Technical Bulletin No.: 05/2023

INTERNATIONAL YEAR OF MILLETS 2023

Division of Crop Improvement

G20 भारत 2023 INDIA



ICAR

ICAR-CENTRAL TOBACCO RESEARCH INSTITUTE
(An ISO 9001:2015 Certified Institute)
RAJAHMUNDRY - 533 105, ANDHRA PRADESH, INDIA

Technical Bulletin No.: 05/2023

Division of Crop Improvement

भाकृअनुप – केन्द्रीय तम्बाकू अनुसंधान संस्थान
ICAR-CENTRAL TOBACCO RESEARCH INSTITUTE
(An ISO 9001:2015 Certified Institute)
RAJAHMUNDRY - 533 105, ANDHRA PRADESH, INDIA

Citation:

Division of Crop Improvement; Tech.Bulletin No: 05/2023, ICAR -Central Tobacco Research Institute, Rajahmundry.

Published by

Dr. M. Sheshu Madhav
Director
ICAR – Central Tobacco Research Institute
Rajahmundry – 533105, Andhra Pradesh, India.
Phone: 0883-2449871-4, Fax: 0883-2448341, 2410555
e-mail :directorctri@gmail.com
Website : https://ctri.icar.gov.in

Compiled by

Dr. K. Sarala
Dr. K. Prabhakara Rao
Dr. M. Sheshu Madhav

Word process & Designing

Md. Elias

All rights reserved. No part of this publication may be reproduced or transmitted in any form by print, microfilm or any other means without the written permission of the Director, ICAR-CTRI.

January, 2023

Preface



Division of Crop Improvement is one of an important and founder disciplines of the ICAR-Central Tobacco Research Institute (CTRI). The Division comprises of three disciplines, Genetics & Plant Breeding, Agricultural Biotechnology, Economic Botany & Plant Genetic Resources.

This Division shoulder the responsibility of taking care of the crop improvement needs of diversified types of tobacco in the country with type and location specific objectives. The Division striving for the improvement of Indian tobacco cultivars in terms of productivity, physical and chemical quality, trade preference, resistance to biotic and abiotic stresses, reducing harmful substances such as tar, tobacco specific nitrosamines, carbon monoxide, etc. The scientists of the Division utilising both conventional breeding methods and biotechnology tools such as tissue culture, genomics, genetic engineering, etc. in developing location specific tobacco cultivars with improved characteristics. Significant achievements of the Division, since its inception are briefed in this bulletin.

As a National Active Germplasm site (NAGS) for tobacco, it is managing more than 3380 number of tobacco germplasm accessions. Identified number of entries possessing disease and pest resistance and quality traits in cultivated tobacco and *Nicotiana* species. The division is instrumental in releasing and recommending 107 tobacco varieties having higher yield, better quality, biotic & abiotic stress resistance and lower levels of harmful substances for their cultivation in different parts of India. As a result, the tobacco productivity has more than doubled compared to the cultivars of 1950s when the

institute established. Very strong pre-breeding programme resulted in transfer of many useful genes viz., resistance to leaf eating caterpillar, aphids, root-knot nematodes and Black shank into pre-breeding populations and tobacco cultivars. Tissue culture lab of the Division is one of the oldest to establish in this area. It is instrumental micropropagation of in large number of elite tobacco lines; development of useful di-haploids, somaclones and transgenics, rescue of inter-specific hybrids, etc. Developed, validated and utilised molecular markers for diversity analysis of tobacco lines, pathogen isolates and DNA finger printing of tobacco cultivar. DNA barcodes developed for the identification of *Nicotiana* species etc. The Division is currently under taking research in the area of gene expression and genome editing for developing stress resistant and less harmful tobacco cultivars.

I am personally thankful to all the scientists worked in the Division, since its inception, for putting the Institute and tobacco crop in India in commanding position with their laudable contributions in the area of crop Improvement. I am thankfully acknowledging the encouragement and constant support rendered by the present and past Directors of the Institute to the programme of the Division. All the technical and supporting staff, current and previous, of the Divisions needs appreciation for their unstinted support in the smooth execution of the research activities of crop improvement. Heads of Divisions/Stations, scientists and staff the other Divisions and Stations for their necessary support in successfully carrying out inter-disciplinary programme of the Division.



Date: 24.01.2023

(M. SHESHU MADHAV)
DIRECTOR

BACKGROUND

Division of Crop Improvement, initially known as Genetics and Plant Breeding, is one of the founder disciplines of the ICAR-Central Tobacco Research Institute (CTRI) since its inception in the year 1947. Currently, the Division comprises of three disciplines, Genetics & Plant Breeding, Agricultural Biotechnology, Economic Botany & Plant Genetic Resources. The tobacco breeding work was initiated under the name Botany while the Cytogenetics research was started in 1952 with the establishment of the Cytology section. Subsequently, the foundation for Biotechnology work was laid with the initiation of tissue culture studies in early 1970's. The Division strives continuously for the improvement of Indian tobacco cultivars in terms of productivity, physical and chemical quality, trade preference, resistance to biotic and abiotic stresses, reducing harmful substances such as tar, tobacco specific nitrosamines, carbon monoxide, etc.

Out of nearly 70 available *Nicotiana* species, ***N. tabaccum*** L. and ***N. rustica*** L. are the cultivated species in India. A unique feature of tobacco production in India is that myriad styles of Flue-cured Virginia (FCV) and non-FCV tobacco are cultivated under widely differing agro-ecological situations. The FCV, ***bidi***, hookah, chewing, cigar-wrapper, cheroot, burley, Oriental, HDBRG, ***Lanka, Pikka, Natu*** etc. are the main types of tobacco grown in the country, with FCV and Burley tobacco being the main exportable types. Breeding objectives for different types of tobacco tend to differ in view of their growing conditions, climatic zones, location specific biotic and abiotic stresses, cultural practices, quality requirements as per their utilisation, traders/consumers preferences etc. This necessitates development of different plant types with desirable characteristics suitable to various zones and end uses. Hence, breeding programmes for each of the various types of tobacco grown in India are different. The Division of Crop Improvement shoulder the responsibility of taking care of the crop improvement needs of diversified types of tobacco in the country with type and location specific objectives. The scientists of the Division complement conventional breeding methods with biotechnology tools such as tissue culture, genomics, genetic engineering, etc. in developing location specific tobacco cultivars with improved characteristics. The Division also undertakes multilocation testing of advanced breeding lines under the All India Network Research Project (Tobacco). Major research thrusts and activities of the Division are briefed below.

