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Testing Seed Quality of Bt Cotton

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The views expressed in this column are his own and not that of Cotton Association of India)

EXPERT's Column

Dr K.R. Kranthi

Bt Cotton

Bt cotton is genetically modified (GM) cotton variety/hybrid that has crystal (Cry) protein toxin producing genes derived from a soil bacteria called Bacillus thuringiensis (Bt). The bacterial species was first discovered by Prof Ishiwata in 1901 in Japan. Many crystal proteins produced by the bacteria are toxic as stomach poison to many species of insects. When insects feed on the toxins, they cause holes in the membrane that lines the insect stomach.

The genetically modified (GM) technology was developed first by Monsanto and released commercially in USA, Mexico and Australia in 1996. Subsequently, the technology was introduced into China (1997), South Africa (1998), Argentina (1998), India (2002), Colombia (2002), Brazil (2005), Costa-Rica (2008), Burkina Faso (2009) and recently in Pakistan and Mynamar in 2010. Thus 13 countries cultivated Bt cotton in 161 lakh hectares in 2012, which accounts for 48% of the global cotton area. Bt Cotton was introduced into India in the year 2002 and became extremely popular to the extent that about 95% of India's cotton area is under Bt cotton hybrids with almost all of the area under Monsanto's Bt technology. Incidentally, cotton is the only GM crop approved for commercial cultivation in India. Bt-cotton hybrids that express Cry1Ac, Cry2Ab, Cry1C and fusion gene (Cry1Ac) have been approved by the GEAC for commercial cultivation in India.

The GEAC approved the following GM Bt cotton events:

1. Monsanto: MON531 (Cry1Ac) event Bollgard;
2. Monsanto: Mon15985 (Cry1Ac+Cry2Ab2) event in Bollgard-II;
3. JK seeds, India: JK Event-1 (Cry1Ac);
4. Chinese Academy of Agricultural Sciences, China: GFM Cry1A (Cry1Ac), introduced by Nath seeds India;
5. NRCPB, New Delhi and UAS Dharwad, India: BNLA601 (Cry1Ac) event;
6. Metahelix, India: Event 9124 (Cry1C).

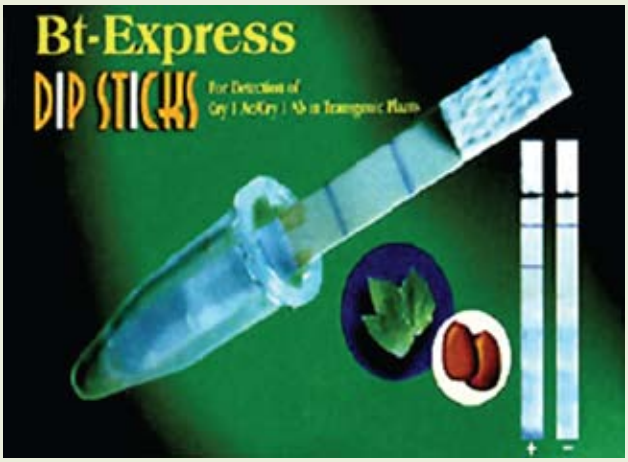
The GEAC has thus far approved the cultivation of 1128 Bt cotton hybrids for commercial cultivation in India. However, about 25 to 30 hybrids developed by 6 to 7 major companies occupy more than 70.0% of the area. It is estimated that about 85% of the current area is under Bollgard-II (Cry1Ac+Cry2Ab) and rest under Bollgard (cry1Ac) being produced and marketed by 44 Indian seed companies.

