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Research Article

MNH-1050: A *Bt*., CLCuV tolerant cotton variety evolved by interspecific hybridization

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Abstract

Cotton Leaf Curl Virus (CLCuV) is serious danger in excellent cotton cultivation in Pakistan. Due to the lack of CLCuV tolerance in upland cotton, the most economical and lasting approach to grab this serious matter is to introgress resistance in upland cotton from wild species. Desi Cotton (*Gossypium arboreum* L.) has resistance against CLCuV. An upland cotton cultivar MNH-1050 (*Bt*.) has been evolved by interspecific hybridization for CLCuV tolerance at Cotton Research Institute (CRI), Multan, Pakistan. It was developed through introgression [BC_5F_6 (*Gossypium hirsutum* L. × *Gossypium arboreum* L.) × MNH-886] × MNH-988 during 2008-2009 at CRI Multan. To manage boll shedding, a combination of gibberellic acid (50 mg/l) and naphthalene acetic (100 mg/l) acid was applied. The variety consistently outperformed than the standard varieties in National Coordinated Varietal Trial (NCVT) Punjab during two consecutive years 2020 and 2021. It produced 8.85 % and 29.7 % higher yield than standard varieties CIM-602 and CYTO-124 respectively in NCVT during 2019-2020. It proved as the more tolerant strain against CLCuV, with 62% and 65% lesser CLCuV incidence than that of standard variety IUB-13 in Preliminary Yield Trial (PYT) and Advance Yield Trial (AYT) during 2018-2019 at CRI Multan. It had relatively lesser attack of Jassid, pink boll worm and complete resistance against other boll worms i.e., army worm, spotted boll worm, american boll worm. MNH-1050 was approved as single gene *Bt.* cotton variety with CLCuV tolerance, heat tolerant, early maturity, low input efficient, specific pest resistance, high yield potential and most adapted for wheat-cotton cropping rotation. It has fluffy opening with ease in picking, high Ginning Out Turn GOT% (40.5%) with refine fiber characters i.e., staple length (28.35 mm), good fiber fineness (4.58 µg/inch) and fiber strength (29.11 g/tex). It fulfills various purposes of industry. It is most suitable for cultivation on marginal land and water deficient areas.

Key words: Upland Cotton (*Gossypium hirsutum* L.), Desi Cotton (*Gossypium arboreum* L.), Interspecific Hybridization, Cotton Leaf Curl Virus (CLCuV), National Coordinated Varietal Trial (NCVT).

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Introduction

Upland Cotton (*G. hirsutum L.*) is a principal fiber crop across the world playing a remarkable role in reducing unemployment pressure, stabilizing national and international economy and providing a huge source of raw material to textile industry [1]. Cotton production significantly fell by 41% to stand at 4.91 million bales compared to 8.33 million bales previous year due to CLCuV, scarcity of water, rise in temperature, insect pest attack, especially Pink bollworm, Whitefly and Thrips [2]. Bollworms of Cotton are seriously destructing crop requiring massive use of chemical pesticide that is imposing severe danger to soil and water health, wellbeing of human, hygenic foodstuffs, marine life and biological control agents in nearly all cotton producing areas of the globe [3, 4]. *Bt.* cotton has been genetically engineered to contain genes of soil bacterium *Bacillus thuringiensis* (*Bt.*) to produce immunity in plants against specific insect pests i.e., cotton bollworms. Adoption of this technology presents to less application of chemical pesticide, less crop destruction, ultimately elevated yields of cotton and entailing health and environmental benefits [5].

As far as global warming is concerned, the frequency and severity of heat waves is increasing in Pakistan's major cotton-growing regions posing a serious natural disaster that influence cotton production. In Pakistan, the average temperature within cotton region prevails 37/25°C that is higher than in other cotton producing areas of the globe [6]. The temperature persists high 40-50°C during earlier growth periods of cotton from May-June, reducing cotton seedling growth, delayed development or even death and increased sowing costs to some extent [7]. The cotton crop is sensitive to heat stress during all the developmental stages but specifically sensitive to reproductive stage. High temperature results in shedding of flowers at the flowering stage and reduces boll weight and ultimately yield [6]. Heat stress adversely affects the morphological, physiological metabolism, quality and ultimately yield of cotton. High Temperature increases the active oxygen content in cotton seedling leaves, activating antioxidant metabolism and increasing its related enzyme activity i.e., ascorbate peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) [7]. These antioxidants detoxify the reactive oxygen species (ROS) in cotton leaves to prevent cellular damage [8]. High Temperature also negatively affects the phenotypic indexes of cotton roots at the seedling stage [9]. Therefore, sustainable approach to manage heat stress is the development of heat tolerant and early maturing cotton cultivars for high quality and yield under heat stress [10].

Among other biotic and abiotic factors, CLCuV is the main threat affecting the production of cotton for decades. White fly (*Bemisia tabici*) is the vector for CLCuV disease that is directly injected by white fly. Yield losses due to CLCuV ranges from 10% to 80%. Infection of CLCuV remarkably alters cotton plant morphological characters including reduction in plant height, bolls count per plant, boll weight, fiber length, strength, and elongation, impacting generally quality

of fiber and ultimately market price [11].

Cotton is backbone of Pakistan's economy, but due to CLCuV its production is lesser than other countries in the world [11]. Cotton Research Institute, Multan, located in the cotton belt of Pakistan, has been played an important role in cotton production of Punjab. It owes the credit of developing quality cotton, high yielding, high ginning out-turn and long staple varieties like MNH- 93, MNH-129, S-12, S-14, MNH-147, MNH-329, CRSM-38, MNH-786 and MNH-886 (*Bt*.), which showed worth performance in the farmer's field. Although all the available approved cultivars have tolerance against CLCuV, nevertheless, the ultimate strategy is to introgress immunity against CLCuV from wild species i.e., *G. arboreum* L.to *G. hirsutum* L. [12].

Numerous researchers performed interspecific hybridization and transferred immune genes for favorable characters from wild diploid species into tetraploid upland cotton e.g., CLCuV tolerant CIM-608 variety was evolved from interspecific hybridization of 2(*G. hirsutum* L. × *G. anomalum* L.) × *3G. hirsutum* [13]. Immune genes for cotton rust spreaded by *Puccinia cacabata* were transfered from *G. anomalum* L. and *G. arboreum* L. into *G. hirsutum* L. by interspecific hybridization. Polyploidy was induced followed by back crossing and continuous screening for immunity [14]. It is valuable to merge the genes for CLCuV, diseases and drought resistance between *G. hirsutum* L. and *G. arboreum* L. [15]. Interspecific hybridization between *G. hirsutum* L. and *G. arboreum* L. was promisingly obtained [16]. In the same way, immune gene B6 from 'A' genome of *G. arboreum* L. for bacterial blight spreaded by *Xanthomonas malvacearum* was transferred into *G. barbadense* L [17 - 18]. Similarly, strategy of transgressive segregation was used to develop a resistant commercial variety 'Auburn 56'against Root knot nematode (*Meloidogyne incognita*) in U.S. Immunity against *Rotylenchulus reniformis* was transfered into the tetraploid 2(AD1) and a tetraploid triple specie hybrid was obtained by crossing a resistant diploid A2-genome *G. arboreum* L. accession (A2-190) with a hexaploid 2[(AD1) D4] bridging line (G 371), [19].

