared with CIM-240 (Virus susceptible variety).

PSC and NCBT Testing: The results of higher yielding ability of A-one planted at PSC Farm, Khanewal, in 2009-10 are presented in Table 12. A-One significantly out yielded (gave 22% higher yield the standard variety) IR-3701. In NCBT testing, A-On**e** produced higher yield in three sites in 2010-11 and seven sites in 2011-12 from out of ten sites of Pakistan. Lint characters of A-One had better micronaire and staple length than standard while other fibre characters are comparable to other varieties. The results of the A-One obtained from progressive growers fields illustrated higher yield potential and gave significant higher mean yield (Table 3). Moreover, confirmation of Bt. Gene Cry1 Ac event (MON-531) presented in NCBT test (Table 15). The above data shows that A-One was approved by Provincial Seed Council, Punjab and registered by FSC&RD, Islamabad for general cultivation in 2011-12.

Table. 2. Yield performance of A-One in Micro Varietal (MVT) and Varietal Trails (VT) from 2005-09

Year	Name of Trial	Location	Yield of Seed Cotton (Kg/ha)			LSD
			A-One	MNH-786	CIM-496	
2005-06	**MVT**	Multan	3106 a	2158 b	-	7.46
	VT	Multan	3726 a	3272 b	2647 c	5.95
		Khanewal	3242 a	2612 b	1031c	4.26
		Vehari	3136 a	2505 b	1551 c	4.41
		Multan	2597 a	1716 b	1070 c	432.9
		Mailsi	4031 a	3402 b	2003 c	4.51
		Jahanian	4318 a	3567 b	2512 c	5.28
		Dunyanpur	4063 a	2792 b	1630 c	4.56
		Average	**3588**	**2838**	**1906**	
2007-08	**VT**	Multan	2456 a	1581 b	1102 c	4.1
		Khanewal	2946 a	2084 b	1603 c	6.211
		Vehari	2245 a	590 b	335 c	6.42
		Mailsi	3666 a	2208 b	2014 c	5.28
		Jahanian	2790 a	1011 b	533 c	5.69
		Dunyanpur	2518 a	1548 b	940 c	5.28
		Average	**2770**	**1504**	**1088**	
		2 years Av.	**3179**	**2171**	**1497**	
2008-09	**VT**	Multan	3232 a	2141 b	2309 c	4.98
		Khanewal	2842 a	1996 b	1739 c	4.41
		Vehari	2192 a	1292 b	1353 c	4.15
		Bahawalpur	2324 a	987 b	1518 c	4.15
		Average	**2648**	**1604**	**1730**	
		4 years Av.	**3028**	**2026**	**1730**	

Table 3. Summary of two years yield performance of A-One in Zonal Varietal Trails during 2007-08 & 2008-09.

Year	No. of Trials	Average Yield of Seed Cotton (Kg/ha)		
		A-One	MNH-786	CIM-496
2007-08	27	3233	1916	1308
2008-09	16	2666	1650	1932
Average	**43**	**2950**	**1783**	**1620**

Table 4: Table 4. Ginning Out Turn % of A-One in Micro and Varietal Trails from 2005-09

Year	Name of Trial	Location	Ginning Out Turn %			
			A-One	MNH-786	CIM-496	LSD
2005-06	**MVT**	Multan	38.7 a	37.9 b	37.6 b	0.299
2006-07	**VT**	Multan	38.6 a	37.7 b	37.5 b	0.3263
		Khanewal	38.5 a	37.6 b	37.1 c	0.515
		Vehari	38.7 a	37.5 b	36.4 c	0.346
		Multan	38.3 a	37.8 a	36.8 b	0.4939
		Mailsi	38.9 a	37.1 b	37.6 c	0.2579
		Jahanian	38.6 a	36.9 b	37.4 b	0.313
		Dunyanpur	38.9 a	37.3 b	36.8 b	0.419
		Average	**38.5**	**37.4**	**37.1**	
2007-08	**VT**	Multan	38.4 a	37.5 b	36.5 c	0.219
		Khanewal	38.6 a	37.1 b	36.9 b	0.372
		Vehari	38.1 a	36.9 b	37.1 b	0.416
		Mailsi	37.9 a	37.2 b	37.2 b	0.491
		Jahanian	39.7 a	37.2 b	36.8 b	0.673
		Dunyanpur	38.2 a	37.5 a	36.7 b	0.412
		Average	**38.5**	**37.2**	**36.9**	
		2 years Av.	**38.5**	**37.3**	**36.9**	
2008-09	**VT**	Multan	38.8 a	37.6 b	37.1 b	0.341
		Khanewal	38.4 a	37.5 b	36.8 c	0.31
		Vehari	38.5 a	37.2 b	36.7 c	0.398
		Bahawalpur	38.1 a	37 b	36.7 b	0.258
		Average	**38.4**	**37.3**	**36.8**	
		4 years Av.	**38.5**	**37.5**	**37.1**	

Table. 5. Summary of two years Ginning Out Turn % of A-One in Zonal Varietal Trails during 2007-08 & 2008-09.

