Tobacco Research 48(2): 81-86, 2022

DEVELOPMENT AND EVALUATION OF FCV TOBACCO CYTOPLASMIC MALE STERILE LINES AND THEIR MAINTAINERS FOR YIELD AND YIELD RELATED TRAITS UNDER KLS

C. NANDA AND P. NAGESH

ICAR-CTRI Research Station, Hunsur, Mysore District, Karnataka-571105.

Recieved on 8th July, 2022 and accepted on 28th Nov, 2022

Hybrids are getting popular in FCV tobacco worldwide as they can be developed very quickly in response to an emergency situation such as outbreak of a disease or to combine multiple disease resistance. For the success of hybrids, it is essential to have workable CMS system that does not compromise the yield and quality. Hence, CMS lines suitable to Karnataka light soils were developed with desirable genetic background using different CMS lines. These CMS lines were evaluated for the influence of male sterile cytoplasm on yield and yield related traits in comparison with their respective maintainers. Study indicated significant differences among the CMS lines tested indicating existence of genetic variation among the lines tested. There were no significant differences between mean performance of the male sterile lines and their respective male fertile line (Maintainer) for most of the yield and yield related traits except for number of leaves. Also, within the same nuclear background, no significant differences were noticed among mean of the different CMS sources for all the traits under study. The results indicate that there was no negative effect of CMS sources on the yield and yield related traits studied.

INTRODUCTION

Flue cured Virginia (FCV) tobacco (*Nicotiana tobaccum* L.) is one of the most important commercial crop grown in marginal and rain fed areas having enormous economic benefits to farmers and national exchequer in comparison to other crops cultivated.India is the second largest producer of FCV tobacco; its cultivation is predominately concentrated in the states of Andhra Pradesh and Karnataka producing 120.98 and 68 millionkg respectively (Anonymous, 2023). In Karnataka, it is cultivated on light soils (in the Southern Transitional Zone) as *Kharif* crop under rain fed conditions, popularly known as Karnataka Light Soils (KLS). Cultivar development programmes in India over the past resulted in development and release of twenty nine varieties and two hybrids (Anonymous, 2019) for various tobacco growing regions, of which two varieties (Kanchan and FCH-222) and one hybrid (CH-3) are recommended for KLS region and are popular among farming community.With the escalating cost of inputs especially fertilizer and labor, farmers are unable to realize higher profits. Hence, development of cultivars with higher yield potential than existing cultivars is of paramount importance to enhance farmer's income.Such a system is available for tobacco in the form of cytoplasmic male sterility. Developing hybrid varieties in self-pollinated crops such as tobacco, requires a stable cytoplasmic system which permits large scale crossing to produce hybrids economically (Legg et al., 1974). Cytoplasm male sterile system is of value in facilitating efficient hybrid production and maintenance of genetic purity (Bohra *et al,* 2016). It is pertinent to mention that hybrids are common in tobacco variety cultivation. Tobacco CMS lines and tobacco hybrids account for more than 4/5 of total global planting area (Mo *et al*, 2023), however, not many FCV hybrids are available for cultivation in India.In tobacco, CMS can be directly deployed without the use of restorers for fertility restoration, as leaf is of economic part (Allard, 1960). Currently not many effective sources of CMS is available for leaf production, which may result in narrowing genetic variability and potential vulnerability to diseases such as black root tolerance (Barbec and DorotaLaskowska, (2004) and blue mold (Berbec (2001). Since the CMS lines suitable to KLS were not available, attempts were made to develop stable CMS linesby transferring cytoplasmic male sterility from different source into elite agronomic background

Key words: FCV tobacco, KLS, yield, Cytoplasmic male sterile, maintainers

through backcross breeding at the ICAR- Central Tobacco Research Institute Research Station (CTRI RS), Hunsur and ICAR-CTRI, Rajahmundry. In order to assess the comparative performance of the newly developed CMS lines and counterpart maintainers in terms of yield and yield related parameters the present study was under taken.

