Exploring the use of testers to maximize selection accuracy of partially inbred S₃ popcorn progenies

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ABSTRACT: The development of superior hybrid cultivars depends on the identification of lines with greater combining ability. This study compared the discrimination and combining ability of four testers when crossed with 43 partially endogamous S₃ popcorn (*Zea mays* L. var. *everta*) progenies by the Residual or Restricted Maximum Likelihood and Best Linear Unbiased Prediction (REML/BLUP) approach. Yield and morphological components of genotypes were evaluated. The experiment was arranged in a complete block design with three replicates at two locations (in the North and Northwestern regions of the State of Rio de Janeiro, Brazil). The REML/BLUP approach was able to efficiently discriminate and estimate the combining abilities for all traits assessed. Testers BRS-Angela and P2 had positive effects on all yield components, indicating their suitability as potential parents of hybrids in crosses with S₃ progenies. The negative genotypic effects were the highest for the testers IAC 125 and UENF 14, indicating that they could be used to adequately identify the progenies with the highest potential among the evaluated genotypes. Overall, at both locations, tester IAC 125 was the best discriminator of the relative merits of S₃ progenies, while BRS-Angela was the tester with the best hybrid yield.

Key words: combining ability; discrimination; REML/BLUP; *Zea mays* L. var. *everta*

Explorando a máxima acurácia seletiva de testadores sobre progênies S₃ parcialmente endogâmicas de milho-pipoca

RESUMO: O desenvolvimento de cultivares híbridas superiores depende da identificação de linhagens com maior capacidade de combinação. Este estudo comparou a discriminação e a capacidade de combinação de quatro testadores quando cruzados com 43 progênies de pipoca S₃ parcialmente endógamas (*Zea mays* L. var. *everta*) pela abordagem de Máxima Verossimilhança Residual ou Restrita e Melhor Previsão Imparcial Linear (REML/BLUP). O rendimento e os componentes morfológicos dos genótipos foram avaliados. O experimento foi organizado em um delineamento de blocos completo, com três repetições, em dois locais (nas regiões Norte e Noroeste do Estado do Rio de Janeiro, Brasil). A abordagem REML/BLUP foi capaz de discriminar e estimar eficientemente as capacidades de combinação de todas as características avaliadas. Os testadores BRS-Angela e P2 tiveram efeitos positivos em todos os componentes de rendimento, indicando sua adequação como potenciais genitores de híbridos em cruzamentos com as progênies S₃. Os efeitos genotípicos negativos foram mais altos para os testadores IAC 125 e UENF 14, indicando que eles poderiam ser usados para identificar adequadamente as progênies com maior potencial entre os genótipos avaliados. No geral, em ambos os locais, o testador IAC 125 foi o melhor discriminador dos méritos relativos das progênies S₃, enquanto BRS-Angela foi o testador com o melhor rendimento híbrido.

Palavras-chave: capacidade combinatória; discriminação; REML/BLUP; *Zea mays* L. var. *everta*

Introduction

In Brazil, a barrier to a greater dissemination of popcorn (*Zea mays* L. var. *everta*) cultivation is the restricted number of cultivars on the market that meet the specific demands of farmers (Amaral Júnior et al., 2013; Ribeiro et al., 2016). Until 2017, only 88 popcorn cultivars were registered by the Ministry of Agriculture, compared to the 3024 registered common maize lines (Brasil, 2017). Curiously, the added value of popcorn cultivation is higher than that of common maize (Ribeiro et al., 2016), due to the high economic potential of microwave popcorn and the high demand for popcorn from cinemas and sport and event centers. In the United States of America, it is estimated that popcorn accounts for a turnover of two billion dollars annually.

Two main breeding strategies are used to develop superior popcorn genotypes for registration and release as new cultivars, specifically recurrent selection and hybridization (Amaral Júnior et al., 2013; Guimarães et al., 2019). Recurrent selection increases the number of favorable alleles gradually, without altering the genetic variability of the population (Ribeiro et al., 2016). With this strategy, more promising inbred lines can be utilized in the breeding of superior hybrids (Hallauer et al., 2010). This strategy was used to obtain S₃ lines from the base population UENF 14, which was registered as a new cultivar in the fifth cycle of intrapopulation recurrent selection for popcorn cultivar production in Brazil (Amaral Júnior et al., 2013).

In functional breeding programs, the line development phase is the most costly part because it involves producing and assessing a large number of genotypes until a satisfactory level of inbreeding is reached (Hallauer et al., 2010). Previous selection of lines makes some aspects of this procedure more efficient, including more economically efficient, since many inferior lines can be eliminated beforehand and the expenses that would be incurred by the maintenance of high numbers of families can be avoided (Marcondes et al., 2016).

A feasible method for the evaluation of lines is the topcross method proposed by Davis (1927). With this method, a large number of lines can be evaluated in crosses with testers. Thus, it is possible to eliminate lines with inferior agronomic performance, and conclusions can also be drawn about the combining abilities of different lines in this way (Hallauer et al., 2010).

The choice of a tester is a key point determining the success of the topcross methodology. A good tester should be highly efficient at discriminating genotypes based on selection targets (Hallauer & Lopes Perez., 1979). The following additional properties are also essential: testers should be easily manipulated, provide information to help classify the relative merits of different lines, and maximize the genetic gain in hybrid breeding (Hallauer et al., 2010).