MAJOR RESEARCH THRUSTS

- Developing tobacco varieties/ hybrids possessing higher leaf yield and acceptable leaf quality, flavor and low levels of harmful substances
- Incorporation of resistance to biotic and abiotic stresses to stabilize productivity
- Tailoring of tobacco plant type for optimizing the seed and phyto-chemical yields
- Production and distribution of pure seed of ruling tobacco varieties
- Germplasm Resource Management
- Biotechnology for tobacco improvement

RESEARCH ACTIVITIES

- Acquisition, maintenance, documentation and distribution of germplasm of different types of cultivated tobacco and wild *Nicotiana* species
- Evaluation of germplasm for important agronomical and resistance traits and developing descriptor list and databases for *Nicotiana* gene pool, including molecular characterisation
- Application of principles of Plant Breeding for developing tobacco cultivars with high productivity and acceptable physical, technological and manufacturing leaf quality
- Exploitation of hybrid vigour for breaking yield barriers and improving quality and resistance traits
- Investigations on producing varieties with acceptable levels of tar, nicotine and reducing sugars, and low levels of tobacco specific nitrosamines
- Developing tobacco cultivars with built-in resistance to biotic and abiotic stresses
- Pre-breeding to transfer resistance factors from wild *Nicotiana* species to tobacco cultivars
- Identification of the pre- and post-fertilization barriers in interspecific hybridization and overcoming them through *in-vitro* techniques for successful transfer of genes of importance to cultivated tobacco

- Cytogenetical studies on the interspecific hybrids and their derivatives to identify the chromosomal basis and mechanism of resistances
- Characterization of pre-breeding materials for their utilisation
- To study inheritance of important morphological, agronomical and resistance traits
- Development of tissue culture protocols for micropropagation, direct organogenesis, somatic embryogenesis, protoplast culture, somatic hybridization, artificial seeds, *Agrobacterium* mediated genetic transformation etc.
- Micropropagation through direct/ indirect organogenesis for the faithful multiplication of interspecific hybrids, non-flowering / seed sterile genetic stocks, maintenance of biochemical mutants and transgenics.
- Anther culture for production of haploids and homozygous lines
- Embryo rescue in wide crosses
- Somaclonal variation for inducing variability for resistance/ tolerance to diseases, insect-pests and abiotic stresses.
- Use of protoplast culture and somatic hybridization for producing desirable distant hybrids.
- Development of tobacco varieties with resistance to biotic and abiotic stresses utilizing biotechnological techniques.
- Development of DUS guidelines and registration of tobacco varieties.
- Characterization and diversity analysis of tobacco cultivars and elite lines using molecular markers.
- Expression analysis of various genes involved in the biotic stresses and other quality traits.
- Identification of molecular markers for resistance to biotic and abiotic stresses.
- DNA barcoding of *Nicotiana* species and ITS sequence characterization of pathogenisolates.
- Genome editing and genetic transformation for development of elite cultivars.

ACHIEVEMENTS

1. GERMPLASM RESOURCES

ICAR-CTRI, Rajahmundry is a National Active Germplasm Site (NAGS) for tobacco under the National Network on Conservation of Plant Genetic Resources. In view of this, Division of Crop Improvement takes the responsibility of collection, conservation, characterization and documentation of tobacco germplasm.

(i) **Maintenance:** A total of 3386 germplasm accessions of *N. tabacum* and *N. rustica*, and 40 wild *Nicotiana* species are being maintained.

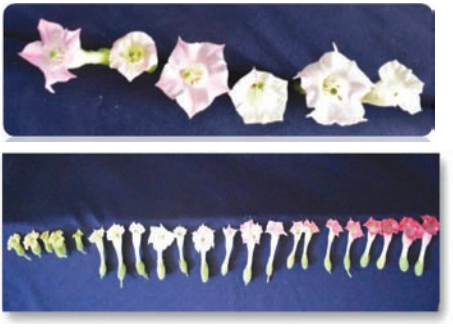
Tobacco germplasm resources at ICAR-CTRI

S.No.	Details	No. of accessions
1	Released/identified varieties	101
2	AINPT lines	109
3	Low nicotine lines	11
4	Root knot resistant	21
5	Disease resistant	21
6	Insect pest resistant lines	167
7	Mutants	36
8	Advanced breeding lines	86
9	New germplasm	127
10	CMS parental lines	114
11	FCV indigenous	41
12	FCV exotic	470
13	Jati tobacco	61
14	Cigar wrapper	75
15	Turkish tobacco	49
16	Bulgaria tobacco	13
17	Exotic air cured	151
18	Japan air cured	54
19	Black tobacco	3
20	Fire cured tobacco	5
21	Air-cured cigarette material	11
22	Burley germplam	121
23	Chewing	178
24	Bidi	230
25	Country cheroot	218
26	Cigar filler	82
27	*N. Rustica*	618
28	Wild *nicotiana* species	213
	Total	3386

(ii) **Evaluation:** Characterization and evaluation of germplasm accessions for morphological descriptors, yield potential, resistance to diseases and insect-pests, and identification of lines suitable for various breeding programmes has been an important activity of the Division. During all these years, over 3000 lines of exotic and indigenous origin have been evaluated for yield, leaf quality, chemical characteristics, resistance to biotic and abiotic stresses etc. and useful lines identified. Elite tobacco entries and *Nicotiana* species were also characterised using isozymes. Around 2100 germplasm accessions were characterised for 25 selected morphological traits. Genetic diversity studied in all the germplasm groups and different tobacco types. Large morphological diversity found to exist in the germplasm collection. A core collection of 305 accessions of *N. tabacum* and *N. rustica* were created and characterised for 25 selected morphological traits. Core collection found to represent the genetic diversity existing in the base collection. Some of the elite tobacco entries identified having resistance to biotic and abiotic stresses and possessing special traits among primary gene pool and wild *Nicotiana* species are given here.



Diversity for leaf shape in *N. tabacum* germplasm



Floral diversity in *N. tabacum* and *N. rustica* germplasm

Resistance sources identified within primary gene pool

Tobacco Mosaic Virus (425 accessions), Tobacco Distorting Virus(36), Tobacco Etch Virus (10), black shank (49), powdery mildew (30), brown spot (9), frog eye spot (26),anthracnose(13), damping off (2) root knot nematode (27), leaf eating caterpillar (15), budworm (12), leaf curl/white fly (8), stem borer (1), aphids (15), and drought tolerance (7).

Cultivar/breeding line	Resistant to
BST x JMR, BST3 x JMR, BSF1-6 x JMR, BSF1-13 x JMR; L-1128 to L-1137; BSRB-1, BSRB-2	Black shank and TMV
BGT-17, BGT x JMR	Caterpillar and TMV
GT-5, GT-9	Damping off
CY 118, CY 113, CM 12, L-621, Oxford 26, CM 16, VA 21	Drought tolerant

Resistance sources among wild *Nicotiana* species

Wild *Nicotiana* species are found to be important sources for resistance to various diseases and insect pests. After rigorous screening, a number of *Nicotiana* species having high degree of resistance/immunity to several biotic stresses were identified.