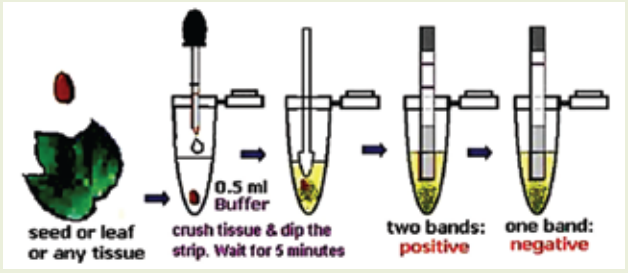
Bt testing kits developed by CICR

Bt detection kits were developed and commercialized by Central Institute for Cotton Research (CICR). Patents for the Bt detection kit were granted in the following countries vide patent numbers (Inventor: Dr K. R. Kranthi: Patents: South Africa, 2007: Patent No. 2004110268. /ZA200410268; China, 2008: Patent No. ZL 03817641.6CN1672049; Mexico, 2008: Patent No. MXPA04011769; Uzbekistan, 2008: Patent No. WO03102208 and South Korea, 2008: Patent No. KR20050026396).

CICR developed simple 'dip-stick' kits and 'ELISA' (Enzyme Linked Immuno-Sorbent Assay) to enable farmers, seed testing officers, researchers, seed companies and regulators detect Bt-seed quality and Bt-toxin expression in plants under field conditions. ELISA test kits have been developed by CICR for detection and quantitative testing of Cry1Ac, Cry2Ab and Cry1C proteins, in the test material such as seeds, leaves or other plant parts. CICR developed PCR tests to detect specific gene and events for five major events based on the data available in public domain. Event specific data was unavailable for Metahelix Event 9124 (Cry1C). CICR developed 'dip-stick-strip test kits' for instantaneous 10-min detection test at 'on-site' conditions for leaves or seeds containing Cry1Ac, Cry2Ab and Cry1C proteins. Additionally another test called "GUS-reporter test" has been developed as a 30-min easy reagent based test to detect the reporter protein/gene associated with Cry2Ab. The dip-stick kits can be used to regulate quality while the ELISA kits are used to quantify the levels of Bt-toxin expression so as to identify the time when Bt-crop shows poor expression of Bt-toxin, when farmers need to take up appropriate control measures.

The Bt-detection kits have been commercialised and became extremely popular with farmers and seed testing agencies, as evidenced by the fact that more than 40,000 kits have been used by stake holders. The Bt-detection kits enabled regulation, streamlining and ensuring Bt-cotton seed quality for farmers in the country. All seed testing laboratories in India have been using the kits and more than 6000 seed lots have been tested using the kits. Legal cases have been filed in courts of several cotton growing states of north, central and south India and are under review. In the absence of the testing kits, illegal Bt-seed would have been rampant and proliferated without any control. It has been widely acknowledged that the kits acted as deterrents for spurious seed traders. It is estimated that the cotton yield losses due to illegal seed trade and Bt-spurious-seed trade would have reached about Rs 250 crores worth each year, if the kits were not available. The kits assisted the technology developers of Bt-cotton to introduce the technology and establish it in the market to an extent of 90% coverage, that resulted in cotton yields doubling to 31.5 million bales (170 kg lint per bale) in just 5 years from a meager 16.5 million bales in 2001. The sub-standard seed samples have now decreased to 5.23% in 2007-08 as compared to 69% in 2003-04 apparently due to the constant vigil and continuous testing. One significant advantage of the simple 'dip-stick test developed by CICR, has been that it empowered farmers, extension workers and seed testing agencies with a rapid test that can be conducted 'on-the-spot' directly in shops or in fields. Therefore it served as a strong deterrent to the manufacturers and traders who would have otherwise continued to produce substandard and spurious seeds which were being





sold either in the name of the GEAC approved brands or most of them as unapproved brands. The role of CICR and ICAR in regulating Bt-seed quality in India has been widely acknowledged.

Seed testing methods of the approved events

Three kinds of tests are used commonly in India to detect the purity of GM seeds.

1. Antibody based tests:
   a. ELISA (Enzyme Linked Immuno-Sorbent Assay) and
   b. Dip-stick-strip immuno-sorbent test
2. PCR (polymerase Chain Reaction) based tests:
   a. Regular PCR
   b. Real-Time qPCR (quantitative PCR)
3. Biochemical tests
   a. GUS (Glucuronidase Assay) to detect the GM reporter enzyme

Gazette notifications for GM seed testing

There are ten main gazette notifications that relate to seed testing of GM crops.

