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Selection of elite *bidi* tobacco (*Nicotiana tabacum* L.) genotypes through G x E interaction studies and Stability assessment

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ABSTRACT

Stability analysis was carried out with 31 drought-tolerant *bidi* tobacco (*Nicotiana tabacum* L.) genotypes evaluated across four environments representing two locations (RARS, Nandyal, Acharya N.G. Ranga Agricultural University and ICAR–CTRI, Research Station, Kandukur) under normal and moisture stress conditions during 2023-24. Combined ANOVA revealed highly significant ($p < 0.001$) effects of genotypes, environments, and genotype × environment interactions (GEI) for cured leaf yield, with environments contributing the highest proportion of variation (82.98%) followed by genotype × environment interaction (12.37%) and genotypes (4.65%). GEI was partitioned employing Additive Main Effects and Multiplicative Interaction (AMMI) model into two significant principal components, explaining 89.7% of interaction variance. AMMI biplots revealed that genotypes ABD 54, ABD 118, NyBD 62 and NyBD 61 were stable and high-yielding across environments, while NyBD 84 and NyBD 85 were specifically adapted to favourable conditions at Nandyal. The integration of AMMI Stability Value (ASV) with the Culling Simultaneous Selection Index (C-SSI) identified ABD 132 (NP2), ABD 54, and NBD 154 as the most stable and high-yielding genotypes. These findings underscore the importance of environment-specific evaluation and AMMI and CSSI based selection for identifying drought-resilient *bidi* tobacco cultivars suited to erratic moisture regimes.

Keywords: *AMMI model and High-yielding genotypes, Bidi tobacco (Nicotiana tabacum L.), Drought tolerance, Genotype × Environment Interaction (GEI), Multi-environment evaluation*

Bidi tobacco (*Nicotiana tobaccum* L.), a smoking type of Non- FCV tobacco 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 (FAOSTAT, 2023). India also the fourth largest producer of *bidi* (Non-FCV) tobacco in the world after China, Brazil and Zimbabwe (FAOSTAT, 2023). In India, *bidi* tobacco is grown in selected states of Andhra Pradesh, Karnataka and Gujarat with an annual production of 300 million kg during 2023-24 (AINPT Annual Report, 2024).

In Andhra Pradesh, bidi tobacco (*Nicotiana tabacum* L.) is predominantly cultivated in the southern agro-climatic zone, particularly in the Nandyal and Kurnool districts, as a rainfed *rabi* crop utilizing residual soil moisture following the withdrawal of the southwest monsoon. The region's semi-arid tropical climate, characterized by low rainfall and dry winter conditions, is highly conducive for producing high-quality bidi tobacco leaves with desirable burning and aroma characteristics suited for the *bidi* industry. This crop occupies approximately an area of 31,000 ha (AINPT Annual Report, 2024) and is mainly grown in deep black cotton and red loamy soils with good drainage and moderate fertility. *Bidi* tobacco is usually transplanted during September to October and harvested between February and March. In *bidi* tobacco, the cured leaf is the economical part and the proportion of leaf quality influences its profitability; therefore, the main aim of breeding programme is increasing cured leaf yield with acceptable limits of leaf quality. 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. Several environmental factors such as temperature, rainfall pattern and quantity, field temperature, sunshine hours, *etc.*, have greatly impacted tobacco yield and quality

(Nanda *et al.,* 2025), leading to huge differences in cured leaf yield and quality 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 termed as Genotypic × Environmental Interaction (GEI). Knowledge of GEI effects on FCV tobacco yield is essential to develop or identify tobacco genotypes stable across temporal environments in order to sustain *bidi* tobacco production in *vertisols* of Andhra Pradesh. Hence, the present investigation was undertaken to understand GEI effects and to identify high-yielding and stable genotypes across environments using the Additive Main effects and Multiplicative Interaction (AMMI) (Gouch, 1988) and Genotype Plus Genotype by Environment Interaction (GGE) biplots (Yan *et al*., 2000).