There is consensus about the allelic frequency of a good tester. Pioneering studies (e.g., Hallauer & Lopes Perez, 1979; Smith, 1986) defined an appropriate tester as being the line with the lowest frequency of favorable alleles. If this allelic condition is met, the full combining potential of the tested lines can then be detected. These results were corroborated by several studies that evaluated the topcross methodology (Marcondes et al., 2016; Lima et al., 2016; Pena et al., 2016). On the other hand, for commercial hybrid production, testers with a high frequency of favorable alleles can identify the best crosses, since hybrids with high heterosis can be obtained with this approach (Lima et al., 2016; Pena et al., 2016).

In this scenario, the success of breeding is associated with the ability of breeders to choose the best plants based on inferences concerning the genetic value of the population (Cruz et al., 2012). To help predict the genetic value of the best genotypes, tools such as the Restricted Maximum Likelihood (REML) and BLUP (Best Linear Unbiased Prediction) estimation methods associated with linear mixed models are very useful (Resende, 2002; Viana & Resende, 2014).

One of the advantages of mixed models is their ability to evaluate phenotypic information from experiments in which the treatment structure involves both fixed and random effects, allowing greater relevance and high accuracy in the selection process (Viana & Resende, 2014). This technique is widely used in the breeding of animals and of forest and perennial plant species (Freitas et al., 2013). In the latter of these subject areas, this approach has been widely used to predict the genetic values of individual plants, leading to great scientific advances and facilitating a more accurate selection of the genotypes (Viana & Resende, 2014). However, there is limited related research on genotype exploration and selection through such estimations of genotype values in annual crops (Pimentel et al., 2014; Torres et al., 2017; Sousa et al., 2019), mainly in maize (Vittorazzi et al., 2017).

Therefore, this study compared the discrimination and combining ability of popcorn testers and partially inbred S₃ popcorn progenies using the REML/BLUP approach for the prediction of genotype values of topcross hybrid lines.

Materials and Methods

Breeding population

Initially, 43 partially inbred S₃ progenies were developed through three generations of self-pollination from the open-pollinated popcorn variety UENF 14 (obtained from the breeding program of the State University of Northern Rio de Janeiro Darcy Ribeiro), following the standard method proposed by Miranda Filho & Viégas (1987). This variety was the result of a cross between a native compound with a North American variety, which was then subjected to mass selection and backcrosses, and has been recommended for cultivation in the North and Northwestern Regions of the State of Rio de Janeiro (RJ) after five cycles of intrapopulation recurrent selection (Amaral Júnior et al., 2013).

As testers, we used the open-pollinated variety BRS-Angela, the topcross hybrid IAC 125, line S₇ (P2), and the open-pollinated variety UENF 14. The genetic basis of three of these four testers was classified as broad (UENF 14, BRS-Angela, and IAC 125), while that of one was narrow (P2). Variety BRS-

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Angela resulted from six cycles of recurrent intrapopulation selection of Embrapa Maize and Sorghum CMS 43 (Pacheco et al., 2001). IAC 125 is a topcross hybrid (a single-cross hybrid rather than a variety), resulting from a cross of the modified single-cross hybrid IAC 112 with a synthetic population derived from a North American hybrid (Sawazaki, 2001). Line P2, named after the UENF popcorn breeding program, was obtained after seven selfing cycles (S7) of the compound CMS-42, and belongs to the Germplasm Bank of Embrapa Maize and Sorghum.

Breeding of topcross hybrids

The S$_3$ progenies were crossed with each of the four testers in a partial diallel mating system. To obtain topcross hybrids, four plots were used per each S$_3$ progeny and one plot was used for each tester. Experimental plots consisting of 6 m-long rows, spaced 1 m apart and with plants spaced 0.40 m apart within each row, were used. At flowering, the crosses were performed by manual pollination. The S$_3$ progenies were used as female and the testers as male parents. The crosses were performed using a pollen mixture obtained from each viable plant of each tester, and then plants of S$_3$ progenies with receptive stigmas were pollinated with these mixtures. For each tester, 43 hybrid combinations were produced, resulting in a total of 172 topcross hybrids. The crosses were carried out between June and July 2014 at the Agricultural College Antônio Sarlo in Campos dos Goytacazes, Rio de Janeiro, Brazil.

Evaluation of topcross hybrids

The topcross hybrids were evaluated in two different environments. The first environment was located at the station of the Agricultural Research Company (PESAGRO) in the city of Itaocara in the Northwestern Region of the State of Rio de Janeiro, Brazil (21°38'50"S, 42°03'46"W; 58 m above sea level a.s.l.). Genotypes in this first location were planted in October of the 2014/2015 growing season, and during this period the total precipitation was 541 mm and the temperature ranged from 19.0 to 37.7 °C. The other location was an experimental area of the Agricultural College Antônio Sarlo in the city of Campos dos Goytacazes (21°42'48"S, 41°20'38"W; 14 m a.s.l.) in the North Region of the State of Rio de Janeiro. In the second location, Genotypes were planted in October of the 2015/2016 growing season, and during this period the total precipitation was 434 mm and the temperature ranged from 21.7 to 32.6 °C.

