



RESEARCH ARTICLE

REML/BLUP IN ANALYSIS OF PRE-COMMERCIAL SIMPLE MAIZE HYBRID

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ABSTRACT

Adoption of mixed models that estimate genetic parameters and predicted genotypic values correctly are important in increasing the efficiency of breeding programs, as decision-making during the selection process. The aim of the present study was to estimate variance components via REML and predict genotypic values of simple maize hybrids via BLUP. Trial was conducted in the city of Frederico Westphalen, RS, Brazil. Pre-commercial hybrids used were coming from the KSP Seeds Ltd. company's breeding program, located in the city of Pato Branco-PR. A randomized complete block design with three replications was used. Twenty-four pre-commercial simple hybrids (KSP Seeds Ltd.) were used in trial's conducting. Assessed traits were: 1. ear insertion height (EH) 2. Plant height (PH) 3. ear diameter (ED) 4. ear length (EL): 5. Average number of rows (NR) 6. number of kernels per row (NKR) 7. grain yield (GY) 8. Hundred-kernels weight (HKW). Deviance analysis was performed with and without heritability estimates thus generating obtaining their deviances, by subtracting the reduced model of the complete model it was obtained the likelihood ratio test (LRT), compared with tabulated value of the chi-square test one degree of freedom. Estimates of genetic parameters were obtained by restricted Maximum Likelihood and Best Linear Unbiased Prediction models (REML / BLUP), using Selegen software (Resende, 2007). Presence of genetic variation among the studied hybrids indicate that can have genetic gain in these maize hybrid's breeding program. KSP22 pre-commercial simple maize hybrid has high performance for grain yield, enabling their inclusion in regional trials with commercial checks.

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INTRODUCTION

Each harvest are produced new maize hybrids in breeding programs of companies, which undergo preliminary assessments. One of the setbacks often faced by breeders in the selection and recommendation of genotypes is to quantify the magnitude of the genotype x environment interaction, noting that it is the breeder's nature the adoption of strategies in order

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to reduce or take advantage of such effects (Cruz and Regazzi, 2001). During preliminary assessments, several genotypes are candidates to be included in the select group, requiring from the breeder the use of appropriate tools to ensure the selection efficacy. The adoption of quantitative genetic methods with consistent statistical methods may result in more accurate estimates of genetic and residual variance components; therefore, improving the predictions of the genotypic value of subject. The use of mixed models or individual BLUP (Resende et al., 1996) introduced changes in the estimation of variance components and genetic parameters; covariance were estimated and interpreted in terms of their mathematical

expectations, generating the variance components. REML/BLUP procedures subsequently developed allow the variance components are estimated directly from the data set, as well as the variances of random effects. Restricted maximum likelihood (REML) method, proposed by (Patterson and Thompson, 1971), became the standard method for estimating the variance and genetic parameters components, mainly for trials with unbalanced data (Filho and De Resende, 2000). The preference for this method stems from its statistical properties superior to the methods of the least squares estimators (Searle et al., 1992).

Mixed models technique have been used by many researchers in the genetic breeding area in crops such as eucalyptus (Resende et al., 1996; Rosy et al., 2012), coffee (Resende et al., 2001), potato (Souza et al., 2005), sugar cane (Zeni-Neto et al., 2008), corn (Arnhold et al., 2012; Dovale et al., 2013), rice (Borges et al., 2009; Dovale et al., 2013). Thus, the standard analytical procedure recommended for studies involving quantitative genetics is the REML / BLUP, with estimates of variance components by restricted maximum likelihood (REML) and Best linear unbiased prediction (BLUP) (Resende et al., 2001). In this context, adoption of models that correctly estimate genetic parameters and predicted genotypic values become important to increase the efficacy of the breeders in decision making during pre-commercial hybrid selection process, especially when there is a select group of superior hybrids and are sought inside of this group, materials with superior performance. The aim of this study was to estimate the variance components by REML and predict the genotypic values of pre-commercial maize hybrids by BLUP.

