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Heterosis and character association studies in CMS Hybrids of FCV tobacco (*Nicotiana tobaccum* L.)

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Abstract

Flue Cured Virginia (FCV) tobacco (*Nicotiana tobaccum* L.) is a promising commercial crop in semi-arid and rain-fed areas delivering economic benefits to farmers in comparison to all other crops cultivated. In India, Andhra Pradesh and Karnataka are the major states cultivating FCV tobacco. In Karnataka, it is cultivated in Karnataka Light Soils (KLS) covering southern transitional zone during kharif season as a rainfed crop. Currently, Kanchan, FCH-222 and CH-3 are the popular cultivars grown in KLS region. Release of improved high yielding varieties/hybrids is the key for varietal diversification and yield enhancement of tobacco in this region. In this connection, commercial exploitation of F1 hybrids through heterosis breeding can play a significant role in enhancing yield and quality. Further very few reports on studies on standard heterosis in FCV Tobacco are available. Hence, the present study involving eight CMS hybrids was undertaken to estimate standard heterosis for yield and yield related traits, and to ascertain interrelationships among the yield related characters and their influence on cured leaf yield. Eight CMS based hybrids (CMS2×A4, CMS6×A4, CMS7×A4, CMS10×A4, CMS2×CY142, CMS6×CY142, CMS7×CY142, CMS10×CY14) were evaluated at ICAR-CTRI, Research Station, Hunsur, Karnataka, India along with checks for three years (2018-21). Significant higher heterosis in desirable direction for cured leaf yield and for various yield attributing characters was observed among the hybrids, which can be exploited commercially. Four promising CMS hybrids were identified, CMS7 × A4; CMS6 × A4; CMS10 × A4 and CMS2 × CY142 which recorded significant positive heterosis for cured leaf, can enhance the productivity limit set by present cultivars and would be the components for varietal diversification under KLS. Positive significant associations were noticed between plant height and number of leaves per plant, and intermodal length. The study revealed direct positive effect of plant height on cured leaf and negative indirect effect of number of leaves, intermodal length and leaf area suggesting that these characters should be judiciously selected for achieving higher yield.

Keywords character association, CMS Hybrids, FCV tobacco, heterosis

Introduction

Flue Cured Virginia (FCV) tobacco (*Nicotiana tobaccum* L) is a commercial crop of globally importance where leaf is the main economical produce. India ranks third in the world with an annual production of around 230 million kg from just 0.24% of the country's total arable land area. FCV tobacco is a highly remunerative crop generating revenue of about

Rs 53,750 corers besides foreign exchange earnings of around Rs. 6,500 crores [1]. It is grown largely in the states of Andhra Pradesh and Karnataka in semi-arid and rain-fed areas. In Karnataka, FCV tobacco is cultivated on light soils in the southern transitional zone, popularly known as Karnataka Light Soils (KLS), during Kharif season under rain fed conditions. The popular cultivars grown are Kanchan, CH-3 and FCH-222, occupying around 68%, 31 % and 1% of the cultivated area under FCV tobacco respectively producing 93.54 mkg of cured leaf [2]. The realization of yield potential of these varieties at farm level is reported to be lower than the reported potential yield of 2000 kg/ha [3], which may be attributed to non-adaptability of the cultivars to frequent climatic fluctuations and various biotic and abiotic factors. Hence there is a need to develop cultivars having high productivity and quality leaf under fluctuating climatic scenario/situations.

