Indian J. Genet. Plant Breed., (2025); 85(1): 157-164
https://doi.org/10.31742/ISGPB.85.1.18
ISSN: 0975-6906
www.isgpb.org

RESEARCH ARTICLE

Assessment of CMS based Flue Cured Virginia tobacco (*Nicoitiana tobaccum* L.) hybrids for stability of marketable yield traits in light soils of Karnataka

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Abstract

Flue-cured Virginia (FCV) tobacco (*Nicoitiana tobaccum* L.) cultivation in the southern transition zone of Karnataka light soils is frequently prone to drastic seasonal variations of climatic factors like rainfall, temperature and sunshine hours. Hence, it is essential to evaluate and identify the cultivars with stable and high mean performance across seasons/environments. In the present study, eight FCV tobacco CMS hybrids and three standard checks were evaluated for yield and yield-related parameters in RCBD with three replications over three crop seasons. AMMI model and GGE biplot were used to detect and characterize genotypic × environmental interaction (GEI) and to identify stable hybrids across the environments. Combined analysis of variance revealed that genotypes and environments differed significantly and GE interaction was significant for cured and bright leaf yield. AMMI II model family was adequate to explain the detected variation attributable to GEI. Two hybrids CMS 7 × A4 and CMS 10 × A4 were found to be stable with high mean performance for marketable yield traits across the seasons based on both stability parameters as well as GGE biplot analysis. Hybrid CMS 7 × A4 with YREM values near unity is likely to maintain its high leaf yield potential across environments even in the presence of cross-over GEI.

Keywords: Genotype x environment interaction, FCV Tobacco, AMMI stability value, stability index, leaf yield

Introduction

Flue-cured Virginia (FCV) tobacco (*Nicoitiana tobaccum* L.) is an economically important crop grown globally on marginal and sub-marginal lands where no other crop can realize profit as much as tobacco. India is the second largest producer of tobacco in the world after China. It is also the fourth largest producer of flue-cured Virginia (FCV) tobacco in the world after China, Brazil and Zimbabwe. In India, FCV tobacco is grown in selected districts of Andhra Pradesh and Karnataka with an annual production of 300 million kg during 2023-24. It accounts for 30% of total tobacco production and 85% of overall tobacco exports in the country. In Karnataka, FCV tobacco is grown as *khraif* crop in the Southern Transition Agroclimatic zone (STZ) on red sandy loam/ red loamy and lateritic soils, popularly known as Karnataka Light Soils (KLS). The tobacco produced in this region has unique quality specific to the region and has great demand in the global market and thus >90% of it is exported.

In FCV tobacco, a cured leaf is the economical part and the proportion of bright leaf influences its profitability, therefore, the main goal of breeding is increasing cured leaf yield with more proportion of bright leaf. The yield in any crop is a complex trait under the influence of polygenes and is considerably affected by the environment. In tobacco also, major yield contributing traits are complex traits under the control of polygenes and are considerably affected by the environment. Several environmental factors such as

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How to cite this article: Nanda C., Nagesh P., Gangadhara K., Sarala K., Rajappa J.J. and Madhav M.S. 2025. Assessment of CMS based Flue Cured Virginia tobacco (*Nicoitiana tobaccum* L.) hybrids for stability of marketable yield traits in light soils of Karnataka. Indian J. Genet., **85**(1): 157-164.

Source of support: Nil

Conflict of interest: None.

Received: June 2024 **Revised:** Dec. 2024 **Accepted:** Feb. 2025

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temperature, rainfall pattern and quantity, field temperature, sunshine hours, etc. have greatly impacted tobacco yield and quality (Liu et al. 2016; Mahadevaswamy et al. 2017; Prasad et al. 2019; Tang et al. 2020), leading to huge differences in cured leaf yield and bright leaf over seasons. However, the performance of the genotype and environment are not always additive, because of the interplay between genetic and non-genetic effects causing differential relative performance of genotypes in different environments and is termed as GEI (Genotypic × Environmental interaction). Knowledge of GEI effects on FCV tobacco yield is essential to develop or identify tobacco genotypes stable across temporal environments in order to sustain FCV tobacco production in KLS. However, no information is available on these aspects for the KLS region of tobacco and hence, the present investigation was undertaken to understand GEI effects and to identify high-yielding and stable genotypes across environments using AMMI (Gauch and Zobel 1989) and GGE-bi-plot technique (Yan et al. 2000).