On the 12th November, 2003, S.O. 1300(E).-In exercise of the powers conferred by Sub-section (1) of Section 4 of the Seeds Act, 1966 (54 of 1966), the Central Government declared the laboratory of Central Institute of Cotton Research (CICR), Indian Council of Agricultural Research (ICAR), Nagpur as the Central Seed Laboratory to carry out the functions of ascertaining the presence or absence of cry1Ac gene in Cotton seeds under the said Act with effect from the date of publication for the whole of lndia. 2. In pursuance of clause (c) of rule 5 of the Seeds Rules, 1968, the Central Government also entrusted the

Central Institute of Cotton Research, Indian Council of Agricultural Research, Nagpur to act as a referral laboratory for Bacillus thuringiensis Cotton seeds (Bt. Cotton seeds).

A sample of 25 gm seeds would be drawn from 450 gm seed packet and ten seeds will be tested for the Cry toxin. The minimum limits of quantification were stipulated at 420 ng/gm seed or 420 ng per sq cm of a leaf disc for the sample to be qualified as positive for Bt.

On 5th November 2005 -S.O. 1567(E)-ln exercise of the powers conferred by Section6 of the Seeds Act, 1966 (Act 54 of 1966), the Central Government, after consultation with the Central Seed Committee specified the purity in terms of quantum of gene express of Bacillus thuringienisis (Bt.) Protein (Toxin) as 90 per cent in Bacillus thuringiensis cotton seed lot for labelling of Bacillus thuringienisis Cotton Seed.

On 21st September 2006, six gazette notifications (G.S.R.584 (E) to 589(E) dated September 21, 2006) were issued to empower all the seed Inspectors/ analysts and laboratories notified under Seed Act, also under EPA, 1986.

On 8th May 2008, S.O.1107(E)- In exercise of the powers conferred by Sub-section (1) of section 4 of the Seeds act, 1966 (54 of 1966), the Central Government made the following amendment in the notification of the Government of India, DAC number S,O, 1300(E) dated 12th November 2003 and published in the Gazette of India, Extraordinary, Part II, Section 3, Sub-section (ii) namely: In the said notification, in paragraph 1, in line No 7, for the words and figures 'cry1Ac', the following words shall be substituted, namely –all types of Bacillus thuringiensis.

Methods to test Bt cotton

A working sample of 25 g should be taken in a random manner from the seed packet.

1. ELISA test / dip-stick-strip test: 90 seeds to be tested from working sample size of 25 g drawn from a single packet of 450 g. A minimum number of 81 seeds tested positive for the test protein, Cry1Ac, Cry2Ab, Cry1C and fusion-gene protein Cry1Ab-Cry1Ac, may be taken as the acceptable value for 90% gene purity.

2. PCR test: 30 seeds to be tested from working sample size of 25 g drawn from a single packet of 450 g. A minimum number of 27 seeds tested positive for primers specific to the gene (cry1Ac, cry2Ab, cry1C and fusion cry1Ac gene (cry1Ab+cry1Ac)) may be taken as the acceptable value for 90% gene purity. If only 25-26 seeds are positive, 30 freshly drawn seeds from the same working sample may be re-tested again on PCR. The total number of positive seeds from the two tests should be equivalent to or more than 54 out of the total 60 seeds tested from the working sample.

3. Event specific PCR (only for referral purposes): 10 seeds to be tested from working sample size of 25 g drawn from a single packet of 450 g. A minimum number of 9 seeds tested positive for the event may be taken as the acceptable value for 90% event purity.

4. Gus-Reporter test: 90 seeds to be tested from working sample size of 25 g drawn from a single packet of 450 g. A minimum number of 81 seeds tested positive for the test protein GUS (ß-Glucuronidase) which is the reporter gene for Bollgard-II may be taken as the acceptable value for 90% gene purity of Cry2Ab.

