USE OF LINE × TESTER MATING DESIGN IN ESTIMATION OF GENETIC EFFECTS OR GENETIC VARIANCE COMPONENTS

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ABSTRACT

The objectives of the present investigation are to derive the ANOVA and test of significance for fixed lines and testers evaluated under random environments, to derive the ANOVA for random set of lines and testers and to estimate the proper components and to discuss the validity of using line × tester mating design for the estimation of type gene action with in a population. Ten new yellow inbred lines namely Nb451 to Nb460, were used for the present study. These lines were testcrossed to two testers; (SC52 and SC155). The resultant 20 testcrosses and two commercial hybrids i.e., TWC352 and TWC353 were evaluated during 2009 season in two locations i.e., Sakha and Nubaria Agriculture Research Station, ARC. For fixed model, the results showed that the differences between the tested locations were highly significant for both models. Only, highly significant differences were observed of locations × lines × testers interaction, suggesting the line × tester interaction should be examined for each location separately. The crosses; Nb457 × SC52, Nb455 × SC155, at Sakha, and Nb460 × SC52 at Nubaria, had grain yield equal or did not significantly differed than the check hybrids. Also, they had significant positive SCA effects (p ≤ 0.05). The results would indicate that SCA was more affected by locations. Therefore, the future evaluation should be conducted over several locations in order to reduce the error of GCA. Also, increasing the number of locations would decrease the variance of the error for SCA. For random model, in order to be used the line × tester mating design for the estimation of genetic variance components, the number of lines and testers should be at least 50 lines (25 × 25). The use of line × tester design for the estimation of genetic variance components is tedious. Other mating designs would be easier. In conclusion, line × tester mating design is mainly recommended as a design to evaluate fixed set of lines crossed to selected testers in order to identify the best hybrid from the possible hybrids under test. Also, the GCA for the lines and SCA of the hybrids could be identified.

Key words: Maize, GCA, SCA, Fixed, Random, Mixed model.

Abbreviations: GCA, general combining ability; SCA, specific combining ability, SC, single-cross; TWC, three-way cross;

INTRODUCTION

Davis (1927) was the first to suggest the evaluation of new inbred lines by crossing them to a common male parent to produce testcrosses of the lines under evaluation. This male parent was designated as a tester. Matzinger (1953) showed that a heterogeneous tester contributes less to line × tester interaction. It was suggested a double-double cross to be more appropriate to differeniate among the lines and to reduce the effect of line × tester interaction.

Hallauer and Miranda, 1980, Russell *et al.*, 1992; Menz *et al.*, 1999 concluded that choice of a suitable tester should be based on simplicity in its use, its ability to classify the relative merit of lines, maximize genetic gain, and give the most accurate information on tested lines. However, it is difficult to identify tester having all these characteristics. The use

of a single-cross as a tester has been reported by Horner *et al.* (1976). The use of an inbred as tester was suggested by Russell and Eberhart (1975) and it has been widely used by breeders (Walejko and Russell, 1977; Darrah, 1985; Horner *et al.*, 1989). Castellanos *et al.* (1998) concluded that the single cross was the best alternative in a breeding program oriented to generate superior three-way hybrids.

Hull (1945) suggested to use of an inbred to select for overdominance and inbred line tester would be efficient than broad base tester. Breeders usually use more than one tester to evaluate new hybrids. Sprague and Tatum (1942) introduced the concepts of general (GCA) and specific (SCA) combining ability of line x tester evaluation. GCA denotes the average performance of 1 of ei parent in crosses, while SCA refers to the deviation of individual cross from the average GCA of the two parents.

Sprague and Tatum (1942) suggested the use of several testers for the evaluation of new lines. They treated the mating design of the line × tester as a two-factor design where the GCA of the lines or testers are the main effects, while the SCA as the interaction of line × tester. Usually the line × tester mating design is evaluated in the field as RCBD and it is repeated over several random environments, i.e., year or location to test the interactions of GCA and SCA with environments.