Materials and methods

Materials used and conduct of experiments

Eight CMS-based hybrids were synthesized by crossing four CMS lines viz., CMS2, CMS 6, CMS7 and CMS10 with two late-maturing germplasm accessions, A4 and CY142. The genotypes/hybrids evaluated were given codes (Table 2). G1-CMS2×A4; G2-CMS6×A4; G3-CMS7×A4; G4-CMS10×A4; G5-CMS2×CY142; G6-CMS6×CY142; G7-CMS7×CY142; G8-CMS10×CY14; G9-Kanchan (Check); G10-FCH-222 (Check) and G11-CH3 (Hybrid Check). 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/environments, 2018-19 (E1), 2019-20 (E2) and 2020-21 (E3). The environmental conditions obtained at each location during the study are provided in Supplementary Table S1.

The experimental location is situated12.31°N 76.29°Eat an elevation of 792 meters (2598 feet). The materials were planted with a spacing of 1.0 m between rows and 0.55 m between plants. Standard agricultural package practices adopted for KLS tobacco were followed to raise healthy crops. Morphological observations like plant height (cm), number of leaves per plant, intermodal length (cm), 5th, 7th and 9th leaf length (cm) and width (cm) were measured on five randomly selected plants and yield with respect to green leaf, cured leaf, bright leaf was recorded on per plot basis and later converted to per plant basis using plant population in each plot. Top grade equivalent (TGE) was estimated using the standard formula. The average leaf area per plant was calculated using the average length and breadth of the 5th, 7th and 9th leaf as suggested by Suggs et al. (1960).

Statistical analysis

The phenotypic data of various yield and yield-related traits recorded on eleven genotypes (including checks), evaluated across three seasons were subjected to combined ANOVA using a mixed linear model (R core team). Based on combined analysis results, replication-wise mean cured and bright leaf yield data of eight hybrids and three checks were subjected to statistical analysis following additive main effects and multiplicative interaction (AMMI) model (Gauch and Zobel 1988) to detect and characterize the patterns of interaction of hybrids with environments represented by three seasons. The sum of squares attributable to a signal-rich component of GEI (GEI $_{signal}$) was computed as per the procedure (Gauch 2013). The additive main effects of hybrids and seasons were fitted by univariate ANOVA followed by fitting by principal component (PC) analysis based on the following AMMI II model implemented using R Studio software v.4.2.1. GGE bi-plot which utilizes a combination of GGE concepts and AMMI bi-plot (Yan et al. 2000) was used analyze GEI patterns and relative stability of test hybrids. AMMI model-based parameters such as AMMI stability value (ASV) (Purchase et al. 2000) and stability index (SI) (Farshadfar 2011) were used to determine the stability of each hybrid. Apart from these, a simple static yield relative to environment maximum (YREM) (Yan 1999) was used to detect crossover GEI and to quantify the reduction in yield potential of test hybrids due to crossover GEI. This analysis was carried out in Microsoft Excel software. The relative stability of the hybrids was assessed based on the AMMI model-based stability parameters as well as through visual interpretation using GGE bi-plot. AMMI Stability Value (ASV) helps to identify stable genotypes across the environments.

Results and discussion

Combined analysis of variance over the seasons revealed significant variation of genotypes (G) and environments (E) for all the traits except leaf number and internode length (Table 1). The results indicate that there are substantial differences among the hybrids tested and seasons evaluated thus justifying the need to study stability and GEI among the tested hybrids. The Box-Whisker plot depicts the range of cured leaf and bright leaf yield of test hybrids and checks across three seasons (Figs.1a and 1b). Hybrid CMS 7 × A4 was the high yielder for both the traits followed by CMS 10 × A4.