The 172 topcross hybrids were evaluated in a complete block design, with three replicates of each topcross per environment. Each experimental unit consisted of one 4.20-m row of plants (with plants spaced 0.30 m apart, and rows spaced 0.60 m apart, resulting in a total of 16 plants per plot). The experimental area was fertilized with 30 kg N ha^{-1} (as urea), 60 kg P$_2$O$_5$ ha^{-1} (as triple superphosphate), and 60 kg K$_2$O ha^{-1} (as potassium chloride). Top dressing was applied 30 days after sowing, which contained 100 kg N ha^{-1} (as urea). Weed, pest, and disease control were carried out whenever necessary.

Evaluated Traits

Prior to harvesting plants, the following variables were measured: mean plant height (PH), measured as the vertical distance in meters from the soil to the flag leaf; mean ear height (EH), measured as the vertical distance in meters from the ground level to the insertion of the highest ear; and plant lodging (PLO), computed as the proportion of lodged and broken plants in relation to the total number of plants per plot. The PH and EH variables were evaluated based on five plants in each plot.

After harvesting, the following traits were evaluated: ear weight (EW, in kg), measured by weighing the husked ears; and grain yield (GY, in kg), estimated by weighing the grains produced in each plot after threshing. The mean hundred-grain weight (100GW, in g) was estimated for a random sample of 100 grains from each plot. Popping expansion (PE) was determined from the average of two grain samples from each plot, for each of which 30 g of grains was weighed, and these grains were then expanded in a microwave oven (Panasonic NN-S65B model, at 1,000 W). The grains were popped for 2.25 min in a special paper bag, and then the popped popcorn volume was measured in a graduated 2 L beaker. Finally, the PE value was determined by dividing the volume expanded by the initial weight (30 g) of the grains. The expanded popcorn volume per hectare (PV, in m^3 ha^{-1}) was estimated by multiplying the mean yield per plot by the popping expansion value for the same plot. This 'super-trait' was proposed by Amaral Júnior et al. (2016) as a means to obtain a simultaneous index of both the gains in grain yield and popping expansion in a given crop.

Multilocation mixed model analysis

For each of the abovementioned traits (PH, EH, PLO, EW, 100GW, PE, and PV), deviance analysis was performed, genetic parameters were estimated, and the genetic values of different genotypes were predicted using analyses with mixed models.

Genetic values were predicted by the mixed models approach, based on the model expressed in Equation 1 below (Littell et al., 2006):

$$y = Xr + Zev + Wt + Qi_1(t) + e \qquad (1)$$

where *y* is the data vector; *r* is the vector of the block effects (assumed to be fixed) added to the overall mean; *ev* is the vector of environmental effects (assumed to be random); *t* is the vector of the additive genetic effects of the individual testers (random); $i_1(t)$ is the vector of the effects of the inbred lines nested within each tester's effect (random); *e* is the error or residual vector (random); *X* is the incidence matrix of fixed effects; *Z* is the incidence matrix of environmental effects and individual genetic effects; *W* is the incidence matrix of individual genetic effects; and *Q* is the incidence matrix of the effects of inbred lines nested within each tester's effects.

The mixed model equation is given by:

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$$\begin{bmatrix} \hat{r} \\ \hat{e} \\ \hat{t} \\ \hat{Il} \end{bmatrix} \begin{bmatrix} X'R^{-1}X & X'R^{-1}Z \\ Z'R^{-1}X & Z'R^{-1}Z+G^{-1} \\ W'R^{-1}X & W'R^{-1}W+G^{-1} \\ Q'R^{-1}X & Q'R^{-1}Q+G^{-1} \end{bmatrix} = \begin{bmatrix} X'R^{-1}y \\ Z'R^{-1}y \\ W'R^{-1}y \\ Q'R^{-1}y \end{bmatrix}$$

$$y\,|\,r,V \sim N(Xr,V)$$

$$e\,|\,I\sigma_e^2 \sim N(0,I\sigma_e^2)$$

$$t\,|\,\sigma_t^2 \sim N(0,I\sigma_t^2)$$

$$il\,|\,\sigma_{il}^2 \sim N(0,I\sigma_{il}^2)$$

$$\varepsilon\,|\,\sigma_\varepsilon^2 \sim N(0,I\sigma_\varepsilon^2)$$

$$V = Z\sigma_\varepsilon^2 Z' + WI\sigma_t^2 W' + QI\sigma_{il}^2 Q' + I\sigma_e^2$$

The genetic parameters were estimated by REML, where we calculated individual heritability in the narrow sense (Equation 2) as follows:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2} \tag{2}$$

Additive genetic variance (Equation 3):

$$\sigma_a^2 = \frac{\left[\hat{a}'A^{-1}\hat{a} + \sigma_e^2 \mathrm{tr}(A^{-1}C^{22}) \right]}{q} \tag{3}$$

Environmental variance (Equation 4):

$$\sigma_e^2 = \frac{\left[y'y - \hat{b}'X'y - \hat{a}'Z'y \right]}{\left[N - r(x) \right]} \tag{4}$$

where: tr: operator matrix; r(x): Matrix rank X; N, q: total observations, respectively; and
Phenotypic variance (Equation 5):

$$\hat{\sigma}_f^2 = \hat{\sigma}_a^2 + \hat{\sigma}_e^2 \tag{5}$$

According to the above model (Viana & Resende, 2014), deviance analysis was computed as follows (Equations 6 and 7).