MATERIALS AND METHODS

Male sterile cytoplasm from sources such as *N.tabacum*, *N.gossei*, GL 26H MS (EC2), PVH 09 MS (IC2) and *N. undulata*was transferred into five elite FCV tobacco lines/varieties viz., Kanchan, FCH 222 and Rathna, and germplasm accessions such as FCH 201and Cocker 371 Gold by backcross method. The details of the sources of CMS, maintainers and their salient features are given in Table 1.The twelve CMS lines thus developed at ICAR-CTRI RS, Hunsur_were evaluated along with another five CMS lines received from CTRI Rajahmundry and their respective maintainers in a RBD trial for their agronomic performance for two years at ICAR-CTRI Research Station, Hunsur.

The materials were planted with spacing of 1.0 m 0.55 m to accommodate 24plants/plotand good agricultural practices (GAP) adopted for KLS tobacco were followed to raise healthy crop. Morphological observations like plant height (cm), number of leaves per plant, intermodal length (cm),

Table 1: Details of the Cytoplasmic male sterile lines, source of CMS and their maintainers used in the study

Sl.No.	Line	Source of CMS	Maintainer	Salient features of maintainer
1	Kan MS1	AP- 1-8 MS *(N.tabacum)*	Kanchan	A high yielding dark cast popular variety
2	Kan MS2	6-6 MS*(N.gossei)*		
3	Kan MS3	GL 26H MS (EC2)		
4	Kan MS4	PVH 09 MS (IC2)		
5	Rathna MS	6-6 MS*(N.gossei)*	Rathna	A high yielding light cast variety with higher bright leaf yield
6	C371G MS	6-6 MS*(N.gossei)*	Cocker371 Gold	Dark green cast line with early flowering
7	FCH 201 MS1	6-6 MS*(N.gossei)*	FCH 201	A light to medium cast high yielding line with higher bright grade yield and brown spot resistance
8	FCH 201 MS2	GL 26H MS (EC2)		
9	FCH 201 MS3	PVH 09 MS (IC2)		
10	FCH 222 MS1	6-6 MS*(N.gossei)*	FCH 222	A wilt resistant medium cast high yielding variety with higher bright grade yield
11	FCH 222 MS2	GL 26H MS (EC2)		
12	FCH 222 MS 3	PVH 09 MS (IC2)		
13	Kanchan MS 5*	*N. undulata*	KNM	A high yielding dark cast line
14	Kanchan MS 6*	*N. undulata*		
15	Kanchan MS 7*	*N. undulata*		
16	Kanchan MS 8*	*N. undulata*		
17	KN MS P1*		P 18-1	A high yielding dark cast line (one of the parent of the popular hybrid CH3)

* CMS lines received from CTRI Rajahmundry
# The source of GL 26H MS (EC2) and PVH 09 MS (IC2) is not known as they were not mentioned when received from CTRI Rajahmundry

5th, 7th and 9th leaf length (cm) and width (cm) were measured on five randomly selected plants and yield in respect of green leaf, cured leaf, bright leaf were recorded on per plot basis. Top Grade Equivalent (TGE) was estimated using the standard formula. Average leaf area per plant (cm^2) was calculated using average length and breadth of 5th, 7th and 9th leaf as suggested by Suggs et al., (1960). The replication wise mean values of each genotype for various characters were used for statistical analysis. Pooled analysis for replicated data was carried out using TNAUSTAT (Manivannan, 2014). The influence of male sterile cytoplasm on different traits was ascertained using critical difference between the mean values of the CMS lines and their corresponding maintainer lines.