Developing hybrid varieties through systematic exploitation of the phenomenon of heterosis [4] using Cytoplasm Male Sterility (CMS) system is fast and simple method. Commercial exploitation of F1 hybrids through heterosis breeding can play a significant role in FCV tobacco breeding endeavors for yield enhancement [5]. Although Flue Cured Virginia (FCV) tobacco (*Nicotiana tobaccum* L) is considered as an autogamous plant, modest levels of heterosis are expressed. Studies on the effect of heterosis for various yield and yield related characters have been reported in FCV tobacco [6-15]. Heterosis has been exploited to produce commercial FCV tobacco hybrids [16-18] in other tobacco growing regions of the world; however, not many FCV hybrids are available for cultivation in India. Often heterosis is estimated as the percentage increase or decrease in mean F1 performance over the average of the two parents (mid parent/average heterosis) or over the better parent (Better parent heterosis) or over the standard checks (Standard heterosis). Standard heterosis is defined from a commercial perspective as the degree of hybrid efficiency over the best available variety [19] is of practical importance and can be a base for enhancing the yield and in turn productivity beyond the limit set by existing popular cultivars grown in the region. Yield is a complex character and is determined by various yield contributing characters which are controlled by multiple genes and environmental factors [20, 21]. Direct selection for high yield in general is difficult due to its complex nature. Therefore, indirect selection through yield contributing components like leaf area index, flowering date, leaf length, plant height, and number of leaves can be an option for increasing tobacco dry leaf yield [22]. Studies reported on standard heterosis in FCV Tobacco in India are very few. The current research was carried out with a primary objective of estimating heterosis over the standard commercial cultivars, Kanchan, FCH 222 and CH3 in respect of eight characters viz., plant height, internodal length, green leaf yield per plant, leaf area per plant, cured leaf yield per plant, bright leaf yield per plant, number of leaves per plant, and total grade equivalent among the eight CMS based hybrids of FCV tobacco. Secondary objective was to generate information on interrelationships among above mentioned yield related characters, their relationship with cured leaf yield per plant as well as their direct and indirect influence on cured leaf yield in CMS hybrids.

Methodology

There were eight CMS-based hybrids in the materials (CMS2×A4, CMS6×A4, CMS7×A4, CMS10×A4, CMS2×CY142, CMS6×CY142, CMS7×CY142, CMS10×CY14) which were synthesized by crossing four CMS lines viz., CMS2, CMS 6, CMS7 and CMS10 with two late maturing germplasm accessions A4 and CY142. The description of the lines utilized in crossing program is presented in Table 1.These hybrids were evaluated at ICAR-CTRI, Research Station, Hunsur, Karnataka along with two varietal checks. Kanchan and FCH-222 and one hybrid check, CH3 in Randomized Block Design (RBD) with three replications for crop seasons, 2018-19, 2019-20 and 2020-21. The materials were planted with spacing of 1.0 m × 0.55 m and 40plants/plot was maintained. Standard agricultural package practices adopted for KLS tobacco were followed to raise healthy crop. Morphological observations like plant height (cm), number of leaves per plant, intermodal length (cm), 5^{th} , 7^{th} 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 and later converted to per plant basis using plant population in each plot.

Table 1. Description of lines used in crossing program

SN.	Line	Salient features
1	CMS2	Cytoplasmic male sterility in Kanchan genetic background. Kanchan is popular released variety with dark green leaves
2	CMS6	Cytoplasmic male sterility in Coker 371 Gold genetic background. Coker 371 Gold is North Carolina line with very dark green leaves
3	CMS7	Cytoplasmic male sterility in FCH201 genetic background. FCH 201 is high yielding, fast ripening line with light green leaves
4	CMS10	Cytoplasmic male sterility in FCH222 genetic background. FCH 222 is wilt resistant high yielding line with medium green leaves
5	A4	Line with more number of leaves, very short internodes, long leaves and late maturing
6	CY142	Line with broad leaves, late maturing

Top Grade Equivalent (TGE) was estimated using the standard formula. Average leaf area per plant was calculated using average length and breadth of 5th, 7th and 9th leaf as suggested by Suggs et al., [23]. For statistical analysis, the replication-wise means of each genotype for each of the different characters were considered. According to the method recommended by Falconer and Mackay, the degree of heterosis was estimated in reference to standard checks for features that indicated considerable variations as given below [24].

$$SH\% = (F1 - SV) * 100/SV$$

Where, F1 = Mean value of a cross
SV = Mean value of standard check
SH= Standard heterosis

Statistical analysis for calculation of correlation was worked out as per Al-Jibouri et al., [25] and path coefficient of various characters was calculated according to Deway and Lu [26].

