Genetics and Molecular Research

Measurements of experimental precision for trials with cowpea (*Vigna unguiculata* L. Walp.) genotypes

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Genet. Mol. Res. 15 (2): gmr.15027991
Received August 8, 2015
Accepted November 26, 2015
Published May 6, 2016
DOI http://dx.doi.org/10.4238/gmr.15027991

ABSTRACT. The aim of this study was to evaluate the suitability of statistics as experimental precision degree measures for trials with cowpea (*Vigna unguiculata* L. Walp.) genotypes. Cowpea genotype yields were evaluated in 29 trials conducted in Brazil between 2005 and 2012. The genotypes were evaluated with a randomized block design with four replications. Ten statistics that were estimated for each trial were compared using descriptive statistics, Pearson correlations, and path analysis. According to the class limits established, selective accuracy and F-test values for genotype, heritability, and the coefficient of determination adequately estimated the degree of experimental precision. Using these statistics, 86.21% of the trials had adequate

experimental precision. Selective accuracy and the F-test values for genotype, heritability, and the coefficient of determination were directly related to each other, and were more suitable than the coefficient of variation and the least significant difference (by the Tukey test) to evaluate experimental precision in trials with cowpea genotypes.

Key words: *Vigna unguiculata* L.; Experimental planning; Quality control

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is one of the most important food sources in tropical and subtropical regions of the world. Currently, Brazil is the third-largest producer of cowpea, which is mainly grown in the north (55,800 ha) and northeast (1.2 million ha), and is the staple diet of those on low incomes (Santos et al., 2014).

However, despite the high Brazilian production, there are always supply shortages in these areas of Brazil. According to Leite et al. (2009), this is because the average Brazilian yield (around 300 kg/ha) is too low, and the genetic potential exceeds 6 t/ha. Santos et al. (2014) stated that genetic improvement is the best way to increase average yield, by selecting highly productive genotypes that are adapted to Brazilian soils and climatic conditions.

In the final stages of plant-breeding programs, it is important to assess genotypes under different environmental conditions in order to identify promising genotypes and those that should be discarded. Therefore, testing should be performed with the maximum possible experimental accuracy, so that small differences between genotypes can be identified (Cargnelutti Filho et al., 2012a).

Cargnelutti Filho and Storck (2007) assessed heritability, the coefficient of determination, and F-test values, and concluded that these statistics are more appropriate than the coefficient of variation (CV) and the least significant difference (LSD) as a percentage of the mean by the Tukey test for the classification of accuracy trials with maize genotypes. Similar studies have been conducted with soybean (Storck et al., 2009), irrigated rice (Cargnelutti Filho et al., 2012b), and sugar cane (Cargnelutti Filho et al., 2012a).

Although the results of these statistics have been similar in genotype competition trials of these crops, Cargnelutti Filho et al. (2012a) suggested that studies should be conducted on other crop species before using these statistics for the evaluation of the experimental precision of genotype competition trials. The aim of this study was to evaluate the suitability of certain statistics as measures of accuracy of experimental trials with cowpea genotypes.

MATERIAL AND METHODS

The grain yield of cowpea genotypes was evaluated in 29 trials conducted in the State of Mato Grosso do Sul, Brazil, between 2005 and 2012. The experimental design was a randomized block with four replications, using 20 genotypes with erect or semi-prostate growth habits. The experimental units were composed of four 5-m long rows separated by 0.5 m, with 0.25 m between the plants in each row.

Initially, a variance analysis were performed for each trial and the following statistics

were calculated: mean square of genotype (MS_G), mean square of error (MS_E), F-test value for genotype (Fc), overall mean of the trial (m), and the CV for percentage and heritability (h^2) (Cruz et al., 2014). The selective accuracy (SA) was then estimated (Resende and Duarte, 2007). Based on the SA, experimental precision was evaluated according to the class limits established by Resende and Duarte (2007).