The objective of this research was to transfer tolerance against CLCuV and heat from Desi cotton (*G. arboreum* L. 2n=26) into cultivated upland *Bt.* cotton (*G. hirsutum* L. 2n = 52) genotypes following conventional breeding techniques.

Materials and Methods

G. arboreum is also known as desi cotton and is a diploid (2n=26) (A genome) species of cotton that exhibits natural resistance to heat, CLCuV and related diseases. Whereas *G. hirsutum* L. is known as upland cotton, a tetraploid (2n=52) (AD genome) species of cotton and is susceptible to CLCuV and other abiotic stresses. To transfer immunity against CLCuV from diploid specie into *G. hirsutum* background, *G. hirsutum* L. as a female (recipient) parent was crossed with *G. arboreum* as a male (donor) parent. Process for evolving immune material against CLCuV is as under:

A cross between *G. hirsutum L.* (2n=52) (AD)1 and *G. arboreum L.* (2n=26) A2 was attempted. Seedling dip (embryo rescue technique) method was adopted to induce chromosome doubling by treating the resultant triploid hybrid with 0.5% aqueous solution of colchicine for 72 hours. To get a stable tetraploid, the resultant hexaploid was then crossed with *G. hirsutum* to produce a pentaploid that was back crossed four times with *G. hirsutum* L. (2n=52) (AD)1.

Virus tolerance against CLCuV was examined in green house through petiole grafting method of the synthesized product

with virus infected petioles of CIM-496 (A higly CLCuV susceptible line). Later on the selected tolerant plants were shifted to field for assessment of their tolerance against CLCuV under field conditions. Plant expression against CLCuV was recorded in the greenhouse as well as under field conditions.

The selected tolerant plants against CLCuV were subjected to further selection by progeny to row trials. Consequently, numerous strains were evolved possessing good CLCuV and heat tolerance.

Parentage/Pedigree

The first approved and novel CLCuV-tolerant and *Bt*. cotton cultivar MNH-1050 at CRI Multan was developed by interspecific hybridization method i.e., BC_5 F_6 (*G. hirsutum* L. × *G. arboreum* L.) × MNH-886 × MNH-988 in 2008-2009 at CRI Multan . By continuous effort it has been derived and its yield and other parameters are improved through back cross, pedigree and gene pyrimiding technique of breeding using diversified parents possessing contrasting genetic make up for CLCuV, heat, high yield and good quality characters. The elite strain was tested in bulk during 2017-2018 and further evaluated for yield comparison and adaptability in PYT, AYT and NCVT trials. Table 1 describes the chronological development of the interspecific variety MNH-1050 (*Bt*.).

Parent age: [BC_5 F_6 (*G .hirsutum* L. × *G .arboreum* L.) × MNH-886] × MNH-988.

Table 1: Chronological development of MNH-1050 (*Bt*.).

Year	**Pedigree**	**Generation**	**Remarks**
	[BC_5 F_6 (VH-148 × 15 mollsoni) × MNH-886] × MNH-988	Cross attempted	Field Experiment
2008-2009	VH-148 × 15 mollisoni	F_1	Field experiment (Normal sowing)
2009-2010	F_1 × MNH-886	BC_1	Single plant selection on the basis of CLCuV tolerance, early maturity and superior yield and fiber quality
2010-2011	BC_1 × MNH-886	BC_2	Single plant selection on the basis of CLCuV tolerance, early maturity and superior yield and fiber quality
2011-2012	BC_2 × MNH-886	BC_3	Single plant selection on the basis of CLCuV tolerance, early maturity and superior yield and fiber quality
2012-2013	BC_3 × MNH-886	BC_4	Families' selection on the basis of CLCuV tolerance, early maturity and superior yield and fiber quality
2013-2014	BC_4 × MNH-988	BC_5F_6	Families' selection on the basis of CLCuV tolerance, early maturity and superior yield and fiber quality
2014-2015	BC_5F_6	BC_5F_7	Bulk testing and single plant, selection from bulk on the basis of CLCuV tolerance, early maturity and superior yield and fiber quality

Year	Pedigree	Generation	Remarks
2015-2016	BC_5F_7	BC_5F_8	Bulk testing and single plant selection from bulk on the basis of CLCuV tolerance, early maturity and superior yield and fiber quality
2016-2017	BC_5F_8	BC_5F_9	Superior single plants selection from bulk on the basis of CLCuV tolerance, early maturity and superior yield and fiber quality
2017-2018	CRI-150	• Preliminary Yield Trials (PYT) • Advanced Yield Trials (AYT) • NBT (National Bio softy trails) • Agronomic trials (sowing date, spacing, fertilizer trials)	
2018-2019	CRI-150	• Preliminary Yield Trials (PYT) • Advanced Yield Trials (AYT) • NBT (National Bio softy trails) • Agronomic trials (sowing date, spacing, fertilizer trials)	
2019-2020	MNH-1050	• Provincial Coordinated Cotton Trial (PCCT). • National Coordinated Varietal Trial (NCVT) • DUS test	
2020-2021	MNH-1050	• Provincial Coordinated Cotton Trial (PCCT) • National Coordinated Varietal Trial (NCVT) • DUS test • 1.25 Acres block at PSC Khanewal for (spot examination).	

Results

Yield performance trials

For yield potential evaluation, MNH-1050 was screened with commercial variety MNH-886 during 2017-2018 and 2018-2019 at Cotton Research Institute, Multan under yield performance trials. MNH-1050 produced outstandingly more yield 3624 and 4025 $kgha^{-1}$ in PYT-3 and in AYT-4 more yield 3855 and 3640 during 2017-2018 and 2018-2019 respectively than standard as shown in Table 2.

National coordinated varietal trials (NCVT)

NCVT 2019-2020

For yield potential evaluation, MNH-1050 was assessed at 7 distinct locations in Punjab under National Coordinated Varietal Trials during 2019-2020 and 2020-2021. It gave 8.85 % and 29.7 % higher yield than that of check varieties CIM-602 and CYTO-124, respectively, during 2019-20 written in Table 3. Seed cotton yield (1408 $kgha^{-1}$) of MNH-1050 was 1.95 % greater than that of 1381 $kgha^{-1}$ of check CIM-602 during 2020-21, as provided in Table 4.