Results and Discussion

Heterosis studies

The standard heterosis or the economic heterosis was estimated as percent increase in hybrid over standard commercial check varieties, Kanchan, FCH222 and CH3 for eight quantitative characters. The analysis of the results (Table 2a and b) revealed that the number of hybrids exhibiting significant standard heterosis (in terms of both magnitude and direction) varied with the traits studied. In general, standard heterosis was in favourable direction for most of the traits studied. When compared to plant morphological traits, cured leaf yield per plant and yield component attributes exhibited higher standard heterosis. Most of the hybrids tested had plant height in between shorter and taller check varieties. Over Kanchan five hybrids showed significantly higher positive heterosis for plant height and all the eight hybrids over CH3 and four hybrids over check FCH222 showed significant positive heterosis. However, three hybrids recorded significant negative heterosis over FCH 222. Ali et al., [27] reported considerable positive heterosis for plant height in tobacco. For the number of leaves per plant three hybrids (CMS 10 × A4, CMS 2 × CY142, and CMS 10 × CY142) showed higher heterosis in desirable direction over all the standard controls. Heterosis in desirable direction for number of leaves per plant could be because of its positive correlation with yield. Standard positive

Table 2a. Standard heterosis of the hybrids for various morphological traits over Kanchan, FCH 222 and CH3

Character	Plant height			Number of leaves			Internodal length			Average leaf area		
Hybrids	Kanchan	FCH 222	CH3	Kanchan	FCH 222	CH3	Kanchan	FCH 222	CH3	Kanchan	FCH 222	CH3
CMS 2 × A4	7.57**	2.48*	13.27**	2.74	1.21	2.58	4.72**	1.31	10.44**	3.31	8.83*	12.62**
CMS 6 × A4	1.09	-3.69**	6.44**	1.67	0.15	1.51	-0.58	-3.82**	4.85**	-1.95	3.29	6.89
CMS 7 × A4	7.66**	2.57*	13.36**	4.57**	3.01*	4.41**	2.91**	-0.44	8.53**	-5.78	-0.75	2.71
CMS 10 × A4	0.92	-3.86**	6.26**	1.04	-0.46	0.89	-0.13	-3.38**	5.32**	1.55	6.98*	10.7**
CMS 2 × CY142	12.57**	7.25**	18.54**	5.05**	3.49*	4.89**	7.12**	3.63**	12.97**	7.65*	13.4**	17.36**
CMS 6 × CY142	5.64**	0.64	11.23**	1.76	0.25	1.6	3.75**	0.38	9.42**	6.72*	12.42**	16.34**
CMS 7 × CY142	1.75	-3.07*	7.14**	1.82	0.31	1.67	-0.06	-3.32**	5.39**	7.57*	13.32**	17.27**
CMS 10 × CY142	9.85**	4.65**	15.67**	4.43**	2.87*	4.27**	5.18**	1.75*	10.92**	14.34**	20.45**	24.65**
No. of significant crosses	5	7	8	3	3	3	5	5	8	4	6	6
Positive	5	4	8	3	3	3	5	2	8	4	6	6
Negative	-	3	-	-	-	-	-	3	-	-	-	-
SE±	**0.9331**			**0.2014**			**0.0290**			**25.1872**		

Table 2b. Standard heterosis of the hybrids for yield traits over Kanchan, FCH 222 and CH3

Character	Green leaf yield			Cured leaf yield			Bright leaf yield			TGE		
Hybrids	Kanchan	FCH 222	CH3	Kanchan	FCH 222	CH3	Kanchan	FCH 222	CH3	Kanchan	FCH 222	CH3
CMS 2 × A4	21.44**	25.38**	16.95**	9.14	18.06**	2.48	2.23	30.06**	1.3	7.8	27.38**	1.44
CMS 6 × A4	14.37**	18.08**	10.14*	16.21**	25.71**	9.12*	9.95	39.88**	8.95	14.22*	34.96**	7.47
CMS 7 × A4	23.58**	27.6**	19.01**	24.65**	34.84**	17.05**	28.67**	63.69**	27.5**	29.37**	52.86**	21.73**
CMS 10 × A4	14.13**	17.84**	9.91*	15.56**	25**	8.51	22.48**	55.81**	21.36**	21.09**	43.09**	13.94*
CMS 2 × CY142	4.31	7.7	0.46	10.77*	19.83**	4.01	11.17	41.43**	10.16	13.29*	33.87**	6.6
CMS 6 × CY142	2.36	5.68	-1.43	2.43	10.81*	-3.81	-6.29	19.21*	-7.15	0.28	18.49*	-5.64
CMS 7 × CY142	2.92	6.26	-0.88	-3.34	4.57	-9.23*	-5.18	20.63*	-6.04	-2.36	15.37*	-8.13
CMS 10 × CY142	-0.62	2.61	-4.3	-0.07	8.1	-6.16	-7.24	18.01*	-8.08	-3.67	13.83	-9.36
No. of significant crosses	4	4	4	4	6	3	2	8	2	4	7	2
Positive	4	4	4	4	6	2	2	8	2	4	7	2
Negative	-	-	-	-	-	1	-	-	-	-	-	-
SE±	**25.7488**			**3.6460**			**3.0311**			**3.3285**		