Subsequently, the LSD between genotype means, calculated by the Tukey test at the 0.05 probability level (expressed as a percentage of the mean), was estimated for each trial. We determined the coefficient of determination (R^2) and the amplitude of means (H) by the difference between means of genotypes with high and low yields. In this way, we obtained 10 statistical indices (MS_G, MS_E, Fc, m, CV, h^2, LSD, R^2, H, and SA) for each trial, conducted a Kolmogorov-Smirnov normality test (Siegel and Castellan Júnior, 2006), and calculated minimum, mean, and maximum values and the CV. We then calculated Pearson correlation coefficients (r) between the statistics, the significance of which were assessed by Student *t*-tests at the 0.05 probability level. We performed multicollinearity diagnostics (Cruz et al., 2014) according to the criteria established by Montgomery and Peck (1982), and a path analysis of the main variables that were candidates for the precision measurements (CV, LSD, Fc, and SA) as a function of the explanatory variables (MS_G, MS_E, m, h^2, R^2, and H).

We calculated the class limits of experimental accuracy based on limits established by Resende and Duarte (2007) and Cargnelutti Filho and Storck (2007), which included the relative trial frequency in each class. In order to compare the means of MS_G, MS_E, and m for each experimental accuracy class, we performed analysis of variance (ANOVA) with the F-test at the 0.05 probability level, and compared the means using the Student *t*-test (LSD) at the 0.05 probability level (Cargnelutti Filho and Storck, 2009). All of the statistical analyses were conducted using the GENES software (Cruz, 2013) following the procedures recommended by Cruz et al. (2014).

RESULTS AND DISCUSSION

Genotype was significant ($P \leq 0.05$) in 24 trials (82.76%), indicating the presence of genetic variability between the cowpea genotypes for grain yield if the genotype effect is considered random, or that there was some difference between genotypes if the genotype effect is considered fixed. In genotype trials of maize (Cargnelutti Filho and Storck, 2007), soybean (Storck et al., 2009), irrigated rice (Cargnelutti Filho et al., 2012b), and sugar cane (Cargnelutti Filho et al., 2012a), genotype was significant for grain yield in 92.08, 79.60, 90.29, and 95.59%, respectively, of trials. In these trials, the F-test values obtained from ANOVA revealed a significant effect of genotype; however, this does not mean that the experiment was accurate, only that the genotype with the highest mean differed from the genotype with the lowest mean, and does not indicate significance differences between means of other genotypes. The fact that significant differences were not detected ($P > 0.05$) between genotypes in five trials may have been due to experimental error. Therefore, Cargnelutti Filho et al. (2012a,b) stated that in addition to using the F-test it is important to classify experiments using experimental precision statistics.

The CV ranged from 32.70 to 148.55% for the 10 statistics in the 29 trials (Table 1). Similar results have been obtained in studies that have assessed the experimental precision of trials of maize (Cargnelutti Filho and Storck, 2007, 2009), soybean (Storck et al., 2009),

irrigated rice (Cargnelutti Filho et al., 2012b), and sugar cane (Cargnelutti Filho et al., 2012a). According to the Kolmogorov-Smirnov test, the data followed a normal distribution for all of the statistics assessed.

Table 1. Descriptive statistics and the results of Kolmogorov-Smirnov normality tests of the statistics of grain yield data (kg/ha) from 29 trials of cowpea (*Vigna unguiculata*) genotypes.

Statistic	Minimum	Mean	Maximum	Standard deviation	CV	P value
MS_G	17,669.94	187,266.30	943,014.36	215,287.81	114.96	0.06
MS_E	6372.80	43,562.68	155,028.20	34,300.14	78.74	0.11
m	117.23	736.66	1491.33	373.03	50.64	0.15
H	222.25	741.76	2078.60	402.33	54.24	0.13
LSD	36.76	83.13	223.09	43.23	52.00	0.06
CV	13.98	31.61	84.83	16.44	52.01	0.07
h^2	1.29	63.31	98.14	28.46	44.96	0.13
R^2	0.62	7.10	53.90	10.55	148.55	0.07
Fc	1.01	7.14	53.90	10.53	147.60	0.08
SA	0.11	0.75	0.99	0.25	32.70	0.18

MS_G = mean square of genotype; MS_E = mean square of error; m = overall mean of the trial (kg/ha); H = amplitude of the means; LSD = least significant difference in the percentage of the mean between genotypes according to the Tukey test at the 0.05 probability level; CV = coefficient of variation (%); h^2 = heritability; R^2 = coefficient of determination; Fc = F-test value for genotype; SA = selective accuracy.