The analysis of variance of the line × tester mating design is similar to that given by Hallauer and Miranda (1980). The test of significance of the components of lines, tester, line × tester and their interaction would depend on the type of the effects of the previous components.

The destiction between fixed (I) and random (II) models is important to derive the expected mean square (EMS) in order to calculate the proper F ratio. The effects of lines or testers are consider fixed when the inferences detected from the evaluations are restricted to the lines or testers under test and each line or tester is considered as a population. On the other hand, the effects will be treated as random effects and the lines or testers represent a random sample from a certain population and the inferences will be given to that population not to the lines under test.

For the fixed model (model I), the objective of the evaluation is the comparisons between the GCA of the lines under test and the SCA of the resulting hybrids. For the random model, the objective is the estimate of variance components due to lines, testers and lines × testers and latter they will be translated in terms of additive and dominance variance with in the studied population. The assumption of both models random and fixed is not valid (Baker, 1978).

Although the analyses of variance are very similar for both models, parameters tested in the two models are very different. The choice of the appropriate model has been left to the breeder.

The objectives of the present investigation are *(1)* to derive the ANOVA and test of significance for fixed lines and testers evaluated under random environments, *(2)* to derive the ANOVA for random set of lines and testers and to estimate the proper components, *(3)* to discuss the validly of using line × tester mating design for the estimation of type gene action with a population.

MATERIALS AND METHODS

Field procedure

To show the difference between the use of model I or model II in the analysis of variance of line × tester mating design, the following field experiment was conducted.

Ten new yellow inbred lines namely Nb451 to Nb460, were isolated from the F_2 population, Nubaria-40, in the breeding nursery at Nubaria Agriculture Research Station, and were used for the present study. Two testers were used to evaluate the performance of these lines using the two single-crosses SC52 and SC155.

In 2008 season, testcrosses were developed using a mixture of pollen grains of male plants of each of the tester. At harvest, all ears from each testcross were shelled in bulk and a part of this seed was used for the evaluation trial. The resultant 20 testcrosses and the two commercial hybrids i.e., three-way crosses 352 and 353 (TWC352 and TWC353) were evaluated during 2009 season at two locations i.e., Sakha and Nubaria Agric. Res. Stations, ARC.

A randomized complete block design, with four replications, was used at each location. Plot consisted of one row, six meter long and 80 cm apart. Hand planting was used; hill spacing was 25 cm along the row. Two kernels per hill were sown and thinned later to one plant per hill to provide a population of, approximately, 50000 plants per hectare. All cultural practices were applied as recommended at the proper time. Data were collected for ears weight per plot (kg), shelled, adjusted to 15.5% grain moisture and converted to grain yield (Mg ha^{-1}).

Statistical analysis

The collected data were statistically analyzed assuming that both lines and tester are fixed while environment was assumed random in order to estimate GCA effects for line and SCA effect for the hybrids and their interaction with environments. The same data were used assuming both the lines and testers were randomly sampled from a certain populations in order to estimate the additive and dominance variance within that population. The two analyses of variance are given below as presented by Steel and Torrie (1980).

Fixed model: in this case, both lines and testers are considered fixed. The outlines of the ANOVA, for the combined analysis, are given in Table (1). It shows the EMS and the proper F ratio. From this table, the LSD value for the line and tester effects is given below the table. The relative importance of GCA and SCA could be tested by calculating the K^2_L, K^2_{LT}. Also, the relative importance of both type of interactions with environments are shown in the table.

Random model: in this case, the objective of the evaluation is to estimate the additive and dominance variances in the population under investigation. The tester should be sampled at random from the same population. In this case, the line × tester mating design is similar to North Carolina Design II (Comstock and Robinson, 1948). The outline of the ANOVA is shown in Table (2). Estimates of Cov HS lines and testers are translated in terms of σ^2_A, while σ^2_{LT} denotes Cov FS − 2Cov HS, which is a function of σ^2_D.