N. umbratica-nesophila, resistant to *Orobanche*

Resistance sources identified among wild *Nicotiana* species

Biotic stress	Resistant species
Tobacco Mosaic Virus	*N. glutinosa, N. goodspeedii, N. ingulba, N. nesophila, N. repanda, N. simulens, N. solanifolia, N. stocktonii, N. undulata, N. wigandioides, N. benthamiana*
Tobacco Distorting Virus	*N. glutinosa, N. nesophila, N. repanda, N. solanifolia, N. plumbaginifolia, N. rustica*
Tobacco Etch Virus	*N. glauca, N. knightiana, N. maritima, N. otophora, N. palmeri, N. paniculata, N. rustica, N. sylvestris, N. trigonophylla, N. undulata*
Powdery Mildew	*N. longiflora, N. maritima, N. nesophila, N. nudicaulis, N. palmeri, N. umbratica, N. gossei, N. ingulba, N. debneyi, N. glauca, N. glutinosa, N. repanda, N. simulens, N. trigonophylla, N. undulata*
Black Shank	*N. longiflora, N. maritima, N. nesophila, N. plumbaginifolia, N. suaveolens*
Root-knot	*N. amplexicaulis, N. nudicaulis, N. repanda, N. longiflora*
Anthracnose	*N. amplexicaulis, N. exigua, N. langsdorffii, N. palmeri,N. simulens, N. stocktonii, N. suaveolens, N. debneyi,N. nudicaulis, N. longiflora*
Brown Spot	*N. exigua, N. glutinosa, N. longiflora, N. repanda,N. plumbaginifolia, N. debneyi*
Frog Eye Spot	*N. longiflora, N. plumbaginifolia, N. repanda, N. alata,N. debneyi, N. undulata, N. nudicaulis*
Aphids	*N. gossei, N. repanda, N. umbratica, N. nesophila*
Leaf Eating Caterpillar	*N. benthamiana, N. gossei, N. umbratica*
Orobanche	*N.umbratica-nesophila, N. benthamiana-repanda, N. nesophila*

Tobacco entries possessing useful chemical quality traits

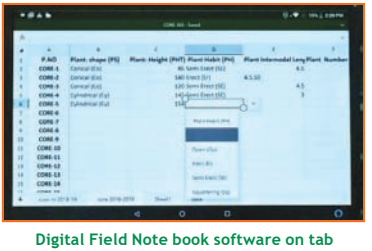
Trait	Tobacco entry
High Solanesol(> 2%)	Abirami, Gauthami, HDBRG, 1/135
Low tar (<20 ppm/ cigarette)	Bell No.61-9-1, Banana leaf, D1, Danadayi, JS-117, JS-78, JS-62, EC 11083, JS 73, JS 115, JS 119, JS 125 and JS 126
Low TSNA (< 2ppm)	Sota 6506, Harrow Velvet, Burley resistant, By 64, By Sota 51, Ky-10, T-117 and VA 510

(iii) Utilization: A number of identified promising germplasm lines have been utilised in tobacco improvement programmes at CTRI and elsewhere for developing improved tobacco lines and understanding various genetic, cytogenetic and molecular basis of biological processes.In India, 75 varieties have so far been bred using indigenous germplasm as parents. Thirteen varieties have one parent of indigenous and other of exotic origin, and six have exotic parents.Thirteen varieties are direct exotic introductions.Head, Division of Crop Improvement acts as the Principal Investigator for all the Crop Improvement activities under the AINRP (T).

Nicotiana species used for production of male sterile lines in various types of tobacco are: *N. undulata, N. rustica, N. debneyi, N. megalosiphon, N. glauca, N. suaveolens, N. gossei*and *N. umbratica.* Utilising the developed CMS lines, two CMS hybrids, CH-1 and CH-3 are released for commercial cultivation.

Multiple biotic stress resistance lines developed: Black shank and TMV (19), black shank and RKN (5), black shank and leaf burn (2), CTP and TMV (3), aphid and budworm (7), aphid and whitefly (1), aphid and caterpillar (4), TMV and aphid (1)

(iv) Documentation: The Division has so far published three germplasm catalogues. In addition, three computer databases viz., Tobacco Germplasm Information System, *Nicotiana* Species Information System and Rainfed *Natu* Tobacco



Digital Field Note book software on tab

germplasm Information System as reference tools for the benefit of tobacco researchers. The passport data of germplasm accessions digitised.

(v) Registration: Eight unique germplasm lines are registered with National Bureau of plant Genetic Resources (NBPGR), New Delhi.

Germplasm registered

Sr. No.	Donor identity	National identity	INGR No.	Pedigree	Novel unique features
1	HV.2006-6	IC0574228	INGR21077	(Abhirami X DWFC) Abhirami	A high yielding caterpillar resistant sun-cured chewing tobacco
2	NLCR 6-10	IC634528	INGR21078	Kanchan	High cured leaf yielding FCV tobacco somaclone with more number of longer and broader curable leaves suitable for irrigated alfisols
3	F6-2-2	IC8099212	INGR21079	A145 x Bhagyalakshmi	High seed yielding chewing tobacco
4	JS-117	IC625211	INGR21080	Kanchan X D-1	Low smoke tar delivering Flue-Cured Virginia (FCV) Tobacco
5	Jayalak-shmi	IC637597	INGR21081	Local collection	White flower and white (cream colour) seed Flue Cured Virginia (FCV) line
6	1/135	IC637598	INGR21082	HDBRG x BY-53	High solanesol (3.43 %).
7	V-4914	IC634529	INGR21083	Siri X VT-1158	High yielding Tobacco Mosaic Virus (TMV) resistant Flue-cured Virginia (FCV) tobacco cultivar
8	BSR-1	IC634526	INGR21084	(VR-2 x Beinhart 1000-1) VR-2	Black shank (*Phytopthora parasitica*) resistant chewing tobacco entry

(vi) Germplasm supply

As tobacco is model plant for biological research, institute regularly receives large number of indents for seeds of cultivated tobacco and wild *Nicotiana* species. Division supplies germplasm accessions on request to other researchers, both public and private, for furthering tobacco research and advancing biological science. As a NAGS for tobacco, facilitates the import of tobacco germplasm by researchers from both public and private organisations through NBPGR, New Delhi.

2. INHERITANCE AND LINKAGE STUDIES

Inheritance of 45 traits, covering morphological, reproductive, and physiological characters was studied. A list of identified genes for various characteristics and linkage groups is presented below.