$$D = -2\ln(L) \tag{6}$$

$$\ln(L) = -\frac{1}{2\ln|X'V^{-1}X|} - \frac{1}{2\ln|V|} - \frac{1}{2(y-Xm)'V^{-1}(y-Xm)} \tag{7}$$

where *ln(L)* is the maximum point of the restricted maximum likelihood (REML) logarithm function; *y* is the vector of the analyzed variable; *m* is the vector of measurement effects (assumed to be fixed), to which all means are added; *X* is the incidence matrix for the fixed effects; and *V* is the variance-covariance matrix of *y*.

The LRT (likelihood ratio test) was used to define the statistical significance of the above effects as follows (Equation 8).

$$LRT :\, | -2\ln(L_{se}) + 2\ln(L_{fm}) \tag{8}$$

where L_{se} is the maximum point of the maximum likelihood function for the reduced model (without effects); and L_{fm} the maximum point of the maximum likelihood function for the full model.

All analyses were performed using SAS (Statistical Analysis System) software, version 9.3 (SAS Institute, 2011). The MIXED procedure (PROC MIXED) command was used to obtain the values estimated by REML/BLUP and LSMEANS was used to calculate least-squares means.

Results and Discussions

Deviance analysis

The chi-square test revealed significant ($p < 0.01$) differences in the studied traits in the two environments (Table 1). This indicates that the topcross hybrids performed differently in different environments (Marcondes et al., 2016).

The effects of the testers on these traits were also significant (chi-square test, $p < 0.01$), indicating that there were different responses of the testers when crossed with the partially inbred S₃ lines. The effects of the inbred lines (nested within the effects of the testers) were also significant (chi-square test, $p < 0.01$), indicating the existence of genetic variability in the responses of the partially inbred S₃ lines when they were crossed with the testers for all studied traits (PH, EH, PLO, EW, GY, 100GW, PE, and PV).

Genetic components

The narrow-sense heritability estimates of the studied traits obtained in the environment of Campos dos Goytacazes ranged from 10 (PLO) to 76% (PE) (Table 2). The high h_a^2 estimate obtained for PE may be explained by the lower environmental and higher additive variance in this trait. The heritability estimate for 100GW was lower than 25% due to the especially strong influence of environmental variance on this trait (Table 2). The heritability estimates for the EW, GY, and PV traits were less strongly affected by the environment, although the environmental effects were still higher than the additive effects on them (41%, 40%, and 40%, respectively) (Table 2). Finally, for PLO, heritability was estimated to be less

Table 1. Deviance analysis of eight traits evaluated for 172 topcross popcorn hybrids at locations in Campos dos Goytacazes and Itaocara, RJ, Brazil.

Effects	Deviance	LRT (χ^2) [2]	Deviance	LRT (χ^2) [2]
		PH		EH
Environment	802.94	1350.22**	1367.89	907.55**
Testers [1]	945.36	1255.56**	2089.36	896.54**
Inbred lines (testers) [1]	1936.04	981.56**	2386.35	855.63**
Full model	2143.36	-	2275.45	-
		PLO		EW
Environment	1510.37	1631.57**	1578.48	680.41**
Testers [1]	1468.73	1345.58**	1598.63	725.63**
Inbred lines (testers) [1]	2739.73	950.43**	2320.85	750.41**
Full model	2541.94	-	2186.89	-
		GY		100GW
Environment	789.94	1250.42**	1467.9	1007.55**
Testers [1]	863.36	1365.56**	1789.36	936.54**
Inbred lines (testers) [1]	2036.04	851.56**	2474.35	785.63**
Full model	2038.36	-	2475.45	-
		PE		PV
Environment	1710.37	1231.57**	1458.48	745.41**
Testers [1]	1568.98	1545.78**	1798.45	825.36**
Inbred lines (testers) [1]	2939.61	1050.23**	1820.85	890.41**
Full model	2941.94	-	1986.89	-

[1] Deviance of the adjusted model, without these effects; [2] LTR, likelihood ratio test, distribution with 1 degree of freedom; ^{ns} = non-significant, and ** = significant at p < 0.01, according to the χ^2 test (p < 0.01 if $\chi^2 > 6.63$, p > 0.05 if $\chi^2 < 3.84$). PH: mean plant height (m); EH: mean ear height (m); PLO: plant lodging; EW: ear weight (kg ha^{-1}): GY: grain yield (kg ha^{-1}); 100GW: mean weight of 100 grains (g); PE: popping expansion (mL g^{-1}); PV: popping volume per hectare (m^3 ha^{-1}).

than 10%, meaning this trait was strongly impacted by the environment. In this environment, the heritability estimates obtained were as expected for each trait: high for PE (Pereira & Amaral Júnior, 2001), which is typically an additive trait; intermediate for EW, GY, and PV (Scapim et al., 2006; Cabral et al., 2015), which characteristically show a predominance of non-additive effects; and low for PLO, which is a trait that is

normally greatly influenced by environmental effects (Freitas et al., 2013).