AMMI model-based detection and characterization of GEI effects

The AMMI analysis of variance (ANOVA) revealed significant MSS for genotypes indicating the existence of significant variation for cured leaf yield and bright leaf yield (Table 2) implying the existence of genetic differences among the tested genotypes. The environmental effects for cured leaf

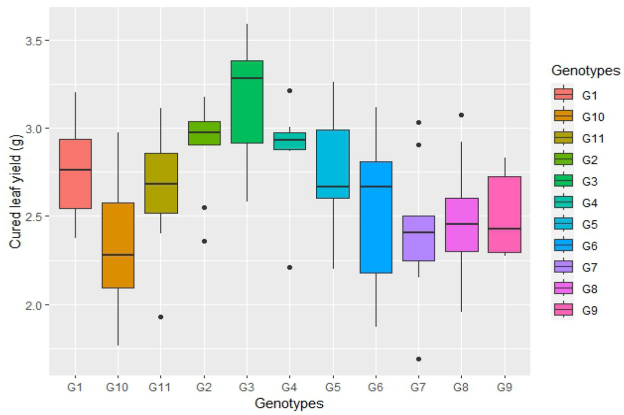

Fig. 1a

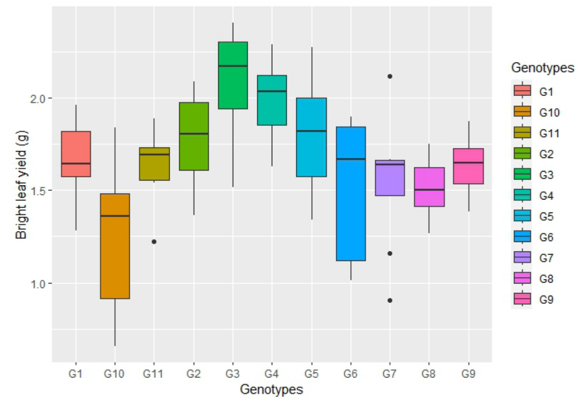

Fig. 1b

Figs. 1a and 1b. Box-Whisker plots showing significant differences among test hybrids for cured leaf yield (Fig. 1a) and bright leaf yield (Fig. 1b)

Table 1. Combined analysis of variance for yield and yield-related traits of FCV tobacco hybrids along with three check cultivars evaluated over three seasons/environments

Traits		PH	NOL	INT	ALA	GLY	CLY	BLY	TGE
Source	Df	Mean Sq	Mean Sq	Mean Sq	Mean Sq	Mean Sq	Mean Sq	Mean Sq	Mean Sq
ENV	2	1186.803**	26.43525**	0.095067	496541.2**	976695**	8221**	3041.6**	2672.7**
REP (ENV)	6	252.9532**	6.62697**	0.070015	23243.4	5013	391	91.5	69.6
GEN	10	299.5621**	2.189515	0.257352	56052.86**	62339**	1108**	991.3**	936.9**
GEN:ENV	20	84.26492	2.030364	0.077922	7564.733	11318	506*	191.7**	152.9*
Residuals	60	81.25666	1.964414	0.150842	16270.66	7889	292	65.5	73.8
CV (%)		7.839819	6.399298	7.395562	11.00523	9.15	14.07	10.62	9.96
Overall mean		114.9803	21.90202	5.251587	1159.053	970.68	121.40	76.21	86.29