MATERIAL AND METHODS

A set of 100 genotypes were evaluated for their drought tolerance at Regional Agricultural Research Station (RARS), Nandyal during 2022-23 and identified 31 best genotypes which performed better under drought conditions with better drought indices. These 31 bidi tobacco germplasm lines viz., NyBD 63, NyBD 56, NyBD 67, NyBD 61, NyBD 68, A119, ABD 118, NyBD 69, NyBD 60, ABD 54, NyBD 72, NyBD 84, NyBD 70, NyBD 59, NyBD 73, NyBD 74, NyBD 85, NyBD 71, NyBD 86, NyBD 62, ABD 84, ABD 132 (NP 2), NyBD 79, ABD 131, NBD 154, NyBD 80, NyBD 75, NyBD 78, NyBD 76, Bhavyasree, and ABD 78 were evaluated during 2023–24 at two locations, at Regional Agricultural Research Station (RARS), Nandyal and at ICAR–CTRI Research Station, Kandukur under two environments (normal and moisture stress conditions) at each location. The experiment was laid out in a Randomized Complete Block Design (RCBD) with two replications across four environments (E1, E2, E3 and E4) representing normal and moisture stress conditions at both locations.

Each entry was transplanted in two rows of 6.75 m in length and 1.5 m of width at a spacing of 0.75 m (between rows) x 0.75 m (between plants) and the crop was raised following recommended package of practices. Fertilizer in the experimental plot was applied at the rate of 110 kg nitrogen per hectare in the form of ammonium sulphate. Out of which 80 kg nitrogen, 70 kg P_2O_5 and 50 kg of K_2O (SOP) was applied as basal and the remaining 30 kg nitrogen was applied as top dressing after two weeks after transplanting. The post-transplanting operations like intercultivation, weeding, plant protection measures, topping and desuckering were done in accordance with the recommended practices during the crop growth period to raise a healthy crop.

Morphological characters *viz*., days to flowering, topped plant height (cm), number of leaves per plant, leaf length (cm), leaf width (cm), and cured leaf yield (kg/ha); physiological traits *viz*., relative water content (%), SPAD chlorophyll meter reading (SCMR), specific leaf area (cm^2/g), root length (cm), and root dry weight (g); and chemical quality traits *viz*., nicotine content (%), reducing sugars (%), and chloride content (%) were recorded on five randomly selected plants from each genotype.

Estimating the selection index (SI) is a useful approach for concurrently selecting genotypes based on cured leaf yield and stability, using cured leaf yield and the AMMI Stability Value (ASV) as dual criteria (Farshadfar and Sutka, 2003). To identify high-yielding and stable genotypes, several parametric selection indices have been proposed, including Kang's Yield Stability Index (Kang, 1993), Bajpai's Index (Bajpai and Prabhakaran, 2000), the Simultaneous Selection Index (Rao and Prabhakaran, 2005), and the Non-Parametric Genotype Selection Index (Farshadfar, 2008). The Culling Simultaneous Selection Index (C-SSI) represents a modification of these earlier approaches, designed to address inherent limitations in genotype ranking, and has been adopted in the present study (Anuradha *et al*., 2022). It is an advanced stability index applied in plant breeding programs to facilitate the simultaneous selection of genotypes with both high yield and superior stability across multiple environments. Unlike traditional selection indices that may inadvertently favour low-yielding but stable genotypes, the C-SSI approach integrates a culling strategy, wherein only genotypes with above-average yield are considered for stability evaluation. This two-stage process enhances the effectiveness of genotype selection under variable environmental conditions, ensuring that the final selections exhibit both agronomic desirability and environmental adaptability (Mahmodi *et al*., 2011).

Statistical Analysis

Phenotypic data of yield and yield-related traits recorded on 31 germplasm lines across two locations were subjected to combined ANOVA using a mixed linear model in R software version 4.5.1 (R Core Team, 2025). Based on combined analysis results, replication-wise mean data of cured and bright leaf yield were subjected to Additive Main Effects and Multiplicative Interaction (AMMI) model (Gauch, 1988) to detect and characterize the genotype × environment interaction (GEI). The sum of squares attributable to the signal-rich component of GEI (GEI signal) was computed following Gauch (2013). AMMI biplots were used to visualize GEI patterns and assess relative stability of the genotypes. AMMI-based stability parameters such as AMMI Stability Value (ASV) (Purchase *et al.*, 2000) and Culling Simultaneous Selection Index (C-SSI) were used to determine the stability of each genotype.