MATERIAL AND METHODS

Trial was conducted in the city of Frederico Westphalen-RS. Pre-commercial hybrids used were coming from the KSP Seeds Ltd. company's breeding program, located in the city of Pato Branco-PR. A randomized complete block design with three replications was used. The experimental plots consisted of two 5-m long cultivar rows, with 42 plants per plot, spaced by 0.70 m between rows, corresponding to a seeding density of 60,000 plants ha⁻¹. Cultural treatments were performed in accordance with the technical recommendations of the maize crop. Rows were opened with mechanized seeder and on the same moment, basic chemical fertilization was performed. In conducting the test, we used 24 simple pre-commercial hybrids (KSP Seeds Ltd.). The hybrids were seeded according to Agroclimatic zoning. In each experimental plot were assessed the following agronomic traits: 1. ear insertion height (EH): refers to the height from the ground to the first ear, given in cm; 2. Plant height (PH): refers to the height from the ground to the insertion node of the last leaf of the plant, given in cm; 3. ear diameter (ED) refers to the average diameter of the middle part three ears held on average for three ears, given in mm; 4. Average ear length (EL): refers to the average length of three cobs, given in centimeters 5. Average number of rows (NR): the count refers to the number of three kernels rows, given in units; 6. Number of kernels per row (NKR): refers to the average number of counting kernels in one row of each three ears, given in units; 7. Grain yield in bags (1 Brazilian

bag = 60 kg) per hectare (RG): refers to the total grain yield in sacks per hectare, adjusted to 13% moisture in a standard stand of 42 plants in two lines; 8. hundred-kernel weight (HKW): refers to the average weight of 100 grains counting performed in three ears, given in grams. For statistical analysis was performed deviance analysis with and without the heritability estimates thus generating obtaining their deviances, by subtracting the reduced model of the complete model, obtaining the likelihood ratio test (LRT), compared with tabulated value Chi-square test with one degree of freedom (DF) at 5% probability of error. Estimates of genetic parameters were obtained by restricted maximum likelihood models and best linear unbiased predictor (REML / BLUP). The used model according to Resende (2007) was:

$$y = Xr + Zg + e, \text{ were:}$$

y = the data vector; r = the vector of replication effects (assumed to be fixed) added to the overall average; g = the vector of genotypic effects (assumed to be random) and e = the vector of errors or noise (random).

Capital letters X and Z are the incidence matrices for the referred effects. Predicted genotypic values unobstructed of all interaction with environments were obtained by $u + g$, where u = average of all local and g = predicted genotypic value. To determine confidence intervals, we used the equation described by Resende (2002):

$$CI = V_G \pm t[(1 - r_{aa}^2)^{1/2}], \text{ were,}$$

CI = Confidence interval; V_G = Genotypic value; t = Student distribution value associated with a certain confidence level ($t=1.96$, to 95% of confidence); r_{aa}^2 = regarding accuracy; σ_G^2 = genetic variance.

Data were submitted to analysis by mixed models, using the model 21 in Selegen software (Resende, 2007). Thus, we obtained estimates of restricted maximum likelihood and the best linear unbiased predictor (REML / BLUP).

RESULTS AND DISCUSSION

Deviance analysis revealed meaningful differences by the LRT at 5% probability error by the chi-square test with 1 DF for all assessed traits (Table 1). Thus, deviance analysis revealed meaningful genetic differences between the 24 pre-commercial hybrids assessed in this study. Variance components and genetic parameters estimates can be seen in table 2. Regarding the estimates of the variance components it can be seen that for PH, 53% of the phenotypic variance were coming from environmental and 47% of genetic nature. For EH trait, genetic component contributes with 61% of phenotypic variance and 39% of environmental nature. The ED trait demonstrates greater participation of the environment component 51.3% in relation to 48.7% which is genetic. The EL trait, shows more pronounced effect of the environment on phenotype (68.7%) compared to 31.3% of the genetic variance component. The NR trait reveals that almost 50% of the phenotypic variance is of genetic origin and 50% of environmental origin, the same behavior is shown by the NKR. Regarding to GY, considered a

trait influenced by several genes as well environmental conditions, reveals that 35% of the variance is of genetic origin and 65% is of nongenetic origin. For HKW, partition of the components of the phenotypic variance in genetic and nongenetic reveals very similar proportions.