RESULTS AND DISCUSSION

The results of the combined analysis of variance pooled over two years (Table -2) depicted high significant variation among the cytoplasmic male sterile(CMS) lines in respect of all the morphological and yield characters except Total Grade Equivalent (TGE), indicating existence of genotypic differences among them. The source of variation for seasons were highly significant implying that seasons were different, but however the source variation for Seasons Genotypic were non-significant indicating the CMS lines and their maintainer lines had enough genetic buffering ability to counter seasonal variations, which is very much desirable in hybrid seed production (Chaplin, 1964; Amankwa, et al 2019; Bowman and Tart, 2000).

The mean performance of CMS lines and their counterpart maintainer lines in respect of plant height (cm); No of leaves per plant; Average leaf area (cm^2); green leaf yield (kg); cured leaf yield (kg);bright grade yield and total grade equivalentwere presented in Table-3. The results indicate that there was no significant differences between the cytoplasmic male sterile lines and their counterpart male fertile lines for plant height (cm); average leaf area (cm^2); green leaf yield (kg); cured leaf yield (kg); bright grade yield and total grade equivalent indicating that the male sterile cytoplasm has no negative effect on these yield and yield attributing traits. Thus, they are as good as their counterpart fertile lines such as Kanchan and FCH 222 which are released varieties.These cytoplasmic lines can be very well used in hybrid development programs without compromising on the yield. Chen, et al (2020) reported similar non significant differences with respect to several agronomic, economic characters and leaf chemical constitutes while comparing the field performance of tobacco cytoplasm line Nat(gla)SK326 and male fertile MF K326.Amankwa et al (2014) reported non-significant differences in plant height, leaf size, yield and grade index in FCV tobacco hybrids with two different sources of CMS.

However, with respect to number of leaves per plant, there was significant difference with respect to CMS Kan Ms1 and CMS RanthaMS over their counterpart maintainers viz.,_Kanchan and Rathna, respectively. The CMS lines had more number of leaves (21.82 and 18.53, respectively) than their counterpart maintainer lines (19.25 and 16.63 respectively) which is a desirable

Table 2: Combined analysis of variance (AVOVA)for yield and yield related traits of CMS lines and their maintainers pooled over two seasons (2018 to 2021) under KLS conditions.

Source	DF	Plant height	No. of leaves	Avg. leaf area	Green leaf yield	Cured leaf yield	Bright leaf yield	TGE
REPLICATION	2	28.1536	4.9419	6567.012	13.6812	0.2473	0.0703	0.138
TREATMENT (T)	23	579.6431**	5.553**	51103.1054**	9.9493*	0.1784*	0.1166*	0.1089
SEASON (S)	1	32416.514**	279.2552**	3919232**	929.3179**	10.2189**	4.9146**	6.6092**
S × T	23	69.9031	0.2652	5088.735	2.5058	0.14	0.0741	0.0891
ERROR	46	111.1598	1.6427	10357.14	5.1976	0.0863	0.0616	0.0717

* Significant @ p=0.05, **Significant @ p=0.01

Table 3: Mean performance of Cytoplasmic male sterile lines and counterpart maintainers in terms of yield and yield related parameters pooled over two years (2018 to 2021) under KLS conditions.

