

Full Length Research Paper

Additive main effects and multiplicative interactions (AMMI) analysis of dry leaf yield in tobacco hybrids across environments

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To determine the yield stability, adaptability and analyze the genotype×environment of Virginia tobacco, 15 hybrids of tobacco including 10 Iranian and 5 international hybrids were evaluated in two different experiments (water stress and normal irrigation) using a randomized completely block design (RCBD) with three replications at two locations including Rasht and Tirtash Tobacco Research Centers, during the growing season of 2006 and 2007 (eight environments). Additive main effects and multiplicative interactions (AMMI) analysis indicated that the dry leaf yield of genotypes were under the major environmental effects of genotype by environment interactions. The first two principal component axes (PCA 1 and 2) were significant ($p \leq 0.01$) and cumulatively contributed to 94.12% of the total genotype by environment interaction. The biplot technique was used to identify appropriate genotype to special locations. Results showed that hybrids PVH03, K394/NC89 and Coker254/NC89 with the lowest interaction, and hybrids ULT109, NC291, Coker254/Coker347 and VE1/Coker347 with the highest interaction were the most stable and unstable hybrids, respectively. Furthermore, hybrids Coker254/K394, NC291 and CC27 were more suitable for Tirtash in non drought stress condition and hybrids NC89/Coker347, K394/Coker347, Coker254/VE1 and ULT109 were more suitable for Rasht in drought stress condition.

Key words: Additive main effects and multiplicative interactions (AMMI), biplot, stability analysis, tobacco.

INTRODUCTION

Genotypes that provide high average yields with minimum genotype by environment interaction (GEI) have been gaining importance over increased yields (Rosielle and Hamblin, 1981; Ceccarelli, 1989; Gauch and Zobel, 1997; Kang, 1998).

Plant breeders invariably encounter genotype x environment interactions (GEIs) when testing varieties across a number of environments. Depending on the magnitude of the interactions or the differential genotypic responses

to environments, the varieties ranking can differ greatly across environments. A combined analysis of variance (ANOVA) can quantify the interactions, and describe the main effects. However, analysis of variance is uninformative for explaining GEI. Other statistical models for describing GEI such as the additive main effects and multiplicative interaction (AMMI) model are useful for understanding GEI. To increase accuracy, AMMI is the model of first choice when main effects and interaction are both important (Zobel et al., 1988). This method integrates analysis of variance and principal component analysis (PCA) into a united approach. The significant feature of this analysis is that adjustment is carried out using information from other locations to refine the estimates within a given location. It removes residual or noise variation from GEI (Crossa et al., 1990a). It has no specific experimental design requirements, except for a two-way data structure (Zobel et al., 1988).

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Abbreviations: PCAs, Principal component axes; MET, multi-environmental trials; AMMI, additive main effects and multiplicative interaction; PCA, principal component analysis; GEIs, genotype x environment interactions.

Table 1. Drought stressed and normal environments, where 15 hybrids were evaluated.

Environment		Site name	Year	Moisture status
Number	Code			
1	TI1	Tirtash	2006	N
2	TI2	Tirtash	2007	N
3	RI1	Rasht	2006	N
4	RI2	Rasht	2007	N
5	TS1	Tirtash	2006	DS
6	TS2	Tirtash	2007	DS
7	RS1	Rasht	2006	DS
8	RS2	Rasht	2007	DS

DS = Drought stress; N = normal.

AMMI analysis provides a graphical representation (biplot) to summarize information on main effects and interactions of both genotypes and environments simultaneously (Crossa, 1990; Crossa et al., 1990a). In AMMI, the additive portion is separated from interaction by ANOVA. Then the PCA, which provides a multiplicative model, is applied to analyze the effect of interaction from the additive ANOVA model. The biplot display of PCA scores plotted against each other provides visual inspection and interpretation of the GEI components. Integrating biplot display and genotypic stability statistics enables genotypes to be grouped based on similarity of performance across diverse environments (Thillainathan and Fernandez, 2001). Previous studies have recommended a combination of stressed and unstressed environments in selection of genotypes that perform well under a wide range of moisture conditions in the tropics (Byrne et al., 1995; Edmeades and Bänziger, 1997; Edmeades et al., 1997a; Vasal et al., 1997). The dependence of crop performance on the genotype and environment as well as their interactions is well established (Gomez and Gomez, 1984).