Estimates of selective accuracy for traits, is related to greater accuracy in assessing the predicted genetic value. It may be noted in this study the values were classified moderate to strong 0.71 0.90 (Cargnelutti-Filho and Storck, 2007), which indicates that the predicted values were close to the true values

Table 1. Deviance analysis by Likelihood Ratio Test (LRT) for the traits plant height (PH, meters), ear height (EH, in centimeters) ear diameter (ED in millimeters), ear length (EL, in centimeters), number of rows (NR in units), number of kernels per row (NKR in units), grain yield (GY in bags ha⁻¹) and hundred-kernel weight (HKW in grams)

Effects	PH	EH	ED	EL	NR	NKR	GY	HKW
Complete	-146.09	-186.05	205.72	144.86	108.84	236.19	393.06	260.27
Reduced	-132.44	-161.37	220.65	151.00	124.43	252.06	398.16	264.58
LRT	-13.65*	-24.68*	-14.93*	-6.14*	-15.59*	-15.87*	-5.10*	-4.31*
Chi-Square	3.84	3.84	3.84	3.84	3.84	3.84	3.84	3.84

*Tabulated Chi-square for the value of 1 degree of freedom considering 5% probability error.

Table 2. Genetic parameters estimated by REML of 24 maize hybrids for the traits plant height (PH, meters), ear height (EH, in centimeters) ear diameter (ED in millimeters), ear length (EL, in centimeters), number of rows (NR in units), number of kernels per row (NKR in units), grain yield (GY in bags ha⁻¹) and hundred-kernel weight (HKW in grams)

Estimates	PH	EH	ED	EL	NR	NKR	GY	HKW
σ^2_G	0.022	0.019	3.804	0.898	0.962	6.170	96.936	3.898
σ^2_E	0.025	0.012	4.040	1.960	0.982	6.196	175.348	10.927
σ^2_P	0.047	0.031	7.844	2.858	1.945	12.365	272.284	14.825
h^2_g	0.465	0.607	0.485	0.314	0.495	0.499	0.356	0.263
h^2_{ml}	0.723	0.823	0.739	0.579	0.746	0.749	0.624	0.517
Ac	0.850	0.907	0.859	0.761	0.864	0.866	0.790	0.719
CVgi(%)	6.738	15.056	3.878	5.159	6.542	6.658	7.430	5.324
CVe(%)	7.229	12.114	3.996	7.620	6.610	6.672	9.993	8.913
PEV	0.006	0.003	0.995	0.378	0.244	1.547	36.463	1.883
SEP	0.078	0.058	0.997	0.615	0.494	1.244	6.038	1.372
Average	2.194	0.910	50.298	18.372	14.995	37.308	132.511	37.087

σ^2_G : genotypic variance, σ^2_E : environmental variance, σ^2_P : Phenotypic variance, h^2_g : heritability of individual plots in the broad sense, ie, the total genotypic effects, Ac: accuracy of genotypes selection, assuming no loss of plots, CVgi(%): genotypic coefficient of variation, CVe(%): residual coefficient of variation, PEV: prediction error variance of genotypic values, assuming no loss of plots e SEP: standard deviation of the predicted genotypic value, assuming no loss of land.