Sl No.	Entry	Plant height (cm)	No. of leaves	ALA (cm^2)	Green Leaf Yield (kg/plot)	Cured leaf Yield (kg/plot)	Bright Grade (kg/plot)	TGE
1	Kan MS1	84.58	21.82*	759.71	13.03	1.57	1.19	1.20
2	Kan MS2	91.46	19.07	629.73	11.54	1.71	1.21	1.31
3	Kan MS3	63.54	17.60	684.84	11.16	1.50	1.27	1.26
4	Kan MS4	81.04	19.43	685.18	11.43	1.69	1.19	1.23
	KAN	**78.33**	**19.25**	**730.31**	**13.22**	**1.74**	**1.41**	**1.42**
5	Rathna MS	91.69	18.53*	777.68	12.43	1.72	1.41	1.38
	RATHNA	**79.50**	**16.63**	**673.55**	**13.79**	**1.58**	**1.29**	**1.27**
6	C371G MS	67.29	17.78	661.77	8.02	1.22	0.81	0.92
	C371G	**81.46**	**19.25**	**708.60**	**9.84**	**1.18**	**0.91**	**0.94**
7	FCH 201 MS1	96.04	20.17	912.28	12.45	1.88	1.31	1.42
8	FCH 201 MS2	95.00	20.53	773.33	13.49	1.77	1.45	1.45
9	FCH 201 MS3	93.96	19.80	877.29	14.06	2.00	1.36	1.44
	FCH201	**101.67**	**20.17**	**901.27**	**12.54**	**1.68**	**1.24**	**1.27**
10	FCH 222 MS1	101.46	20.53	973.79	14.85	1.99	1.31	1.31
11	FCH 222 MS2	101.46	20.53	1002.00	13.94	1.92	1.45	1.58
12	FCH 222 MS 3	94.38	19.98	881.32	13.38	1.88	1.32	1.45
	FCH222	**92.29**	**20.53**	**945.78**	**13.03**	**1.75**	**1.40**	**1.41**
13	Kanchan MS 5	83.33	18.15	742.11	12.63	1.73	1.22	1.31
14	Kanchan MS 6	80.00	19.98	705.83	13.85	1.67	1.34	1.37
15	Kanchan MS 7	91.04	18.88	717.71	11.84	1.44	1.12	1.14
16	Kanchan MS 8	93.13	20.35	780.35	12.24	1.69	1.34	1.32
	KNM	**81.67**	**19.07**	**670.55**	**12.50**	**1.69**	**1.35**	**1.35**
17	KN MS P1	67.29	18.70	824.40	14.63	2.06	1.54	1.56
	P18-1	**59.38**	**18.33**	**957.79**	**14.89**	**1.92**	**1.54**	**1.54**
	S.E.D	7.4552	0.9063	71.9623	1.6121	0.2077	0.1755	0.1893
	CD at 5%	14.985	1.8216	144.644	3.2403	0.4174	0.3528	0.3804
	CV%	12.34	6.61	12.87	17.95	17.33	19.24	20.18
Seasons								
	2023	67.08	17.67	588.66	9.59	1.37	1.06	1.06
	2018	103.83	21.08	992.77	15.81	2.02	1.52	1.59
	S.E.D	2.1521	0.2616	20.7737	0.4654	0.0599	0.0507	0.0546
	CD at 5%	4.3258	0.5259	41.7552	0.9354	0.1205	0.1018	0.1098
S	Treatments							
	S.E.D	10.543	1.2817	101.77	2.2798	0.2937	0.2482	0.2677
	CD at 5%	NS	NS	NS	NS	NS	NS	NS

* Significant at p=0.05 over their corresponding maintainers

trait.Similar results were reported by Legg et al., (1974) in burley tobacco.

From the Table 3, it is also evident that, there are no significant differences among the different sources of CMS lines viz., *N.tabacum, N.gossei,* GL 26H MS (EC2), PVH 09 MS (IC2) and *N. undulata* with respect to all the yield and yield attributing traits compared within single agronomic background like Kanchan, FCH 201 and FCH 222. This is clearly enunciating that these sources can be equally and effectively utilized in developing new CMS lines thus broadening the genetic basis of the CMS lines as well as the genetic basis of the hybrids derived utilizing them. However, CMS may be susceptible to diseases (Amankwa et al., 2014) hence; CMS lines are to be screened against major prevalent disease under KLS.

The study revealed significant genotypic differences among the lines tested indicating distinctness of the nuclear background chosen. Comparison of the mean performance of the male sterile lines and their corresponding male fertile lines, indicated that there were no significant differences between the two for all the yield and yield related traits except for number of leaves per plant. The study also revealed that mean performance of the lines with different source of male sterile cytoplasm such as *N. tabacum, N. gossei,* GL 26H MS (EC2), PVH 09 MS (IC2) and *N. undulata* but same genetic background (Kanchan, FCH 201 and FCH 222) were comparable indicating that these sources had no negative influence on the traits studied and can be utilized effectively in the plant breeding programs. However, further studies should be made to ascertain the effect of sterile cytoplasm on other traits such as reactionto major prevalent diseases of KLS.