Table 2: Yield ($kgha^{-1}$) performance of MNH-1050 in preliminary yield trials (PYT) and advance yield trials (AYT) at CRI Multan during 2018 and 2019.

Trial	Variety	2017-2018	2018-2019
PYT-3	MNH-1050	3624	4025
	MNH-886	2840	2245
AYT-4	MNH-1050	3855	3640
	MNH-886	2145	2249

Table 3: Comparative yield (kgha^{-1}) performance of MNH-1050 with check CIM-602 and CYTO-124 in NCVT during 2019-2020.

Institute/Station	Variety		Increase/ Decrease (%)	CYTO-124 (Check-2)	Increase/ Decrease (%)
	MNH-1050	CIM-602 (Check-1)			
CCRI Multan	1526	1682	-9.27	1541	-0.97
Neelum seeds Mul.	2487	2410	3.91	1736	43.26
CRS Bahawalpur	1277	1162	9.89	990	28.89
CRS Sahiwal	2305	3024	-23.77	1965	17.30
CRS Khanpur	2338	849	175.38	1427	63.84
CRS Vehari	649	751	-13.6	188	245.2
NIBGE Fsd.	2176	1751	24.27	1914	13.69
Punjab (Avg.)	1808	1661	8.85	1394	29.7

Table 4: Comparative yield (kg ha^{-1}) performance of MNH-1050 with check CIM-602 in NCVT during 2020-2021.

Institute/Station	Variety		Increase/ Decrease (%)
	MNH-1050	CIM-602 (Check)	
CCRI Multan	505	859	-41.2
CRI Multan	419	622	-32.6
CRS Bahawalpur	1790	1181	51.57
CRS Sahiwal	943	1010	-6.63
CRS Khanpur	2256	2574	-12.35
CRS Vehari	1815	1292	40.48
NIAB Fsd.	2129	2129	0.0
Punjab (Avg.)	1408	1381	1.95

The candidate cotton variety MNH-1050 occupied 67th position among 99 different varieties (Annexure 2, 3 and 4). It also occupied 22nd and 16th positions out of 27 varieties in its respective NCVT set-D and set-C, respectively, during 2019 and 2020, as attached in Annexure 2 and 3.

Bt. protein testing

Bt. protein of MNH-1050 was tested against CIM-602 in four labs, which are ABRI (FSD), CEMB (LHR), NIGAB (ISBD) and NIBGE (FSD). The *Bt.* protein concentration of MNH-1050 ranged from 1.72-2.252 µg/gram of fresh weight of leaf, compared to that of CIM-602, ranging from 1.08-2.465 µgm/gm values provided in Table 5 and Annexure 5.

Table 5: *Bt.* Protein quantification in leaf of MNH-1050 through ELISA after 80 days of sowing µgm/gm of fresh weight.

Character	MNH-1050			CIM-602		
	ABRI Fsd., NIGAB Isbd., NIBGE Fsd. (Δ of 3 Labs)	CEMB	Avg.	ABRI Fsd., NIGAB Isbd., NIBGE Fsd. (Δ of 3 Labs)	CEMB	Avg.
Event	MON-531	MON-531	-	MON-531	MON-531	-
Gene	Cry1Ac	Cry1Ac	-	Cry1Ac	Cry1Ac	-
Purity (%)	100	100	100	100	100	100
Protein concentration (µgm/gm)	1.72	2.252	1.986	1.08	2.465	1.77

CLCuV infestation

We compared CLCuV infestation on MNH-1050 with IUB-13 (check) in PYT and AYT at CRI Multan during 2018 and 2019. MNH-1050 had 62% and 65% lower CLCuV incidence than that of IUB-13 in PYTs and AYTs during 2017-2018 and 2018-2019, respectively expressed in Table 6.

Table 6: Comparison of CLCuV infestation on MNH-1050 with IUB-13 (check) in preliminary yield trials (PYT) and advance yield trials (AYT) at CRI Multan during 2018 and 2019.

Year	Trials/ Location	CLCuV (%)		Inc. /Dec. (%)
		MNH-1050	IUB-13	Over Check
2017-2018	PYT-3	15	40	62
2018-2019	AYT-4	12	35	65

Spot examination report of MNH-1050, 23. 10. 2020

The line is very good, highly tolerant to CLCuV and drought-tolerant, medium to early maturing, with a bigger boll size than normal and normal leaf shape, medium compact fruiting, good bearing, very good and fluffy opening, has uniform maturity and creamy pollen. Plant shape is semi spreading semi compact moderately resistant against sucking insect pest complex and spotted bollworm of cotton.

Fiber quality characteristics

MNH-1050 possesses a harmonious combination of fiber traits, i.e., GOT = 40.5%, staple length = 28.35 mm, fiber fineness = 4.58 µgm/inch and fiber strength = 29.11 gm/tex (Annexure 6).

Morphological characters

MNH-1050 was evaluated for morphological characters during two consecutive years in NCVT. The observed characters include plant height (140-200 cm), monopodial branches per plant (1-4), sympodia branches per plant (38-45), bolls per plant (60-70) and boll weight (4.0-4.5 gm) Annexure 1. .

Entomological studies

Entomological studies on MNH-1050 were conducted at Cotton Research Institute, Multan to assess its tolerance level against sucking pests (Jassid, whitefly, thrips), red cotton bug (RCB), dusky cotton bug (DCB) and bollworms (ABW, SBW and PBW) attack as compared to commercial varieties MNH-1027, 1035, 1045 and IUB-13 during 2017-18 and 2018-19. The new variety MNH-1050 had relatively lesser attack of Jassid and complete resistance against boll worms (ABW, SBW, American BW) except Pink boll worm whose attack is little more as compare to others (Table 7 and 8).

Table 7: Comparative response of MNH-1050 against sucking and bollworm infestation at CRI Multan 2017-2018.

Sr. No.	Variety	Yield (kg/ha)	PH (cm)	CLCuV (%)	White Fly	Jassid	Thrips	AW	SBW	American BW	PBW (%)
1	MNH-1027	2538	115	23	7.6	1.35	8.5	0	0	0	19
2	MNH-1035	2940	165	20	9.8	1.80	8.9	0	0	0	21
3	MNH-1045	2801	185	15	5.5	1.70	7.5	0	0	0	7
4	MNH-1050	2650	190	20	9.4	1.20	7.6	0	0	0	25
5	IUB-13	2390	152	30	13.2	1.2	7.5	0	0	0	35

Table 8: Comparative response of MNH-1050 against sucking and bollworm infestation at CRI Multan 2018-2019.