Table 3. Genotypic estimates by best linear unbiased predictor for 24 maize hybrids for the traits plant height (PH), ear height (EH), ear diameter (ED) and ear length (EL)

Order	Hybrid	PH u+g	Hybrid	EH u+g	Hybrid	ED u+g	Hybrid	EL u+g
1	KSP18	2.36	KSP19	1.09	KSP20	52.78	KSP5	20.10
2	KSP4	2.36	KSP17	1.09	KSP17	52.51	KSP22	19.51
3	KSP21	2.36	KSP21	1.04	KSP18	52.46	KSP7	19.30
4	KSP19	2.34	KSP22	1.03	KSP8	52.25	KSP13	19.21
5	KSP20	2.31	KSP18	1.02	KSP22	52.20	KSP4	19.09
6	KSP23	2.30	KSP20	1.02	KSP19	51.88	KSP8	18.88
7	KSP17	2.29	KSP8	1.02	KSP16	51.57	KSP10	18.77
8	KSP22	2.29	KSP4	1.01	KSP15	51.45	KSP3	18.71
9	KSP14	2.26	KSP24	0.98	KSP12	51.35	KSP12	18.56
10	KSP24	2.25	KSP14	0.98	KSP24	51.13	KSP16	18.45
11	KSP12	2.22	KSP6	0.93	KSP14	50.51	KSP18	18.40
12	KSP8	2.19	KSP16	0.90	KSP3	50.46	KSP20	18.36
13	KSP6	2.18	KSP23	0.90	KSP21	50.25	KSP23	18.22
14	KSP9	2.15	KSP13	0.89	KSP2	49.63	KSP14	18.16
15	KSP7	2.14	KSP12	0.88	KSP5	49.58	KSP6	17.98
16	KSP11	2.11	KSP1	0.83	KSP9	49.32	KSP11	17.97
17	KSP10	2.11	KSP3	0.83	KSP13	49.29	KSP2	17.88
18	KSP2	2.11	KSP10	0.83	KSP1	49.26	KSP24	17.85
19	KSP3	2.11	KSP15	0.82	KSP7	49.17	KSP15	17.83
20	KSP16	2.09	KSP2	0.78	KSP4	48.83	KSP17	17.78
21	KSP13	2.04	KSP11	0.75	KSP6	48.35	KSP21	17.73
22	KSP15	2.03	KSP7	0.72	KSP23	48.09	KSP9	17.39
23	KSP1	2.00	KSP5	0.71	KSP10	47.95	KSP1	17.35
24	KSP5	1.93	KSP9	0.68	KSP11	46.77	KSP19	17.33
CI		±0.161		±0.219		±2.068		±1.246

u+g: predicted average associated to genotypic value; CI: confidence interval limit.

of genetic hybrids. These results are similar to those obtained by Nardino *et al.* (2016), which worked with evaluation of different maize hybrids grown in different sites of southern of Brazil. Heritability in the broad sense of individual plots (h^2_g) revealed low (0.26) and moderate magnitudes (0.60). This estimate is obtained by the ratio between the $^2G / ^2F$ and their respective standard deviations, indicating that the environmental effects to some traits, especially the low magnitude were more pronounced.

Standard deviation of the predicted genotypic value, assuming no loss of plots, stand out low for most of the traits, but to the GY deviations are most evident, however this trait presents a quantitative inheritance, and the nonhereditary effects are more active on this component. Table 3 shows the results of the predicted genotypic average (BLUP) for traits plant height (PH), ear height (EH), ear diameter (ED) and ear length (EL). Regarding to PH trait, KSP18, KSP4 and KSP21 hybrids, showed higher plant height than hybrid ranked from the 11th

Table 4. Genotypic estimates by best linear unbiased predictor for 24 maize hybrids for the traits number of rows (NR), number of kernels per row (NKR), grain yield (GY) and hundred-kernel weight (HKW)