PH = Plant height (cm); NOL = No. of leaves; INT= Internodal length (cm); ALA = Average leaf area (cm^2); GLY = Green leaf yield (gm/plant); CLY = Cured leaf yield (gm/plant); BLY = Bright leaf yield (gm/plant) and TGE = Top grade equivalent

yield and bright leaf yield were also significant suggesting that environmental factors like field temperature, rainfall, and sunshine hours had profound effects in influencing these traits. Differences among the tested genotypes and environments are a prerequisite for the existence of GEI. In the present study, highly significant GEI indicated that the response of test hybrids varied across the tested environments. The sum of squares (SS) attributable to GEI, was further portioned into those attributable to (a) GE_{signal} attributable to repeatable and predictable components and (b) GE_{noise} attributable to non-repeatable and unpredictable components. The contribution of GE_{signal} to SS GEI with respect to cured leaf yield and of bright were 42.24 and 65.82 percent, respectively indicating that the major portion of the detected GEI effects are repeatable and hence, predictable. The significant GEI necessitated the need to identify stable hybrids with high marketable yield as the location is a non-variable factor from the KLS farmer's point of view (as a majority are small and marginal farmers) and thus the consistency of marketable leaf yield of test hybrids over years ensures the stability of its performance even under seasonal variations of environmental factors. AMMI model-based stability parameters estimated have been presented in Table 3. AMMI Stability Value (ASV) helps to identify stable hybrids across the three seasons and was estimated as the distance from zero in a two-dimensional scatter-plot of IPC1 score against IPC2 score (as IPC1 and IPC2 contributed significantly towards GEI). In the present study, hybrids CMS 7 × A4 (G3) with a lower magnitude of ASV were considered to be a genotype with stable cured leaf yield across three seasons, while for bright leaf yield, CMS 2 × Cy142 (G5) was considered as stable (Table 3).

Stability analysis requires AMMI model diagnosis and in the present study, more than 99.99% of SS due to GEI was explained by the first two IPC's viz., ICP1 and ICP2. Further, the significance of MSS of ICP1 and ICP2 indicates the adequacy of the AMMI 2 model with respect to both cured leaf and bright leaf yield. Significant GEI for tobacco leaf yield has been earlier reported by researchers (Ahemd et al. 2019; Fashalami et al. 2022; Kurt 2020, 2023), while Justify et al (2018) for bright leaf yield using AMMI analysis. Sadeghi et al. (2011) reported the significance of GEI for dry tobacco leaf yield.

Assessment of Stability Based on GGE Bi-plot

The stability of the test hybrids over years/seasons is important as it will help in reducing susceptibility to unpredictable components of GEI effects. The ICP1 and ICP2 with respect to cured leaf yield and bright leaf data of eight hybrid genotypes with three checks across three environments were used in the construction of GGE bi-plots. GGE bi-plots graphically display the interaction between each genotype and each environment. There are various ways to use and interpret GEI bi-plot of which four views are relevant (Segherloo et al. 2010) and thus used in the present investigation. The results of the four views of the GGE plot are discussed below.

AEC view based on environment-focused scaling for interpreting the mean performance of the genotypes vs stability patterns

Average Environment Co-ordinate (AEC) based GGE bi-plot was used in the estimation of yield and stability of genotypes in which the mean performance of the genotypes can be visualized based on the location of genotypes in relation to the AEC Line. A single arrowed line that passes through the origin of the biplot and the center of the circle is called an average environment coordinate (AEC) and it points to the higher mean performance of genotypes across test environments (Yan 1999). The average environment is defined as the average values of PC1 and PC2 for all the environments and is presented with a circle at the end of the arrow (Yan and Tinker 2006). The genotypes with their points located towards the AEC arrow are considered to have a higher mean yield, while, the genotypes with their points located opposite to the AEC arrow are considered to have a poor mean yield. Further, the relative lengths of projections of the genotypes from AEC are indicative of their relative stability. The shorter the length of the projections of genotypes from AEC, the greater the stability of the genotypes. Yan and Kang (2003) observed longer the projections of genotypes poorer in their stability. In our study, two hybrids CMS10 × A4 (G4) and CMS10 × A4 were identified as highly stable genotypes across the test environments with higher cured lea yield (Fig. 2a) as well as higher bright leaf yield (Fig. 2b).