Suggestions:

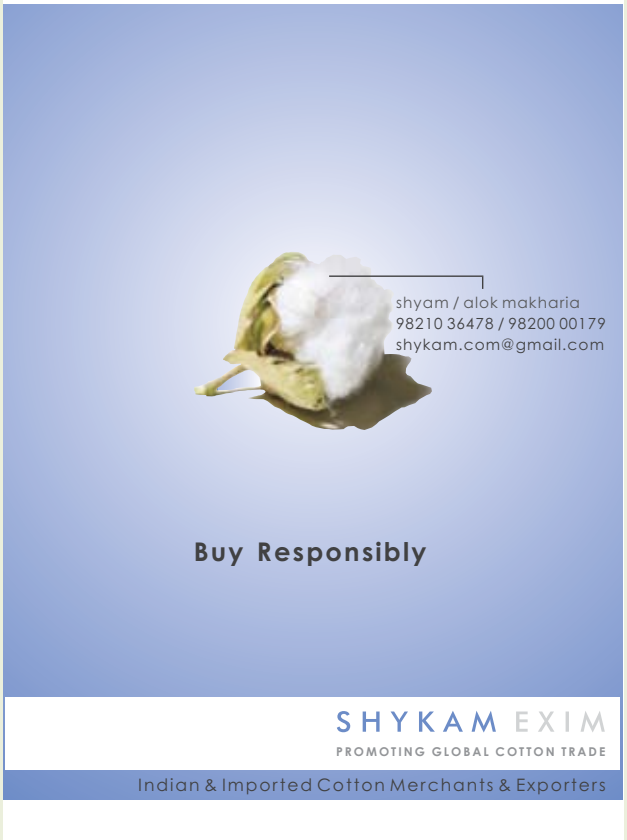
The current gazette notification does not include essential requirement of 90% purity for each of the Cry toxins in Bollgard-II. It only mentions that the hybrid seeds should contain 90% of Bt toxins, which, in effect are present even in F-2 seeds. This should be rectified. The state seed testing laboratories must be provided with Bt testing fw acilities. Seed analysts should be trained in Bt testing methods. Only Andhra Pradesh has been able to set up such facilities. Gazette notifications must be issued for DNA based PCR testing to include all GM events approved in India. Unless and until seed testing is done rigorously, there is every possibility that the crop suffers in farmers' feilds. Therefore, the seed testing laboratories in all the cotton growing states must develop Bt testing facilities and test for the presence of Bt in random samples to ensure that the best quality seed reaches farmers.

Cotton Consumption - Cotton Year-wise

(In Lakh Bales)

Month	2006-07	2007-08	2008-09	2009-2010	2010-11	2011-12	2012-13 (P) (Oct-Aug)
October	17.33	18.32	16.54	18.13	22.09	17.77	21.84
November	17.81	16.94	16.94	18.47	21.09	18.34	21.09
December	18.49	18.86	17.98	19.49	22.57	20.13	22.63
January	18.22	18.54	16.93	19.54	22.1	20.33	23.3
February	17.11	18.14	16.23	18.81	20.23	20.31	22.24
March	18.39	18.45	17.51	20.01	21.77	20.38	23.61
0April	18.06	17.98	17.12	20.53	20.17	20.31	23.24
May	17.89	18.95	17.83	20.93	18.64	21.27	22.84
June	17.85	18.55	18.01	20.71	18.23	21.17	22.76
July	18.42	18.5	18.98	22.11	19	22.14	24.01
August	18.58	17.62	18.59	21.73	18.64	22.08	24.05
September	18.03	16.9	18.29	21.42	21.71	21.46	
Total	216.18	217.75	210.96	241.88	246.23	245.47	251.81

(Source: Office of the Textile Commissioner)