By exposing a number of genotypes to a set of contrasting environments, it is possible to identify genotypes with a high average yield and low GEI (Ceccarelli, 1989). For this reason, testing selected materials over diverse environments to ensure that forthcoming genotypes have stable performance over a range of environments is a universal practice. However, differential genotypic responses to variable environmental conditions associated with GEI may limit accurate yield estimates and identification of high yielding stable genotypes (Crossa et al., 1991; Basford and Cooper, 1998; Kang, 1998). Various biotic and abiotic stresses have been implicated as causes of GEI. Consequently, improving genotype resistance/tolerance to different stresses to which they would likely be exposed might minimize GEI (Kang, 1998). Selection under managed drought stress at flowering stage is an effective means of increasing tolerance to a number of stresses occurring near flowering (Edmeades and Bänziger, 1997). Thus in mid-season, drought tolerant

genotypes that perform well under variable moisture regimes (Chapman et al., 1997) and N levels (Bänziger et al., 1999) are expected to give better yield with reduced GEI across variable environments as compared to conventionally selected genotypes. Concerning the use of AMMI in multi-environmental trials (MET) data analysis, which partitions the GEI matrix into individual genotypic and environmental scores, an example was provided by Zobel et al. (1988), who studied the GEI of a soybean MET. Another example was provided by Annicchiarico and Perenzin (1994), who showed that earliness x cold stress and plant height x drought interactions for wheat were responsible for the observed GEIs. Yan et al. (2000) applied AMMI analysis to the yield data of winter wheat performance trials, and suggested two winter wheat mega-environments in Ontario.

Yan and Rajcan (2002) applied to genotype by trait biplot analysis, soybean multiple traits and MET data and found that selection for seed yield alone was not only the simplest, but also the most effective strategy in the early stages of soybean breeding. The objectives of this study were to (i) interpret GEI obtained by AMMI analysis of dry leaf yield of 15 Virginia tobacco hybrids over eight environments, (ii) visually assess how to vary yield performances across environments based on the biplot and (iii) determine genotypes with high yields, depending on the differential genotypic responses to environments.

MATERIALS AND METHODS

This study was carried out to determine the dry leaf yield (ton ha^{-1}) performances of 15 tobacco hybrids across eight environments, including drought stress and irrigated conditions separately, for both Tirtash and Rasht locations during the growing season in the years 2006 and 2007 (Table 1). Of the 15 hybrids used, ten varieties including Coker347/VE1, Coker347/NC89, Coker347/K394, Coker347/Coker254, 5-VE1/NC89, VE1/K394, VE1/Coker254, NC89/K394, NC89/Coker254 and Coker254/K394 were derived from the Iranian hybrids, and five varieties including ULT109, PVH03, CC27, NC291 and NC55 were international hybrids.

All experiments were arranged in accordance with a randomized completely block design (RCBD) with 3 replicates. The experimental

Table 2. AMMI analysis for dry leaf yield of 15 hybrids evaluated in 8 environments in Iran.

Source	df	Sum of squares	Mean of squares	F
Genotype (G)	14	39.709	2.836	
Environment (E)	7	1478.973	211.280	
Genotype × Environment	98	129.774	1.324	
IPCA 1	20	105.787	5.289	**36.986
IPCA 2	18	16.355	0.908	**6.349
IPCA 3	16	5.375	0.336	**2.349
IPCA 4	14	1.292	0.092	0.643
IPCA Residual	30	0.955	0.032	
Pooled error	240	34.254	0.143	
Total	359	1682.714		

** Significant at the 0.01 probability level; df = degree of freedom; F = tabulated frequency.

plots consisted of 6 rows, each 5 m in length with 50-cm row spacing. All agronomic application such as, hoeing, weeding and fertilizing were practiced uniformly except irrigation which was only applied to experiment conducted under irrigated conditions. SAS software (1996) was applied to perform data analysis of AMMI on the values of dry leaf yield obtained per plot across environments. The AMMI model equation according to Gauch and Zobel (1996) is:

$$Y_{ger} = \mu + \alpha_g + \beta_e + \sum_n \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + \varepsilon_{ger}$$

Where Y_{ger} = the observed yield of g^{th} genotype in e^{th} environment for r^{th} replicate; μ = the grand mean; α_g = the deviation of mean of the g^{th} genotype from the grand mean m ; β_e = the deviation of mean of the e^{th} environment from the grand mean m ; λ_n = the singular value for the n^{th} interaction principal component axis (PCA); γ_{gn} = the genotype eigenvector for n^{th} (PCA) axis; δ_{en} = the environment eigenvector values for the n^{th} PCA axis; ρ_{ge} = the residual effects; and ε_{ger} = the error term.