In Campos dos Goytacazes, the environmental variance values found were higher than those of the additive variance for the EW, GY, 100GW, PV, EH, and PLO traits. Exceptions were observed for PE and PH (Table 2). The values of phenotypic, additive, and environmental variances were close to zero for PH and EH (Table 2). In these cases, it appears that either the genotypes tested have low genetic variability in these traits or that there are weak environmental effects on these traits (Sousa et al., 2019).

For the environment of Itaocara, the heritability estimates of the studied traits obtained ranged from 6% (PLO) to 91% (PE) (Table 3). The 91% heritability estimate for PE was attributed to the higher additive (26.51%) than environmental variance (2.59%) in this trait. For PH and EH, heritability was estimated as 60% and 45%, respectively. Unlike Campos dos Goytacazes, in Itaocara the estimates of trait heritability obtained were relatively high overall. This demonstrates that there was a weak effect of the Itaocara environment on PH and EH, allowing for the full potential expression of different genotypes for these traits there, while in Campos dos Goytacazes the results were driven by the strong effects of this environment on the variation in these traits.

For the EW, GY, 100GW, PV, and PLO traits, the environmental variance was higher than the additive variance in Itaocara (Table 3), which negatively impacted the heritability of these traits estimated there. The EW and GY traits had the highest heritability estimates (44% and 39%, respectively) among the variables adversely affected by environmental variance. On the other hand, the lowest heritability values were found for 100GW, PV, and PLO, with values of 21%, 13%, and 6%, respectively. In the case of PV, the strong environmental influence of the environment at Itaocara on this trait resulted in a greater decrease in its heritability estimate there compared to that at Campos dos Goytacazes. Finally, for PLO, the environmental effect predominated in

Table 2. Estimates of narrow-sense heritability (h_a^2), phenotypic variance, additive variance, and environmental variance in eight trait variables in 172 topcross popcorn hybrids evaluated in the environment of Campos dos Goytacazes, RJ, Brazil.

Componente	EW	GY	100GW	PE	PV	PH	EH	PLO
h_a^2	0.41	0.40	0.24	0.76	0.40	0.41	0.31	0.10
σ_f^2	1022378.40	859660.90	9.94	36.63	901.60	0.02	0.014	2.83
σ_a^2	422771.77	350051.60	2.45	27.63	363.77	0.01	0.004	0.28
σ_e^2	599606.60	509609.30	7.48	8.60	537.83	0.01	0.009	2.55

PH: mean plant height (m); EH: mean ear height (m); PLO: plant lodging; EW: ear weight (kg ha^{-1}): GY: grain yield (kg ha^{-1}); 100GW: mean weight of 100 grains (g); PE: popping expansion (mL g^{-1}); PV: popping volume per hectare (m^3 ha^{-1}). h_a^2: narrow-sense heritability; σ_f^2: phenotypic variance; σ_a^2: additive variance; σ_e^2: environmental variance.

Table 3. Estimates of narrow-sense heritability (h_a^2), phenotypic variance, additive variance, and environmental variance in eight trait variables in 172 topcross popcorn hybrids evaluated in the environment of Itaocara, RJ, Brazil.

Componente	EW	GY	100GW	PE	PV	PH	EH	PLO
h_a^2	0.44	0.39	0.21	0.91	0.13	0.60	0.45	0.06
σ_f^2	135606.20	98701.14	4.54	29.11	118.31	0.05	0.03	2.25
σ_a^2	59667.51	39201.65	0.94	26.51	16.35	0.03	0.01	0.12
σ_e^2	75938.69	59499.48	3.59	2.59	101.96	0.02	0.01	2.12

PH: mean plant height (m); EH: mean ear height (m); PLO: plant lodging; EW: ear weight (kg ha^{-1}); GY: grain yield (kg ha^{-1}); 100GW: mean weight of 100 grains (g); PE: popping expansion (mL g^{-1}); PV: popping volume per hectare (m^3 ha^{-1}). h_a^2: narrow-sense heritability; σ_f^2: phenotypic variance; σ_a^2: additive variance; σ_e^2: environmental variance.

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Itaocara, resulting in the lowest heritability estimates being obtained for this trait there. In general, these results were similar to those found at Campos dos Goytacazes, with high estimates found for PE, intermediate for EW and GY, and low for PLO.

Heritability is the proportion of phenotypic variance that is genetically determined, and, therefore, higher heritability values indicate there being greater chances of success for plant selection for particular traits (Hallauer et al., 2010). Among the evaluated traits, PE was the only one with high h_a^2 estimates ($h_a^2 > 0.50$) in both environments (Tables 2 and 3) (Resende, 2002). This can be explained by the higher estimated impact of additive genetic than environmental variance on this trait (Scapim et al., 2006). Moreover, this finding is in line with the superior additive genetic effects previously found on the expression of PE by Pereira & Amaral Júnior (2001).

According to the classification of Resende (2002), the h_a^2 values of PH and EH were intermediate ($0.15 < h_a^2 < 0.50$), and high heritability was found only for PH in Itaocara, RJ. These traits are mostly controlled by additive genetic effects (Scapim et al., 2006), but the low h_a^2 estimates found for them may be related to there being greater impacts of environmental variance on them than the estimated additive effects at this location. This assumption is supported by the fact that any change in the variance components of a trait affects its heritability (Batista et al., 2012).