RESULTS AND DISCUSSION

ANOVA

The data of 31 drought tolerant bidi tobacco genotypes evaluated in four environments of two locations were subjected to combined ANOVA and AMMI analysis after validating the homogeneity of error variance through the Bartlett test ($p>0.05$). Results from combined ANOVA reported that genotypes (G), environments (E) and genotype × environment interaction (GEI) effects were highly significant ($p < 0.001$), which are presented in Table 1.

Among the source of variance governing the total treatment variation (G+E+GE), highest proportion of variation was explained by environments (82.98%) followed by genotype × environment interaction (12.37%) and genotypes (4.65%). The predominance of environmental variance reflected strong sensitivity of genotypes studied to location-specific factors (Falconer and Mackey, 1996). Significant GEI indicate that there was cross over among genotypes with respect to cured leaf yield in four different environments These results altogether suggest the necessity of breeding bidi tobacco varieties for specific regions as the cured leaf yields were responding to environmental fluctuations. Meanwhile, the limited genotypic contribution suggests that breeding progress will depend heavily on the careful exploitation of GE interaction and the identification of genotypes with broad or specific adaptation.

The presence of genotype-environment interaction (GEI) was clearly demonstrated by the AMMI model, when the interaction was partitioned among the first two interaction principal component axis (IPCA) as they were significant in postdictive assessment. The IPC1 explained 64.2% and the second principal component axis (IPC2) explained a further 25.5% of the GEI sum of squares (Table 1). This implied that the interaction of the tobacco genotypes with four environments was predicted by the first two components of genotypes and environments, which are in agreement with Nanda *et al*. (2025) who reported significant effects of genotypes, environments, and their interactions (GEI) for cured and bright leaf yield in FCV tobacco.

Biplot analysis is possibly the most powerful interpretive tool for AMMI models. There are two basic AMMI biplots, the AMMI 1 biplot where the main effects (genotype mean and environment mean) and IPCA1 scores for both genotypes and environments are plotted against each other (Figure 1). On the other hand, the second biplot is AMMI 2 biplot (Figure 2) where scores for IPCA1 and IPCA2 are plotted (Table 2). The mean cured leaf yield value of genotypes averaged over environments indicated that the genotypes ABD 84 and A 119 had the highest (1576 kg/ha) and the lowest (1226 kg/ha) yield, respectively (Table 2). Different genotypes showed inconsistent performance across all environments.

Biplots were plotted by numbering the drought tolerant tobacco genotypes serially from 1 to 31 instead of original names for clarity in depiction of the graphs. In the AMMI 1 biplot (Figure 1), displacement along the horizontal axis (abscissa) represents differences in the main additive effects (mean performance of genotypes or environments), while displacement along the vertical axis (ordinate) reflects differences in genotype × environment interaction effects. Genotypes that cluster together in the biplot exhibit similar adaptation patterns, and environments grouped together exert similar influences on the genotypes. When a genotype or environment has an Interaction Principal Component Axis 1 (IPCA1) score near zero, it indicates minimal interaction effects and suggests stability across environments. Furthermore, if the genotype and environment have the same sign on the IPCA1 axis, their interaction is positive (favourable), whereas opposite signs indicate a negative (unfavourable)

Table 1. AMMI ANOVA for 31 drought tolerant bidi tobacco genotypes across three locations for cured leaf yield