Order	Hybrid	NR u+g	Hybrid	NKR+ u+g	Hybrid	GY u+g	Hybrid	HKW u+g
1	KSP17	16.57	KSP5	41.32	KSP22	151.18	KSP24	39.37
2	KSP19	16.40	KSP4	40.74	KSP13	141.96	KSP7	38.74
3	KSP18	15.90	KSP23	40.57	KSP12	140.48	KSP8	38.55
4	KSP3	15.83	KSP10	40.15	KSP8	139.76	KSP22	38.35
5	KSP22	15.74	KSP8	39.24	KSP24	138.12	KSP13	38.21
6	KSP14	15.58	KSP7	38.99	KSP14	137.97	KSP2	37.92
7	KSP16	15.57	KSP18	38.90	KSP4	137.54	KSP5	37.74
8	KSP20	15.57	KSP21	38.57	KSP17	137.33	KSP18	37.61
9	KSP11	15.41	KSP13	38.07	KSP5	136.75	KSP19	37.59
10	KSP12	15.24	KSP20	37.32	KSP15	135.08	KSP12	37.50
11	KSP24	15.24	KSP1	37.24	KSP18	133.46	KSP6	37.42
12	KSP6	15.24	KSP9	36.99	KSP20	133.18	KSP17	37.35
13	KSP9	15.08	KSP12	36.91	KSP10	131.86	KSP3	37.25
14	KSP8	14.75	KSP11	36.74	KSP6	129.56	KSP15	37.20
15	KSP4	14.58	KSP3	36.70	KSP9	129.50	KSP23	37.06
16	KSP15	14.58	KSP16	36.57	KSP11	128.89	KSP16	36.97
17	KSP5	14.41	KSP14	36.07	KSP23	128.89	KSP20	36.77
18	KSP7	14.41	KSP22	35.74	KSP16	127.74	KSP1	36.60
19	KSP1	14.25	KSP2	35.66	KSP7	127.43	KSP4	36.45
20	KSP2	14.25	KSP15	35.32	KSP19	125.70	KSP10	36.22
21	KSP21	14.25	KSP6	34.91	KSP21	125.60	KSP21	36.20
22	KSP13	14.08	KSP24	34.74	KSP2	121.46	KSP14	36.19
23	KSP10	13.58	KSP17	34.57	KSP1	121.01	KSP9	33.82
24	KSP23	13.25	KSP19	33.24	KSP3	119.72	KSP11	32.89
IC		±0.996		±2.585		±16.605		±2.749

u+g: predicted average associated to genotypic value; CI: confidence interval limit.

The mean heritability (h^2_{ml}) revealed moderate (0.51) and high (0.82) estimates. The magnitude of the heritability parameter in this study indicate that the experimental arrangement was adequate to control the effects of the environment and that the predicted genotypic values were properly estimated. Genetic coefficient of variation (CV_{gi}) indicates how much of genotypic fraction represents the total variation, since the residual variation coefficient (CV_e) reveals how environmental fraction is contributing to the total variation. The magnitudes of these two factors are reflected in the selective accuracy; in this sense, the selective accuracy will be higher as minors were the absolute differences between the genetic parametric values and estimated/predicted genotypic values. Similarly, it can be inferred that close estimates of the coefficients, with close relationship to 1, shows that the experimental quality was adequate, which is consistent with the accuracy of estimates outlined above. According to Vencovsky (1987), when a ratio between CV_{gi} / CV_e close to 1 or more, a favorable situation for selection gains occur. Estimates of prediction error variance of genotypic values (PEV), assuming no loss of plots presented low magnitudes for most of the traits; however, the largest variance that was observed for GY trait, was expected, due of its nature presenting more pronounced effects from environment on it.

ranked (KSP12). However, in current hybrids, plant breeding programs has been looking for the reduction in PH; thus, KSP1, KSP5 and KSP15 hybrids shows itself most promising from maize genetic breeding viewpoint. Reduction in plant size is a goal for selection, because according to De Jesus Freitas *et al.* (2013) plants with larger size are more susceptible to breakage and lodging, especially when it comes to regions with high incidence of winds. For EH trait, KSP19 and KSP17 hybrids showed higher genotypic mean by BLUP prediction. EH is carefully evaluated by maize breeders, which are currently looking for plants with lower gravity center, both regarding to source-sink, as to avoid losses due to lodging. Thus KSP9, KSP5 and KSP7 hybrids revealed lower genotypic average between the hybrids' set.