ICAC'S Cotton This Month

According to the latest release of October 10, 2013 of International Cotton Advisory Committee, world cotton production is expected to be lower in 2013/14 than the past three seasons, but world cotton demand is not rising. During 2013/14, world cotton production is projected at 25.5 million tons. China's production is projected at 6.4 million tons, 500,000 tons less than in 2012/13, due to fewer planted hectares as farmers switched to other crops and unfavorable weather earlier in the season. U.S. production is projected at 2.8 million tons, nearly one million tons less than in 2012/13, because of a decrease in planted area and drought in Texas. However, production in India is expected to increase due to a southwest monsoon in early June, reaching a record of 6.4 million tons, on an area of 11.9 million hectares, and a higher national yield of 526 kilograms per hectare. Pakistan is projected to produce just over 2 million tons this season, while production in Uzbekistan is projected at 1 million tons.

Cotton plantings in the southern hemisphere start in large scale in October. Cotton area in this part of the world is projected to be approximately 2.7 million hectares, similar to 2012/13. However, higher yields are expected to result in an increase in South American production.

During 2013/14, world cotton mill use is expected to remain around 23.5 million tons with consumption shifting from China to other countries. China's consumption this season is projected to decrease by 250,000 tons due to the higher price of cotton relative to polyester. However, mill use in other countries, notably India, Pakistan and Turkey is expected to rise, off-setting China's reduced demand this season.

World cotton trade is projected at 8.5 million tons during 2013/14, approximately 1 million tons less than last season, largely accounted for by a decrease in imports by China. The decrease in U.S. production will lead to a decrease in exports this season. Although Indian production will be record high this season, its exports are projected at 1.3 million tons, 400,000 tons less than last season. At the end of September, the Indian government announced that it is withdrawing the export incentives for cotton.

World cotton ending stocks for 2013/14 are forecast at 20.4 million tons, an increase of two million tons from the previous season. In September, the Chinese government started buying cotton for its national reserve, amounting to just over 100,000 tons at the end of September. In 2013/14, China's reserves are expected to increase to 11.4 million tons, up by almost 2 million tons from last season. However, world ending stocks outside China will increase by less than 200,000 tons, so that at the end of the current season, China will hold nearly 60 percent of world stocks. The ratio of world ending stocks outside China to world use outside China is projected at 0.58, the same as last season.

The forecast for the season average Cotlook A Index in 2013/14 ranges from 76 to 106 cents per pound,with a midpoint of 90 cents per pound. The main reason behind the decline in projected prices from a week ago is that ending stocks outside China are estimated 443,000 tons higher for 2012/13 and projected 779,000 tons higher for 2013/14 than a week ago.

The world cotton demand and supply, as drawn up by the ICAC, is given below.

(in mt)

	2011-12	2012-13	2013-14
Beginning Stock	10.08	15.23	18.27
Production	28.04	26.88	25.54
Consumption	22.80	23.48	23.51
Imports	9.76	9.75	8.50
Exports	9.87	10.10	8.50
Ending Stocks	15.23	18.27	20.30
Cotlook A Index	100.01	87.98	-

(Source: ICAC Monthly - 10.10.2013)