Source of variation	df	Sum of squares	Mean sum of squares	Variation (%)
Total	247	43376416.9	175613.02	
Treatment Design	123	40369038.1	328203.56	
Genotypes (i)	30	1877883.7	62596.12**	4.65
Environments (j)	3	33497417	11165805.65**	82.98
Genotypes x Environments (GEI)	90	4993737.4	55485.97**	12.37
Interaction Principal Component 1 (IPC1)	32	3203710.5	100115.95	64.20 of GEI
Interaction Principal Component 2 (IPC2)	30	1272328.6	42410.95	25.50 of GEI
Experimental Design	124	3007378.8	24253.05	
Replications (with in environment) (r)	4	215268.5	53817.12	
Error	120	2792110.3	23267.59	

'**' 0.01%, '*' 0.05% level of significance

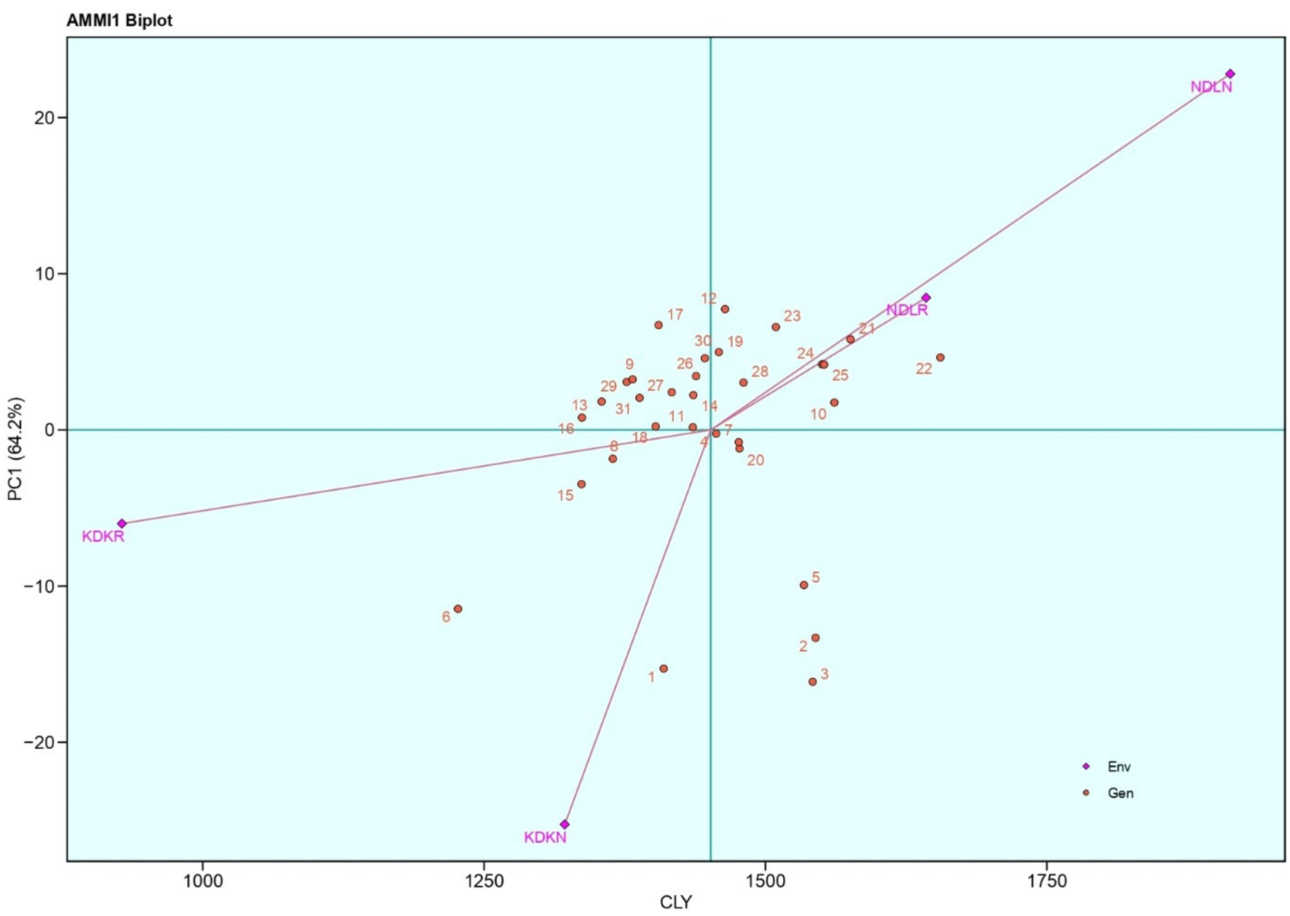

Figure 1. AMMI 1 Biplot (cured leaf yield vs IPC 1) for cured leaf yield (CLY) of 31 drought tolerant bidi tobacco genotypes across four environments