Table (1): Outline of ANOVA table for line x tester model when both factors are fixed repeated over random environments.

Source of variation	Df	Expected MS	MS	F-test
Environments (E)	$(e-1)$			
Block / E	$(r-1)e$			
Lines (L)	$(l-1)$	$\sigma^2_e + rt\,\sigma^2_{LE} + ert\,K^2_L$	M_7	M_7/M_6
L × E	$(l-1)(e-1)$	$\sigma^2_e + rt\,\sigma^2_{LE}$	M_6	M_6/M_1
Tester (T)	$(t-1)$	$\sigma^2_e + rl\,\sigma^2_{TE} + erl\,K^2_T$	M_5	M_5/M_4
T × E	$(t-1)(e-1)$	$\sigma^2_e + rl\,\sigma^2_{TE}$	M_4	M_4/M_1
L × T	$(l-1)(t-1)$	$\sigma^2_e + r\,\sigma^2_{LTE} + er\,K^2_{LT}$	M_3	M_3/M_2
L × T ×E	$(l-1)(t-1)(e-1)$	$\sigma^2_e + r\,\sigma^2_{LTE}$	M_2	M_2/M_1
Combined error	$(r-1)(lt-1)e$	σ^2_e	M_1	

Where r, l, t, e are no. of blocks, no. of lines, no. of testers and no. of environments, respectively.

Statistics:

$LSD_{(0.05)}$ for Lines means $= [2\,M_6/ert]^{1/2} \times t_{0.05}$

$LSD_{(0.05)}$ for Testers means $= [2\,M_4/erl]^{1/2} \times t_{0.05}$

$LSD_{(0.05)}$ for Line x Tester means $= [2\,M_2/er]^{1/2} \times t_{0.05}$

k^2_L (k^2 GCA for lines) $= [M_7 - M_6]/ert$

k^2_T (k^2 GCA for testers) $= [M_5 - M_4]/erl$

k^2_{LT} (k^2 SCA $= [M_3 - M_2]/er$

Relative contribution GCA : SCA $= k^2_L / k^2_{LT}$

Relative contribution GCA × E : SCA × E $= \sigma^2_{LE} / \sigma^2_{LTE}$

Table (2): Outline of ANOVA table for line x tester model when both factors are random repeated over random environments.

Source of variation	Df	Expected MS	MS	F-test
Environments (E)	$(e-1)$			
Block / E	$(r-1)e$			
Lines (L)	$(l-1)$	$\sigma^2_e + r\,\sigma^2_{LTE} + rt\,\sigma^2_{LE} + re\,\sigma^2_{LT} + ert\,\sigma^2_L$	M_7	$[M_7+M_2]/[M_6+M_3]$
L x E	$(l-1)(e-1)$	$\sigma^2_e + r\,\sigma^2_{LTE} + rt\,\sigma^2_{LE}$	M_6	M_6/M_2
Tester (T)	$(t-1)$	$\sigma^2_e + r\,\sigma^2_{LTE} + rl\,\sigma^2_{TE} + er\,\sigma^2_{LT} + erl\,\sigma^2_T$	M_5	$[M_5+M_2]/[M_3+M_4]$
T × E	$(t-1)(e-1)$	$\sigma^2_e + r\,\sigma^2_{LTE} + rl\,\sigma^2_{TE}$	M_4	M_4/M_2
L × T	$(l-1)(t-1)$	$\sigma^2_e + r\,\sigma^2_{LTE} + er\,\sigma^2_{LT}$	M_3	M_3/M_2
L × T × E	$(l-1)(t-1)(e-1)$	$\sigma^2_e + r\,\sigma^2_{LTE}$	M_2	M_2/M_1
Combined error	$(r-1)(lt-1)e$	σ^2_e	M_1	

Where F = inbreeding coefficients.