Furthermore, AMMI's stability value (ASV) was calculated in order to rank genotypes in terms of stability using the formula suggested by Purchase (1997) as shown below:

AMMI stability value (ASV) =

$$\sqrt{\left[\frac{SS_{IPCA1}}{SS_{IPCA2}} (IPCA1score) \right]^2 + [IPCA2score]^2}$$

where: SS = Sum of squares; IPCA1 = interaction principal component analysis axis 1; IPCA2 = interaction principal component analysis axis 2

In general, an absolute AMMI stability value (ASV) was determined using a procedure that combines IPCA1 and IPCA2. NCSS 2000 software (Hintze, 1998) was used in estimating their association. In addition to these, the AMMI adjusted mean dry leaf yield (ton ha^{-1}) for each hybrid was estimated from untransformed (original) data to demonstrate mean performance.

PROC GLM of SAS was run to calculate genotype by environment interactions. For each genotype and environment, genotypic and environmental scores were obtained by PROC IML of SAS. In addition, principal component axes (PCAs) were extracted and statistically tested by Gollob (1968) F-test procedure (Vargas and Crossa, 2000). These components were used to obtain a biplot by SAS Gplot procedure (Burgueno et al., 2001). To assess fitting

AMMI model, predictive and postdictive approaches offered by Zobel et al. (1988) were applied to the data analysis.

RESULTS AND DISCUSSION

The AMMI analysis of variance of dry leaf yield (ton ha^{-1}) of the 15 hybrids tested in eight environments showed that 87.89% of the total sum of squares was attributable to environmental effects, only 2.36% to genotypic effects and 7.72% to GEI effects (Table 2). A large sum of squares for environments indicated that the environments were diverse, with large differences among environmental means causing most of the variation in dry leaf yield. The magnitude of the GEI sum of squares was 3.3 times larger than that for genotypes, indicating that there were substantial differences in genotypic response across environments. Results from AMMI analysis (Table 2) also showed that the first principal component axis (PCA 1) of the interaction captured 81.52% of the interaction sum of squares in 20.41% of the interaction degrees of freedom. Similarly, the second principal component axis (PCA 2) explained a further 12.60% of the GEI sum of squares. Furthermore, PCA 1 and PCA 2 had sums of squares greater than that of genotypes.

The mean squares for the PCA 1 and PCA 2 were significant at $P = 0.01$ and cumulatively contributed to 94.12% of the total GEI. Therefore, the post-dictive evaluation using an F-test at $P \leq 0.01$ suggested that two principal component axes of the interaction were significant for the model with 38 degrees of freedom. However, the prediction assessment indicated that AMMI with only two interaction principal component axes was the best predictive model (Zobel et al., 1988). This model (AMMI 1 and AMMI 2) had 38 degrees of freedom. Further interaction principal component axes captured mostly noise and therefore did not help to predict validation observations. Thus, the interaction of the 15 genotypes with eight environments was best predicted by the first two principal components of genotypes and environments. The most accurate model for AMMI can be predicted by