Sr. No.	Variety	Yield (kg/ha)	PH (cm)	CLCuV (%)	White Fly	Jassid	Thrips	AW	SBW	American BW	PBW (%)
1	MNH-1027	2938	120	16	9.05	1.35	7.4	0	0	0	23
2	MNH-1035	3194	175	18	10.51	1.50	6.4	0	0	0	25
3	MNH-1045	3301	185	20	6.07	1.41	6.0	0	0	0	10
4	MNH-1050	2850	192	22	13.04	1.24	6.1	0	0	0	27
5	IUB-13	2790	152	28	16.21	1.46	5.8	0	0	0	40

Agronomic studies

Plant spacing trial

During 2017-2018 and 2018-2019 MNH-1050 and IUB-13 were screened at different plant distances to determine distance response of MNH-1050 at CRI Multan. MNH-1050 observed higher seed cotton yield of 2521, 2535 kg ha^{-1} when planted on 18 and 12 inches plant to plant distance during 2018-2019 and 2019-2020 respectively shown in Table 10.

Fertilizer studies

During 2017-2018 and 2018-2019 MNH-1050 and IUB-13 were screened at different rates of fertilizer application to determine fertilizer response of MNH-1050 at CRI, Multan. MNH-1050 observed higher seed cotton yield 2175 and 2643 kg ha^{-1} with the use of 114 kg hai^{-1} of fertilizer during 2018-2019 and 2019-2020 respectively in Table 9.

Sowing date trial

During 2017-2018 and 2018-2019 MNH-1050 and IUB-13 were screened at different sowing dates with 15 days' interval from 1st April to 16th June to see the effect of time of sowing on productivity of advanced genotypes. MNH-1050 observed higher seed cotton yield of 4126, 3022 kg ha^{-1} when planted on 16th April during 2018-2019 and 2019-2020 respectively in Table 11.

Table 9: Field trial to determine fertilizer response of MNH-1050 conducted at CRI Multan.

Sr. No.				Yield (kgha^{-1})			
	N (kg/ha)	P (kg/ha)	K (kg/ha)	MNH-1050		IUB-13	
				2017-2018	2018-2019	2017-2018	2018-2019
1	114	0	0	1832	2389	992	1943
2	114	57	0	2175	2643	1088	1699
3	198	57	94	1859	2482	1148	2161
4	250	57	94	1566	2576	1195	1810
5	306	57	94	1698	2599	1291	1591
Mean				1826	2537	1142	1840

Table 10: Plant spacing trial to determine distance response of MNH-1050 conducted at CRI Multan.

Sr. No.	Spacing	Yield (kg ha^{-1})			
		MNH-1050		IUB-13	
		2017-2018	2018-2019	2017-2018	2018-2019
1	9"	2081	2353	1626	2029
2	12"	1937	2535	1530	1997
3	15"	2420	1984	1686	1918
4	18"	2521	1838	1489	1820
Mean		2239	2177	1582	1941

Table 11: Sowing date trial to determine response of MNH-1050 conducted at CRI Multan.

Sr.No.	Sowing Dates	Yield (kg ha^{-1})			
		MNH-1050		IUB-13	
		2017-2018	2018-2019	2017-2018	2018-2019
1	01-April	3336	2884	2931	2548
2	16-April	4126	3022	2225	2459
3	01-May	3157	2523	1465	1366
4	16-May	1471	1161	750	630
5	01-June	968	685	426	396
6	16-June	538	360	412	367
Mean		2266	1772	1368	1261

Discussion

MNH-1050 was approved as interspecific, *Bt*., CLCuV tolerant, heat tolerant, early maturing and low input efficient variety with dynamic yield potential on the basis of overall performance of yield trials, NCVT and technology tests from well-known bio-labs. MNH-1050 expressed tolerance for sucking pests and has improved fiber traits with favorable GOT percentage. Research connected to production technology for MNH-1050 investigated that different levels of planting distances, fertilizers and cultural practices were adopted to synchronize the maximum yield potential of variety and its feedback to inputs is specific to adaptability. Cotton Research Institute, Multan is acknowledged to present various auspicious varieties i.e., MNH- 93, MNH-129, S-12, S-14, MNH-147, MNH-329, CRSM-38, MNH-786 and MNH-886 (*Bt*.), which showed worth performance in the farmer's field.

G. hirsutum L. has narrow genetic diversity and deficient in resistance against CLCuV whereas its wild diploid species possesses capability for resistance against various calamities i.e., insect, pests, diseases and numerous abiotic stresses. Therefore, there is urgent necessity for utilization of this source for evolving tolerance against CLCuV in cultivated tetraploid species [12]. The main cause restricts the success of interspecific hybridization in cotton is deficit in retaining crossed bolls. Tremendous efforts had made to achieve hybrids in results of interspecific hybridization for decades [20], however it was realized that it is futile to achieve hybrids under *in situ* conditions owing to numerous incompatibility elements e.g., hybrid unviability, hybrid breakdown and self-incompatibility hurdles [11]. Results revealed that ovule growth was unsuccessful between the cross *G. hirsutum* L. × *G. arboreum* L. on account of deranged effect of hybrid embryos on endosperm growth, whereas anomalous mitosis in endosperm nuclei and inappropriate embryo differentiation were the key reason in reciprocal cross [21 - 22]. Similarly, in upland cotton accessions, indirect effect of embryo on endosperm was noticed as compared to desi cotton [23]. In this study, interspecific hybridization of cotton was facilitated with use of exogenous hormone following pollination. The plant hormones accelerate growth of pollen tube [24]. The exogenous hormone application solely can control specific crossing hurdles within *Gossypium* [25 - 26]. In this study the Gibberellic acid was applied as growth regulator to attain interspecific hybrids between *G. hirsutum* L. and *G. arboreum* L. species of cotton [27]. Although, countless introgressed hybrids were evolved through ovule and embryo culture in *Gossypium* [28, 29, 20, 30, 31, 32], nevertheless traditional breeding techniques were discovered to be better to further propagate such introgressed material [33].

The introgressed, evolved *Bt*. variety

MNH-1050 has a massive capability to defeat the CLCuV disease. This developed hybrid from *G. hirsutum* L. and *G. arborium* L. expressed strong tolerance against CLCuV disclosing that biotic stress like disease can be introgressed in *G. hirsutum* L. from *G. arborium* L. using backcrossing, gibberellic acid and naphthalene acetic acid. It is also explored that the auto tetraploids can be utilized to transfer desirable traits in *G. hirsutum* L. from *G. arborium* L. and more sustainable tolerance against CLCuV can be developed in the accessible cotton germplasm through conventional breeding techniques.

