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Full Length Article

Assessment of Flue-Cured Tobacco Recombinant Inbred Lines under Multi-Environment Yield Trials

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Abstract

Cross-over interaction is always a major concern for plant breeders when recommending a crop cultivar for different agro-ecologies. Hundred flue-cured tobacco recombinant inbred lines (RILs) derived from $F_{4:5}/F_{4:6}/F_{4:7}$ populations along with three parental checks were evaluated to determine genotype by environment (GE) interaction. The experiments were conducted for three consecutive years (2012–2013, 2013–2014 and 2014–2015) at two different locations *i.e.*, Mardan (plain area) and Mansehra (hilly area), Pakistan using alpha lattice design with three replications in each environment. Six distinct environments were generated in combination of three years and two locations. Results obtained from additive main effect and multiplicative interaction (AMMI) analysis revealed that 46.5% of phenotypic variation in yield was contributed by environmental effects while 45.0% was explained by GE interaction. First four principal components (PCs) were significant and cumulatively explained 89.9% of variation due to GE interaction. For instance, based on AMMI-1 model, genotype G11 was identified as high yielding (2669 kg ha^{-1}) followed by G86 (2586 kg ha^{-1}) and G28 (2563 kg ha^{-1}). Likewise, in AMMI-2 model, G11, G5 and G56 were identified as most stable genotypes. Generally, performance of inbred lines at Mardan was consistent. Mansehra had the most discriminating and erratic environments over years for FCV lines. Genotypes G11 and G86 appeared as high yielding elite tobacco lines possessing dynamic stability. The mentioned FCV lines were superior to standard checks in yield and stability; hence could be recommended for diverse environments. This study puts emphasis on the significance of conducting multi-environment yield trials to screen not only best performing lines but also to reduce breeding cycles for new tobacco cultivars.

Keywords: Genotype by environment Interaction (GEI); AMMI analysis; Stability analysis; Inbred lines; Tobacco

Introduction

Tobacco (*Nicotiana tabacum* L.) is one of the most important nonfood cash crops widely grown for its commercial utility. Its farming, product manufacturing, sale and distribution result in huge economic activities in various economies of the world. In Pakistan, tobacco is a source of income and generates a valuable foreign exchange for the country. In Pakistan, being a single major contributor to federal excise duty, tobacco industry is expected to contribute over US$ 1 billion to the federal exchequer which is more than any other crop (Yasmeen and Khalid, 2017). During 2017–2018, export of tobacco and its by-products earned over US$ 25 million for the country. Tobacco is a high labour-intensive crop and usually requires a lot of inputs. It has been estimated that about 80,000 persons are engaged in its cultivation. Similarly, tobacco processing and cigarette manufacturing factories have provided jobs for 50,000 persons whereas around one million find indirect employment through tobacco industry in Pakistan (PTB, 2019). However, no considerable efforts have been carried out to address breeding priorities in tobacco such as development of high yielding and disease resistant tobacco cultivars in the country. Due to lack of appropriate cultivars, cultivation of susceptible tobacco cultivars with modest yield is in practice (Ahmed and Mohammad, 2017). Consequently, there had been complete reliance on introduced genetic material for commercial cultivation. In Pakistan, the leading tobacco companies relied on import of tobacco cultivars (seeds) from Brazil and U.S.A. every year to achieve yield targets (Ahmed and Mohammad, 2017). This necessitated the initiation of indigenous tobacco breeding program to

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address the issues of the tobacco industry.

Tobacco is predominantly (≥90%) grown in Khyber Pakhtunkhwa province of Pakistan which has distinct agro-ecological zones (Table 1). These zones covering diverse climatic conditions ranging from Southern Piedmont Plains (50°C) to Eastern Wet Mountains (-15°C). Given this climatic diversity, a decline in both productivity and quality of tobacco has greatly affected the farming community in the region. Therefore, cultivation of heat and drought tolerant tobacco cultivars is essential to reduce risk factors in achieving goals of sustainable agriculture (Su *et al*., 2017). Development of high yielding tobacco cultivars with wider adaptability is an integral part of plant breeding program. However, the genotype by environment interaction (GEI) aggravates the recommendation of a cultivar for a range of environments. Cross-over interaction, resulting in change of genotypes ranking across environments, is a serious concern to plant breeders in cultivar development as it restricts a specific cultivar to a specific environment (Mafouasson *et al*., 2018). Therefore, testing of breeding material in diverse environments is a crucial practice in plant breeding to identify line(s) that could express its true yield and quality potential (Montesinos-López *et al*., 2018).