There were significant, positive associations ($P \leq 0.05$) between the following pairs: MS_G and H, MS_G and h^2, MS_G and R^2, MS_G and Fc, MS_G and SA, MS_E and m, m and H, m and LSD, m and CV, H and LSD, H and CV, H and h^2, H and R^2, H and Fc, H and SA, LSD and CV, h^2 and R^2, h^2 and Fc, h^2 and SA, R^2 and Fc, R^2 and SA, and Fc and SA. There were significant, negative correlations ($P \leq 0.05$) between MS_G and LSD, MS_G and CV, MS_E and h^2, and MS_E and SA (Table 2). Similar results were obtained by Cargnelutti Filho and Storck (2007, 2009) and Cargnelutti Filho et al. (2012a) when assessing experimental precision measurements in trials with sugar cane. The condition number ranged between 6.928 and 20.049 (Table 3), indicating low collinearity (Montgomery and Peck, 1982). In cases of high collinearity, the use of cluster analysis would not be appropriate, because the statistics would be highly correlated; therefore, path analysis could be performed using this dataset (Cruz et al., 2014).

Table 2. Pearson correlation coefficient estimates between the statistics for grain yield data (kg/ha) from 29 trials of cowpea (*Vigna unguiculata*) genotypes.

Statistic	MS_E	M	H	LSD	CV	h^2	R^2	Fc	SA
MS_G	0.015	0.361	0.962*	-0.381	-0.381	0.464*	0.809*	0.808*	0.405*
MS_E		0.700*	0.053	0.413*	0.413*	-0.588*	-0.350	-0.348	-0.586*
M			0.440*	0.658*	0.658*	-0.250	-0.013	-0.011	-0.268
H				0.483*	0.483*	0.485*	0.790*	0.788*	0.437*
LSD					1.000*	-0.207	-0.330	-0.332	-0.128
CV						-0.207	-0.330	-0.332	-0.128
h^2							0.565*	0.562*	0.838*
R^2								1.000*	0.483*
Fc									0.479*

*Significant according to the Student *t*-test at the 0.05 probability level with 27 degrees of freedom. MS_G = mean square of genotype; MS_E = mean square of error; m = overall mean of the trial (kg/ha); H = amplitude of the means; LSD = least significant difference in the percentage of the mean between genotypes according to the Tukey test at the 0.05 probability level; CV = coefficient of variation (%); h^2 = heritability; R^2 = coefficient of determination; Fc = F-test value for genotype; SA = selective accuracy.

Table 3. Pearson correlation coefficient estimates and direct and indirect effects of mean square of genotype (MS_G), mean square of error (MS_E), overall mean of the trial (m), amplitude of the means (H), heritability (h^2), and coefficient of determination (R^2) on the coefficient of variation (CV), least significant difference between genotypes (LSD), F-test value for genotype (Fc), and selective accuracy (SA) for grain yield data (kg/ha) from 29 trials of cowpea (*Vigna unguiculata*) genotypes.