Conclusion

Cotton Leaf Curl Virus is major threat for cotton crop. Due to lack of CLCuV tolerant genotypes in upland cotton, the sole solution is to introgress this tolerance in upland cotton from its wild relatives. A highly promising *Bt*. variety MNH-1050 of Cotton Research Institute CRI, Multan has been developed by introgression between *G. hirsutum* L and *G. arborium* L. It is tolerant to CLCuV disease and early maturing possesses higher degree of heat tolerance. Its harmonious combinations of fiber traits under low input will stabilize and increase cotton production and boost up the economy of the country.

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Annexure 1

Results of NCVT Trial 2019-2020 in Punjab, Khyber P K, Sindh and Balochistan (Seed-Cotton yield kg/ha).

Pakistan Central Cotton Committee (PCCC), Multan

Directorate of Agriculture Research

2019- NCVT Field Trial Results

Annexure-IV: Average Seed-Cotton/Phutti Yield (kg/hectare) of Set-D at Provinces and Country Levels (Descending Order on Pakistan Basis)

Sr.	Code	Denomination	PUNJAB								SINDH					BALOCHISTAN				KPK	*PAKISTAN Average
			CCRI	NEELUM	CRS	CRS	J.SC	CRS	NIBGE	Punjab	CCRI	CRS	TASSCO	SAU	Sindh	CRS	CRS	ARI	Balochistan	CRS	
			Multan	Multan	B.pur	Sahiwal	R.Y.Khan	Vehari	F.abad	Average	Sakrand	Ghotki	T. A.YAR	T.Jam	Average	Lasbella	Sibbi	Khuzdar	Average	D.I. Khan	
1	PC-1988	Bt-CIM-775	3059	3458	1621	3402	2043	1551	1827	2423	2507	1752	2479	2587	2331	3910	2132	3942	3328	2552	2588
2	PC-2000	Sahara-Klean-5	2087	1840	1980	3288	2748	1344	1869	2165	3275	1875	3190	2515	2714	3378	2777	3356	3170	2102	2508
3	PC-1980	CEMB-Klean-Cotton-4	1623	3233	1119	3477	2292	1351	1451	2078	2761	2124	2585	2716	2547	3492	2032	3480	3001	2268	2400
4	PC-1981	CEMB-Klean-Cotton-3	1756	3290	1205	3666	2023	973	2216	2161	2662	1911	2493	2839	2476	2541	3029	2621	2730	2144	2358
5	PC-1979	CEMB-Klean-Cotton-5	1810	2980	1176	3515	2495	1229	1455	2094	2440	2043	2529	2406	2355	3456	2029	3547	3011	2188	2353
6	PC-1978	CEMB-Klean-Cotton-6	1442	3611	1147	3364	2199	1220	1778	2109	2311	2046	2341	2814	2378	3007	2320	3062	2796	2102	2318
7	PC-2001	Sahara-300	1668	2026	1693	3364	2265	1217	2243	2068	2720	2016	2754	1991	2370	2840	2923	2860	2874	2008	2306
8	PC-1996	NIA-88	2013	2478	1147	2494	2678	615	2414	1977	3117	965	2402	2544	2257	3480	2735	3530	3248	1908	2301
9	PC-1990	Bt-CIM-789	1992	3405	961	2910	1239	1266	2127	1986	2151	1889	1918	2149	2027	3486	2863	3476	3275	2441	2285
10	PC-1985	Bt-Cyto-535	1593	2439	1478	3402	1684	1260	1873	1961	2378	2311	2145	1454	2072	3468	1846	3543	2952	2583	2230
11	PC-1986	Bt-Cyto-533	1598	2975	1621	2759	2386	716	2048	2015	2014	1864	2391	1765	2009	2750	2972	2784	2835	2731	2225
12	PC-1983	CRIS-673	2112	3061	1205	2986	2101	1338	1834	2091	2318	1847	2429	1948	2136	2804	2057	2852	2571	2148	2203
13	PC-1989	Bt-Cyto-511	2187	3324	1421	3099	1228	1656	1576	2070	2232	1692	2054	1380	1840	2595	3119	2815	2843	2335	2181
14	PC-1984	CRIS-671	1914	2865	1248	3364	1583	714	1931	1946	1945	2395	2052	2453	2211	2768	1948	2929	2548	2331	2163
15	PC-1987	Bt-CIM-785	1575	2989	975	2835	1614	774	2049	1830	1691	1808	2057	1437	1748	3510	2840	3524	3291	2573	2150
16	PC-1991	Bt-CIM-678	3024	2607	975	2683	896	588	1606	1768	2672	1730	2060	1319	1945	3767	1931	3745	3148	2378	2132
17	PC-1977	MNH-1035	1998	3205	1190	2797	2405	1365	1276	2034	2433	1631	1401	1176	1660	2816	2962	2822	2867	2115	2106
18	PC-1998	CRIS-644	1651	2731	1004	3137	1356	534	1812	1746	2504	1355	2702	2231	2198	2678	2975	2752	2802	1851	2085
19	PC-1995	NIAB-929	2122	2468	1033	3288	2187	1118	2598	2116	2097	1160	1743	1464	1616	2696	2434	2669	2600	2082	2077
20	PC-2002	MZM-7	1585	2676	1421	3515	1906	769	1793	1952	1961	1619	1932	1424	1734	2661	2876	2765	2767	1971	2058
21	PC-1982	CRIS-638	1757	2814	1234	3402	1953	522	1757	1920	2262	2030	1094	1195	1645	2714	3066	2848	2876	2151	2053
22	PC-1976	MNH-1050	1526	2487	1277	2305	2238	649	2176	1808	2266	2108	2033	1721	2032	2571	2736	2585	2631	1930	2041
23	PC-1992	Bt-CIM-303	2041	2578	1076	2872	896	1437	1639	1791	1200	1800	1630	1420	1513	3300	1734	3334	2789	2419	1958
24	**PC-1993**	**CIM-602 (Bt-Standard)**	**1682**	**2410**	**1162**	**3024**	**849**	**751**	**1751**	**1661**	**1982**	**1046**	**2640**	**2126**	**1949**	**2702**	**1814**	**2742**	**2419**	**1647**	**1889**
25	PC-1999	Cyto-226	2081	2319	1750	2305	1719	399	1927	1786	1355	1898	1931	1533	1679	1847	2835	1928	2203	2425	1883
26	PC-1997	PC-1997	1775	2291	1162	2608	1840	837	1386	1700	1253	1032	1306	808	1100	1871	2936	1962	2256	2090	1677
27	**PC-1994**	**Cyto-124 (Non-Bt Standard)**	**1541**	**1736**	**990**	**1965**	**1427**	**188**	**1914**	**1394**	**1237**	**1549**	**1792**	**1497**	**1519**	**1877**	**2139**	**2010**	**2009**	**1890**	**1583**
		Average:	**1897**	**2752**	**1269**	**3031**	**1861**	**977**	**1864**	**1950**	**2213**	**1759**	**2151**	**1886**	**2002**	**2925**	**2521**	**2981**	**2809**	**2199**	**2152**
Statistical Analysis Results																					
1		CD 5%	77	636	144	297	400	489	149	'''	694	217	454	718	'''	607	205	500	'''	199	'''
2		CD 1%	103	848	192	396	533	650	199	'''	925	290	605	954	'''	810	273	666	'''	265	'''
3		Significance	**	**	**	**	**	**	**	'''	**	**	**	**	'''	**	**	**	'''	**	'''
4		CV %	2	4	6	6	13	16	4	'''	19	7	12	12	'''	12	4	10	'''	5	'''

Notes:

1. * "Pakistan-Average" has been worked out on the basis averages of all fifteen (15) locations for more accuracy.
2. ** NCVT "Field trials results should be associated with Fiber-Testing and Biochemical-Testing results of reference labs.
3. Errors and ommissions are accepted. Detailed Final results shall be released in due course.