S. No.	CHARACTER	GENE(S)	LINKED GENE (recombination)
1.	Petiolate leaf	Pta, Ptb, Ptc (compl.)	Iau (0.20)
2.	Auricle	Au1, Au2 (dupli.)	Bw (0.32)
3.	Auricle inhibitor	Iau	
4.	Broad winged petiole	Bw*	
5.	Enlarged corolla	Lc	
6.	Pink petal colour	Pka, Pkb (compl.)	Pka-Fg (0.36) Pka-Thg(0.40)
7.	Pink colour intensifiers	Pki1, Pki2 (dupli.)	
8.	Fading of petal colour	Fd	
9.	Pink colour filament	Fpa, Fpb (compl.)	
10.	Pink filament inhibitor	Itp	
11.	Pink tip of filament	Ftp	
12.	Red pigment in leaf	rp1, rp2 (dupli.)*	
13.	Crumpled leaf dwarf	Icr	
14.	Hooked leaf tip	Lta*	
15.	Creamy plant colour	Crp	
16.	Green vs white throat	Thg, thg	Thg-Cr (0.06)
17.	Shape of corolla throat	Thfl : fluted the : constricted thf : funnel shaped	Ist (0.37) Lht (0.45)
18.	Protruded style	Sta, Stb (compl.)	
19.	Style length inhibitor	Ist	Lht (0.38)
20.	Narrow anthers	anr1, anr2	
21.	Green vs pale yellow filament	Fg - fg	Fg-Thg (0.20) Fg-Cr (0.30)
22.	Ribbon leaf	Rb	
23.	Crinkled leaf	Cr	

3. CULTIVAR DEVELOPMENT

Tobacco is one of the classic self-pollinated species with relatively low (4-10%) natural cross-pollination. Significant levels of inbreeding depression and limited heterosis with predominance of additive genetic variance made the breeders to focus largely on the development of pure line cultivars. Also, for populations with large amounts of non-additive genetic variance and rare crosses exhibiting high heterosis, hybrids were developed to maximize genetic improvements. Though tobacco is highly amenable for transformation, transgenic are not accepted by traders, hence, transgenic varieties are not bred at the division.

Breeding of tobacco in all these years was mainly focused for developing high yielding, better quality and stress tolerant genotypes suitable for its traditional uses. Also, emphasis is being given to develop varieties having low levels of tar and other harmful substances, and developing tobacco for value added products. Evolving genotypes for alternative uses received modest attention till date. Traditional methods of breeding viz., Introduction, pureline selection, pedigree, back-cross, recurrent selection, mutation breeding, polyploidy etc. are mainly used in evolving tobacco cultivars. However, pureline selection followed by back-cross breeding are relatively widely used ones. A number of improved varieties have been developed combining higher leaf yields, desirable quality, and disease and pest resistance.

4. EVALUATION OF BREEDING METHODS

Through a study of bi-parental matings, it was found that pedigree method is the best to improve FCV tobacco cultivars for yield and quality characteristics. In addition, multiparent crosses, convergent crosses, selective diallel mating, triallel analysis, generation mean analysis etc. were tested. Detailed studies on genetic variability, correlations, multiple regression analyses, cytoplasmic male sterility, heterosis breeding, varietal mixtures etc. were made to identify suitable methods for tobacco improvement.

5. INTERSPECIFIC HYBRIDIZATION

Wild *Nicotiana* species are important sources for resistance to various diseases and insect pests. After intensive systematic

screening, *Nicotiana* species having high degree of resistance/ immunity to several diseases were identified. Through pre-breeding, many useful genes viz., resistance to leaf eating caterpillar (*N. gossei* and *N. benthamiana*), aphids (*N. gossei*), root-knot nematodes (*N. longiflora* and *N. amplexicaulis*) and Black shank (*N. plumbaginifolia*) have been incorporated into pre-breeding populations and tobacco cultivars through interspecific hybridization. Detailed biochemical and chromosome analysis studies on interspecific hybrids and derivatives are made for pursuing appropriate selection methods and develop stable resistant lines.

Species incompatibility found to come in the way of utilization of interspecific hybridization for incorporation of resistance in cultivated tobacco. Incompatibility barriers encountered have been successfully overcome through utilization of hormones, sesquiploid and bridge-cross techniques and *in-vitro* embryo rescue methods as mentioned below.

(i) Direct / hormone-aided hybridization: *N. gossei*, *N. amplexicaulis*, *N. megalosiphon*, *N. debneyi, N. umbratica, N. africana, N. glauca, N. alata, N. glutinosa, N. repanda, N. trigonophylla, N. rustica, N. plumbaginifolia, N. longiflora*

(ii) Bridge-cross technique: *N. x benthamiana-glutinosa, N. x benthamiana-repanda, N. x umbratica-nesophila, N. x gosseiexcelsior, N. x glutinosa-trigonophylla*

(iii) Auto tetraploidization of resultant hybrids: *N.tabacum* cv. Delcrest x *N. plumbaginifolia*

Number of hybrids were micropropagated under *in vitro* to overcome death of the hybrids seedlings in the initial stages. *In-vitro* embryo rescue methods were found to be successful in the production of following hybrids.

Inter-specific hybrids rescued through embryo rescue/ anther culture

Hybrids rescued	Day of embryo abortion
N. benthamiana x N. tabacum	13
N. rustica x N. benthamiana	8
N. gossei x N. tabacum	8
N. amplexicaulis x N. tabacum	13
N. tabacum x N. longiflora	10
N. megalosiphon x N. tabacum	13
N. rustica x N. tabacum	10
N. debneyi x N. tabacum	Different stages
N. umbratica x N. tabacum	10

Further, detailed biochemical and chromosome analysis on interspecific hybrids and derivatives were made for pursuing appropriate selection methods and developing stable resistant lines.

Need based pre breeding activities involving vast repository of tobacco germplasm collection at ICAR-CTRI covering wild relatives, promising landraces, and popular cultivars has resulted in the development of number of pre-breeding lines resistance to various pests and diseases viz., leaf eating cater pillar, aphid, TMV, budworm, black shank, root-knot Nematodes etc. Recently released FCV variety, CTRI Sulakshana is an interspecific cross derivative in which the resistance to TMV and tolerance to aphid are transferred from *N. gossei*.



CTRI Sulakshana: A TMV resistant and aphid tolerant variety developed through pre-breeding

Table : Resistance factors transferred from wild *Nicotiana* species to tobacco cultivars

Wild species	Resistance transferred	Cultivars fortified
N. gossei	Leaf eating caterpillar and aphids	Jayasri, Hema, VT 1158, Gauthami, CM-12, Bhavya,
	TMV and aphids	CTRI Sulakhana
N. benthamiana	Leaf eating caterpillar	Jayasri (MR)
N. longiflora	Root-knot nematode (*Meloidogyne arenaria, M. incognita* and *M. javanica*)	Bhavya and 16/ 103
	Budworm	Bhavya, Jayasri, 16/103
N. amplexicaulis	Root-Knot Nematodes (*Meloidogyne arenaria, M. incognita* and *M. javanica*)	Bhavya, 16/103, Mc Nair-12 and CM-12
N. plumbaginifolia	Black shank	BSRB-2

6. MUTATION RESEARCH

Variability for morphological and important agronomic traits was created through physical and chemical mutagenesis. Physical mutagens viz., X-rays, gamma rays and fast neutrons and chemical mutagens like EMS, MES, MMS, SA and NMU were used for induction of mutations. Some of the economically important induced mutants include bright to pale coloured leaves, low nicotine content, increased leaf area, increased leaf number etc. These mutants were used in hybridizations with other cultivars to develop varieties, Jayasri, Jayasri (MR), CM-12 (KA) and Cy-79 (Kanthi).