Various methods have been proposed to measure the stability of genotypes over a wide range of environments. However, fewer methods can adequately explain cultivar performance across environments (Dehghani *et al*., 2006). Additive Main Effect and Multiplicative Interaction (AMMI) analysis proved to be capable of extracting a large part of GE interaction and was found efficient in analysing the interaction patterns (Gauch and Zobel, 1989). The AMMI analysis is the hybrid method to assess multi-environment trials (METs) which unifies analysis of variance and principal component analysis (Gauch, 1988). Many well cited publications including Gauch and Zobel (1988), Zobel *et al*. (1988) and Crossa *et al.* (1990) advocated the use of AMMI analysis for multivariate analysis. Gauch and Zobel (1988) compared the AMMI analysis, simple ANOVA approach and regression approach in interpreting GE interaction and reported that ANOVA failed to expose significant interaction component while the regression approach explained only a fraction of interaction sum of squares. Conversely, principal component analysis (PCA) was inefficient to explain main effects of GE interaction. However, AMMI analysis was effective to gain insight of complex GE interaction. The outcomes from AMMI can be drawn into useful biplots. Each genotype is assigned a particular score in regard to its stability over environments. Plotting of PCA scores against each other provides visual inspection and interpretation of complex patterns of GE interaction. Keeping in view the above narrated facts, the aims to this study were to; a) interpret GE interaction obtained by AMMI analysis for yield performance of 100 recombinant inbred lines in FCV tobacco over environments and b) identify high yielding line(s) based on genotypic response to environments.

Materials and Methods

Germplasm, Experimental Design and Procedure

Field experiments were conducted during three consecutive years *i.e*., 2012-2013, 2013-2014 and 2014-2015 on Flue Cured Virginia (FCV) tobacco at Tobacco Research Station, Khan Garhi, District Mardan (plain area) and Tobacco Research Sub-Station, District Mansehra (hilly area), Khyber Pakhtunkhwa - Pakistan. Plant material included 100 $F_{4:5}/F_{4:6}/F_{4:7}$ recombinant inbred lines derived from Speight G-28 × Speight G-126, Speight G-126 × Speight G-28, Speight G-28 × NC-606 and Speight G-126 × NC-606 (Table 2). At both locations during three years, all the experiments were planted using alpha lattice (20 × 5) design. Experimental units were randomly allotted to blocks in three replicates. Six environments *i.e*., Mardan during 2012-2013, Mansehra during 2012-2013, Mardan during 2013-2014, Mansehra during 2013-2014, Mardan during 2014-2015 and Mansehra during 2014-2015 were considered as E-1, E-2, E-3, E-4, E-5 and E-6, respectively for analysis of GE interaction. Description of environmental conditions at each location is presented in Table 3.

Nursery Raising

Nurseries were raised from December 5 to 10 each year at hilly area (Mansehra, Pakistan) while at plain area (Mardan, Pakistan) from December 15 to 20. Virginia-tobacco seedlings were raised on seed beds surrounded by polythene bags to avoid frost injuries. Size of seedbeds was 10 m^2 (1 m × 10 m) raised about 15 cm above the ground level. To ensure good water holding capacity, farm yard manure was applied over the surface of seedbeds. One gram of seed from each entry was mixed with dry fine sand and evenly distributed on the seedbed. Garden watering cane was used to shower water periodically to the seedlings. For stem thickening purpose, the tips of leaves were removed when the seedlings reached 4 to 6 leaves stage. This not only improves the survival rate of transplanted seedlings in the field but also encourages nitrogen and dry matter accumulation in the leaves while reducing the nicotine content (Xie *et al*., 2017). In this way, its industrial value is increased.

Transplantation

Seedlings were transplanted from March 01 to 05 and March 15 to 20 at Mansehra and Mardan, respectively during each year. Each genotype consisted of two rows having 6 m length, with row to row and plant to plant spacing for 90 and 60 cm, respectively. Even-sized seedlings of 5–8 inches height and pencil thickness having complete roots were transplanted to the field. Diseased and

Table 1: Agro-ecological zones of Khyber Pakhtunkhwa province of Pakistan

Zone	Description	Districts
A	Higher Northern mountains, Northern mountains	Buner, Shangla, Dir/Lower and Upper, Swat and Chitral
B	Sub-humid Eastern mountains and wet mountains	Haripur, Batagram, Mansehra, Abbottabad, Kohistan, Torghar
C	Central Valley Plain	Peshawar, Mardan, Charsadda, Nowshera, Swabi, Kohat, Hangu
D	Piedmont plain, Suleiman piedmont	Bannu, Karak, Lakki Marwat, Tank, D.I. Khan

Source: Khyber Pakhtunkhwa Climate Change Policy. 2016. Page 4

Table 2: List of parent cultivars (checks) and RILs with parentage

Code	Cultivars/lines	Parentage	Main features	
Chk1	Speight G-28	(Coker-139 × Oxford 1-181) and NC-95	i)	Recommended cultivar for several decades in Pakistan, having modest yield and quality
			ii)	Plants are shorter than many cultivars which bear 25 leaves per plant
			iii)	Medium to late maturity
Chk2	Speight G-126	K-326 × Speight G-96	i)	Moderate yield with inferior cured leaf quality
			ii)	Late maturing than most of the cultivars
			iii)	Good holding ability
Chk3	NC-606	NC-729 × NC-82	i)	Produces 30 good quality leaves per plant
			ii)	Taller plants with longer internodal length.
G1 – G100	G1 – G25	Spt G-28 × Spt G-126	i)	Segregating populations were advanced in bulk till F_4 generation
	G26 – G50	Spt G-126 × Spt G-28	ii)	Single plant selection in F_4 generation was made under rainfed condition
	G51 – G75	Spt G-28 × NC-606		
	G76 – G100	Spt G-126 × NC-606		