20th July 2020

(Dr.Tassawar Hussain Malik)
Director Research

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Annexure 2

Results of NCVT Trial 2020-2021 in Punjab, Khyber P K, Sindh and Balochistan. (Seed-Cotton yield kg/ha).

Pakistan Central Cotton Committee (PCCC), Multan

Directorate of Agriculture Research

2020- NCVT Provisional Field Trial Results in Punjab & KPK

Annexure-III: Average Seed-Cotton/Phutti Yield (kg/hectare) of Set-C

Sr.	Code	Denomination	PUNJAB								KPK
			CCRI	CRI	CRS	CRS	CRI	CRS	NIAB	Punjab	CRS
			Multan	Multan	B.pur	Sahiwal	Khanpur	Vehari	F.abad	Average	D.I. Khan
1	2	3	4	5	6	7	8	9	10	11	12
1	PC-2035	Rustam-SP	661	383	907	1545	1701	501	2487	1169	1867
2	PC-2036	BH-224	817	347	943	972	1898	611	1938	1075	2078
3	PC-2037	Saim-102	870	1040	1790	1180	2579	1114	3205	1683	2112
4	PC-2038	NIAB-SANAB-M	1404	1220	1921	1283	3326	1579	2392	1875	1675
5	PC-2039	Sahara-300	1374	849	1539	923	1849	1270	3420	1603	1891
6	PC-2040	IR-NIBGE-15	719	371	1098	1938	2805	849	2033	1402	1921
7	PC-2041	PC-2041									
8	PC-2042	Sahara-Klean-5	577	514	1241	846	2589	1365	2607	1391	1897
9	PC-2043	NIAB-989	651	741	1360	1517	2558	1238	3157	1603	2171
10	PC-2044	Ghauri-2	282	163	418	966	1387	152	1124	642	2171
11	PC-2045	IR-NIBGE-16	872	419	1337	1887	2150	905	2153	1389	2220
12	PC-2046	Tara-CII-222	863	801	1444	1064	2301	2137	2727	1620	1920
13	PC-2047	Cyto-536	1433	1064	1157	1302	2552	1778	1938	1604	2631
14	PC-2048	ASPL-710	1141	909	1194	1094	2370	1693	2511	1559	1988
15	PC-2049	FH-492	1353	945	1647	1081	2462	1524	2129	1592	2121
16	PC-2050	MNH-1050	505	419	1790	943	2256	1815	2129	1408	2410
17	PC-2051	PC-2051									
18	PC-2052	GH-Sultan	1421	873	1050	1385	2798	1851	2201	1654	2500
19	PC-2053	Tahafuz-15	744	562	1361	947	2378	1301	2272	1366	2007
20	PC-2054	Tara-CKC-333	666	586	919	883	2236	1740	1937	1281	1968
21	PC-2055	PB-93	496	454	1157	1068	2565	1139	2248	1304	2018
22	PC-2056	Rawal-222	1169	885	1563	1371	2666	1412	2153	1603	1977
23	PC-2057	MNH-1086	1028	443	1396	902	1906	1249	1937	1266	2141
24	PC-2058	Ghauri-3	601	347	1540	1032	2698	638	2081	1277	2033
25	PC-2059	SLH-Chandi	786	861	1802	921	2799	1453	2416	1577	2064
26	PC-2060	WEAL-AG-10	1016	1040	1623	1771	2915	1670	2727	1823	1966
27	PC-2061	CIM-602	859	622	1181	1010	2574	1292	2129	1381	2103
		Average:	892	674	1335	1193	2413	1291	2322	1446	2074
Statistical Analysis Results											
	1	CD 5%	111.5	204.6	379.2	171.1	756.1	382.1	670.7	``	130.4
	2	CD 1%	148.5	272.6	505.3	227.9	1007.5	509.1	893.6	``	173.7
	3	Significance	**	**	**	**	**	**	**	``	**
	4	CV %	8	19	17	9	19	18	17	``	4
Notes:		1	NCVT "Field trials results should be associated with Fiber-Testing and Biochemical-Testing results of reference labs.								
		2	Errors and ommissions are accepted.								
		3	Results are preliminary and Final set of results will be released in due course with maximum information.								

(Dr.Tassawar Hussain Malik)

Director Research Headquarters

Updated: 12-4-2021 Page-3/17

Annexure 3

Yield performance of the candidate variety MNH-1050 at PSC, Khanewal During 2020.

PUNJAB SEED CORPORATION SEED FARMS, KHANEWAL.
FINAL PICKING REPORT OF SPOT EXAMINATION COTTON TRIAL SOWN AT PSC SEED FARM 81-82/10 R, KHANEWAL DURING KHARIF-2020