The estimated heritability values of PLO were low ($h_a^2 < 0.15$), according to the classification proposed by Resende (2002). This indicates a weak influence of the genetic compositions of the testers on the expression of this trait, which was thus almost exclusively due to environmental effects on the plants (Freitas et al., 2013).

According to Resende's (2002) classification, the h_a^2 values found for EW, GY, 100GW, and PV were medium, except in Itaocara. These traits are strongly influenced by dominant genetic effects, and the use of crosses for plant evaluation (topcross hybrids) favored the expression of these effects (Cabral et al., 2015). These traits show a greater variance due to dominance than additive genetic effects, which leads to a reduction in their narrow-sense heritability. In this way, greater gains can be obtained by exploiting the heterosis of these traits (Wei et al., 2016).

At Itaocara, the strong environmental influence on PV was detrimental to its estimated heritability (0.13). This drastically reduces the possibility of obtaining gains in this trait by breeding, and these conditions do not favor the selection of genotypes for PV. However, PV was used so that simultaneous selection for GY and PE could be possible (Amaral Júnior et al., 2016). Under these conditions, direct selection is indicated as the most likely means to successfully breed for higher selection gains in each of these traits (Amaral Júnior et al., 2016; Lima et al., 2016).

Discrimination of testers

For the discrimination of testers, each tester's genotypic effect was estimated via Best Linear Unbiased Prediction (BLUP) and associated with its crossing mean. According to Littell et al. (2006), this procedure allows for the precise characterization of the testers, meaning one can estimate their potential effects on crossing and use this estimate as a threshold measurement of the central phenotypic tendency of each tester. To obtain genetic gains in future generations and ensure the selection of good inbred lines with high combining ability (generating high-yield hybrids), testers should exhibit high average values (Cancellier et al., 2016), in addition to having good combining ability or strong genotypic effects.

Two main types of testers may be considered the most important in hybrid breeding programs. The first type is an inbred lines discriminator, which, according to Hallauer & Lopes Perez (1979) and Smith (1986), should be the tester that has the lowest frequency of favorable alleles, so that the entire potential manifested in the hybrid resulting from crossing is only that of the inbred line evaluated. The second type is an elite tester with high combining ability and a predominance of favorable genes, which can facilitate the identification of inbred lines that, when crossed with it, generate hybrids with high productivity (Hallauer & Lopes Perez, 1979; Smith, 1986). The genotypic effects predicted by BLUP on PH and EH in Campos dos Goytacazes were close to each other and in the same direction of selection, with each tester having either negative or positive values for both traits. Thus, testers BRS-Angela (PH = -0.07 and EH = -0.06) and UENF 14 (PH = -0.07 and EH = -0.03) had negative genotypic effects; whereas P2 had positive effects (PH = 0.13 and EH = 0.09), and IAC 125 had negligible effects (PH = 0.001 and EH = 0.00), meaning that IAC 125 can be considered unable to sufficiently discriminate lines in terms of these traits (Table 4).

Regarding the location of Itaocara, the genotypic effects on PH and EH were also similar and in the same direction of selection for each tester (Table 5). The genotypic effects of testers BRS-Angela (PH = -0.09 and EH = -0.05) and UENF 14 (PH = -0.16 and EH = -0.10) were negative. On the other hand, those of the testers P2 (PH = 0.20 and EH = 0.14) and IAC 125 (PH = 0.05 and EH = 0.01) were positive, and P2 was the tester with the highest values for these traits (Table 5).

The traits PH and EH are somewhat peculiar, since selection for them can occur in either a positive or negative direction, depending on the tolerance of plants to lodging under particular climatic conditions. In this study, positive selection for these traits was considered favorable, since the mean plant height was close to the standard for this crop (2 m). However, Freitas et al. (2013) emphasized the fact that negative selection for this trait may be of interest when the mean plant height exceeds 2 m, particularly at locations with a high incidence of strong winds, which would increase the plant lodging rate (although this was not the case in the present study). Thus, the BRS-Angela and UENF 14 testers would be good potential discriminators, and P2 and IAC 125 would be testers of high combining ability, for the above traits.

The phenotypic means of PLO in Campos dos Goytacazes varied from 2.34% (IAC 125) to 2.76% (BRS-Angela), indicating low rates of plant lodging in the studied plots (Table 4). The

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Table 4. Genetic effects predicted by BLUP and the phenotypic means of four testers derived from crosses with 43 partially endogenous S_3 popcorn progenies in a partial diallel mating system for the environment of Campos dos Goytacazes, RJ, Brazil.