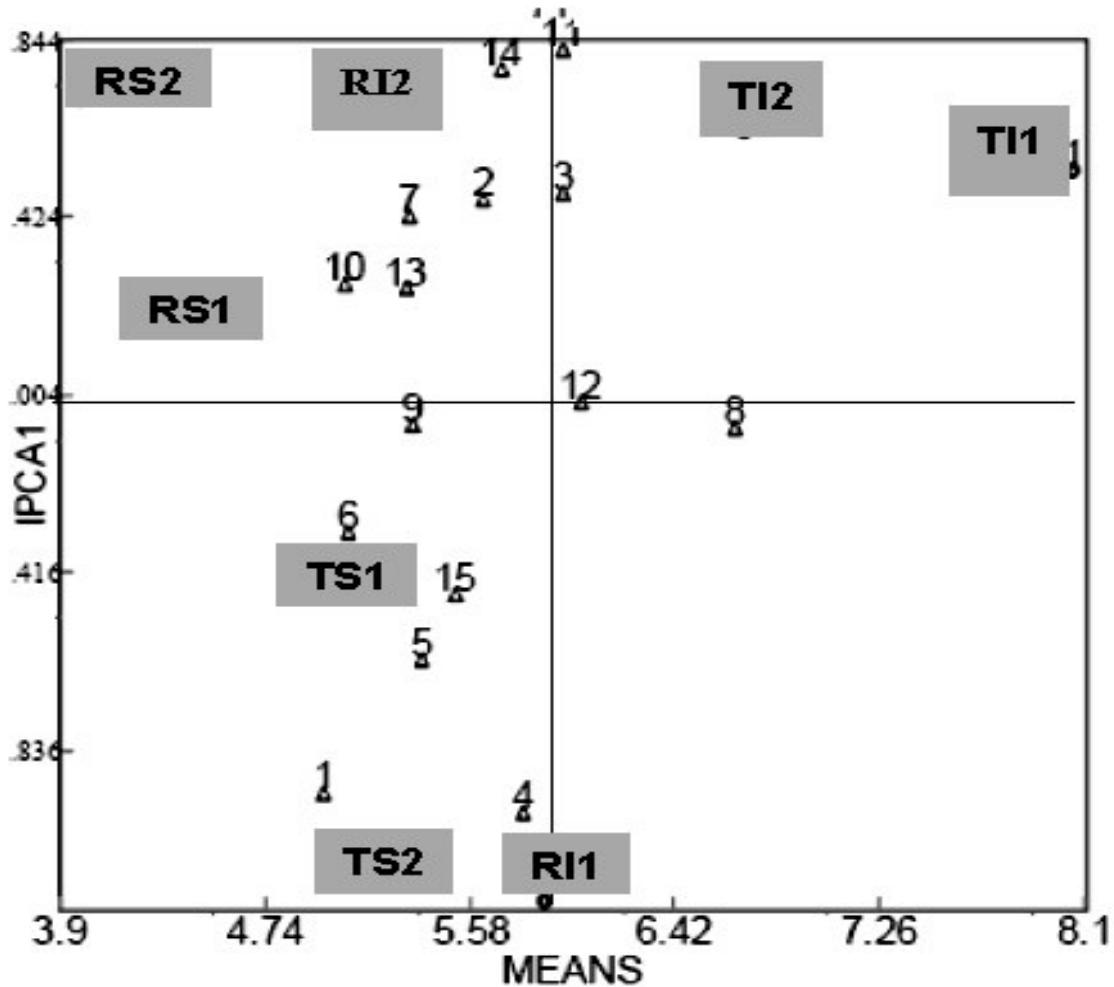


Figure 1. AMMI model 2 biplot of the 15 hybrids (Δ) evaluated in 8 environments.

Table 3. Mean of dry leaf yield together with first and second interaction principal component environment.

Environment	Mean of yield	IPCA-1j*	IPCA-2j**
TI1	8.050	0.534	0.982
TI2	6.698	0.629	0.377
RI1	5.881	-1.119	0.144
RI2	5.362	0.722	-0.247
TS1	5.139	-0.453	-0.064
TS2	5.129	-1.112	-0.036
RS1	4.515	0.181	-0.417
RS2	3.992	0.703	-0.680

* ** are first and second interaction principal component environment, respectively.

terms to be included in an AMMI model cannot be specified a priori without first trying AMMI predictive assessment. In general, factors like type of crop, diversity of the germplasm and range of environmental conditions will affect the degree of complexity of the best predictive model (Crossa et al., 1990b). The AMMI model 2 biplot of the hybrid trials was demonstrated in Figure 1. The environments showed much variability in both main effects and interactions (Table 3). However, the high potential environments were distributed evenly in quadrant II (TI1, TI2) with minimum interaction effects, while the lower potential environments were sparsely distributed in quadrants I (RI2, RS1 and RS2) and IV (TS1, TS2 and RI1) with high IPCA1 values. The lowest yielding environments, RS2 and RS1 demonstrated the highest positive interaction IPCA1 scores. These two environments were characterized by managed drought stress during vegetative propagation. This biplot also indicated TI1 as the highest yielding environment. The hybrids showed less variability in mean yield than in interaction scores (Figure 1). From this biplot, three groups of hybrids were

by using the first two PCAs (Gauch and Zobel, 1996; Yan and Rajcan, 2002). Conversely, Sivapalan et al. (2000) recommended a predictive AMMI model with the first four PCAs. These results indicate that the number of the

Table 4. AMMI adjusted mean Dry leaf yield ($t\ ha^{-1}$) based on untransformed data, AMMI stability values (ASV), and ranking orders of the 15 Hybrids tested across 8 environments.