Table 3: Description of climatic conditions at the studied sites

Months	Mardan 2013	Mansehra 2013	Mardan 2014	Mansehra 2014	Mardan 2015	Mansehra 2015
	(E-1)	(E-2)	(E-3)	(E-4)	(E-5)	(E-6)
			Rainfall (mm)			
Mar	70	92	162	197	81	4
Apr	53	40	54	148	214	0
May	6	35	11	0	76	1
Jun	24	104	7	11	0	9
Jul	231	225	62	24	231	225
Aug	128	172	75	8	128	172
Total	512	668	371	388	730	411
			Temperature (Min—Max °C)			
Mar	9—27	11—22	8—24	8—19	10—25	17—20
Apr	14—32	13—27	13—31	13—27	15—31	7—36
May	18—36	18—32	17—38	16—31	17—36	15—27
Jun	19—39	21—39	22—43	21—39	22—39	15—27
Jul	21—38	22—35	24—41	17—25	21—38	22—35
Aug	22—38	22—32	23—38	22—32	22—38	22—32
Mean	17—35	18—31	18—38	16—29	19—35	16—30

Source: Data recorded by weather stations installed at Tobacco Research Station, Mardan and Tobacco Research sub-Station, Mansehra

weak seedlings were discarded. Fertilization for tobacco crop was based on the recommended dose of Pakistan Tobacco Board for each location: 45:90:90 NPK kg ha^{-1} at Mardan and 60:90:90 NPK kg ha^{-1} at Mansehra. Removal of flowers (topping) and small unproductive leaves (suckers) was done manually. Ripened leaves were hand harvested (picking of 2–4 matured leaves) from bottom to top in 4 to 5 steps at weekly interval. Harvested leaves were submitted to barns for curing. Other cultural practices and crop management including tillage, hoeing, irrigation and pesticides application were done as per routine practice for tobacco crop.

Statistical Analysis

Yield data across years and locations were subjected to analysis of variance using the appropriate model for alpha lattice design to assess the significance of genotypes, environments and their interaction (Steel *et al*., 1997). The S.A.S. software was used to carry out all analysis of variance procedures (S.A.S., 2009). For significance, genotypes (G) were tested against genotype by environment (GE) interaction while GE interaction was tested against main error. Significant GE interaction for yield justified the use of AMMI model for interpretation of GE interaction. The AMMI analysis was carried out using GenStat v. 12 computer software (GenStat, 2009). Biplots were constructed using PC scores. Each location over years was considered as discrete environment. Yield means were adjusted for blocks and replications in each environment before subjecting to AMMI analysis. Three parental check cultivars Speight G-28, Speight G-126 and NC-606

(designated as Chk1, Chk2 and Chk3, respectively) were used to compare their performance with 100 RILs (G1 to G100).

The AMMI stability value for genotypes was calculated using the following formula as proposed by Purchase *et al*. (2000).

$$ASVi = \sqrt{[\frac{SS_{IPCA1}}{SS_{IPCA2}}(IPCA1\ Score)]^2 + (IPCA2\ Score)^2}$$

Where SSIPCA1/SSIPCA2 is the weight given to the interaction principal component axis 1 (IPCA1) value by dividing the IPCA1 sum of squares by the interaction principal component axis 2 (IPCA2) sum of squares.

The AMMI model offers no provision for quantifying the extent of stability which is important to rank genotypes according to their stability. This issue was solved by Purchase *et al*. (2000) who proposed AMMI stability value (ASV) using principal components scores of each genotype. The IPCA1 score has always a larger share in explaining GE interaction sum of squares. Therefore, it has to be adjusted by the proportional differences between IPCA1 and IPCA2 scores to balance the relative share of IPCA1 and IPCA2 in total GE interaction sum of squares. The distance from zero (origin) is then measured using Pythagoras theorem (Purchase *et al*., 2000). The AMMI stability value is the relative distance from the origin in an AMMI2 biplot *i.e*., PC1 vs PC2. Genotypes having large ASV were considered unstable or specifically adapted whereas genotypes with small ASV were consistent in performance across environments and are widely adapted.

Results

Additive Main Effect and Multiplicative Interaction (AMMI) Analysis

Significant differences were detected for genotypes, environments and genotype by environment interactions. Interactions due to GE were further split into four principal components (Table 4). The AMMI analysis indicated that 46.51% of the variation was due to environments. Genotypes added small but significant portion (8.50%) to the total sum of squares. Out of total variance, almost equal contribution of environments (45.0%) and GE interactions (46.5%) was observed for yield.