'Which-won-where' view or polygon view pattern of GGE bi-plot

Which-won-where or polygon view pattern of GGE bi-plot was reported to be the best technique to identify GEI and to effectively interpret bi-plot (Yan and Kang 2003). In this technique, a polygon is drawn on genotypes positioned away from the origin of the bi-plot such that all other genotypes are within a polygon. Perpendicular lines called equity lines, originating from the bi-plot origin are drawn to each side of the polygon, thus dividing the bi-plot into sectors. The vertex genotype in each sector is the winning genotype at environments whose points fall into the respective sector (Yan et al. 2000). Thus, environments whose points fall in the sector will have the same winning genotypes, while environments of different sectors have different winning genotypes. Environments that share the same best genotypes are considered Mega environments. Thus, the polygon view of the GGE biplot indicates the presence or absence of crossover GEI. In the present study,

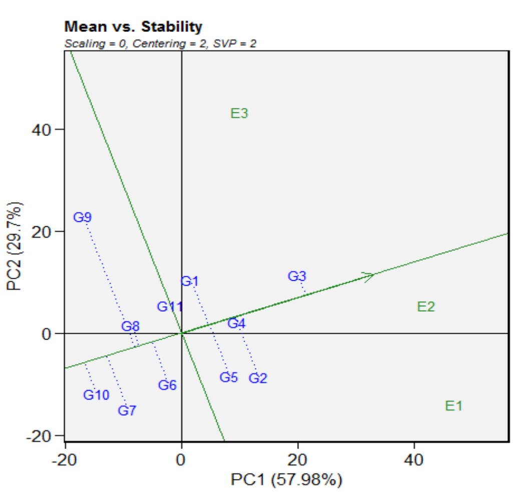

Fig. 2a

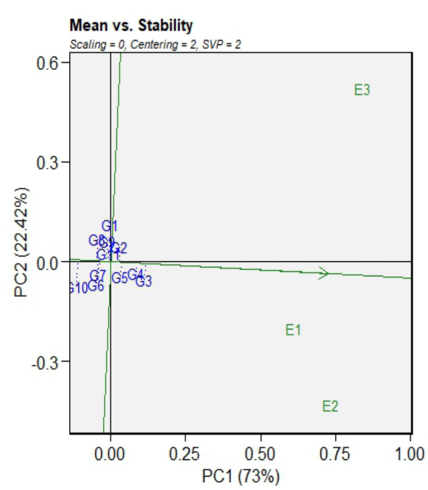

Fig. 2b

Fig. 2a & 2b: Average environment coordination (AEC) view of GGE-biplot based on environment-focused scaling for the mean performance *vs.* stability of test genotypes for cured leaf yield (Fig. 2a) and bright leaf yield (Fig. 2b)

Table 2. Analysis of variance based on AMMI model for cured leaf yield and bright leaf yield of FCV tobacco hybrids along with three check cultivars evaluated over three seasons/environments

Source of variation	DF	Cured leaf yield				Bright leaf yield			
		Sum of Squares	Mean Squares	G × E explained (%)	Cumulative (%)	Sum of Squares	Mean Squares	G × E explained (%)	Cumulative (%)
GEN	10	11085	1108**			9913	991.3**		
ENV	2	16443	8221**			6083	3041.6**		
GEN:ENV	20	10112	506*			3833	191.7**		
PC1	11	7446	677*	73.6	73.6	3142	285.7**	82	82
PC2	9	2666	296	26.4	100	691	76.8	18	100
Residuals	60	17515	292			3928	65.5		
GEI signal	-	4272				2523			
GEI noise	-	5840				1310			

**, * Significant at $p \leq 0.01$ and $p \leq 0.05$, respectively

test hybrids CMS 7 × A4 (G3) occupied the vertices of the polygon for cured leaf yield (Fig. 3a) as well as bright leaf yield (Fig.3b). Test hybrids, CMS 7 × A4 (G3) and CMS10 × A4 (G4) were winners during *kharif* 2019-20 & 2020-21, test hybrids CMS6 × A4 (G2) were winners in *kharif* 2018-19 for cured leaf yield, while, CMS 7 × A4 (G3) and CMS10 × A4 (G4) were winners in *kharif* 2018-19& 2019-20, CMS2×A4 (G1) and CMS6 × A4 (G2) were winners in *kharif* 2020-21.