	Hybrid	Dry leaf yield ($t\ ha^{-1}$)		IPCA1	IPCA2	ASV	
		Mean	Rank			Value	Rank
1	VE1/Coker347	4.988	15	-0.941	-0.219	6.098	14
2	NC89/Coker347	5.629	7	0.466	-0.382	3.039	9
3	K394/Coker347	5.960	3	0.481	-0.268	3.130	10
4	Coker254/Coker347	5.799	5	-0.981	0.518	6.406	15
5	NC89/VE1	5.381	9	-0.618	-0.562	4.037	11
6	K394/VE1	5.084	13	-0.323	0.030	2.090	7
7	Coker254/VE1	5.334	11	0.425	-0.029	2.749	8
8	K394/NC89	6.668	1	-0.072	-0.107	0.478	3
9	Coker254/NC89	5.346	10	-0.066	0.147	0.452	2
10	Coker254/K394	5.065	14	0.266	0.431	1.775	6
11	ULT109	5.957	4	0.821	-0.314	5.321	13
12	PVH03	6.042	2	-0.016	-0.034	0.109	1
13	CC27	5.086	12	0.256	0.567	1.751	5
14	NC291	5.708	6	0.776	0.477	5.043	12
15	NC55	5.517	8	-0.076	-0.061	0.495	4

IPCA = Interaction principal component analysis axis.

identified. Group one includes hybrids PVH03, K394/Coker347, ULT109 and Coker254/Coker347 that showed similar main effects (mean yield) to the grand mean. K394/Coker347, Coker254/Coker347 and ULT109 hybrids showed high interaction scores that varied in direction. Coker254/Coker347 had a positive direction. Whatever the direction is, the greater the IPCA scores, the more specifically adapted these hybrids were to certain environments (Zobel et al., 1988; Crossa et al., 1990a, 1997). Their high interaction with environments was also confirmed by high ASV and rank (Table 4), suggesting erratic (unstable) yield across environments.

Furthermore, ULT109 and K394/Coker347 performed well in RI2 and RI1 environments where they interacted positively. Similarly, TS2 and RI1 favored Coker254/Coker347, which interacted with them positively because all their interaction scores had similar signs (Zobel et al., 1988; Crossa et al., 1997). On the contrary, PVH03 had an IPCA1 score close to zero and ranked first (least) in ASV value, reflecting minimum GEI or stable yield over the environments. NC291, Coker254/Coker347 and ULT109 were well adapted across non-drought stressed environments. Group 2 consisted of hybrids K394/NC89. It exhibited the highest mean yield and IPCA1 score close to zero. K394/NC89 showed three in ASV value. This indicated that K394/NC89 was stable across environments. NC291 and ULT109 were well adapted across non-drought stressed environments. Group 3 included NC55, Coker254/NC89, K394/VE1, NC89/VE1, Coker254/Coker347 and VE1/Coker347. They were relatively the lowest in mean yield. Their interaction scores

were negative, which allowed them to perform well in environments with negative interaction values (TS1, TS2 and RI1). NC55, NC89/VE1 and K394/VE1 were well adapted to drought stress conditions in Tirtash. Their interaction scores were negative, which allowed them to perform well in environments with negative interaction values. In the biplot showing mean yield against IPCA1 scores, PVH03 appeared to be the best in terms of mean yield as well as in minimum GEI, followed by K394/NC89 and Coker254/NC89. However, for the AMMI 2 model, IPCA2 scores was considered in interpreting GEI that captured 12.6% of the interaction sum of squares as suggested by Gauch and Zobel (1996). A biplot is generated using genotypic and environmental scores of the first two AMMI components (Vargas and Crossa, 2000). Furthermore, when IPCA1 was plotted against IPCA2, Purchase (1997) pointed out that the closer the genotypes score to the center of the biplot (Figure 2), the more stable they are. According to this figure, PVH03, K394/NC89 and Coker254/NC89 were close to the center. Coker254/Coker347, VE1/Coker347, NC291 and ULT109 remained in their previous positions, and were unstable in performance, as indicated in both biplots. The best hybrids with respect to sites TI2 and TI1 were hybrids NC291, CC27 and Coker254/K394. Hybrids Coker254/VE1, K394/Coker347, NC89/Coker347 and ULT109 were best for sites RI2, RS2 and RS1; hybrids NC55, K394/NC89 and NC89/Coker347 were best for site TS1; and for RI1 the best hybrid was Coker254/Coker347. Thus, based on Figure 2 and ASV ranking as well as in mean yield (Table 4), PVH03 and K394/NC89 were identified to be superior