interaction (Gauch and Zobel, 1996; Olivoto *et al*. 2019).

From the AMMI 1 biplot, the genotypes ABD 54, ABD 118, NyBD 62 and NyBD 61 were identified as stable and high yielders with desired IPC scores of 1.75, -0.78, -1.19 and -0.24, respectively, which were nearer to the ordinate. Genotypes (NyBD 61, NyBD 86, NyBD 84, ABD 118, NyBD 62, NyBD 78, NyBD 79, NyBD 68, NyBD 67, NyBD 56, ABD 131, NBD 154, ABD 54, ABD 84, ABD 132 (NP2)) that were on the right side of the ordinate were more than average yielders, while the genotypes (A119, NyBD 73, NyBD 74, NyBD 70, NyBD 69, NyBD 76, NyBD 60, ABD 78, NyBD 71, NyBD 85, NyBD 63, NyBD 75, NyBD 72, NyBD 59, NyBD 80) that were on the left side of the ordinate are below average yielders (Crossa and Cornelius, 1997). Both the environmental conditions of Nandyal (normal and stress conditions) fell on the right side of the ordinate, indicating higher cured leaf yields than the mean cured leaf yield of genotypes (1451 kg/ha) over the four environments. The yield of tobacco genotypes at Kandukur under normal (1322 kg/ha) and stress conditions (928 kg/ha) was below the average yield (Table 2) and hence fell towards the left side of the ordinate. Hence, Nandyal was considered as favourable location under both normal and moisture stress conditions for expression of tobacco leaf yield compared to the Kandukur location (Figure 1 & Table 2). Higher discriminating power of genotypes was observed at Nandyal under normal conditions and at Kandukur under moisture stress conditions as seen from its longer vector length. The AMMI 1 biplot statistical model has been used to diagnose the G x E interaction pattern of grain yield of *bidi* tobacco. The genotypes ABD 54, ABD 118, NyBD 62 and NyBD 61 were hardly affected by the G x E interaction and thus will perform well across a wide range of environments.

In AMMI 2 biplot, the environmental scores are joined to the origin by vector lines. Sites with short vectors do not exert strong interactive forces. Those with long vectors exert strong interaction. AMMI 2 biplot demonstrated the genotypes closer to the centre of the biplot were more stable than the genotypes placed away from the centre. Hence, the genotypes near the origin are not sensitive to environmental interaction and those distant from the origins are sensitive and have large interaction.

From the AMMI 2 biplot genotypes, *i.e.*, NyBD 62, NyBD 74 and NyBD 61, were considered as the most stable drought-tolerant genotypes since they were very close to the origin (Figure 2). Among specifically adapted drought-tolerant tobacco genotypes, NyBD 84 (mean yield over environments: 1464 kg/ha; yield at Nandyal under normal conditions: 2168 kg/ha; IPC1 = 7.73; & IPC2 = 5.32) and NyBD 85 (mean yield over environments: 1405 kg/ha; yield at Nandyal under normal conditions: 2199 kg/ha; IPC1 = 6.71; & IPC2 = 9.63) were specifically adapted to the Nandyal location under normal conditions that were far away from the ordinate and were nearer to the NDLN vector. At Kandukur, A119 (mean yield over environments: 1226 kg/ha; yield at Nandyal under normal conditions: 1374 kg/ha; IPC1 = -11.50; & IPC2 = 3.30), NyBD 67 (mean yield over environments: 1542 kg/ha; yield at Nandyal under normal conditions: 1841 kg/ha; IPC1 = -16.10; & IPC2 = -0.42), and NyBD 56 (mean yield over environments: 1543 kg/ha; yield at Nandyal under normal conditions: 1833 kg/ha; IPC1 = -13.3d at Nandyal under normal conditions: 1721 kg/ha; IPC1 = -15.30; & IPC2 = -3.38) under normal conditions performed better than at other environments (Figure 2 and Table 2).