Mean yield obtained from six environments was plotted against the scores of first principal component (PC1) to evaluate the response of environments based on the mean yield of genotypes (Fig. 1). One hundred RILs were codified as G1 to G100, whereas, three check cultivars Speight G-28, Speight G-126 and NC-606 were codified as Chk1, Chk2 and Chk3, respectively. It can be seen that E-6 was the most productive environment followed by E-4 (Fig. 1). The long distance of E-6 from the origin indicates that E-6 was the most responsive environment.

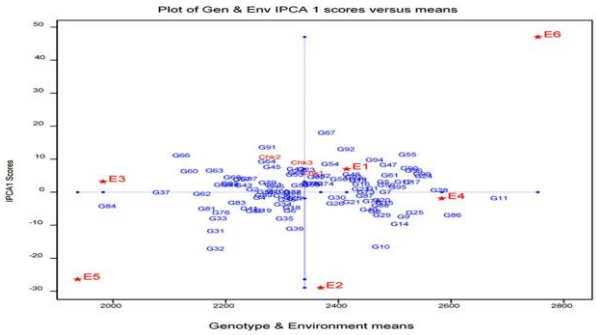

Fig. 1: AMMI1 biplot of 100 RILs along with three check cultivars and six environments based on their IPCA1 scores against mean yield. Genotypes/environments on the right are high productive while on the left are low productive. Origin line (IPCA1) represents stability. Genotypes/environments lying in the vicinity of origin line are stable/consistent

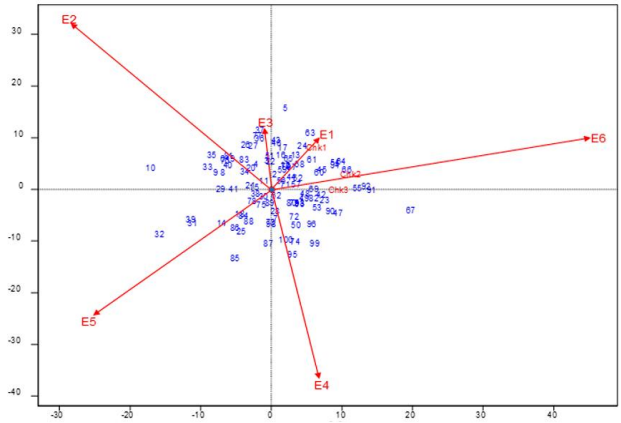

Fig. 2: AMMI2 biplot of 100 RILs along with three check cultivars and six environments based on their IPCA1 against IPCA2 scores. Genotypes lying near the origin are stable. Long environmental vectors indicating discriminating environments

Genotypes and environments located on the positive x-axis had positive association while those located on the negative x-axis had negative association (Fig. 1). The GE interaction biplot insinuated that genotypes G99, G96 and G24 had positive interaction with E-4 as indicated by their proximity to E-4 (Fig. 1). Similarly, genotypes G48, G49 and G93 responded well to E-1. The lowest yielding genotype G84 negatively responded to E-3 (Fig. 1). Genotype G10 had positive interaction with E-2 as both shared the same quadrant.

AMMI2 biplot was constructed based on IPCA1 and IPCA2 scores, two principal components explained 51.7% of the GE interaction thus making the AMMI2 model more fit than AMMI1 (Fig. 2). Mean yield data over years ranged between 1937–2414 and 2368–2754 kg ha^{-1} at Mardan and

Table 4: Analysis of variance based on AMMI model for yield of 100 RILs along with three check cultivars of FCV tobacco evaluated across six environments during 2012–2013, 2013–2014 and 2014–2015

Source of variation	Degree of freedom	Sum of Squares	Mean Squares	Total variation explained (%)	G × E explained (%)	Cumulative (%)
Total	617	116738872	-	-	-	-
Genotypes	102	9929238	97345*	8.51	-	-
Environments	5	54296690	10859338**	46.51	-	-
GE Interactions	510	52512944	102967**	44.98	-	-
IPCA1	106	14514964	136934**	-	27.64	27.64
IPCA2	104	12649041	121625**	-	24.09	51.73
IPCA3	102	11263975	110431**	-	21.45	73.18
IPCA4	100	8772740	87727**	-	16.71	89.88
IPCA Residuals	98	5312225	54206	-	-	-
Grand mean = 2339.79			$R^2 = 0.62$	-	CV = 20.58	-

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

Table 5: List of top four high yielding FCV tobacco genotypes, based on AMMI model, evaluated across six environments during 2012–2013, 2013–2014 and 2014–2015

Environments	Mean yield (kg ha^{-1})	Score		Top 4 genotypes at each environment			
		IPCA1	IPCA2	1st	2nd	3rd	4th
E-1	2414	6.999	8.745	G86	G83	G93	G9
E-2	2368	-28.943	32.518	G10	G5	G14	G11
E-3	1982	3.213	14.093	G11	G17	G5	G24
E-4	2583	-1.905	-43.171	G14	G25	G86	G67
E-5	1937	-26.399	-18.052	G96	G87	G28	G39
E-6	2754	47.035	5.866	G96	G67	G55	G92
Mardan (3 years)	2111	-	-	G86	G17	G87	G73
Mansehra (3 years)	2568	-	-	G11	G1	G14	G25

Mansehra, respectively. List of top four high yielding genotypes at each environment is presented in Table 5.