heterosis for number of leaves per plant was reported by several researchers [14, 27-35]. Significant standard heterosis in both positive and negative direction was noticed for inter nodal length among the hybrids tested. Five hybrids recorded significant positive heterosis over Kanchan, two hybrids over FCH222 and all the hybrids over check CH3. While three hybrids found to have significant negative heterosis for intermodal length over FCH222. Significant standard negative heterosis was reported by Megha Ganachari et al., [14], while Qaizar and Mohammad [36] reported larger internodes in hybrids over parents in FCV tobacco. Significant positive heterosis was observed for average leaf area. Four hybrids over Kanchan and six hybrids over FCH222 and CH3, recorded significant positive heterosis. The highest significant heterosis was recorded on the hybrid CMS 10 × CY142 (24.65) over hybrid check CH3. Qaizar and Mohammad [36] reported positive significant heterosis in studies of tobacco exotic hybrids under varied environments. Wajahat Ali et al., [32] reported 47.6% more leaf area in hybrids over check variety. MeghaGanachari, et al., [14] reported standard heterosis in range of 11.28 to 37.85 in many crosses. Several researchers found similar results for leaf area [31, 37-39]. Significant heterosis mostly in positive direction was noticed for cured leaf yield, bright leaf yield green leaf yield, and TGE. Four hybrids CMS2 × A4, CMS6 × A4, CMS7 × A4 and CMS10 × A4 recorded significant positive heterosis over all the standard checks. Hybrids exhibit considerable positive heterosis for green leaf yield, according to other researchers also [27, 35, 40-41]. In case of cured leaf yield, six hybrids exhibited significant positive heterosis over FCH222, four hybrids over Kanchan and two hybrids over CH3 while only one hybrid registered significant negative heterosis. Similar trend was observed for TGE. For bright leaf yield, all the eight hybrids tested recorded significant positive heterosis over check FCH222. While over Kanchan and CH3 only two hybrids (CMS7 × A4 and CMS 10 × A4) recorded significant positive heterosis for bright leaf yield. Ramachandra et al., [42], Lalitha et al., [35], Aleksoska and Alksoski [29] and Ramanarao et al., [11] reported positive significant heterosis over check for total cured leaf yield. MeghaGanachari et al., [14] reported standard positive and negative heterosis in ten crosses which ranged from-45.14 to 15.77. Estimates of heterobeltosis for cured leaf yield in range of -16.0 to 9.94 were reported by Qaizar Ahmed et al., [43]. 19.33 percent increase in cured leaf yield per plot over check variety was reported by Wajahat Ali et al., [32]. MeghaGanachari et al., [14] reported positive significant standard heterois for TGE in their breeding studies. The hybrids which manifested higher heterotic effects for cured leaf yield per plant also showed significant positive and desirable heterosis for various yield attributing characters. Existence of significant heterosis in desirable direction for all the traits indicates scope for exploiting the same for commercial purpose.

Character association studies

Efforts were made to understand the interrelation among various characters and their influence on cured leaf yield per plant in CMS hybrids by estimating genotypic and phenotypic correlation coefficients. Magnitude of genotypic correlations for characters studied was higher than their corresponding phenotypic correlations implying significance of genetic coherence in the expression of these traits (Table 3). Correlation between different characters displayed that plant height had consequential positive association with number of leaves per plant (rg = 0.996, rp= 0.8123) and intermodal length (rg = 0.999, rp= 0.0.9303); while intermodal length exhibited remarkable positive association with average leaf area (rg = 0.6326, rp= 0.5092). Similar significant positive association of plant height with number of leaves was observed by Shah et al., [44] and Shubha et al., [45]. Sherzahmed and Fidaahmed [46] reported significant positive association of plant height with intermodal length and significant association of intermodal length with leaf area. None of the morphological traits had significant correlation with the cured lead yield. However, green leaf yield, bright leaf yield and TGE had high significant correlation with cured leaf yield. Significant positive correlation of green leaf yield with cured leaf yield was earlier reported by several researchers [44-50]. Similarly, significant positive correlations for bright leaf yield with cured leaf yield were reported by different researchers [44-47, 49] and Shubha et al., [45] observed significant positive correlations of TGE with cured leaf.