By looking at the cosine angle between any two environmental vectors in an AMMI 2 biplot, one can determine the relationship between the two environments (Figure 2). Between environments, an angle of 0° indicates perfect positive correlation (+1), 90° indicates no connection, and 180° indicates complete negative correlation (-1) (Gower and Hand 1996). Among the environments, both the environments of Kandukur *i.e*., normal and moisture stress conditions recorded negative association with Nandyal location conditions thus exhibiting cross over type of interaction for cured leaf yield. By observing both the biplots, the genotype, NyBD 62 can be considered as the best drought tolerant genotype for high cured leaf yield and very high stability.

Estimating the selection index (SI) is a useful approach for concurrently selecting genotypes based on cured leaf yield and stability, using cured leaf yield and the AMMI Stability Value (ASV) as dual criteria (Farshadfar and Sutka, 2003). To identify high-yielding and stable genotypes, several parametric selection indices have been proposed, including Kang's Yield Stability Index (Kang, 1993), Bajpai's

Table 2. Data on Cured leaf yield (CLY), AMMI stability values (ASV) and Simultaneous Selection Index with culling strategy (c-SSI) for 31 drought tolerant *bidi* tobacco genotypes across four environments in *bidi* tobacco (*Nicotiana tabacum* L.)

S. No.	Genotype	CLY at NDLN (kg/ha)	CLY at NDLMS (kg/ha)	CLY at KDKN (kg/ha)	CLY at KDKMS (kg/ha)	Average	PC1	PC2	ASV	c-SSI rank
1	NyBD 63	1511	1568	1721	838	1410	-15.30	-3.38	38.70	
2	NyBD 56	1893	1342	1833	1104	1543	-13.30	12.30	35.70	
3	NyBD 67	1643	1613	1841	1071	1542.0	-16.10	-0.42	40.57	
4	NyBD 61	1869	1710	1316	930	1456	-0.24	-2.82	2.88	9
5	NyBD 68	1683	1738	1599	1117	1534	-9.93	-4.56	25.44	
6	A119	1459	1237	1374	835	1226	-11.50	3.30	29.17	
7	ABD 118	1941	1598	1351	1015	1476	-0.78	2.39	3.10	7
8	NyBD 69	1722	1628	1255	852	1364	-1.85	-3.85	6.05	19
9	NyBD 60	2007	1375	1135	1011	1382.0	3.23	8.83	12.01	17
10	ABD54	1909	2045	1366	925	1561	1.75	-11.60	12.41	2
11	NyBD 72	1960	1502	1294	986	1436	0.17	5.08	5.10	13
12	NyBD 84	2168	1591	1140	958	1464	7.73	5.32	20.19	
13	NyBD 70	1742	1705	1114	857	1355	1.82	-6.54	7.99	20
14	NyBD 59	1875	1693	1175	1001	1436.0	2.23	-2.69	6.23	12
15	NyBD 73	1670	1525	1240	911	1337.0	-3.47	-1.63	8.90	22
16	NyBD 74	1815	1550	1196	787	1337.0	0.79	-0.50	2.04	21
17	NyBD 85	2199	1446	1218	757	1405	6.71	9.63	19.46	
18	NyBD 71	1786	1723	1239	862	1403	0.22	-5.49	5.52	15
19	NyBD 86	2021	1731	1214	868	1459	4.98	-1.49	12.64	8
20	NyBD 62	1841	1665	1277	1125	1477	-1.19	-1.31	3.27	6
21	ABD 84	2202	1818	1349	934	1576	5.80	0.46	14.62	
22	ABD 132(NP2)	2146	1928	1326	1221	1655	4.64	-2.59	11.98	1
23	NyBD 79	2111	1777	1214	935	1509	6.59	-0.84	16.63	
24	ABD 131	2172	1740	1383	905	1550	4.20	2.23	10.82	4
25	NBD 154	2158	1719	1344	988	1552	4.18	2.75	10.89	3
26	NyBD 80	2060	1528	1245	922	1438	3.44	5.60	10.32	11
27	NyBD 75	1865	1754	1217	832	1417	2.41	-5.20	8.00	14
28	NyBD 78	1987	1796	1312	827	1481	3.03	-3.61	8.45	5
29	NyBD 76	1877	1682	1188	760	1377	3.07	-3.40	8.45	18
30	Bhavyasree	2023	1709	1240	813	1446	4.59	-0.91	11.60	10
31	ABD 78	1982	1490	1254	827	1388	2.05	4.88	7.11	16
Mean		**1912.8**	**1642.7**	**1321.7**	**928.2**	**1451**			**13.4**	