Year	No. of Trials	Average Ginning Out Turn %		
		A-One	MNH-786	CIM-496
2007-08	27	38.40	37.20	35.60
2008-09	16	38.42	36.64	36.15
Average	**43**	**38.41**	**36.92**	**35.88**

Table. 6. Staple Length of A-One in Micro and Varietal Trails from 2005-09

Year	Name of Trial	Location	Staple Length (mm)			
			A-One	MNH-786	CIM-496	LSD
2005-06	**MVT**	Multan	28.1 b	26.9 c	29.7 a	0.345
2006-07	**VT**	Multan	27.8 b	26.4 c	29.4 a	0.294
		Khanewal	27.6 b	26.8 c	29.3 a	0.458
		Vehari	27.9 b	26.8 c	29.2 a	0.491
		Multan	27.4 b	26.2 c	28.6 a	0.298
		Mailsi	27.7 b	27.1 b	29.5 a	0.451
		Jahanian	28.1	26.3	28.9	0.423
		Dunyanpur	27.9 b	26.3 c	29.4 a	0.311
		Average	27.8	26.5	29.2	
2007-08	**VT**	Multan	27.5 a	25.6 b	27.7 a	0.456
		Khanewal	26.6 b	25.6 b	29.7 a	0.71
		Vehari	27.1 b	26.7 c	28.7 a	0.34
		Mailsi	27.3 b	26.2 c	29.5 a	0.371
		Jahanian	27.8 b	25.5 c	29.6 a	0.324
		Dunyanpur	26.9 b	26.2 c	29.7 a	0.211
		Average	27.2	25.9	29.2	
		2 years Av.	27.5	26.3	29.2	
2008-09	**VT**	Multan	27.4 b	26.4 c	29.6 a	0.42
		Khanewal	27.9 b	26.3 c	29.2 a	0.35
		Vehari	26.5 b	25.4 c	28.6 a	0.342
		Bahawalpur	27.1 b	25.7c	28.9 a	0.234
		Average	27.2	25.9	29.1	
		4 years Av.	27.6	26.3	29.3	

Table. 7. Summary of two years Staple Length of A-One in Zonal Varietal Trails during 2007-08 & 2008-09.

Year	No. of Trials	Average Staple Length (mm)		
		A-One	MNH-786	CIM-496
2007-08	27	27.3	26.2	29.1
2008-09	16	27.5	26.4	29.2
Average	**43**	27.4	26.3	29.15

Table. 8. Micronaire value & staple strength of A-One in Micro and Varietal Trails from 2005-09.

Year	Name of Experiment	Micronaire Value			Fibre strength (tppsi)		
		A-One	MNH-786	CIM-496	A-One	MNH-786	CIM-496
2005-06	MVT	4.48	4.98	4.84	96.4	94.6	97.3
2006-07	VT	4.52	4.97	4.81	96.3	94.7	97.5
2007-08	VT	4.58	5.07	4.78	96.7	94.8	97.7
2008-09	VT	4.50	4.99	4.8	96.5	94.2	97.4
2 year average		**4.52**	**5.00**	**4.81**	**96.5**	**94.5**	**97.5**
4 year average		**4.55**	**5.02**	**4.79**	**96.5**	**94.7**	**97.6**

Table 9: Plant Characters of A-One During 2007-09.

Plant Character	Average of Varietal trials 2007-8 & 2008-9			
	A-One	MNH-786	CIM-496	LSD
Plant Height (cm)	159.4 (a)	161.45 (a)	135.55 (b)	2.24
No. of Monopodial Branches per Plant	1.65 (a)	1.6 (a)	2.25 (a)	0.5919
No. of Sympodial Branches per Plant	22 (a)	21.2 (a)	19.9 (a)	2.42
Maximum Boll weight (g)	4.4 (a)	4.35 (a)	3.65 (b)	0.216

Table 10. Pest population on A-One under un-aprayed conditions.

Variety	Year	Jassid	Whitefly	Thrips	Bollworm damage (%)
		Number of insects per leaf			
	2006	0.7	2.5	2	0.25
	2007	3	0.35	1.1	0.3
A-One	2008	0.91	8.45	7.91	0.4
	2009	0.8	3.25	5.6	0.35
	Average	**1.35 (c)**	**3.64 (c)**	**4.15 (c)**	**0.33 (c)**
	2006	1.5	7.5	7.5	8.5
	2007	3.93	0.4	0.18	7.98
MNH-786	2008	2.59	11.49	3.09	1.67
	2009	2.24	4.5	9.6	10.8
	Average	**2.24 (a)**	**5.97 (a)**	**5.09 (b)**	**7.24 (a)**
	2006	1.25	5.75	7.5	7.58
	2007	2.99	0.64	1.08	6.56
CIM-496	2008	1.53	0.97	4.4	3.18
	2009	0.75	7.5	10.75	10
	Average	**1.63 (b)**	**3.72 (b)**	**5.93 (a)**	**6.83 (b)**
LSD		0.083	0.037	0.0581	0.0346

Table 11. Screening of A-One against Cotton Leaf Curl Virus at Allah Din Research Centre, BZU. Multan.