REFERENCES

Allard, R.W .1960. Principals of Plant breeding. John Wily and Sons. Inc., New York.

Amankwa, G. A., S. Mishra, A.D. Shearer, C. Saude, D.L.VanHooren and M. D.Richmond. 2019. CTH144 flue cured tobacco F_1 hybrid. **Can. J. Plant Sci.** 99: 966-968.

Amankwa, G. A., S. Mishra, A.D. Shearer, C. Saude, D.L.VanHooren. 2014. Evaluation of two flue cured tobacco F_1 hybrids with different source of male sterile cytoplasm. CORESTA Congress, Quebec, 2014, Agronomy/Phytopathology Groups, APPOST 02.

Anonymous, 2019. Training manual on breeding of FCV tobacco production, CTRI Rajahmundry (https://ctri.icar.gov.in/)

Anonymous,2023: A. Minhas, Statistica, Consumer goods and FMG21, March, 2023

Berbe ìc, A.; Laskowska, D. 2004.Agronomic Performance of Flue-Cured Tobacco F1 Hybrids Obtained with Different Sources of MaleSterile Cytoplasm. Beitr. **Table Int**. 21, 234–239.

Berbec A. 2001. Floral Morphology and Some Other Characteristics of Iso-genomic Alloplasmics of *Nicotiana tabacum* L. Contributions to Tobacco & Nicotine Research: 19 (6):309-314.

Berbeæ A.; Berbeæ T. 2018. *Nicotiana* species as sources of cytoplasmic male sterility in tobacco breeding.CORESTA Congress, Kunming, 2018, Agronomy/Phytopathology Groups, APPOST 26

Bhora, A, U C. Jha, P. Adhimoolam, D. Bisht, and N. P. Singh. 2016. Cytoplasmic Male S (CMS) in hybrid breeding in field crops. **Plant cell rep** 35, 967-993.

Bowman, D.T and A.G. Tart. 2000. North Carolina measured crop performance: Tobacco: 2000. **N. C. Agric. Res. Serv. Crop Sci. Res. Rep**. No 187.

Chaplin, J.F.1964. Use of male sterile tobaccos in the production of hybrid seed; **Tob. Sci**. 8 105–109.

Legg Paul D., G. B. Collins, and C. C. Litton.1974. Cytoplasmic male sterility and theutillzatlon of hybrids in burley tobacco (*Nicotiana tabacum* L.). **TobSci..**18-58: 160-162

Manivannan,N. 2014. TNAUSTAT-Statistical package. Retrieved from **https://sites.google.com/site/tnaustat**

Mo, Z., Ke, Y., Huang, Y., Duan, L., Wang, P., Luo, W., Que, Y., et al. 2023. Integrated Transcriptome and Proteome Analysis Provides Insights into the Mechanism of Cytoplasmic Male Sterility (CMS) in Tobacco (*Nicotiana tabacum* L.).**J. of Proteomics**, 275:104825.

Suggs C. W. ,J. F. Beeman and W. E. Splinter. 1960. Physical properties of green Virginiatype tobacco leaves Part III, relation of leaf length and width to area. **Tob. Sci**., 4**:** 194-197

Chen X J, Zhi-Jun Tong, Bing-Guang Xiao, Yong-PingLi, Fang-Chan Jiao, YongLi, Tao Pang Dun-Huang Fang,Xing-Fu Wu, Yi-Han Zhang, HeXie, GeBai and Da-Hai Yang. 2020. Identification and evaluation of tobacco cytoplasmic male sterile line NTA(Gla)Sk326 generated from Asymatric protoplast fusion with *N. tabacum* K326. **Plant Cell Tissue and Organ culture (PCTOC)**: 1-15