Variable	Effect	CV	LSD	Fc	SA
MS_G	Direct	-0.007	-0.007	0.745	0.771
	Indirect through MS_E	-0.693	-0.693	-0.001	-0.329
	Indirect through m	0.202	0.202	-0.017	-0.028
	Indirect through H	0.828	0.828	0.173	0.136
	Indirect through h^2	-0.083	-0.083	0.023	0.182
	Indirect through R^2	-0.280	-0.280	-0.115	-0.327
	Pearson correlation (r)	-0.381	-0.381	0.808*	0.405*
MS_E	Direct	0.391	0.391	-0.099	-0.203
	Indirect through MS_G	0.251	0.251	0.003	0.001
	Indirect through m	-0.501	-0.501	-0.003	-0.054
	Indirect through H	0.046	0.046	0.010	0.008
	Indirect through h^2	0.105	0.105	-0.030	-0.231
	Indirect through R^2	0.121	0.121	-0.149	-0.004
	Pearson correlation (r)	0.413*	0.413*	-0.348	-0.586*
m	Direct	0.379	0.379	-0.048	-0.077
	Indirect through MS_G	-0.250	-0.250	0.068	0.026
	Indirect through MS_E	0.558	0.558	-0.069	-0.142
	Indirect through H	-0.357	-0.357	0.079	0.062
	Indirect through h^2	0.045	0.045	-0.013	-0.098
	Indirect through R^2	0.005	0.005	-0.006	0.001
	Pearson correlation (r)	0.658*	0.658*	-0.011	-0.268
H	Direct	0.861	0.861	0.180	0.141
	Indirect through MS_G	-0.667	-0.667	0.183	0.068
	Indirect through MS_E	-0.027	-0.027	-0.005	-0.010
	Indirect through m	0.246	0.246	-0.021	-0.034
	Indirect through h^2	-0.086	-0.086	0.024	0.191
	Indirect through R^2	0.273	0.273	0.337	0.010
	Pearson correlation (r)	0.483*	0.483*	0.788*	0.437*
h^2	Direct	-0.178	-0.178	0.241	0.394
	Indirect through MS_G	-0.321	-0.321	0.088	0.033
	Indirect through MS_E	0.300	0.300	0.058	0.120
	Indirect through m	-0.140	-0.140	0.011	0.019
	Indirect through H	0.417	0.417	0.087	0.068
	Indirect through R^2	-0.196	-0.196	0.050	0.007
	Pearson correlation (r)	-0.207	-0.207	0.562*	0.838*
R^2	Direct	-0.346	-0.346	0.427	0.222
	Indirect through MS_G	-0.561	-0.561	0.154	0.057
	Indirect through MS_E	0.179	0.179	0.035	0.071
	Indirect through m	-0.007	-0.007	0.001	0.001
	Indirect through H	0.680	0.680	0.142	0.111
	Indirect through h^2	-0.100	-0.100	0.028	0.013
	Pearson correlation (r)	-0.330	-0.330	1.000*	0.483*
CV		0.811	0.811	0.785	0.866
Residual variable		0.223	0.223	0.463	0.658
Condition number		6.928	6.928	17.117	20.049

*Significant according to the Student *t*-test at the 0.05 probability level with 27 degrees of freedom.

Using path analysis, we found that the main variables formed two groups, with similar results within the groups and different results between them. The CV and LSD formed the first group and the Fc and SA the second (Table 3). The significant, positive linear association between CV and LSD (r = 1.000), the moderate, linear relationship between Fc and SA (r = 0.479), and the weak associations between statistics in different groups, i.e., between CV and SA (r = -0.128), CV and Fc (r = -0.332), LSD and SA (r = -0.128), and LSD and Fc (r = -0.332), confirmed the existence of two groups. These results support those obtained by Resende (2002), Resende and Duarte (2007), Cargnelutti Filho and Storck (2007, 2009), and Cargnelutti Filho et al. (2012a), who found that the SA and Fc are better suited than the CV

and the LSD to evaluate trial experimental precision with maize, common bean, soybean, irrigated rice, and sugar cane genotypes.

MS_G was not significantly correlated with the CV and LSD (r = -0.381), and had little direct effect on them (-0.007). Therefore, it can be inferred that there is no linear relationship between MS_G and the CV and LSD, and that the classification of experimental precision for these statistics is independent of genetic variability. MS_E, m, and H were positively correlated with the LSD and CV (r = 0.413, 0.658, and 0.483, respectively), and had similar direct effects on them (0.391, 0.379, and 0.861, respectively), suggesting that MS_E, m, and H affect the CV and LSD. h^2 and R^2 were negatively correlated with the CV and LSD (r = -0.207 and -0.330, respectively), and had similar direct effects on them (-0.178 and -0.346, respectively), suggesting that h^2 and R^2 affect the CV and LSD.

Based on these results, we can infer that accurate trials (with low CV and LSD values) are associated with low residual variances and grain yield means and ranges, and a high heritability and coefficient of genotypic determination, and are independent of the genetic variability of the group of genotypes in the trial. These results corroborate those obtained for testing grain yield with genotypes of maize (Cargnelutti Filho and Storck, 2007, 2009), soybean (Storck et al., 2009), rice (Cargnelutti Filho et al., 2012b), and sugar cane (Cargnelutti Filho et al., 2012a).