Year	Varieties	
	Virus Infestation (%)	
	A-One	CIM-240
2006	2.4 (d)	10.74 (a)
2007	12.2 (c	87.5 (c
2008	15.6 (b)	93 (a)
2009	17.6 (a)	89 (b)
LSD	1.41	1.65

Table 12: Final yield results of Bt. Cotton varieties per acre Trials at Punjab Seed Corporation Seed Farm, Khanawal, (2009-10).

S. No.	Variety	Yield/Acre
1	MNH-886	1159 (a)
2	CRS-2007	767.71 (i)
3	ASR-703	721.67 (k)
4	ASR-802	857.44 (g)
5	NS-131	1137.9 (b)
6	NS-121	784.95 (i)
7	KZ-181	1120.4 (c
8	KZ-182	926.77 (f)
9	CIM-496	361.21 (0)
10	SITARA-008	591.31 (n)
11	**A-ONE**	960.95 (e)
12	FH-113	715.33 (k)
13	FH-114	802.56 (h)
14	IB-BWR	1040.4 (d)
15	MG-6	720.44 (k)
16	**IR-3701 Std.**	740.83 (j)
17	IR-1524	414.39 (o)
18	IR-2381	647.67 (l)
19	CEMB-I	860.1 (g)
20	CEMB-II	625.15 (m)
	LSD	**15.31**

Table 13. Performance of Bt. Strains in National Coordinated Bt. Trial by Pakistan Central Cotton Committee, 2010-11 (10 sites of Pakistan).

Strains	Seed cotton (Kg/ha)	Lint %	Staple length (mm)	Micronaire value (ug/inch)	Fibre strength (tppsi)	Plant Pop./ha
A-One	1811 e	37.5 g	27.8 b	5 abc	98.2 de	39991 e
NS-131	2829 a	41.5 d	26.4 c	5 abc	103.2 a	42502 a
NS-141	2044 d	42.8 c	25.5 d	4.7 c	102.1 b	41426 b
KZ-181	2197 c	40.4 f	25.4 d	5.6 a	101 c	40798 cd
KZ-191	1704 f	41.5 d	25.5 d	5.6 a	101.1 c	40619 d
Sitara-09	1626 g	40.2 f	25.7 d	5.4 ab	100.7 c	39633 e
CBS-2	2448 b	43.3 b	25.5 d	4.8 bc	98.8 d	42861 a
CBS-1	1982 d	40.8 e	28.6 a	4.5 c	97.8 e	41067 bcd
Tarzan-1	1775 e	40.4 f	28.4 a	4.6 c	93.7 f	39902 e
IR-3701 (Std)	**2466 e**	**44.6 a**	**24.7 e**	**5.5 a**	**94 f**	**41336 bc**
LSD	**65.17**	**0.3186**	**0.3613**	**0.635**	**0.843**	**581.69**

Table 14. Performance of Bt. Strains in National Coordinated Bt. Trial by Pakistan Central Cotton Committee, 2011-12 (10 sites of Pakistan).

Strains	Seed cotton (Kg/ha)	Lint %	Staple length (mm)	Micronaire value (ug/inch)	Fibre strength (tppsi)	Plant Pop./ha
A-One	2403 ef	39.1 g	27.6 a	5 c	97.3 d	42233 b
NS-131	2530 cd	42.6 b	27.6 a	4.6 e	101.3 a	43219 a
NS-141	2660 b	43.3 a	26.1 d	5 c	99.7 c	42143 bc
KZ-181	2409 ef	43.4 a	25.8 e	5.4 ab	94.2 e	41336 e
KZ-191	2582 bc	41.9 d	26.5 c	4.9 cd	93.3 f	41067 f
Sitara-09	2469 de	41.4 e	25.8 e	5.3 b	100.6 b	41605 d
CBS-2	2608 bc	41.2 e	26.3 cd	4.7 de	91.9 h	41964 c
CBS-1	2290 g	39.6 f	27.3 b	4.6 e	92.6 g	41336 e
Tarzan-1	2822 a	43.5 a	27.1 b	5 c	91.6 i	39364 g
IR-3701 (Std)	**2352 fg**	**42.2 c**	**25.1 f**	**5.6 a**	**99.7 c**	**41516 de**
	94.04	0.2799	0.2468	0.2693	0.269	234.16

Table 15. Performance of Bt. Strains for confirmation of "Cry1Ac" event (MON-531) by National Coordinated Bt. Trial by Pakistan Central Cotton Committee, 2011-12.

Strains	PCR Test	ELISA Test	Purity Test (Immunostrip %)
A-One	+	1.093 b	80 c
NS-131	+	0.932 d	100 a
NS-141	+	1.062 c	80 c
KZ-181	+	0.461 g	70 d
KZ-191	+	1.049 c	80 c
Sitara-09	+	0.807 e	40 e
CBS-2	+	1.191 a	90 b
CBS-1	+	0.581 f	80 c
Tarzan-1	+	0.804 e	80 c
IR-3701 (Std)	**+**	**0.92 d**	**70 d**
LSD		0.0171	4.87

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