Genotype(s) relative to ideal genotype

The average environment coordinate (AEC) view is based on the scaling of test genotypes relative to the ideal genotype. A genotype with high mean performance and high stability across the test environments is considered an ideal genotype. An ideal genotype is present at the center of concentric circles with AEC passing through it in a positive direction and has a vector length equal to the longest vector of the genotype on the positive side of AEC. Using the ideal genotype as a center, several concentric circles are drawn around to help in easy visualization of the distance between each test genotype and the ideal genotype. Stable genotypes are those which are located closer to the ideal genotype which in turn is located in origin (Kaya et al. 2006). Test hybrid genotype, CMS10 × A4 (G4), and hybrid check CH 3 (G11) were identified as near ideal ones for cured leaf yield as they were close to the origin (Fig. 4a). Similarly, for bright leaf yield (Fig.4b), test hybrids CMS6 × A4 (G2), CMS2 × CY142 (G5) and CMS10 × A4 (G4) were identified as near ideal ones along with the checks CH 3 (G11) and Kanchan (G9) although most of the test genotypes were near to the origin, Also assessment through YREM also indicated similar outcome that the stable hybrids' interaction with the three seasons is

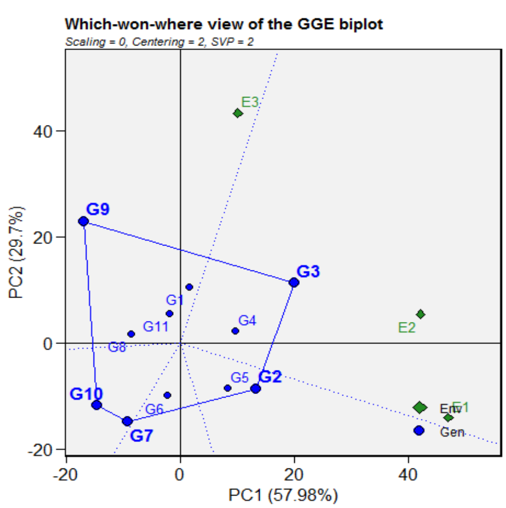

Fig. 3a

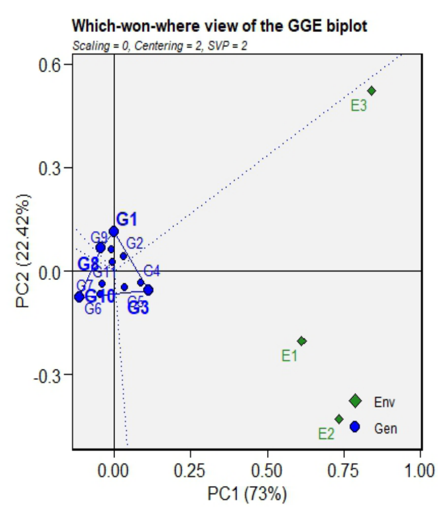

Fig. 3b

Figs. 3a and 3b. Polygon view of GGE-biplot based on the symmetrical scaling for "which won-where" pattern of test genotypes and environments for cured leaf yield (Fig. 3a) and bright leaf yield (Fig. 3b)