Table 3. Genotypic and phenotypic correlation coefficients for yield and yield related traits in CMS hybrids of FCV tobacco

Genotypic/ Phenotypic	PH	NOL	INT	ALA	GLY	BLY	TGE	CLY
PH	1.000 **	0.9976 **	0.999 **	0.5931	0.0859	0.0385	0.0428	0.0766
NOL	0.8123 **	1.000 **	0.9935 **	0.498	0.2675	0.3398	0.3444	0.3509
INT	0.9303 **	0.5419 **	1.000 **	0.6326 *	0.0122	-0.0923	-0.0878	-0.0416
ALA	0.5344 **	0.4124 *	0.5092 **	1.000**	-0.355	-0.2323	-0.2971	-0.343
GLY	0.1304	0.2694	0.0224	-0.1641	1.000 **	0.7811**	0.8274 **	0.8758 **
BLY	0.0507	0.2167	-0.0633	-0.1777	0.7565**	1.000 **	0.9997 **	0.9675 **
TGE	0.0813	0.2633	-0.0483	-0.1584	0.8085**	0.9702 **	1.000 **	0.9959 **
CLY	0.1187	0.3178	-0.0279	-0.2339	0.8254**	0.9064 **	0.9493 **	1.000**

PH- Plant height, NOL- Number of leaves, INT- Internodal length, ALA- Average leaf area,
GLY- Green leaf yield, CLY- Cured leaf yield,
BLY-Bright leaf yield, TGE-Top Grade Equivalent
*** Significant at 5%,**
**** Significant at 1%**

Table 4. Estimates of direct and indirect effects of yield components on cured leaf yield at genotypic level in CMS hybrids of FCV Tobacco

Genotypic	PH	NOL	INT	ALA	GLY	BLY	TGE
PH	3.6539	-1.01134	-2.4856	-0.12935	0.02768	0.05398	-0.03266
NOL	3.64517	-1.01376	-2.47176	-0.10859	0.08623	0.4763	-0.26265
INT	3.6501	-1.00707	-2.48819	-0.13796	0.00393	-0.12934	0.06693
ALA	2.16727	-0.50481	-1.57403	-0.21808	-0.1144	-0.3256	0.22662
GLY	0.31372	-0.27118	-0.03031	0.07737	0.32234	1.09491	-0.63102
BLY	0.14071	-0.34448	0.22958	0.05066	0.25179	1.40172	-0.76248
TGE	0.15646	-0.34911	0.21836	0.0648	0.2667	1.40134	-0.76269

Residual-0.0186, PH- Plant height, NOL-Number of leaves, INT-Internodal length,
ALA-Average leaf area, GLY-Green leaf yield, CLY-Cured leaf yield, BLY-Bright leaf yield,
TGE-Top Grade Equivalent

To ascertain the magnitude and direction of direct and indirect effects of characters influencing cured leaf yield, the correlation coefficients were partitioned through path coefficient analysis. The results revealed that plant height had direct positive effect on cured leaf while number of leaves, intermodal length and leaf area had negative direct effect indicating that optimizing of these traits are necessary for achieving higher yield (Table 4). Similarly, green leaf and bright leaf had direct positive effect on cured leaf yield.

Conclusion

The hybrids manifested higher heterotic effects for cured leaf yield per plant as well as for various yield attributing characters. Existence of significant heterosis in desirable direction for all the traits indicates scope for exploiting the same for commercial purpose. Four CMS hybrids CMS7 × A4; CMS6 × A4; CMS10 × A4 and CMS2 × CY142 exhibited significant heterosis for cured leaf. These CMS hybrids can enhance productivity beyond the limit set by present popular varieties Kanchan, FCH222 and CH3 and aid in varietal diversification under Karnataka light soils of India. Plant height had significant positive association with number of leaves per plant and intermodal length; while intermodal length exhibited significant positive association with average leaf area. From the path coefficient analysis it was known that the plant height had direct positive effect on cured leaf while number of leaves, intermodal length and leaf area had negative direct effect indicating that optimizing of these traits are necessary for achieving higher yield.

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