The principal component scores obtained for each genotype specify the stability and steadiness of that genotype over environments (Table 6). Fig. 2 shows that genotypes G56, G71, G62 G2 and G11 were in the proximity of origin. The small distance of these lines from the origin shows that these lines were insensitive to environmental interactive forces and thus can be considered as widely adaptable to diverse environments. The superior ranking (1st) of G11 based on mean yield makes it more reasonable to be considered as widely adapted genotype. The long distances of G67 (right lower quadrant), G85 and G32 (left lower quadrant), G10 (left upper quadrant) and G5 and G63 (right upper quadrant) from the origin suggested the response of these genotypes to specific environments and thus restricts their cultivation in specific environments (Fig. 2).

Genotypes sharing the same quadrant have close relation while those in opposite quadrant have no association. The angle between the environment vectors suggests the association of environments. The six environmental vectors were spread into all four quadrants; however, those sharing the same quadrant indicated the similar response towards genotypes of that particular quadrant (Fig. 2). Likewise, the widespread of some genotypes suggests that these genotypes were responding to fluctuating environments and interacted well with specific environment. It can be seen that environment E-1 has the shortest vector followed by E-3 which indicates their low discriminating power (Fig. 2). It can also be inferred that less force was exerted on genotypes to deviate from mean yield in these environments. However, the very strong discriminating nature of these environments makes them inadequate for consideration as representative environments. Genotype G10 and G95 interacted well with E-2 and E-4, respectively. Similarly, environment E-6 may have triggered certain alleles in G67 which enhanced its yield as compared to other environments. Majority of the genotypes clustered around E-3 and E-1 which were relatively stable environments. Superior performance of some genotypes in particular environment restricts their use in other environments and hence, could be regarded as specifically adapted.

All the three check cultivars clustered away from the origin suggesting their inconsistent yield performance. Several genotypes were identified having better stability and yield performance than check cultivars. The wide spread of environmental vectors in all four quadrants indicated the lack of association among these environments. It is pertinent to mention that environments were grouped irrespective of their geographical location (Mardan and Mansehra) which suggests the unpredictable nature of agro-climatic conditions at these two locations.

AMMI Stability Value

The G56, G71, G62, G2 and G11 appeared to be the most stable genotypes as evidenced by their small AMMI stability value **(**ASV) (Table 7). However, stability alone cannot be the sole criterion for selection, as a highly stable line may not necessarily be a high yielding line. Therefore,

Table 6: Mean yield performance based on AMMI model of 100 RILs of FCV tobacco evaluated across six environments during 2012–2013, 2013–2014 and 2014–2015