CM-12: Developed through chemical mutagenesis

Recently, several promising lines were developed through irradiation of three FCV (Siri, Kanchan and FCJ-11) and two (Banket A1 and YB-22) cultivars with different doses (300 Gy, 400 Gy and 500 Gy) of 10 MeV electron beam. Currently they are in the advanced stages of testing in replicated trials.

7. BIOTECHNOLOGICAL STUDIES

The Division with various biotechnological tools complementing the conventional breeding programmes in characterizing the tobacco genetic resources for useful traits and identifying molecular basis of stress resistance for developing improved tobacco cultivars having higher yields, desirable quality and resistance to biotic and abiotic stresses.

(i) Tissue culture: Protocols for *in-vitro* culture of various explants were standardized / developed for diverse genotypes of cultivated tobacco and intra- and interspecific hybrids. These include micropropagation and somatic embryogenesis, production of synthetic seeds, anther culture, *in-vitro* gynogenesis, pollen culture, protoplast culture, somatic hybridization, *in-vitro* embryo rescue, etc.

Lines developed:

a. Micropropagation: Number of interspecific hybrids, non-flowering / seed sterile genetic stocks, biochemical mutants transgenics etc. were micropropagated through direct/indirect organogenesis.

b. Anther culture: Number of dihaploid lines were developed through anther culture and evaluated under field condition. A dihaploid line, D-1 having high yield potential was produced through anther culture and utilised in breeding programmes.



Anther Culture



FCJ-11: A promising somaclone identified for release to NLS area

c.Somaclonal development: Somaclonal variation was generated for creating variability for desirable traits. Somaclones of line D-1 having tolerance to budworm and those from cultivar Candel having tolerance to leaf curl were developed. Somaclones of Kanchan and VT-1158 were generated from the leafcurl infected leaf explants. Among them, somaclones viz., FCJ-11, Tobios-6, Tobios-2, NLCR-10, VTCMV-1-15-14, VLCR-12-15-14-5 etc. found to be promising in advanced field trials.The somaclone, FCJ-11 having an yield potential of 3300 kg/ha was identified for release to Northern Light Soil of AP.

d. Embryo rescue: A number of interspecific hybrids were rescued at critical stage(s) of embryo abortion through embryo rescue. Thus, lines having resistance to caterpillar, aphid and rootknot nematodes were developed.

(ii) Genetic transformation

a. Direct genetic transformation: It was demonstrated that recipient parent could be transformed by injecting donor DNA through the pollen tube/carpellary septum pathway. This way, the donor parent characters viz. red flower colour and brown seed colour of variety "Red Russian Carmine", was transferred to 'Jayalakshmi' producing white flowers and white seed.

b. Development of transgenic (Bt) tobacco lines toxic to leaf eating caterpillar and budworm: BT tobacco transgenic having Cry 1 A(b) (toxic to *Helicoverpa armigera*) and Cry 1 C (toxic to *Spodoptera litura*) were developed under Hema and Jayasri backgrounds. Evaluation of these transgenic tobacco lines in screen house and limited open experimental field trials confirmed their toxic nature to leaf eating caterpillar and budworm. However, due to non-preference of transgenics in the tobacco trade, the transgenic work was discontinued at the Division.

iii) Tobacco Genomics

- To remove the scarcity of molecular markers, 70 microsatellite markers were developed and validated their applicability in differentiating different types of tobacco and diverse cultivars of FCV tobacco, and their transferability in a wide range of *Nicotiana* species. These SSR maker sequences were deposited which are being used in tobacco molecular breeding. Also, designed and demonstrated the utility of organellar SSR markers (10 cpSSRs and 10 Mt SSRs).

SSR markers specific for identification of *Nicotiana* species and various *N. tabacum* types/group

Species/Types Species	SSR Markers
N. gluaca	TbM44, TbM46, TbM59
N. paniculata	TbM3, TbM29, TbM41
N. thrisiflora	TbM32, TbM41
N. rustica	TbM60
N. trigonophylla	TbM52
N. plumbaginifolia	TbM61
N. sylvestris	TbM49
N. rependa	TbM33
N. corymbosa	TbM60
N. acuminatae	TbM41
N. clevlandii	TbM35, TbM45, TbM47
N. nudicaulis	TbM16, TbM30, TbM31, TbM33 TbM47
N. suaveolense	TbM7, TbM31
N. gossei	TbM4, TbM20, TbM33, TbM35
N. maritama	TbM43, TbM47
N. velutiana	TbM20, TbM43
N. occidentalis	TbM31, TbM44, TbM48, TbM57
N. simulans	TbM43, TbM46
N. goodspeedii	TbM37, TbM44
N. rosulata	TbM21, TbM46, TbM46, TbM52
Types	
FCV	TbM1, TbM9, TbM17, TbM25,TbM33
Chewing	TbM6, TbM14, TbM22, TbM30
Chewing (rustica)and Hookah	TbM8, TbM16, TbM24, TbM32
Burley	TbM2, TbM10, TbM18, TbM26,TbM34
Cheroot	TbM4, TbM12, TbM20, TbM28
Bidi	TbM7, TbM15, TbM23, TbM31
Cigar wrapper	TbM5, TbM13, TbM21, TbM29
Natu	TbM3, TbM11, TbM19, TbM27, TbM35

Designed and validated tobacco chloroplast primers

Name	sequence of Forward primer	Sequence of Reverse primer
NtcpSSR-1	GTAGAAAGACGAAAGTGGATTCG	AATACCCTACCCTGTTCATCTGG
NtcpSSR-2	TAGCTACCGAGATCAATGCAGTC	CATTGGATCTCCTGTCTCATCTC
NtcpSSR-3	CTTTCCGTACCTTCGCTTAATTC	CCCCCATTTTTGTATCATAGACC
NtcpSSR-4	AGACCTTCTCGGTAAAACAGGTC	GCTCCCAAATAATGAATCAGAGC
NtcpSSR-5	TGCAAGAAAATAACCTCTCCTTC	TGGCCTAGTCTATAGGAGGTTTTG
NtcpSSR-6	AATTAAGAACAAAAGCTCGTTGC	TAAAGTTTGGAAGACCACGACTG
NtcpSSR-7	TATATATGTTCTGGGACGGAAGG	CATCGCAAAATCCTAGTACCAATC
NtcpSSR-8	CCATATCAAATGCAGCCTCTATC	CTGAGTTCTTAGCCAAAATTGACG
NtcpSSR-9	TTCGTCGTCGAGAATTGAATAG	ACTGGAAGTGGAAGAGCTATTTG
NtcpSSR-10	CTTTGCCAAGGAGAAGATGC	ATCACTACACTATCACGGCCAAC
NtcpSSR-11	AAAAGAAGAGGTGGTCCGAATAG	AGTCGTCAACATGAAAGCGTAAG
NtcpSSR-12	CCTATTTTGGGTGGATTTAAACTG	TCAAGGTCAATCTATTCACTCGTC