SUPPLY AND DISTRIBUTION OF COTTON

October 10, 2013

Seasons begin on August 1 — Million Metric Tons

	2008/09	2009/10	2010/11	2011/12 Est.	2012/13 Proj.	2013/14 Proj.
BEGINNING STOCKS						
WORLD TOTAL	**12.257**	**11.942**	**8.676**	**10.081**	**15.23**	**18.27**
China (Mainland)	3.321	3.585	2.688	2.087	6.18	9.61
USA	2.188	1.380	0.642	0.566	0.73	0.85
PRODUCTION						
WORLD TOTAL	**23.503**	**22.247**	**25.869**	**28.041**	**26.88**	**25.54**
China (Mainland)	8.025	6.925	6.400	7.400	7.30	6.70
India	4.930	5.185	5.865	6.354	6.09	6.37
USA	2.790	2.654	3.942	3.391	3.77	2.81
Brazil	1.214	1.194	1.960	1.877	1.28	2.03
Pakistan	1.926	2.070	1.907	2.311	2.20	1.50
Uzbekistan	1.000	0.850	0.910	0.880	1.00	1.00
Others	3.617	3.369	4.385	5.827	5.23	5.13
CONSUMPTION*						
WORLD TOTAL	**23.862**	**25.520**	**24.502**	**22.796**	**23.48**	**23.51**
China (Mainland)	9.265	10.192	9.580	8.635	8.29	8.04
India	3.872	4.300	4.509	4.340	4.93	5.02
Pakistan	2.519	2.393	2.100	2.217	2.42	2.46
East Asia & Australia	1.714	1.892	1.796	1.646	1.86	1.92
Europe & Turkey	1.458	1.600	1.549	1.495	1.53	1.58
Brazil	1.000	1.024	0.958	0.888	0.89	0.85
USA	0.771	0.773	0.849	0.718	0.75	0.76
CIS	0.596	0.604	0.577	0.550	0.58	0.60
Others	2.666	2.743	2.583	2.306	2.24	2.28
EXPORTS						
WORLD TOTAL	**6.609**	**7.798**	**7.636**	**9.870**	**10.10**	**8.50**
USA	2.887	2.621	3.130	2.526	2.90	2.26
India	0.515	1.420	1.085	2.159	1.73	1.33
Brazil	0.596	0.433	0.435	1.043	0.94	0.86
Australia	0.261	0.460	0.545	1.010	1.35	1.00
CFA Zone	0.469	0.560	0.476	0.597	0.79	0.91
Uzbekistan	0.650	0.820	0.600	0.550	0.65	0.57
IMPORTS						
WORLD TOTAL	**6.647**	**7.928**	**7.725**	**9.760**	**9.75**	**8.50**
China	1.523	2.374	2.609	5.342	4.43	3.18
East Asia & Australia	1.714	1.989	1.825	1.998	2.19	2.26
Europe & Turkey	0.862	1.170	0.972	0.724	1.00	0.74
Pakistan	0.417	0.342	0.314	0.173	0.43	0.46
CIS	0.231	0.209	0.132	0.098	0.07	0.08
TRADE IMBALANCE 1/	**0.038**	**0.130**	**0.089**	**-0.111**	**-0.35**	**0.00**
STOCK ADJUSTMENT 2/	**0.007**	**-0.122**	**-0.051**	**0.013**	**0.00**	**0.00**
ENDING STOCKS						
WORLD TOTAL	**11.942**	**8.676**	**10.081**	**15.227**	**18.27**	**20.30**
China (Mainland)	3.585	2.688	2.087	6.181	9.61	11.44
USA	1.380	0.642	0.566	0.729	0.85	0.63
ENDING STOCKS/MILL USE (%)						
WORLD-LESS-CHINA(M) 3/	**57**	**39**	**54**	**64**	**57**	**57**
CHINA (MAINLAND) 4/	**39**	**26**	**22**	**72**	**116**	**142**
Cotlook A Index 5/	**61.20**	**77.54**	**164.26**	**100.01**	**87.98**	

1/ The inclusion of linters and waste, changes in weight during transit, differences in reporting period and measurement error account for difference between world imports and exports.
2/ Difference between calculated stocks and actual; amounts for forward seasons are anticipated.
3/ World-less-China's ending stocks divided by World-less-China's mill use, multiplied by 100.
4/ China's ending stocks divided by China's mill use, multiplied by 100.
5/ U.S. Cents per pound
(Source : ICAC Monthly October 2013)

UPCOUNTRY SPOT RATES

(Rs./Qtl)