Lines	Yield (kg ha^{-1})	IPCA1	IPCA2	Yield rank	Lines	Yield (kg ha^{-1})	IPCA1	IPCA2	Yield rank
G1	2448	0.241	2.232	26	G53	2306	4.454	-5.573	59
G2	2235	-0.072	2.087	82	G54	2369	7.621	2.533	43
G3	2276	-0.799	4.500	69	G55	2506	10.534	-2.427	10
G4	2249	-2.524	3.958	80	G56	2178	1.330	1.139	92
G5	2467	2.192	13.823	19	G57	2212	2.970	0.122	87
G6	2302	-6.455	6.207	60	G58	2384	3.086	-3.305	40
G7	2471	-0.838	-6.583	17	G59	2315	1.162	2.916	53
G8	2452	-6.690	3.531	25	G60	2119	5.486	0.991	100
G9	2504	-8.320	3.176	11	G61	2474	4.245	3.172	15
G10	2459	-17.41	5.351	22	G62	2142	-1.356	-3.260	99
G11	2669	-2.667	0.343	1	G63	2164	5.650	9.216	97
G12	2499	2.243	4.513	12	G64	2257	8.432	2.454	77
G13	2418	2.597	5.069	34	G65	2191	1.845	4.672	91
G14	2492	-10.500	-8.789	13	G66	2104	10.272	2.291	101
G15	2465	-4.101	-1.188	20	G67	2363	17.123	-8.161	44
G16	2424	1.550	6.114	32	G68	2195	3.624	3.964	89
G17	2514	2.149	7.588	8	G69	2309	4.973	-1.415	55
G18	2300	-5.548	-4.712	64	G70	2258	2.006	3.822	75
G19	2249	-6.400	5.363	81	G71	2268	1.438	0.173	72
G20	2459	-3.289	4.086	23	G72	2340	1.748	-6.641	48
G21	2407	-3.871	0.050	37	G73	2432	-0.905	-6.125	30
G22	2423	0.073	5.084	33	G74	2359	1.654	-10.767	45
G23	2326	5.938	-4.065	52	G75	2302	-2.588	-3.474	61
G24	2534	3.960	6.801	4	G76	2175	-7.097	5.059	93
G25	2519	-7.097	-9.559	6	G77	2302	-0.914	10.766	62
G26	2378	-4.339	7.396	42	G78	2443	-3.597	-2.570	28
G27	2307	-2.837	7.663	56	G79	2334	1.648	-3.934	51
G28	2563	-0.293	-4.011	3	G80	2335	1.336	-3.978	49
G29	2461	-7.827	0.412	21	G81	2150	-5.874	6.429	98
G30	2381	-2.513	-2.157	41	G82	2354	4.098	-3.748	46
G31	2167	-12.642	-6.013	95	G83	2203	-4.075	5.290	88
G32	2165	-17.979	-7.708	96	G84	1973	-5.100	-4.907	103
G33	2170	-8.862	5.404	94	G85	2235	-6.591	-12.508	83
G34	2285	-4.547	2.674	67	G86	2586	-7.841	-8.632	2
G35	2288	-8.904	6.373	66	G87	2430	-1.996	-10.071	31
G36	2284	-1.991	8.927	68	G88	2458	-4.910	-6.331	24
G37	2070	-0.921	11.159	102	G89	2251	-1.820	-3.712	79
G38	2292	-3.161	-1.240	65	G90	2509	6.374	-6.208	9
G39	2306	-11.974	-4.288	58	G91	2261	12.707	-2.746	74
G40	2438	-6.141	4.996	29	G92	2397	12.149	-1.620	39
G41	2226	-5.799	0.463	84	G93	2410	3.701	-2.641	36
G42	2307	6.148	-2.170	57	G94	2448	8.877	3.474	27
G43	2214	1.189	9.539	86	G95	2488	0.674	-13.343	14
G44	2191	1.353	0.544	90	G96	2517	5.666	-5.696	7
G45	2266	6.717	2.499	73	G97	2225	3.166	4.099	85
G46	2272	0.846	8.258	70	G98	2302	-1.133	-6.429	63
G47	2472	7.386	-6.774	16	G99	2532	4.586	-11.101	5
G48	2407	4.529	-1.139	38	G100	2271	-0.697	-11.187	71
G49	2418	3.066	-3.434	35	Chk1	2335	4.959	7.379	50
G50	2468	1.310	-8.446	18	Chk2	2258	9.746	2.107	76
G51	2254	-0.794	5.359	78	Chk3	2315	8.056	-0.864	54
G52	2343	3.715	2.041	47	Grand mean = 2341 kg ha^{-1}		-	-	

Highest yielder = G11 (2669 kg ha^{-1}), Lowest yielder = G84 (1973 kg ha^{-1})

it is always recommended that stability measures should be accompanied by critical observation of yield performance. Hence, genotype G11 could be the best choice for diverse environments as it ranked 5th based on stability and 1st based on the mean yield performance (Table 7). On the other hand, highly unstable genotypes were G67, G32 G10, G85 and G5 where genotypes G5 and G10 exceeded (ranked 19th and 22nd, respectively) in yield performance than three check cultivars and thus were specifically adapted.

Mean yield of genotypes was plotted against ASV for better visualization of stability and yield performance (Fig. 3). It can be inferred from Fig. 3 that G11 and G28 were high yielding genotypes with better stability ranks. Similarly, genotype G86 was also amongst the top yielding genotypes but was relatively responsive to environments than genotypes G28 and G11. The three check cultivars (Speight G-28, Speight G-126 and NC-606) used in the study had moderate stability and yield performance (Fig. 3). Numerous RILs out-performed the three check cultivars based on their stability and mean yield indicated adequate scope available for commercial cultivar development.

Table 7: Ranking of genotypes based on AMMI Stability Value (ASV) and mean yield (kg ha^{-1}) of 100 RILs of FCV tobacco evaluated across six environments during 2012–2013, 2013–2014 and 2014–2015