However, in genotype competition trials, it is important that statistical experimental precision accounts for genetic variability (Resende and Duarte, 2007; Cruz et al., 2014), and is independent of the mean. Therefore, the LSD and CV, which are traditionally used for this purpose, may be replaced by the SA and Fc, which have been identified as the most appropriate (Resende and Duarte, 2007; Cargnelutti Filho and Storck, 2007, 2009; Storck et al., 2009; Cargnelutti Filho et al., 2012a,b).

MS_G was positively correlated with the Fc and SA (r = 0.808 and 0.405, respectively), and had a strong, positive direct effect on them (0.745 and 0.771, respectively). MS_E was positively correlated with the Fc and SA (r = -0.348 and -0.586, respectively), and had a weak, negative direct effect on them (-0.099 and 0.203, respectively). There was no association between m and the Fc and SA (r = -0.011 and -0.268, respectively), and had little direct effect on them (-0.048 and -0.077, respectively). H, h^2, and R^2 were significantly correlated with the Fc and SA, and had moderate direct effects on them. These results confirm those of Cargnelutti Filho and Storck (2007, 2009): that H, h^2, and R^2, together with the Fc and SA, can accurately identify the best genotypes for grain yield.

Therefore, it is possible to infer that accurate trials (with high SA and Fc values) are associated with high genetic variances, ranges of means, heritabilities, and coefficients of determination and low residual variances, being independent of the mean grain yield. Our results confirm the suitability of the SA and Fc as experimental precision measures for trials with cowpea genotypes, as found in maize (Cargnelutti Filho and Storck, 2007, 2009), soybean (Storck et al., 2009), irrigated rice (Cargnelutti Filho et al., 2012b), and sugar cane (Cargnelutti Filho et al., 2012a).

Based on the limits of experimental accuracy that were established by Resende and Duarte (2007) and Cargnelutti Filho and Storck (2007), 25 trials (86.21%) had adequate experimental precision and four (13.79%) could be discarded due to insufficient experimental precision (Table 4). Variability in experimental precision based on SA, Fc, h^2, and R^2 values has been observed in trials with genotypes of maize (Cargnelutti Filho and Storck, 2007,

2009), soybean (Storck et al., 2009), irrigated rice (Cargnelutti Filho et al., 2012b), and sugar cane (Cargnelutti Filho et al., 2012a).

Table 4. Class limits of experimental precision of selective accuracy (SA), F-test value for genotype (Fc), heritability (h^2), coefficient of determination (R^2), and single (f_i) and relative frequencies (f_{ri}) in relation to grain yield (kg/ha) from 29 trials of cowpea (*Vigna unguiculata*) genotypes.

Experimental precision	SA	Fc	h^2	R^2	f_i	f_{ri} (%)
Very high	≥0.90	≥5.26	≥0.81	≥0.84	11	37.93
High	≥0.70 and <0.90	≥1.96 and <5.26	≥0.49 and <0.81	≥0.66 and <0.84	7	24.14
Moderate	≥0.50 and <0.70	≥1.33 and <1.96	≥0.25 and <0 .49	≥0.57 and <0.66	7	24.14
Low	<0.50	<1.33	<0.25	<0.57	4	13.79

We verified that, by measuring MS_G, MS_E, and m (Table 5), accurate trials (those that accurately distinguish between different genotypes) have high genetic variability (MS_G) and low residual variation (MS_E) regardless of the mean of the trials, confirming the results obtained by Cargnelutti Filho and Storck (2007, 2009).

Table 5. Mean square of genotype (MS_G), mean square of error (MS_E), and overall mean of the trial (m) in each experimental precision class from 29 trials of cowpea (*Vigna unguiculata*) genotypes.

Experimental precision	MS_G	MS_E	m
Very high	308,740.90[a]	30,481.67[b]	644.22[NS]
High	106,827.84[b]	33,693.51[b]	543.30
Moderate	112,599.35[b]	66,912.23[a]	838.52
Low	68,541.34[c]	70,850.09[a]	949.55

Means followed by different lowercase letters in the same column significantly differed according to the Student *t*-test at the 0.05 probability level. NS, not significant.

Therefore, Fc, SA, h^2, and R^2 are the most appropriate statistics to assess the experimental precision of trials with cowpea genotypes, because they all take into account this information.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) and the National Council for Scientific and Technological Development (CNPq) for financial support.

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