Statistics:

$\sigma^2_L = [M_7 + M_2 - M_6 - M_3]/ert = \text{Cov HS} = [(1+F)/4]\,\sigma^2_A$

$\sigma^2_T = [M_5 + M_2 - M_3 - M_4]/erl = \text{Cov HS} = [(1+F)/4]\,\sigma^2_A$

$\sigma^2_{LT} = [M_3 - M_2]/er = \text{Cov FS} - 2\,\text{Cov HS} = [(1+F)/2]^2\,\sigma^2_D$

$\sigma^2_{LE} = [M_6 - M_2]/rt = [(1+F)/4]\,\sigma^2_{AE}$

$\sigma^2_{TE} = [M_3 - M_2]/re = [(1+F)/4]\,\sigma^2_{AE}$

$\sigma^2_{LTE} = [M_2 - M_1]/r = [(1+F)/2]^2\,\sigma^2_{DE}$

Combined analysis of data was done using Proc ANOVA for both models (SAS system, 2003).

RESULTS AND DISCUSSION

The data from the line × tester mating design were analyzed into two forms, when the lines and testers are fixed and when the two factors are considered random.

1- Fixed effects (lines and testers):

Analysis of variance for grain yield using mixed models (random environments & fixed effects for lines and testers) is presented in Table (3). The results showed that the differences between the tested locations were highly significant for both models. Only, highly significant differences were observed of locations × lines × testers interaction, suggest the line × tester interaction should be examined for each location separately.

Means of grain yield for the tested lines and testers at each location are presented in Table (4). The results showed that the crosses, Nb457 × SC52, Nb455 × SC155, at Sakha, and Nb460 × SC52 at Nubaria, had grain yield equal or did not significantly different than the check hybrids. Also, they had significant positive SCA effects ($p \leq 0.05$). in addition, the crosses, Nb451 × SC52, Nb452 × SC52, Nb456 × SC52, Nb458 × SC155 and Nb460 × SC155 at Sakha, and Nb452 × SC52, Nb451 × SC155 and Nb457 × SC155, at Nubaria, equal or did not significantly different than the check hybrid and, at the same time had high positive SCA effects.

The results would indicate that SCA was more affected by locations. Therefore, the evaluation should be planned over several locations in order to reduce the error of GCA as the error of test in as variance of $GCA = \dfrac{\sigma^2 e}{re} + \dfrac{\sigma^2 LE}{e}$, also, increasing the number of locations would decrease the variance of the error for SCA as it is: Variance of $SCA = \dfrac{\sigma^2 e}{re}$

If the environment effect was considered fixed, wrong inference will be detected as shown in the last column of Table (3).

Table (3): Analysis of variance of grain yield using fixed line x tester model for ten lines testcrosses evaluated in two random locations at 2009.

Source of variation	df	Mean square	Valid F-value Random env.	Unvalid F-value Fixed env.
Locations (Loc)	1	48.96	63.65 **	76.07 **
Replications / Loc	6	0.76		
Lines (Lin)	9	1.64	1.00 ns	2.56 *
Testers (Tes)	1	14.14	16.10 ns	21.98 **
Lin × Tes	9	1.08	0.51 ns	1.68 ns
Loc × Lin	9	1.63	0.77 ns	2.55 *
Loc × Tes	1	0.88	0.41 ns	1.37 ns
Loc × Lin × Tes	9	2.11	3.30 **	3.28 **
Error	126	0.64		
CV		10.2		

*, ** indicate significance at the 0.05 and 0.01 level of probability, respectively.

Table (4): Means of grain yield (Mg ha^{-1}) for 20 testcrosses and their SCA resulting from the 10 lines × two testers evaluated under Sakha and Nubaria locations.