Lines	ASV	ASV rank	Mean yield	Yield rank	Lines	ASV	ASV rank	Mean yield	Yield rank
56	1.20	1	2178	92	18	7.95	53	2300	64
71	1.43	2	2268	72	23	7.98	54	2326	52
62	1.91	3	2142	99	88	8.16	55	2458	24
2	2.11	4	2235	82	8	8.18	56	2452	25
11	2.12	5	2669	1	46	8.19	57	2272	70
44	2.77	6	2191	90	50	8.34	58	2468	18
30	2.93	7	2381	41	40	8.40	59	2438	29
57	3.05	8	2212	87	27	8.48	60	2307	56
59	3.24	9	2315	53	29	8.58	61	2461	21
15	3.42	10	2465	20	24	8.72	62	2534	4
89	3.60	11	2251	79	19	8.75	63	2249	81
38	3.63	12	2292	65	Chk3	8.82	64	2315	54
52	3.64	13	2343	47	43	8.82	65	2214	86
21	3.97	14	2407	37	Chk1	9.15	66	2335	50
70	4.01	15	2258	75	26	9.18	67	2378	42
12	4.17	16	2499	12	96	9.19	68	2517	7
1	4.18	17	2448	26	76	9.23	69	2175	93
80	4.21	18	2335	49	81	9.31	70	2150	98
97	4.31	19	2225	85	9	9.36	71	2504	11
79	4.31	20	2334	51	36	9.42	72	2284	68
75	4.40	21	2302	61	6	9.43	73	2302	60
22	4.64	22	2423	33	90	9.73	74	2509	9
48	4.71	23	2407	38	94	9.83	75	2448	27
78	4.86	24	2443	28	54	10.06	76	2369	43
3	4.93	25	2276	69	77	10.10	77	2302	62
58	4.95	26	2384	40	86	10.41	78	2586	2
49	4.98	27	2418	35	25	10.47	79	2519	6
28	4.99	28	2563	3	100	10.56	80	2271	71
4	5.03	29	2249	80	Chk2	10.83	81	2258	76
93	5.13	30	2410	36	47	10.93	82	2472	16
20	5.21	31	2459	23	37	11.01	83	2070	102
65	5.52	32	2191	91	64	11.09	84	2257	77
68	5.53	33	2195	89	14	11.14	85	2492	13
34	5.55	34	2285	67	33	11.14	86	2170	94
51	5.81	35	2254	78	74	11.26	87	2359	45
69	5.85	36	2309	55	87	11.32	88	2430	31
16	5.91	37	2424	32	66	11.36	89	2104	101
82	6.30	38	2354	46	63	11.51	90	2164	97
7	6.46	39	2471	17	35	11.53	91	2288	66
41	6.62	40	2226	84	55	12.51	92	2506	10
13	6.65	41	2418	34	99	12.68	93	2532	5
72	6.69	42	2340	48	95	13.66	94	2488	14
83	7.00	43	2203	88	92	13.88	95	2397	39
60	7.10	44	2119	100	39	14.70	96	2306	58
73	7.16	45	2432	30	91	14.77	97	2261	74
42	7.18	46	2307	57	31	14.80	98	2167	95
17	7.34	47	2514	8	5	15.05	99	2467	19
61	7.49	48	2474	15	85	15.41	100	2235	83
98	7.51	49	2302	63	10	19.68	101	2459	22
53	7.60	50	2306	59	32	20.37	102	2165	96
45	7.69	51	2266	73	67	21.31	103	2363	44
84	7.73	52	1973	103	Mean	8.01	-	2340	-

Discussion

In the present study, environments and GE interaction were the driving factors in causing most of the variation in yield. Sum of squares for GE interactions were five times larger than that for genotypes suggesting the possible existence of environment groups (Yan and Kang, 2003; Kadhem and Baktash, 2016). Large sum of squares of environment indicated the diverse nature of environments which caused most of the variation in yield (Tarakanovas and Ruzgas, 2006). The GE interaction captured 44.98% of the total sum of squares which suggested the significant response of genotypes over environments (Mohammad *et al.*, 2011). Sizable proportion of environment and GE interaction in total variation implies the existence of different mega-environments having different sets of high yielding genotypes (Gauch and Zobel, 1996). This offers great impediments to development of stable cultivar for FCV tobacco which could be due to the masking effect of variant environments. The GE interaction was partitioned into four

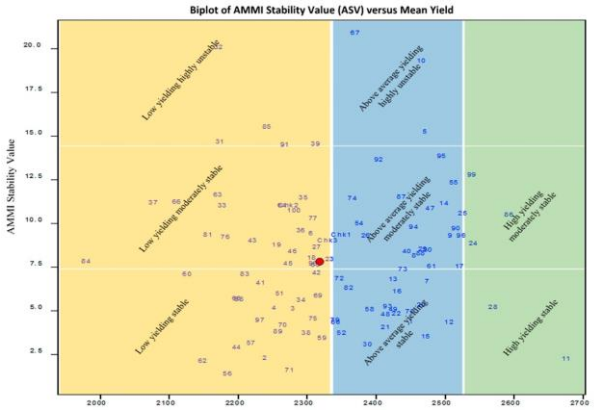

Fig. 3: Biplot of AMMI stability value *vs* mean yield of 100 RILs and three checks of FCV tobacco evaluated across six environments. Red spot represents mean of ASV and yield. Genotypes on the right bottom are stable and high yielding

PCs. Generally, first two PCs explain more variation due to GE interaction. Biplots based on AMMI2 model (first two IPCAs) were more meaningful and credible for stability of lines. Similarly, AMMI3 biplot could be used by plotting IPCA2 and IPCA3; however, higher axes are dominated by noise and have little predictive value (Purchase *et al*., 2000). Therefore, Crossa *et al*. (1991) and Gauch *et al*. (2008) advocated the use of AMMI2 for its practicality and accuracy in exploring patterns of GE interaction than AMMI1. The more IPCA score comes closer to zero (origin), the more stable the genotypes will be in performance. In contrast, genotypes having higher scores of IPCAs indicate differential yield performance across environments which may lead to cross over interaction. (Hagos and Abay, 2013). Significant cross over interaction reduces the efficacy of cultivars by altering their yield ranking across environments (Adugna and Abuschagne, 2002). In the current study, significant GE interaction signaled the need of an in-depth analysis of yield performance of flue-cured tobacco to determine adaptation pattern and stability across diverse environments.