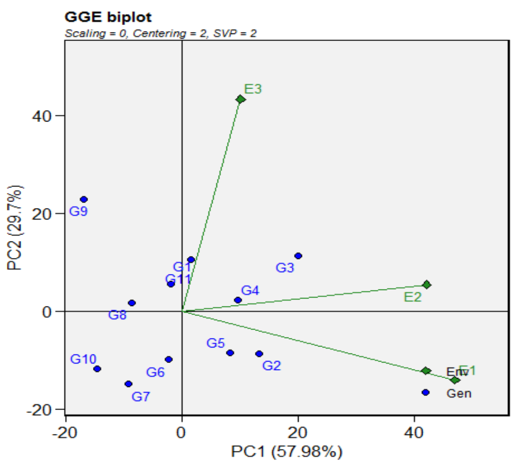

Fig. 4a

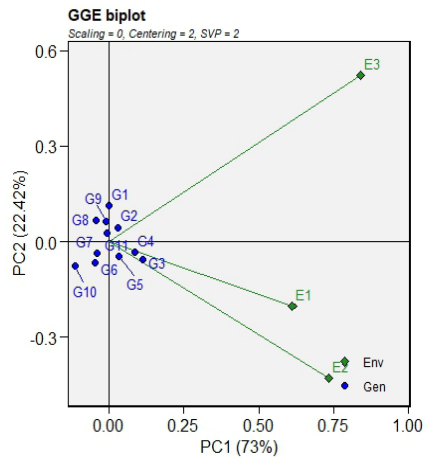

Fig. 4b

Figs. 4a and 4b. Average environment coordination (AEC) view of GGE-biplot for identification of test genotypes relative to ideal genotypes for cured leaf yield (Fig.4a) and bright leaf yield (Fig. 4b)

Table 3. Ranking of genotypes based on AMMI Stability Value (ASV) and mean leaf yield of FCV tobacco hybrids evaluated over three seasons

Genotype name	Cured leaf yield						Bright leaf yield					
	Mean. (g/plant)	RY	ASV	RASV	SI	Average YREM	Mean. (g/plant)	RY	ASV	RASV	SI	Average YREM
G1	125.0	5	4.8207	8	11	0.8816	75.49	5	6.9696	11	16	0.8080
G2	129.7	3	5.7002	10	13	0.9268	81.19	4	4.9972	8	12	0.8612
G3	142.6	1	0.9984	1	2	1.0000	95.01	1	2.9361	3	4	1.0000
G4	131.2	2	5.1978	9	11	0.9238	90.44	2	3.2019	4	6	0.9535
G5	125.8	4	3.8234	7	13	0.8821	82.09	3	2.4383	1	4	0.8613
G6	116.3	7	2.4839	3	10	0.8115	69.19	9	5.0921	9	18	0.7194
G7	108.6	10	2.7450	4	14	0.7682	70.02	8	2.7593	2	10	0.7321
G8	113.4	9	3.7824	6	15	0.7955	68.49	10	3.5796	5	15	0.7279
Checks												
Kanchan	113.5	8	9.0822	11	19	0.8019	73.84	7	4.4672	6	13	0.7856
FCH 222	104.9	11	2.9293	5	16	0.7349	58.05	11	5.5556	10	21	0.5987
CH3	120.9	6	2.2294	2	8	0.8492	74.52	6	4.5419	6	12	0.7892

of non-cross over type and that it remained highest yielder in all the three seasons. Thus, these hybrids could be used as potential cultivars for KLS conditions.

GGE bi-plot view based on discriminative ability and representativeness of test environments

Dotted lines connecting the test environment pointing to the origin are called environmental vectors. The length of the environmental vectors and the angle between the respective environment vectors with AEC help in identifying discriminating ability and representatives of the test environments. A discriminative environment is one which has the ability to discriminate between test genotypes while a representative environment should represent an average of three environments. Shorter and longer environment vectors indicate the lower and higher discriminative ability of environments, respectively. Small and large angles between environment vectors and AEA indicate the most and least representativeness of environments. The acute and obtuse angles between the test environment vectors indicate similarity and dissimilarity between the test environments, respectively. Based on the preceding description, *kharif* 2020-21 (E3) is found to be more discriminative as its environmental vector is longer than others for both cured (Fig. 5a) and bright leaf yield (Fig. 5b). While *kharif* 2019-20 (E2) was the representative environment for cured leaf yield and *kharif* 2018-19 (E1) was the most representative environment for bright leaf yield.