S NO	VARIETY	AREA IN ACRES	PRODUCE (KGS)	Y.P.A (Mds)
1	CIM-343	0.083	20	6.02
2	CIM-663	0.083	15	4.52
3	CIM-678	0.083	9	2.71
4	CIM-785	0.083	11	3.31
5	CIM-775	0.083	14	4.22
6	CIM-303	0.083	18	5.42
7	CYTO-511	0.083	21	6.33
8	CYTO-533	0.083	9	2.71
9	CYTO-535	0.083	7	2.11
10	NS-211	0.083	13	3.92
11	FH-492	0.083	20	6.02
12	FH-ANMOL	0.083	23	6.93
13	GHAURI-2	0.083	15	4.52
14	EAGLE-4	0.083	19	5.72
15	ASPL-709	0.083	24	7.23
16	ASPL-710	0.083	32	9.64
17	ROHI-1	0.083	13	3.92
18	ROHI-2	0.083	21	6.33
19	CEMB-100	0.083	15	4.52
20	CKC 5	0.083	20	6.02
21	CKC 6	0.083	17	5.12
22	EYE-20	0.083	22	6.63
23	RUSTAM-11	0.083	17	5.12
24	SUN CROP-3	0.083	16	4.82
25	TAHAFUZ-15	0.083	19	5.72
26	CH SUNCROP	0.083	39	11.75
27	CH TAHAFUZ 12	0.083	15	4.52
28	DIAMOND-2	0.083	22	6.63
29	BS-15	0.083	28	8.43
30	MNH-1086	0.083	40	12.05
31	MNH-1050	0.083	39	11.75
32	YAQEEN 7	0.083	42	12.65
33	ICI-2121	0.083	27	8.13
34	YBG-2222	0.083	26	7.83
35	YBG-2323	0.083	15	4.52
36	TAHAFUZ-10	0.083	41	12.35
37	BAKHTAWER-1	0.083	30	9.04
38	RH-AFNAN II	0.083	35	10.54
39	IR-NIBGE-14	0.083	22	6.63
40	IR-NIBGE-15	0.083	26	7.83
41	SAHARA KLEAN-5	0.083	49	14.76
42	SAHARA-300	0.083	64	19.28
43	SAIM-102	0.083	53	15.96
44	THAKAR-22	0.083	47	14.16
45	CKC THAKAR-76	0.083	34	10.24
46	GS ALI-II (NON BT)	0.083	15	4.52
47	CYTO-226 (NON BT)	0.083	32	9.64
48	MNH-1086	0.083	39	11.75
49	NIAB-SANAB-M	0.083	44	13.25
50	NIAB-512	0.083	41	12.35
51	NIAB-135	0.083	61	18.37
52	NIAB-898	0.083	26	7.83
53	BH-221	0.083	39	11.75
54	BH-224	0.083	12	3.61
55	WEAL AG 9	0.083	21	6.33
56	WEAL AG 10	0.083	37	11.14
57	WEAL AG CKC 301	0.083	21	6.33
58	WEAL AG 201 (H)	0.083	17	5.12
59	SAYBAN-209	0.083	46	13.86
	Total:-	4.90	1575	8.04

Annexure 4

NCVT Bt test results from reference labs during 2020.

2020 NCVT - BCT Test Results from Reference Labs

Annexure-VII: Test-3: Testing Results for CP4-EPSPS Gene (Event MON-1445) RR Technology

Sr.	Codes	Denomination	Bt/Non-Bt	Claim (Gene/ Event/Technology)	1- NIBGE-Faisalabad			3- ABRI-Faisalabad			Average of 3 Labs			Remarks
					Trait Confirmation (+/-) PCR	Trait Purity (%)	Quantification ELISA (µg/g)	Trait Confirmation (+/-) PCR	Trait Purity (%)	Quantification ELISA (µg/g)	Trait Confirmation (+/-) PCR	Trait Purity (%)	Quantification ELISA (µg/g)	
1	2	3	4	5	6	7	8	12	13	14	15	16	17	18
45	PC-2045	IR-NIBGE-16	Bt	Cry1Ac/MON-531/BG-I	-	0	NT	-	0	1.48	-	0	1.48	
46	PC-2046	Tara-CII-222	Bt	Cry1Ac+Cry2A/C-II	-	0	NT	-	0	2.60	-	0	2.6	
47	PC-2047	Cyto-536	Bt	Cry1Ac/MON-531/BG-I	-	0	NT	-	0	3.34	-	0	3.34	
48	PC-2048	ASPL-710	Bt	Cry1Ac/MON-531/BG-I	-	0	NT	-	0	2.52	-	0	2.52	
49	PC-2049	FH-492	Bt	Cry1Ac/MON-531/BG-I	-	0	NT	+	60	2.87	(+/-)	30	2.87	
50	PC-2050	MNH-1050	Bt	Cry1Ac/MON-531/BG-I	-	0	NT	-	0	1.72	-	0	1.72	
51	PC-2051	PC-2051												
52	PC-2052	GH-Sultan	Bt	Cry1Ac/MON-531/BG-I	-	0	NT	-	0	2.62	-	0	2.62	
53	PC-2053	Tahafuz-15	Bt	Cry1Ac+Cry2A+GTG/C-III/CKC	+	100	NT	-	100	3.44		100	3.44	????
54	PC-2054	Tara-CKC-333	Bt	Cry1Ac+Cry2A+GTG/C-III/CKC	+	100	NT	+	100	2.30	+	100	2.3	????
55	PC-2055	PB-93	Bt	Cry1Ac/MON-531/BG-I	-	0	NT	-	0	1.76	-	0	1.76	
56	PC-2056	Rawal-222	Bt	Cry1Ac/MON-531/BG-I	-	0	NT	-	0	1.55	-	0	1.55	
57	PC-2057	MNH-1086	Bt	Cry1Ac/MON-531/BG-I	-	0	NT	-	0	0.39	-	0	0.39	
58	PC-2058	Ghauri-3	Bt	Cry1Ac+Cry2A+GTG/C-III/CKC	+	100	NT	+	80	5.30	+	90	5.3	????
59	PC-2059	SLH-Chandi	Bt	Cry1Ac/MON-531/BG-I	-	0	NT	-	0	5.79	-	0	5.79	
60	PC-2060	WEAL-AG-10	Bt	Cry1Ac/MON-531/BG-I	-	0	NT	-	0	1.68	-	0	1.68	
61	PC-2061	CIM-602	Bt	Cry1Ac/MON-531/BG-I	-	0	NT	-	0	1.08	-	0	1.08	
62	PC-2062	CKC-6	Bt	Cry1Ac+Cry2A+GTG/C-III/CKC	+	90	NT	+	100	2.88	+	95	2.88	
63	PC-2063	Suncrop-3	Bt	Cry1Ac+Cry2A+GTG/C-III/CKC	+	100	NT	+	100	1.38	+	100	1.38	????