The plain (Mardan) and hilly (Mansehra) area locations were used in this study belong to different climatological zones and experiments were repeated over three years. Therefore, differences among environments were expected. However, significant variance among environments indicated that both locations were not consistent across years. This could be due to differential distribution of rain showers across years as is the case in this study (Table 2). Sadeghi *et al*. (2011) also reported that environments were contributing 87.88% of variance in cured leaf yield of flue-cured tobacco indicating diverse nature of environments. Hence, repeating experiments over years were important for credible assessment of yield stability. Lack of repeatable yield performance in a location poses serious hurdle in breeding for specific location because much of this variance is unpredictable. In such case, breeders are more interested in consistency of yield performance across diverse environments to minimize the crop failure and risk of yield losses.

Past studies reported that assessment of stability in yield performance could be increased by involving multiple locations and years (Ali *et al*., 2017; Koundinyaa *et al*., 2019). Piepho (1998) stated that farmers from marginal lands are more conscious of stable yield performance due to sporadic environmental conditions that cause significant yield losses. Therefore, breeders need to develop cultivars which could perform reasonably well in a diverse range of environments.

Overall, the mean yield at Mansehra (2568 kg ha^{-1}) was higher than Mardan (2111 kg ha^{-1}). Higher yield at Mansehra might be due cooler environment and low temperature throughout the crop season resulting longer period for growth and development. Moreover, lower rain showers in early stage of growth and development at Mansehra might have favourable effects on tobacco yield. This suggests that the ecological conditions of hilly area (Mansehra) are well suited for selection of RILs with higher yield in FCV tobacco. The outcomes of present study got support from findings of Ahmed *et al*. (2014) who also mentioned that Mansehra was more productive in terms of yield than Mardan for FCV tobacco. Generally, Mardan (plain) had better tendency than Mansehra (hilly) to repeat the results based on the yield performance of genotypes which is evident by the small environmental vectors of E-1 and E-3 (Mardan during 2012–2013, 2013–2014) suggesting steady environments (Fig. 2). Contrarily, Mansehra (hilly) had long environmental vectors (E-2, E-4 and E-6) suggesting the capricious climatic conditions at Mansehra. However, environments at Mansehra were more productive even for the poor yielding genotypes of Mardan such as genotype G67 ranked 89th and 99th at E-3 (Mardan during 2013–2014) and E-5 (Mardan during 2014–2015) while ranked 4th and 2nd at E-4 (Mansehra during 2013-2014) and E-6 (Mansehra during 2014–2015), respectively. This shows the affinity of some genotypes towards Mansehra than Mardan. The inconsistent environment of Mansehra could be attributed to its high elevation (1088 m) as compared to Mardan (315 m). High elevation usually brings unpredictable pattern of precipitations and unsteady average temperatures. However, due to long vegetative phase at Mansehra, genotypes produced higher mean yields as compared to Mardan.

Stability is generally classified into two concepts: static and dynamic (Sudaric *et al*., 2006). Static concept of stability refers to consistence performance of genotype across diverse environments while dynamic stability is the relative performance of genotype with respect to mean yield of environment. However, static concept of stability becomes difficult to achieve when there exists yearly variance. In the current study, mean yield of environments were significantly different, therefore breeding for dynamic concept of stability would be more meaningful and realistic. A number of genotypes attained higher mean yield than the check cultivars and possessed better stability. Due to the close

position of G11 to the origin of an AMMI2 biplot, it can be inferred that G11 possesses dynamic stability. The ASV was used as scale to classify genotypes based on their stability. Calculation of ASV is more valuable when dealing with large number of genotypes. In present study, stability of G11 was further confirmed by ASV vs. mean yield biplot. Check cultivars Speight G-28, Speight G-126 and NC-606 had modest stability and yield performance and hence should be replaced by high yielding stable genotypes to ensure better economic profitability in FCV tobacco.

Conclusion

AMMI analysis was efficient in understanding GE interaction. Generally, tobacco genotypes were inconsistent in yield performance across diverse environments which resulted in cross over interaction. Greater fluctuations in environmental conditions in hilly areas provide greater opportunity to breed for stable cultivars. Based on present findings, it can be concluded that G11 (derived from Spt G-28 × Spt G-126) was the highest yielding stable recombinant inbred line. Hence, genotype G11 has the potential to substitute the check cultivars.

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