Standard Descriptions with Basic Grade & Staple in Millimetres based on Upper Half Mean Length [By law 66 (A) (a) (4)]							Spot Rate (Upcountry) 2012-13 Crop OCTOBER 2013					
Sr. No.	Growth	Grade Standard	Grade	Staple	Micronaire	Strength /GPT	7th	8th	9th	10th	11th	12th
1	P/H/R	ICS-101	Fine	Below 22mm	5.0 – 7.0	15	10489 (37300)	10545 (37500)	10545 (37500)	10545 (37500)	10404 (37000)	10264 (36500)
2	P/H/R	ICS-201	Fine	Below 22mm	5.0 – 7.0	15	10770 (38300)	10826 (38500)	10826 (38500)	10826 (38500)	10686 (38000)	10545 (37500)
3	GUJ	ICS-102	Fine	22mm	4.0 – 6.0	20	8492 (30200)	8436 (30000)	8436 (30000)	8436 (30000)	8492 (30200)	8492 (30200)
4	KAR	ICS-103	Fine	23mm	4.0 – 5.5	21	9589 (34100)	9533 (33900)	9533 (33900)	9533 (33900)	9533 (33900)	9533 (33900)
5	M/M	ICS-104	Fine	24mm	4.0 – 5.5	23	11135 (39600)	11135 (39600)	11051 (39300)	11051 (39300)	11051 (39300)	11051 (39300)
6	P/H/R	ICS-202	Fine	26mm	3.5 – 4.9	26	11923 (42400)	11923 (42400)	N.Q.	N.Q.	N.Q.	N.Q.
7	M/M/A	ICS-105	Fine	26mm	3.0 – 3.4	25	12092 (43000)	12092 (43000)	N.Q.	N.Q.	N.Q.	N.Q.
8	M/M/A	ICS-105	Fine	26mm	3.5 – 4.9	25	12429 (44200)	12429 (44200)	N.Q.	N.Q.	N.Q.	N.Q.
9	P/H/R	ICS-105	Fine	27mm	3.5 – 4.9	26	12204 (43400)	12007 (42700)	11923 (42400)	11923 (42400)	12148 (43200)	12176 (43300)
10	M/M/A	ICS-105	Fine	27mm	3.0 – 3.4	26	12401 (44100)	12401 (44100)	N.Q.	N.Q.	N.Q.	N.Q.
11	M/M/A	ICS-105	Fine	27mm	3.5 – 4.9	26	12654 (45000)	12654 (45000)	N.Q.	N.Q.	N.Q.	N.Q.
12	P/H/R	ICS-105	Fine	28mm	3.5 – 4.9	27	12345 (43900)	12204 (43400)	12204 (43400)	12204 (43400)	12429 (44200)	12457 (44300)
13	M/M/A	ICS-105	Fine	28mm	3.5 – 4.9	27	13273 (47200)	13132 (46700)	12795 (45500)	12795 (45500)	12879 (45800)	12879 (45800)
14	GUJ	ICS-105	Fine	28mm	3.5 – 4.9	27	13216 (47000)	13076 (46500)	12513 (44500)	12513 (44500)	12598 (44800)	12541 (44600)
15	M/M/A/K	ICS-105	Fine	29mm	3.5 – 4.9	28	13441 (47800)	13301 (47300)	12935 (46000)	12935 (46000)	13020 (46300)	13020 (46300)
16	GUJ	ICS-105	Fine	29mm	3.5 – 4.9	28	13357 (47500)	13216 (47000)	12795 (45500)	12795 (45500)	12879 (45800)	12823 (45600)
17	M/M/ A/K	ICS-105	Fine	30mm	3.5 – 4.9	29	13385 (47600)	13329 (47400)	13216 (47000)	13216 (47000)	13301 (47300)	13301 (47300)
18	M/M/A/K/T/O	ICS-105	Fine	31mm	3.5 – 4.9	30	13469 (47900)	13385 (47600)	13076 (46500)	13076 (46500)	13160 (46800)	13160 (46800)
19	K/A/T/O	ICS-106	Fine	32mm	3.5 – 4.9	31	13638 (48500)	13582 (48300)	N.Q.	N.Q.	N.Q.	N.Q.
20	M(P)/K/T	ICS-107	Fine	34mm	3.0 - 3.8	33	16169 (57500)	16169 (57500)	15888 (56500)	15888 (56500)	16028 (57000)	16028 (57000)

(Note: Figures in bracket indicate prices in Rs./Candy)