Testing Results for Cry1Ac Gene (C-I Event), Cry1ac + Cry2A Genes (C-II Event); and Cry1Ac+Cry2A+GTG Genes (C-III Event) CKC-Technology

Sr.	Codes	Denomination	t/Non-Bt	Claim (Gene/ Event/Technology)	*BCT Test Results (CEMB)									Remarks
					Cry1Ac (C-I)			Cry2A (C-II)			ry1Ac+Cry2A+GTG(C-III/CK			
					Trait Confirmation (+/-) PCR	Trait Purity (%)	Quantification ELISA (µg/g)	Trait Confirmation (+/-) PCR	Trait Purity (%)	Quantification ELISA (µg/g)	Trait Confirmation (+/-) PCR	Trait Purity (%)	Quantification ELISA (µg/g)	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
41	PC-2041	PC-2041												
42	PC-2042	Sahara-Klean-5	Bt	Cry1Ac+Cry2A+GTG/C-III/CKC	+	100	3.192	+	100	2.432	+	100	2.332	CKC-Confirmed
43	PC-2043	NIAB-989	Bt	Cry1Ac/MON-531/BG-I	+	100	2.920	+	100	2.320	-	0	0.000	
44	PC-2044	Ghauri-2	Bt	Cry1Ac+Cry2A+GTG/C-III/CKC	+	100	2.977	+	100	2.332	+	100	2.232	CKC-Confirmed
45	PC-2045	IR-NIBGE-16	Bt	Cry1Ac/MON-531/BG-I	+	100	2.153	+	100	2.153	+	100	2.253	CKC-Confirmed
46	PC-2046	Tara-CII-222	Bt	Cry1Ac+Cry2A/C-II	+	100	3.170	-	0	0.000	-	0	0.000	
47	PC-2047	Cyto-536	Bt	Cry1Ac/MON-531/BG-I	-	0	0.000	-	0	0.000	-	0	0.000	
48	PC-2048	ASPL-710	Bt	Cry1Ac/MON-531/BG-I	+	100	2.341	+	100	2.662	-	0	0.000	
49	PC-2049	FH-492	Bt	Cry1Ac/MON-531/BG-I	+	100	2.405	+	100	2.434	-	0	0.000	
50	PC-2050	MNH-1050	Bt	Cry1Ac/MON-531/BG-I	+	100	2.252	-	0	0.000	-	0	0.000	
51	PC-2051	PC-2051												
52	PC-2052	GH-Sultan	Bt	Cry1Ac/MON-531/BG-I	+	100	3.192	-	0	0.000	-	0	0.000	
53	PC-2053	Tahafuz-15	Bt	Cry1Ac+Cry2A+GTG/C-III/CKC	+	100	3.388	+	100	2.434	+	100	3.335	CKC-Confirmed

Annexure 5

Spot examination of Bt and non Bt cotton candidate lines during 2020 at PSCF Khanewal.

Spot Examination of BT & Non BT Cotton Candidate Lines Kharif-2020 at Punjab Seed Corporation Farms, Khanewal on 23.10.2020

Fibre Quality Report (Summary)

Sr. No.	Genotype Name	GOT (%age)	Mic. (Microgram / Inch)	Staple Length (mm)	Fibre Strength (g/tex)	Fibre Uniformity Index (%age)
1.	NIAB-135	38.0	5.05	26.97	30.55	80.82
2.	Weal AG 201 (II)	40.5	5.75	26.51	30.47	82.03
3.	NIAB-898	41.0	4.71	28.84	28.56	83.80
4.	SAHARA Klean-5	40.3	4.81	27.46	27.04	81.63
5.	YBG-2222	40.7	4.56	27.8	31.42	81.86
6.	YAQEEN-7	39.6	4.98	26.0	29.23	82.05
7.	Rohi-2	39.0	4.83	29.39	29.38	82.82
8.	CII Sucrop	39.5	4.34	26.9	29.84	81.53
9.	CEMB-100	41.9	4.63	28.59	29.19	81.34
10.	Rohi-1	38.5	4.06	28.04	30.21	82.47
11.	CIM-663	40.0	4.28	28.43	29.17	81.64
12.	Eagle-4	38.6	4.65	26.67	28.27	81.99
13.	Sucrop-3	39.0	4.62	27.85	30.53	80.83
14.	ASPL-709	38.7	4.48	27.32	28.72	81.86
15.	MNH-1050	40.5	4.58	27.74	29.11	83.12
16.	BH-221	38.7	4.62	26.91	28.95	81.52
17.	GS ALI-II (Non BT)	41.3	4.00	28.69	31.12	82.80
18.	Rustam-II	40.0	5.19	24.90	27.33	80.54
19.	IUH AFNAN-II	38.5	5.11	25.33	27.23	80.44
20.	CIM-343	41.0	5.58	27.98	28.44	84.01
21.	CKC Thakar-76	40.0	4.38	29.28	30.91	82.25
22.	IR NIBGE-15	38.5	4.87	28.22	31.8	81.68
23.	NIAB SANAB-M	42.0	4.83	28.76	28.38	82.67
24.	WEAL AG-10	39.5	4.62	26.74	32.0	79.89
25	CKC-6	38.0	4.25	27.61	30.33	80.82
26.	CYTO-226 (Non BT)	40.3	4.89	29.60	28.44	82.83
27.	EYE-20	41.0	4.95	25.58	29.69	81.73
28.	YBG-2323	38.5	4.45	27.01	30.58	78.81
29.	FH-492	42.0	4.79	29.80	31.05	82.53
30.	WEAL AG CKC-301	40.0	5.09	26.51	29.41	81.68
31.	CIM-303	39.5	4.87	26.55	30.48	81.55
32.	DIAMOND-7	38.5	4.63	26.13	30.48	80.35
33.	TAHAFUZ-15	38.0	3.35	26.44	29.63	79.55
34.	CIM-375	42.3	4.84	27.08	28.58	81.67
35.	CYTO-333	41.0	3.98	28.04	27.45	83.35

36.	SAYBAN-209	38.0	4.36	26.67	28.54	81.48
37.	BS-15	39.5	4.18	25.34	29.98	79.25
38.	NS-211	40.5	4.64	26.91	28.72	81.75
39.	WEAL AG-9	41.0	4.39	27.35	30.72	81.17
40.	CYTO-535	41.5	3.97	28.41	28.85	82.31
41.	GHAURI-2	39.5	4.95	28.47	29.77	81.54
42.	FH-ANMOL	40.0	4.75	29.69	30.88	82.06
43.	CKC-5	41.0	4.76	26.71	26.97	81.16
44.	ICI-2121	39.0	4.82	26.80	31.18	82.33
45.	BH-224	38.5	5.02	28.08	28.98	82.56
46.	MNH-1086	39.0	5.01	27.36	29.39	81.33
47.	SAHARA-300	38.5	4.67	26.98	29.06	81.55
48.	TAHAFUZ-10	40.0	3.64	28.24	28.71	80.72
49.	IR NIBGE-14	38.0	3.86	27.86	33.49	80.87
50.	CII.TAHAFUZ-12	41.0	4.22	26.86	29.17	81.37
51.	BAKHTAWER-I	39.5	4.26	27.50	28.74	81.50
52.	CIM-785	40.0	4.71	28.76	31.18	83.27
53.	SAIM-102	42.0	4.34	27.07	29.24	81.40
54.	ASPL-710	38.5	4.91	25.13	28.46	80.66
55.	CYTO-511	39.0	4.76	27.63	29.95	82.17
56.	CIM-678	40.0	4.19	28.33	29.90	81.84
57.	NIAB-512	38.0	4.69	28.68	28.61	82.75
58.	THAKAR-22	38.1	4.30	26.07	30.68	80.99

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