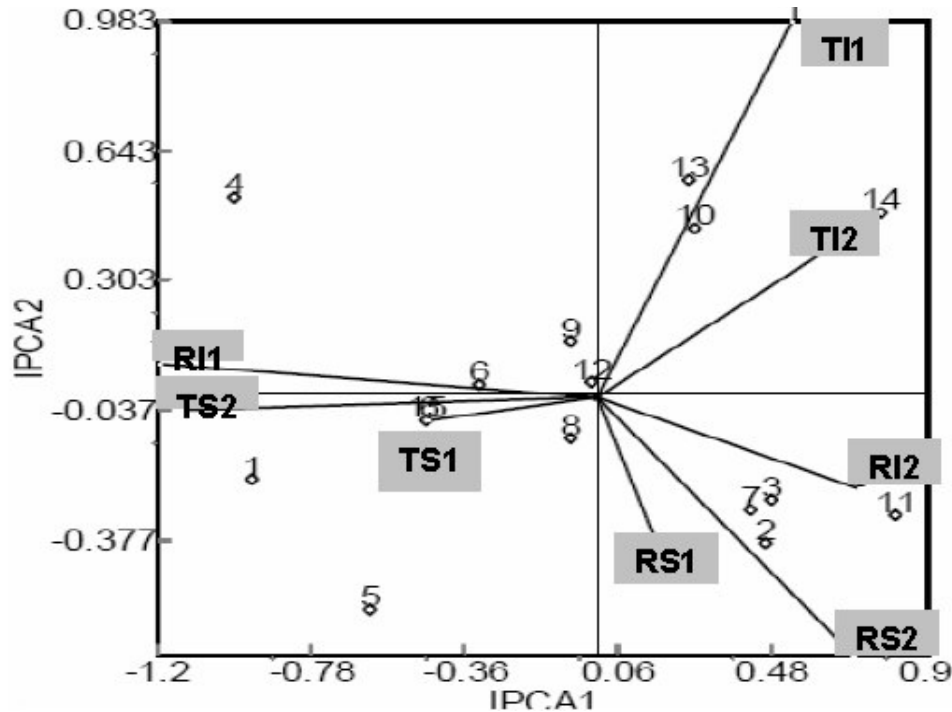


Figure 2. Biplot of 15 hybrids and eight environments for dry leaf yield using genotypic and environmental scores.

followed by Coker254/NC89 in yield stability.