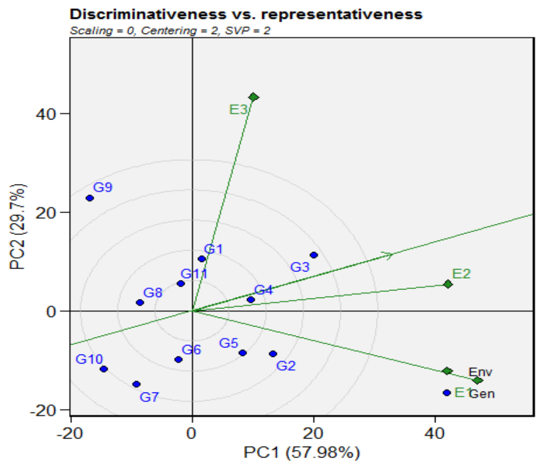

Fig. 5a

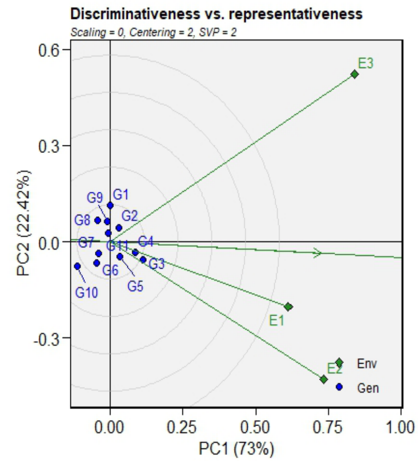

Fig. 5b

Figs. 5a and 5b: Discriminative vs. representativeness view of GGE-biplot for cured leaf yield (Fig. 5a) and bright leaf yield (Fig. 5b)

The results of stability analysis based on AMMI and GGE biplot indicated that the genotypes CMS 7 × A4 (G3) and CMS 10 × A4 (G4) as promising and stable. Stability Index (SI) takes into account both mean yield and stability, genotypes with low SI are regarded as both stable and high yielders. Several studies have been conducted earlier and the results on the effectiveness of AMMI and GGE biplot analysis of GEI to identify the stable and high-yielding lines in tobacco are reported (Sadeghi et al. 2011; Justify et al. 2018; Ahemd et al. 2019; Fashalami et al. 2022; Kurt 2023). The stable and higher mean yield of these hybrids can be attributable to the genetic homeostasis resulting from a higher degree of genetic variation, making them capable of withstanding environmental fluctuations and producing high and stable marketable yields.

Authors' contribution

Conceptualization of research (CN, KS); Designing of the experiments (CN, PN); Contribution of experimental materials (CN); Execution of field/lab experiments and data collection (CN, PN); Analysis of data and interpretation (CN, KG); Preparation and editing of the manuscript (CN, PN, JJR, KS, MSM).

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Supplementary Table S1. Description of climatic conditions over environments during crop period

Months	E1 (2018-19)					E2 (2019-20)					E3 (2020-21)				
	Temp (°C)		Rainfall (mm)	No. of Rainy days	Sun shine (hrs)	Temp (°C)		Rainfall (mm)	No. of Rainy days	Sun shine (hrs)	Temp (°C)		Rainfall (mm)	No. of Rainy days	Sun shine (hrs)
	Max	Min				Max	Min				Max	Min			
Apr	33.8	16.4	88.2	8	8.5	36.5	20.1	31.5	5	8.6	34.8	14.8	54.6	3	8.4
May	35.6	15.7	194.2	13	5.4	34.7	19.2	84.9	7	8.1	34.5	15.6	137.1	7	7.9
June	27.5	14	79	12	5.9	31.2	19.2	97.1	5	7.7	30.7	14.8	65.9	7	3.6
July	27.1	13.7	106	13	2.9	27.9	18.3	98.9	11	4.7	28.3	14.6	82.1	10	3.3
Aug	27.2	14	118.2	14	3.7	26.7	17.9	272	15	3.1	27.2	13.4	97.5	12	1.8
Sept	28.2	14.8	83	4	6.4	29.3	16.4	78.4	11	7	28.1	13.1	136.7	10	3