| Line | Sakha | | | | Nubaria | | | |
| | SC52 | | SC155 | | SC52 | | SC155 | |
	GY	SCA	GY	SCA	GY	SCA	GY	SCA
Nb451	7.84†	0.37	6.35	-0.37	8.51†	-0.45	8.96†	0.45
Nb452	7.91†	0.42	6.32	-0.42	9.64†	0.26	8.67†	-0.26
Nb453	7.18	-0.17	6.78	0.17	8.77†	0.04	8.24†	-0.04
Nb454	7.96†	0.27	6.68	-0.27	8.66†	0.14	7.94	-0.14
Nb455	6.66	-0.86	7.64†	0.86*	7.89	-0.14	7.72	0.14
Nb456	7.93†	0.29	6.61	-0.29	9.06†	0.09	8.42†	-0.09
Nb457	9.53†	0.85*	7.08†	-0.85	8.30†	-0.46	8.77†	0.46
Nb458	6.44	-0.56	6.82†	0.56	8.42†	-0.09	8.14	0.09
Nb459	7.40	-0.06	6.79	0.06	7.88	-0.07	7.57	0.07
Nb460	7.48	-0.55	7.85†	0.55	8.81†	0.66*	7.05	-0.66
SC52	8.12							
SC155	7.52							
SE (s$_{ij}$)			0.35				0.43	
SE (s$_{ij}$ – s$_{kl}$)			0.50				0.62	

† Did not significant different than the two check hybrids.

* Significantly different from zero.

2- Random model (line × tester mating design for the estimation of the additive and dominance variances:

If the lines and testers were randomly chosen from a certain population with the objective of estimation of σ^2_A, σ^2_D prevailing in this population the ANOVA table will be similar to Table (2). In this case, lines, testers and locations were assumed random. The MS and F ratios for the sources of the variations are given in Table (5).

Table (5): MS and F ratio of the line x tester mating design (both random) evaluated under two different locations (random).

Source of variation	df	Expected MS	MS	Cal. F
Environments (E)	1		48.96	
Block / E	6		0.77	
Lines (L)	9	$\sigma^2_e + 2\sigma^2_{LTE} + 4\sigma^2_{LT} + 4\sigma^2_{LE} + 8\sigma^2_L$	1.65	1.4 ^{ns}
Tester (T)	1	$\sigma^2_e + 2\sigma^2_{LTE} + 4\sigma^2_{LT} + 20\sigma^2_{TE} + 40\sigma^2_T$	14.15	8.3 *
L × T	9	$\sigma^2_e + 2\sigma^2_{LTE} + 4\sigma^2_{LT}$	1.08	1.2 ^{ns}
L × E	9	$\sigma^2_e + 2\sigma^2_{LTE} + 4\sigma^2_{LE}$	1.64	1.9 ^{ns}
T × E	1	$\sigma^2_e + 2\sigma^2_{LTE} + 20\sigma^2_{TE}$	0.88	0.4 ^{ns}
L × T × E	9	$\sigma^2_e + 2\sigma^2_{LTE}$	2.11	3.3 **
Combined error	126	σ^2_e	0.64	

ns, *, ** denote insignificance and significance at the 0.05 and 0.01 levels, respectively.

The test of significance for the previous table indicates that both the testers and tester × line × environment were significant sources of variation. From the above table, also, the various variance components were:

$\sigma^2_L = 0.13$, $\sigma^2_T = 0.36$, $\sigma^2_{LT} = -0.28$ ($\approx$ o), $\sigma^2_{LE} = -0.12$ ($\approx$ o), $\sigma^2_{TE} = -0.06$ ($\approx$ o) and $\sigma^2_{LTE} = 0.74$. The previous negative estimates were considered zero.

In order to translate the previous estimates in terms of σ^2_A, σ^2_D the following assumptions should be full field:

(1) Both the lines and testers should be chosen at random from the population under investigations.

(2) Epistasis is assumed to be negligible accordingly

$$\sigma_L^2 + \sigma_T^2 = 2\left(\frac{1+F}{4}\right)\sigma_A^2$$
$$= 0.13 + 0.36 = \sigma_A^2 = 0.49$$
$$\sigma_{LT}^2 = \sigma_D^2 = -0.28 \approx 0$$

Both σ_T^2 and σ_{LTE}^2 were significant and they were estimates of σ_A^2 and σ_{AE}^2, respectively.