REFERENCES

- Annicchiarico P, Perenzin M (1994). Adaptation patterns and definition of macro-environments for selection and recommendation of common wheat genotypes in Italy. *Plant Breed.* 113: 197-205.
- Bünziger M, Edmeades GO, Laitte HR (1999). Selection for drought tolerance increases maize yield across a range of nitrogen levels. *Crop Sci.* 39: 1035-1040.
- Basford KE, Cooper M (1998). Genotype x environment interactions and some considerations of their implications for wheat breeding in Australia. *Aust. J. Agric. Res.* 49: 153-174.
- Burgueno J, Crossa J, Vargas M (2001). SAS programs for graphing GE and GGE biplots. Biometrics and Statistics Unit, CIMMYT.
- Byrne PF, Bolaños J, Edmeades GO, Eaton DL (1995). Gains from selection under drought versus multilocation testing in related tropical maize populations. *Crop Sci.* 35: 63-69.
- Ceccarelli S (1989) Wide adaptation: How wide? *Euphytica*, 40: 197-205.
- Chapman SC, Crossa J, Edmeades GO (1997). Genotype by environment effects and selection for drought tolerance in tropical maize. I. Two mode pattern analysis of yield. *Euphytica*, 95: 1-9.
- Crossa J (1990). Statistical analyses of multilocation trials. *Adv. Agron.* 4: 55-85.
- Crossa J, Gauch HG, Zobel RW (1990a). Additive main effects and multiplicative interaction analysis of two international maize cultivar trials. *Crop Sci.* 30: 493-500.
- Crossa J, Vasal SK, Beck DL (1990b). Combining ability estimates of CIMMYT's late yellow maize germplasm. *Maydic.* 35: 279-185.
- Crossa J, Fox PN, Pfeiffer WH, Rajaram S, Gauch HG (1991) AMMI adjustment for statistical analysis of an international wheat yield trial. *Theor. Appl. Genet.* 81: 27-37.
- Crossa J, Franco J, Edmeades GO (1997). Experimental design and the analysis of multilocation trials of maize grown under drought stress. In Edmeades GO, Bünziger M, Mickelson HR, and Pena-Valdiva CB (eds). *Developing Drought and Low N-Tolerant Maize. Proceedings of a References/PhD dissertation 156 Symposium, March 25-29, 1996, CIMMYT, El Batan, Mexico.* CIMMYT, pp. 524-536.
- Edmeades GO, Bünziger M (1997). Conclusion: What have we learned and where do we go? In Edmeades GO Bünziger M, Mickelson HR and Pena-Valdiva CB (eds.). *Developing Drought and Low N-Tolerant Maize. Proceedings of a Symposium, March 25-29, 1996, CIMMYT, El Batan, Mexico.* CIMMYT, pp. 557-563.
- Edmeades GO, Bünziger M, Beck DL, Bolaños J, Ortega A (1997a). Development and per se performance of CIMMYT maize populations as drought-tolerant sources. In Edmeades GO, Bünziger M, Mickelson HR, Pena-Valdiva CB (eds.). *Developing Drought and Low N-Tolerant Maize. Proceedings of a Symposium, March 25-29, 1996, CIMMYT, El Batan, Mexico.* CIMMYT, pp. 254-262.
- Gauch HG, Zobel RW (1996). AMMI analyses of yield trials. In Kang MS and Gauch HG (eds.). *Genotype by Environment Interaction.* CRC. Boca Raton, Florida, pp. 85-122.
- Gauch HG, Zobel RW (1997). Identifying mega-environments and targeting genotypes. *Crop Sci.* 37: 311-326.
- Gollob HF (1968). A statistical model which combines features of factor analytic and analysis of variance techniques. *Psychometrik*, 33: 73-115.
- Gomez KA, Gomez AA (1984). *Statistical Procedures for Agricultural Research.* 3rd Edition. John Wiley. New York.
- Hintze JL (1998). *NCSS-2000 Statistical System for Windows.* Number Cruncher Statistical Systems. Keyville, UT.
- Kang MS (1998). Using genotype by environment interaction for crop cultivar development. *Adv. Agron.* 62: 199-246.
- Purchase JL (1997). Parametric analysis to describe G x E interaction and yield stability in winter wheat. PhD Thesis. Department of Agronomy, Faculty of Agriculture, University of the Orange Free State, Bloemfontein, South Africa.
- Rosielle AA, Hamblin J (1981). Theoretical aspects of selection for yield in stress and non-stress environments. *Crop Sci.* 21: 943-946.

- SAS Institute (1996). SAS/STAT user's guide, second edition. SAS Institute Inc., Cary, NC.
- Sivapalan S, Brien LO, Ferrara GO, Hollamby GL, Barclay I, Martin PJ (2000). An adaptation analysis of Australian and CIMMYT/ICARDA wheat germplasm in Australian production environments. *Aust. J. Agric. Res.* 51: 903-915.
- Thillainathan M, Fernandez GCJ (2001). SAS applications for Tai's stability analysis and AMMI model in genotype x environmental interaction (GEI) effects. *J. Hered.* 92: 367-371.
- Vargas M, Crossa J (2000). The AMMI analysis and graphing the biplot. Biometrics and Statistics Unit, CIMMYT.
- Vasal SK, Cordova H, Beck DL, Edmeades GO (1997). Choices among breeding procedures and strategies for developing stress tolerant maize germplasm. In: Edmeades GO, Bänziger M, Mickelson HR and Pena-Valdiva CB (eds.). *Developing Drought and Low N-Tolerant Maize. Proceedings of a Symposium, March 25-29, 1996, CIMMYT, El Batan, Mexico.* CIMMYT, pp. 263-273.
- Yan W, Hunt LA, Sheng Q, Szlavnic Z (2000). Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Sci.* 40: 597-605.
- Yan W, Rajcan I (2002). Biplots analysis of the test sites and trait relations of soybean in Ontario. *Crop Sci.* 42: 11-20.
- Zobel RW, Wright MS, Gauch HG (1988). Statistical analysis of a yield trial. *Agron J.* 80: 388-393.