Testador	BLUP	LSMEAN	Standard error	BLUP	LSMEAN	Standard error
		PH			EH	
BRS Angela	-0.07	2.06	0.16**	-0.06	1.13	0.08**
IAC 125	0.001	2.1	0.16**	0.00	1.16	0.09**
P2	0.13	2.17	0.16**	0.09	1.21	0.09**
UENF 14	-0.07	2.07	0.16**	-0.03	1.15	0.08**
		PLO			EW	
BRS Angela	0.61	2.76	0.21**	798.24	3639.91	283.91**
IAC 125	-0.46	2.34	0.18**	-624.7	2858.79	222.98**
P2	-0.17	2.53	0.19**	183.2	3305.13	257.80**
UENF 14	0.02	2.54	0.19**	-356.8	3016.91	235.31**
		GY			100GW	
BRS Angela	678.02	2940.39	229.35**	1.68	13	1.01**
IAC 125	-608.1	2241.44	174.83**	-1.75	11.04	0.86**
P2	239.65	2702.37	210.78**	0.61	12.38	0.96**
UENF 14	-309.5	2407.08	187.75**	-0.53	11.73	0.91**
		PE			PV	
BRS Angela	1.85	31.49	2.45**	26.12	92.62	7.22**
IAC 125	3.08	32.21	2.51**	-10.61	72.83	5.68**
P2	-8.86	26.03	2.03**	-15.00	70.52	5.50**
UENF 14	3.92	32.52	2.53**	-0.51	78.18	6.09**

PH: mean plant height (m); EH: mean ear height (m); PLO: plant lodging; EW: ear weight (kg ha⁻¹): GY: grain yield (kg ha⁻¹); 100GW: mean weight of 100 grains (g); PE: popping expansion (mL g⁻¹); PV: popping volume per hectare (m³ ha⁻¹).

Table 5. Genetic effects predicted by BLUP and the phenotypic means of four testers, derived from crosses with 43 partially endogenous S_3 popcorn progenies, in a partial diallel mating system, for the environment of Itaocara, RJ, Brazil.

Testador	BLUP	LSMEAN	Standard error	BLUP	LSMEAN	Standard error
		PH			EH	
BRS Angela	-0.09	1.99	0.15**	-0.05	1.13	0.08**
IAC 125	0.05	2.06	0.16**	0.01	1.17	0.09**
P2	0.20	2.14	0.16**	0.14	1.24	0.09**
UENF 14	-0.16	1.95	0.15**	-0.10	1.11	0.08**
		PLO			EW	
BRS Angela	-0.1	1.49	0.11**	97.12	1593.02	124.25**
IAC 125	-0.26	1.35	0.10**	-162.6	1445.14	112.72**
P2	0.31	2.14	0.16**	283.29	1691.87	131.96**
UENF 14	0.06	1.75	0.13**	-217.8	1410.37	110.00**
		GY			100GW	
BRS Angela	34.84	1266.49	98.78**	0.32	15.33	1.19**
IAC 125	-118.7	1177.79	91.86**	-0.67	14.72	1.14**
P2	251.13	1384.73	108.00**	1.11	15.81	1.23**
UENF 14	-167.3	1150.12	89.70**	-0.76	14.68	1.14**
		PE			PV	
BRS Angela	2.95	31.08	2.42**	4.02	39.48	3.07**
IAC 125	4.35	31.82	2.48**	1.57	37.76	2.94**
P2	-7.54	25.77	2.01**	-1.63	35.78	2.79**
UENF 14	0.23	29.72	2.31**	-3.95	34.09	2.65**

PH: mean plant height (m); EH: mean ear height (m); PLO: plant lodging; EW: ear weight (kg ha⁻¹): GY: grain yield (kg ha⁻¹); 100GW: mean weight of 100 grains (g); PE: popping expansion (mL g⁻¹); PV: popping volume per hectare (m³ ha⁻¹).

genotypic effects on PLO, which are considered adequate when negative, were appropriate for IAC 125 (-0.46) and P2 (-0.17). The genotypic effect of tester BRS-Angela was positive (0.61), indicating a higher probability of lodging, whereas the estimate for tester UENF 14 (0.02) was positive but very close to zero (Table 4).

For the environment of Itaocara, the phenotypic means of PLO ranged from 1.49% (IAC 125) to 2.14% (P2), indicating low lodging rates for these genotypes at this location (Table

5). For this variable, testers P2 (0.31) and UENF 14 (0.06) had positive genotypic effects (i.e., led to increased plant lodging and breaking), whereas the genotypic effects were negative for BRS-Angela (-0.10) and IAC 125 (-0.26), indicating that the latter testers led to a reduction in the incidence of this adverse effect among their respective hybrids.

In relation to the above considerations, it should be emphasized that, since negative values are of most interest in selection for PLO (Cabral et al., 2015), the tester with the

greatest negative effect is consequently the one with the best genotypic potential for this trait. Therefore, tester P2 was the best potential discriminator, and the BRS-Angela and IAC 125 testers were those with the highest combining ability, for this trait.

The results for EW, GY, and 100GW were similar in the environment of Campos dos Goytacazes, especially in relation to the direction of selection for these traits for each tester (Table 4). For these three traits, the genotypic effects were simultaneously negative for IAC 125 (EW = -624.7, GY = -608.1, and 100GW = -1.75) and UENF 14 (EW = -356.8, GY = -309.5, and 100GW = -0.53), resulting in lower phenotypic means (Table 4). Conversely, positive genotypic effects were observed for testers BRS-Angela (EW = 798.24, GY = 678.02, and 100GW = 1.68) and P2 (EW = 183.2, GY = 239.65, and 100GW = 0.61), resulting in higher phenotypic means (Table 4).