In predicted genotypic value analysis for ED trait, KSP20, KSP17, KSP18, KSP8 and KSP22 hybrids showed largest genotypic values than hybrid ranked from the 13th ranked (KSP21). Hybrids with lower genotypic values were KSP11 and KSP10. Larger ED, in many situations are sought by plant breeding, by enabling cobs with largest number of kernel rows; however, it is important to be noted the kernel depth and ear length, so that there is a balance among these three traits and thus, largest productivity levels. Regarding to EL, KSP5, KSP22, KSP7 and KSP13 hybrids are higher than genotype

ranked from 17th ranked (KSP2). It can be inferred that the hybrids with largest EL, generally do not have the larger ED, where that ears with largest length allow the formation of rows with more kernels, which in turn positively relates with GY. Table 4 shows the results of the predicted genotypic average (BLUP) for traits number of rows (NR), number of kernels per row (NKR), grain yield (GY) and hundred-kernel weight (HKW). For NR trait, BLUP genotypic values of KSP17 and KSP19 hybrids were largest than the hybrid ranked from 8th ranked (KSP8), considering a confidence interval for separation of hybrids. Maize hybrids with highest NR per ear can enable a larger number of kernels per ear, however a balance between NR, NKR and HKW it is necessary, in order to give a higher GY per area. Regarding to NKR trait, KSP5, KSP4, KSP23 and KSP10 hybrids were higher considering a confidence interval, then hybrid ranked from 7th ranked (KSP16), it can be inferred that hybrids with higher NR, not were the same as shown more NKR, indicating that ears with largest diameter and NR, presents smallest length and therefore smallest NKR.

Grain yield is the target trait among the studied traits by the genetic breeding of all plant breeders, because it is directly associated with economic return. Adopting methods such as mixed models for evaluation of pre-commercial hybrids that are already considered as a select group by breeders is crucial to maximize the accuracy of prediction of actual genotypic value of individuals, removing the biased information from nongenetic effects. Once defined the superior hybrids within the pre-commercial group, these are directed to final testing and registration. Genetic-statistical models that include simultaneously random and fixed components as mixed models, is becoming more popular in the current scenario of research, as can be seen in the works of (Baretta *et al.*, 2016; Boer *et al.*, 2007, Souza *et al.*, 2015).

BLUP genotypic estimates of hybrids indicated that KSP22, KSP13 and KSP12 showed high performance for the GY trait, due their genotypic averages were higher than hybrids ranked from 22th ranked. Among these we can highlight KSP22 hybrid, with GY greater than 150 sc ha^{-1} , being much higher than national average GY. This hybrid shows potential to be tested in more sites growing, along with others already commercial hybrids to assess their agronomic performance and if, in fact is evidenced high performance, will be possibly launched commercially by KSP Seeds Ltd. company's breeding program. KSP1 and KSP3 hybrids revealed lower GY than expected for a pre-commercial hybrid; these hybrids possibly will comprise tests in other growing sites, where not necessarily be eliminated from the program because maybe the effects of Frederico Westphalen's environment where were evaluated, penalized their performance. An alternative is assayed them in other environment and if their low performance stays, there are liable to be eliminated of the pre-commercial hybrid test group. Regarding to HKW, trait that reveals association with GY (Nardino *et al.*, 2016; Souza *et al.*, 2015) KSP24, KSP7, KSP8 and KSP22 hybrids had the largest magnitudes HKW, can highlight the predicted genotypic values for this trait are in agreement with other studies as mentioned above, regarding to the GY in association with HKW.

Conclusion

The presence of genetic variation among the studied hybrids indicate that can have gain by genetic improvement of these maize hybrids. KSP22 pre-commercial maize hybrid has high performance for grain yield, enabling their inclusion in regional trials with commercial checks.

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