CLY at NDLN-Cured Leaf Yield at Nandyal under normal conditions
CLY at NDLMS-Cured Leaf Yield at Nandyal under Moisture stress conditions
CLY at KDKN-Cured Leaf Yield at CTRI, Research Station, Kandukur under normal conditions
CLY at KDKMS-Cured Leaf Yield at CTRI, Research Station, Kandukur under moisture stress conditions
PC1- Principal Component Axis 1
PC2- Principal Component Axis 2
C-SSI-Simultaneous Selection Index with culling strategy
ASV- AMMI stability values

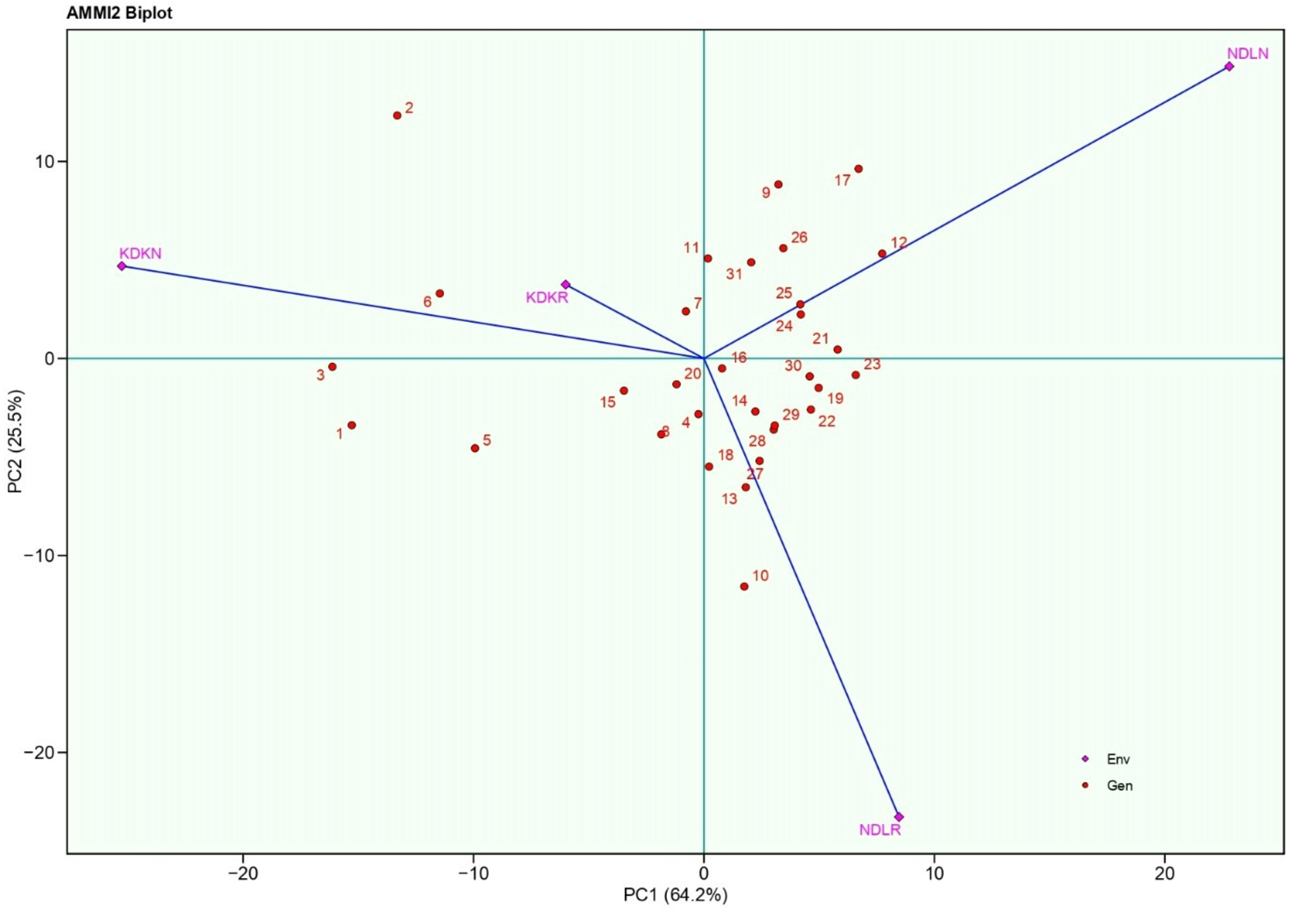

Figure 2. AMMI 2 Biplot (IPC 1 vs IPC 2) for cured leaf yield of 31drought tolerant bidi tobacco genotypes across four environments

Index (Bajpai and Prabhakaran, 2000), the Simultaneous Selection Index (Rao and Prabhakaran, 2005), and the Non-Parametric Genotype Selection Index (Farshadfar, 2008). The Culling Simultaneous Selection Index (C-SSI) represents a modification of these earlier approaches, designed to address inherent limitations in genotype ranking, and has been adopted in the present study (Anuradha *et al*., 2022). It is an advanced stability index applied in plant breeding programs to facilitate the simultaneous selection of genotypes with both high yield and superior stability across multiple environments. Unlike traditional selection indices that may inadvertently favour low-yielding but stable genotypes, the C-SSI approach integrates a culling strategy, wherein only genotypes with above-average yield are considered for stability evaluation. This two-stage process enhances the effectiveness of genotype selection under variable environmental conditions, ensuring that the final selections exhibit both agronomic desirability and environmental adaptability (Mahmodi *et al*., 2011).