- Molecular diversity of Indian tobacco types as well as several species of *Nicotina* were done with RAPD, SSR markers and AFLP markers.
- ***Development of Mapping Population:*** Trait specific mapping populations were developed for important traits viz., solanesol, nicotine, TSNA, high biomass, seed characters which can be exploited for development of markers through linkage and association mapping approaches.

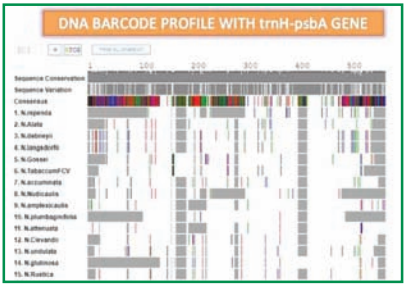
(iv) Molecular biology: Molecular diversity analysis of various tobacco types, Cataloguing of *Nicotiana* species through DNA barcoding, Gene expression analysis under stresses were carried out. The work on genome editing was initiated to develop safer tobaccos with lower level of harmful substances.

- ***Molecular characterization of damping-off pathogen:*** Damping-off is a serious problem in tobacco nurseries. Molecular characterization of pathogen isolates collected from tobacco nursery fields of CTRI Research stations Rajahmundry, Jeelugumilli, Dinhata and Hunsur using ITS (Internal Transribed Spacer) was carried out and the majority of the pathogen isolates were found to be *Pythium aphanidermatum* and a very few as *P. myriotylum*. The ITS gene regions comprising ITS1, 5.8S and

ITS2 were sequenced from isolates are deposited in NCBI Gen Bank (Accession Nos. JX473000, KF425540, KF425541 and KF425542).

- In the gene expression studies, the transcripts of selective candidate genes, Mitogen-Activated Protein Kinase 2 (NtMEK2), Pathogenesis-Related protein (PR1a), Phenylalanine Ammonia-Lyase 1 (PAL1) and Beta-1, 3-glucanase gene (Glunse) are found to be elevated in damping off tolerant tobacco genotypes GT9 and GT5.

- **Cataloguing of genus *Nicotiana* using DNA barcoding:** Species specific DNA barcodes were developed for 24 *Nicotiana* species which belong to 13 different sections of sub genus rustica, tabacum and petunioide using trnH-PsbA and ycf1 loci. The sequence of trnH-PsbA with accessions No. MK072595, MK072596, MK072597, MK075951, MK114101, MK114102 and Ycf1 sequence MT123534, MT101750 were deposited in NCBI Gen Bank.



- **Molecular markers for CMS:** Two CMS specific primers targeting ATP synthase were identified and validated for differentiating CMS and fertile lines.

- ***ITS Sequence Characterization of Orobanche***: Identification of *Orobanche*species, infecting different tobacco types in Andhra Pradesh was carried out based on the sequence characterization of their Internal Transcribed Spacer (ITS) regions and found that *Orobanchecernua* is a predominant species in the tobacco growing regions of Andhra Pradesh.

- ***Detection and characterization of tobacco leaf curl virus:***Tobacco leaf curl disease is widespread in several states in India causing economic losses to farmers. PCR primers specific to coat protein gene (cp) of tobacco leaf curl virus were designed and validated for molecular identification of the disease.

- A computational algorithm for prediction of micro-RNA in tobacco was developed using shell scripting under UNIX environment. It was validated with EST, cDNA and GSS (Genome Survey Sequences).

(v) Genome editing

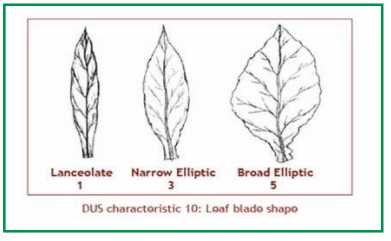
- Burley tobacco contain tobacco-specific nitrosamines (TSNAs) that are considered to be associated with health risks of tobacco consumption. These potent carcinogens are the nitrosated products of nicotine and nornicotine, the major secondary metabolites reported in tobacco. The process of nitrosation is mediated by a key enzyme N-demethylase, a member of CYP (Cytochrome P450) gene family. In order to regulate the TSNA content in burley, various basic studies were conducted initially. The CYP gene variants/homologues related to TSNA formation were analyzed in five *Nicotiana* sequenced genomes. In the *In silico* expression analysis of CYP genes in the database of micro array and RNA sequencing experiments revealed that the expression of CYP82E4 gene coding demethylase is more prominent in high TSNA yielding genotypes than other CYP genes. Further, the *in vitro* expression studies in various burley genotypes revealed that the high converter burley genotypes recorded higher total nitrogen content and have relatively higher expression of CYP82E4 compared to low converters.
- In order to moderate the levels of TSNA in burley tobacco, it was targeted to edit the CYP82E4 gene through CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/ CRISPR-associated protein 9) genome editing tools.
- The guide RNAs targeting the CYP82E4 gene were designed to shutdown the expression of CYP82E4 gene. Analysis of the sequenced database and the expression database of available burley tobacco from Sol genomics networks and other web sources were for the alternate targets using CRISPR tools indicated that the designed gRNAs are in the accepted off target limits. Transformation studies with designed gRNAs are in progress.

8. SEED PRODUCTION

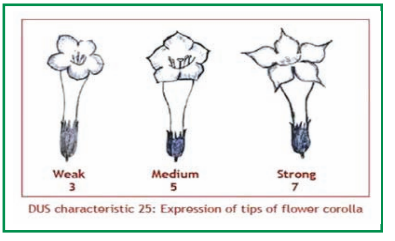
Division takes the responsibility of producing breeder seed of released varieties. Supplies the truthfully labelled seed of released varieties to farmers, thus, meeting 90% seed requirement of tobacco farmers.

9. DEVELOPMENT OF DUS GUIDELINES FOR FCV AND BIDI TOBACCO

The DUS guidelines were developed with 28 characteristics for registration of FCV and *bidi* varieties. These guidelines were notified in the Gazette of India by PPV&FR Authority, New Delhi through an amendment to its earlier notification related to registration of released crop varieties.