In order to use the line × tester mating design for the estimation of genetic variance components: *(1)* the number of lines and testers should be at least 50 lines (25 × 25), *(2)* to reduce the number of crosses, the sampled lines and testers would be split in five sets each set consisted of five lines and five testers. Therefore, the use of line × tester design for the estimation of genetic variance components is tedious. Other mating designs would be easier.

In conclusion, line x tester mating design is mainly a design to evaluate fixed set of lines crossed to selected testers in order to identify the best hybrid from possible hybrids under test. Also, the effects of GCA for the lines and SCA of the hybrids could be estimated.

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الملخص العربى
استخدام تحليل السلالة فى الكشاف لتقدير التأثيرات الوراثية أو مكونات التباين الوراثى

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تهدف هذه الدراسة الى عمل جدول تحليل التباين واختبار المعنوية عند تقييم السلالات والكشافات ذات التأثير الثابت تحت البيئات عشوائية التأثير، وكذلك عمل جدول تحليل التباين عند تقييم السلالات والكشافات ذات التأثير العشوائى بالاضافة الى تقدير المكونات الوراثية المناسبة.

تم استنباط عشر سلالات صفراء جديدة من الذرة الشامية (من Nb451 الى Nb460)، وفى موسم ٢٠٠٨ تم عمل الهجن الاختبارية لهذه السلالات مع الكشافات (SC52 and SC155). فى موسم ٢٠٠٩ تم تقييم الهجن الاختبارية الناتجة مع هجينين تجاريين كهجن مقارنة (TWC352 and TWC353) فى كلا من محطتى البحوث الزراعية بسخا والنوبارية.

أوضحت النتائج وجود فروق معنوية بين البيئات المختبرة لكلا الطريقتين (الثابت والعشوائى). كما ظهرت فروق معنوية فقط للتفاعل الثلاثى (Loc x L x T) عند استخدام التأثير العشوائى مما يؤكد أنة يجب تقييم السلالات والكشافات فى عدد أكبر من البيئات المختلفة.

أعطى الهجينان Nb457 × SC52, Nb455 × SC155 أعلى محصول ولايختلف معنويا عن هجن المقارنة فى سخا، وكذلك أعطى الهجين Nb460 × SC52 محصولا لايختلف معنويا عن هجين المقارنة فى النوبارية. أيضا أعطت هذه الهجن قيم معنوية لتأثير SCA.

أظهرت النتائج أن SCA تأثرت أكثر بواسطة البيئات المختلفة وعلى ذلك يجب اجراء التقييم فى عدد من البيئات المختلفة وذلك لتقليل الخطأ الخاص بقيم GCA. أيضا سوف ينعكس ذلك على خفض الخطأ الخاص بقيم SCA.

عند استخدام التأثير العشوائى – وعند استخدام تصميم السلالة فى الكشاف لتقدير مكونات التباين الوراثى – يجب أن يكون عدد السلالات والكشافات حوالى ٥٠ سلالة على الأقل تقسم الى مجموعتين. أظهرت النتائج أن استخدام تصميم السلالة فى الكشاف لتقدير مكونات التباين الوراثى غير عملى وأن التصميمات الأخرى أكثر سهولة.

يمكن القول أن تصميم السلالة فى الكشاف يستخدم أساسا لتقييم مجموعة من السلالات ذات التأثير الثابت ويتم تهجينها الى كشافات منخبة بهدف انتخاب أفضل هجين من الهجن الممكن الحصول عليها تحت الاختبار. كما يمكن الحصول على القدرة العامة على التآلف (GCA) للسلالات المختبرة وكذلك القدرة الخاصة على التآلف (SCA) للهجن تحت الاختبار. وعند تقييمها فى عدد من البيئات عشوائية التأثير يجب اختبار كل من GCA و SCA وكذلك التفاعل Env × GCA و Env × SCA على الترتيب وتجنب اختبارهم باستعمال الخطأ التجريبى.