To rank genotypes using the C-SSI, the AMMI Stability Value (ASV) was first estimated for each genotype, with the study average ASV score calculated as 13.42 (Table 2). The higher the ASV score, the less stable the genotype and hence the more specifically adapted to a particular environment, while a lower score of ASV implies higher stability of a genotype across environments (Purchase *et al*., 2000). A total of 22 out of 31 drought-tolerant bidi tobacco genotypes were recorded with lesser scores than the mean ASV score. As a result, only those 22 genotypes were taken into account for the final ranking based on cured leaf yield in C-SSI (Table 2). Genotypes ABD 132 (NP2), ABD 54 and NBD 154 were considered as the top three best drought-tolerant tobacco genotypes with e”1550 kg/ha cured leaf yield and with less than 13.42 ASV score.

CONCLUSION

AMMI and biplot analyses identified ABD 54, ABD 118, NyBD 62, and NyBD 61 as stable and high-yielding bidi tobacco genotypes across environments (Nandyal and Kandukur), whereas NyBD 84 and NyBD 85 were found specifically adapted to favorable (normal) conditions at Nandyal. The C-SSI–based ranking further emphasized ABD 132 (NP2), ABD 54, and NBD 154 as the most promising genotypes for combined yield performance and stability. Integrating AMMI and advanced selection indices like C-SSI was helpful in identifying genotypes with high productivity coupled with environmental resilience, thereby enhancing the breeding efficiency and sustainability of *bidi* tobacco cultivation under moisture limited conditions.

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