DUS characteristic 10: Leaf blade shape



DUS characteristic 25: Expression of tips of flower corolla

10. COPYRIGHTS OBTAINED

Three copyrights were obtained for software's dealing with germplasm characterisation and management from Copyright Authority, Government of India.

- A software titled, *Nicotiana* Species Information System was developed with 90 descriptors for storing, updating and retrieving information on *Nicitiana* species along with images. A copyright obtained for this software system (R. No.: SW-8169/2014).

- A copyright obtained for the software entitled "Rainfed *Natu* Tobacco germplasm Information System(R. No.: SW-13101/2019)" for storing, updating and retrieving information on 40 parameters for various rainfed *Natu* tobacco accessions.

- A user friendly menu driven Digital notebook software was developed, for recording morphological data of tobacco lines in field using mobile, tab and laptop for easy recording, analysis and data export. A copyright obtained for this software (R. No.: SW-13893/2020)

Research Impact

With its concerted research efforts in the area of germplasm management, tobacco breeding and biotechnology, the Division is responsible for releasing/identifying/recommending 107 tobacco varieties including 34 FCV and 73 non-FCV varieties, since inception, for commercial cultivation. In addition to higher yield, some of released varieties possess speciality traits viz., stress resistance and quality traits.

List of released / identified varieties of various tobacco types for cultivation in India

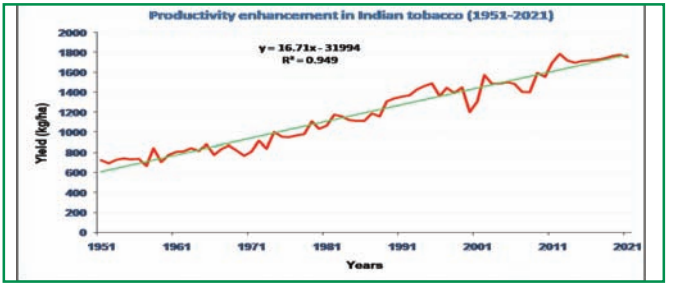
S. No.	Tobacco type	Varieties released / identified
1	Flue-cured tobacco (34 No.)	HarrissonSpl., Chatam, Delcrest, Kanakaprabha, Dhanadayi, CTRI Special,Jayasri, CTRI Spl. (MR), 16/103, FCV special, Godavari Spl., Swarna, Mc Nair 12, Jayasri (MR), Hema, Bhavya, Gauthami, CM 12 (KA), VT 1158, Kanchan, Thrupthi, Rathna, Kanthi, Hemadri, Siri, Sahyadri,FCH222, LT Kanchan, CH-1, N-98, CH-3, CTRI Sulakshana, FCJ-11*, FCR-15*
2	Bidi tobacco (19)	GT 4, NPN 190, Anand 119, Anand 2, Spoorthy (PL 5),GT 5, GT 7, GTH1, Bhavyasree, GT 9, NBD 43, MRGTH-1, ABT 10, Vedaganga 1, GABT-11, Nadyala Pogaku-1,NBD 209, ABD-132*, GABTH-2*
3	Chewing tobacco (23)	Chama, Podali, DP 401, GandakBahar, Sona, Vairam, Thangam,Bhagyalakshmi, Maragadham, Prabha, PT 76, Meenakshi, Vaishali Special, Lichchavi, Manasi, Abirami, Kaviri, Meenakshi (CR), Sangami, Kamatchi, Abirami (CR), DJ-1*, BSR-1*
4	Hookah and Chewing (*Rustica*) Tobacco (11)	DD 437, Sonar Motihari, GC 1,GT 6, GCT 2, GT 8, GCT3, Dharla, Azad Kanchan, ArR-27*, GCT 5
5	OrientalTobacco (1)	Tungabhadra*
6	*Motihari*tobacco (2)	Manasi, Torsa
7	*Natu* tobacco (5)	Prabhat, Vishwanath, *Natu* Special, Gajapati, Bhairavi
8	Cheroot tobacco (4)	DR 1, Bhavani Special, Lanka Special, Sendarapatty Special
9	Cigar-wrapper /filler tobacco (4)	Dixie Shade, S 5, Olor, Krishna
10	Burley tobacco (4)	Burley 21, Banket A1, HDBRG (Dark burley), YB-22*

* Identified for release

Tobacco cultivars possessing speciality traits

Specialty trait	Tobacco cultivar
Biotic stress resistance	
TMV	CTRI Spl. (MR),GodavariSpl., Jayasri (MR), VT 1158, CTRI Sulakshana, FCR-15, MRGTH-1, Banket A1, YB-22, Prabhat
Black shank	Mc Nair 12, CM 12 (KA),Bhavya, Rathna, Kanchan, BSR-1, Vaishali Special, Anand-119, GT-5
Powdery mildew	Swarna
Fusarium wilt	FCH222
Root-knot nematode	Bhavya, Kanchan, ABT 10, NBD-209, Vaishali Special, Anand-119, GT-5
Aphid	CTRI Sulakshana
Caterpiller	Meenakshi (CR), Abirami (CR)
Abiotic stress resistance	
Drought	Thrupthi, Kanthi, Sahyadri, N-98, CM-12
Wetfoot	Rathna
Quality	
Flavorful hybrids	CH-1, CH-3
Low tar	LT Kanchan, ABD-132

All the tobacco varieties cultivated till date in India are either directly released by ICAR-CTRI or facilitated for their release. As a result of crop improvement programmes of ICAR-CTRI, the productivity of various types of tobaccos in vogue is more than doubled (around 2.3 times) compared to those cultivated in 1950s. The productivity potential of FCV reached to 3300 kg/ha and non-FCV to



around 5000 kg/ha. In a case study with FCV tobacco, the rate of gain in cured leaf yield is estimated to be 26 kg/ha out of which > 46 was attributable to genotypic improvement. The tobacco improvement efforts mainly led by the Division has resulted in increase of total tobacco productivity from around 725 kg/ha to 1750 kg/ha. This amount to an improvement of about 17 kg/ha/year since inception of the institute.



Siri: A ruling FCV variety of BS/SLS areas



Kanchan: Pupular FCV variety of NLS



Abirami: Widely cultivated Chewing tobacco variety of TN



FCH-222: A *Fusarium* wilt resistant FCV tobacco variety

The genetic resources supplied by the institute to other organizations has helped in furthering the biological research substantially. The basic studies conducted at the division generated lot of information on various genetical, biochemical and biotechnological aspects relevent to biological research.

In addition, the Division played a great role in skill sharpening and capacity building of thousands of students and other stake holders in the area of Crop Improvement. Since 2001, more than 200 post-graduate/ Ph.D. students successfully completed their research programmes at the Division as part of fulfilling their academic requirements. Number of under-graduate students visit the division regularly and are getting motivated to take up further studies in biology.

FACILITIES AVAILABLE

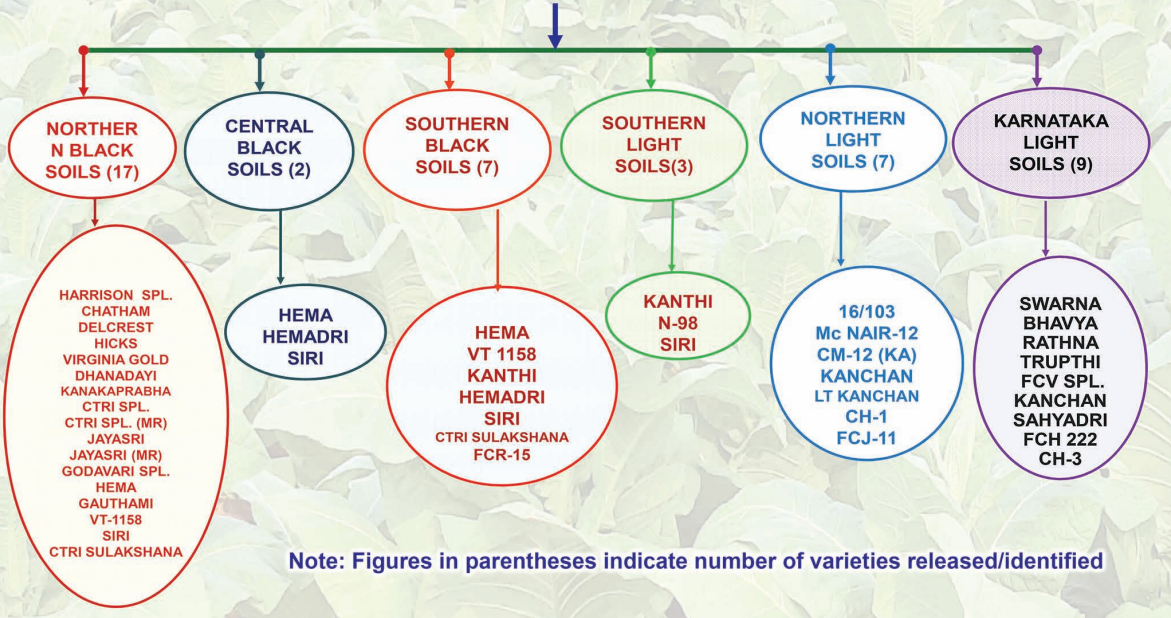
- Ultra-precision balances
- Inverted, research, stereo and phase-contrast microscopes
- Orbital shakers
- Incubator shaker
- Deep freezers (-80°C, -20°C)
- Microwave oven
- Refrigerated centrifuge
- Microcentrifuges
- Ultra-centrifuge
- Ozone generator
- Growth chamber
- Tissue culture facility
- Cell manipulator
- Plate reader
- Elisa reader
- Electrophoresis systems
- Gel documentation system
- Thermal cycler
- Real time PCR
- Transgenic screen house
- Bio-analyser
- Incinerator



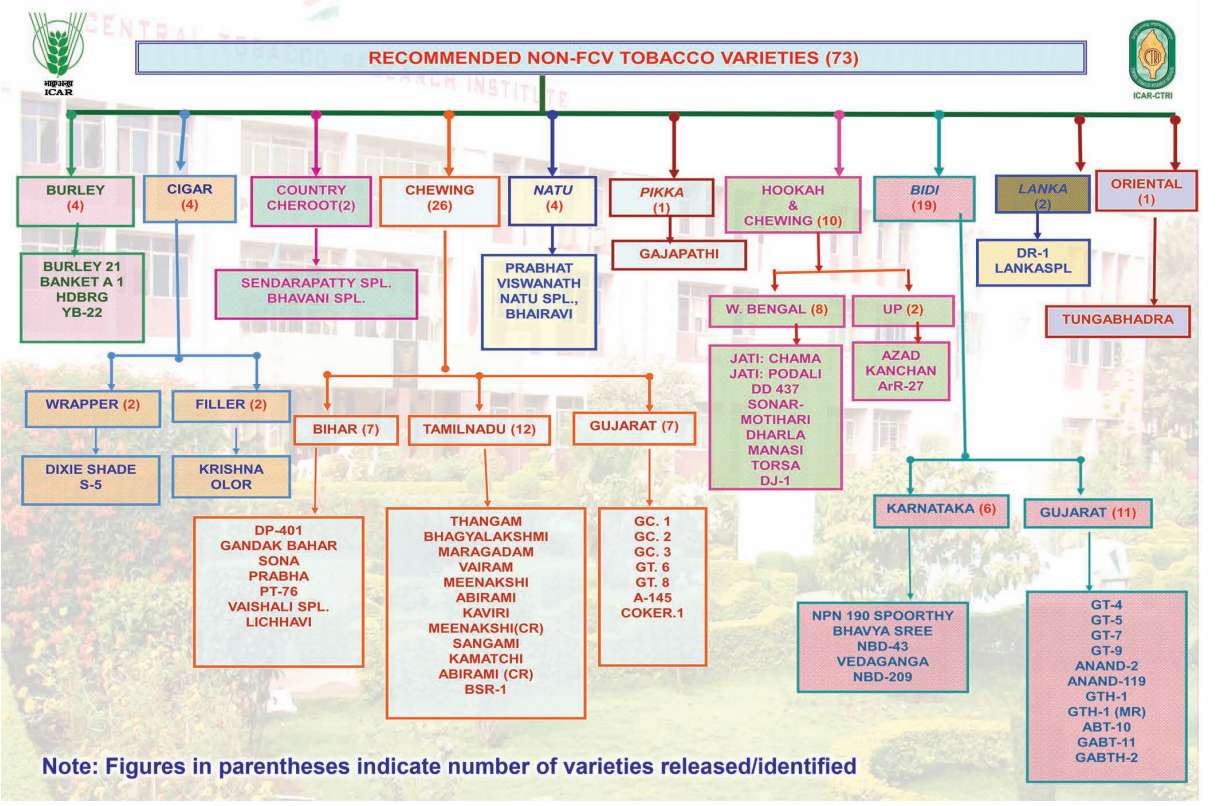




RECOMMENDED FCV TOBACCO VARIETIES (34)*



Note: Figures in parentheses indicate number of varieties released/identified



Note: Figures in parentheses indicate number of varieties released/identified









ICAR - Central Tobacco Research Institute
(An ISO 9001: 2015 Certified Institute)
Rajahmundry - 533 105, Andhra Pradesh, India
Phone: 0883 - 2449871-4, Fax: 0883 - 2448341
e-mail : directorctri@gmail.com, director.ctri@icar.gov.in
website : https://ctri.icar.gov.in