Similar to results for Campos dos Goytacazes, the genotypic effects on EW, GY, and 100GW were negative in the environment of Itaocara for both IAC 125 (EW = -162.6, GY = -118.7, and 100GW = -0.67) and UENF 14 (EW = -217.8, GY = -167.3, and 100GW = -0.76), resulting in lower mean values of these traits in these testers' hybrids. However, the BRS-Angela (EW = 97.12, GY = 34.84, and 100GW = 34.84) and P2 (EW = 283.29, GY = 251.13, and 100GW = 1.11) testers had positive genotypic effects, resulting in higher phenotypic means in their hybrids. Thus, the IAC 125 and UENF 14 testers would be potential discriminators and BRS-Angela and P2 would be testers with high combining ability for these traits in both environments. The phenotypic means of trait PE ranged from 26.03 (P2) to 32.52 mL g^{-1} (UENF 14) (Table 4). Only tester P2 had a negative BLUP estimate for this trait (-8.86), while the estimates of the other testers were positive, in the following increasing order: BRS-Angela (1.85), IAC 125 (3.08), and UENF 14 (3.92) (Table 4). The phenotypic means of trait PV varied from 70.52 (P2) to 92.62 m³ ha^{-1} (BRS-Angela). BRS-Angela was the only tester with a positive BLUP estimate for this trait (26.12), and P2 had the lowest BLUP estimate (-15.00) for PV (Table 4).

In Itaocara, the genotypic effect on PE was negative only for tester P2 (-7.54), and the phenotypic mean value (25.77 mL g^{-1}) there with this tester was far below the commercial recommendation for popcorn (30.00 mL g^{-1}). For the other

testers, the genotypic effects were positive, leading to an increased PE in the hybrids derived from these testers. Finally, for PV, testers P2 (-1.63) and UENF 14 (-3.95) presented negative genotypic effect values, resulting in lower popcorn volumes, while the genotypic effects for BRS-Angela (4.02) and IAC 125 (1.57) were positive, increasing the popcorn yield potential in the resulting hybrids. Therefore, tester P2 would be a good potential discriminators for the PE and PV traits, while BRS-Angela and IAC 125 would be testers with high combining ability for PE and PV, in both environments. In summary, the testers with the highest and lowest genotypic potential in crosses with S$_3$ progenies were identified herein for each trait in each environment (Table 6).

Based on the above genotypic effects estimated by BLUP, the effects of testers BRS-Angela and P2 were positive for almost all traits. This fact, associated with the higher phenotypic means found for these testers, indicates that they have the best combining ability when crossed with the partially inbred S$_3$ progenies. They are therefore of potential interest for use in the breeding of superior hybrids (Tables 4 and 5), which agrees with the assumption that testers with a predominance of favorable alleles can be used to develop promising hybrids (Hallauer & Lopes Perez, 1979; Smith, 1986).

Testers IAC 125 and UENF 14 had the highest concentrations of negative genotypic effects, as estimated by BLUP. Thus, the full genotypic potential of the hybrids resulting from cross with these testers is presumably due to the potential of the S$_3$ progenies themselves. Therefore, the use of testers IAC 125 and UENF 14 should be preferred when the goal is to reliably identify the most promising progenies among the evaluated genotypes. This classification of the testers was based on the assumption that the tester with the lowest frequency of favorable alleles is the most appropriate to use to discriminate the potentials of genotypes in crosses (Hallauer & Lopes Perez, 1979; Smith, 1986). According to Guimarães et al. (2012), the high genetic variability among the hybrids produced by testcrosses using a particular tester and the low frequency of favorable alleles therein are essential traits of a good tester.

Considering the importance of exploiting topcross hybrids in selective breeding, BRS-Angela was the only tester with positive and high BLUP estimates for the three key traits for

Table 6. Identification of the tester selected to best discriminate the heterocyclic potential of the genotypes in crosses with S$_3$ progenies for each trait in each environment.

Trait	Lowest genotypic potential		Highest genotypic potential	
	Campos	Itaocara	Campos	Campos
PH	BRS-Angela	UENF 14	P2	P2
EH	BRS-Angela	UENF 14	P2	P2
PLO	BRS-Angela	P2	IAC 125	IAC 125
EW	IAC 125	UENF 14	BRS-Angela	P2
GY	IAC 125	UENF 14	BRS-Angela	P2
100GW	IAC 125	UENF 14	BRS-Angela	P2
PE	P2	P2	UENF 14	IAC 125
PV	P2	UENF 14	BRS-Angela	BRS-Angela

PH: mean plant height (m); EH: mean ear height (m); PLO: plant lodging; EW: ear weight (kg ha^{-1}): GY: grain yield (kg ha^{-1}); 100GW: mean weight of 100 grains (g); PE: popping expansion (mL g^{-1}); PV: popping volume per hectare (m³ ha^{-1}).

popcorn breeding (PE, GY, and PV) in both environments. Thus, this tester was characterized as the best to use to obtain improved topcross hybrids with partially inbred S$_3$ progenies. On the other hand, tester BRS-Angela is an open-pollinated variety, so obtaining lines from this population will possibly generate candidate hybrids with greater heterotic potential when crossed with the lines derived from S$_3$ progenies.

In the analysis of the testers with the lowest genotypic effects on the studied traits, IAC 125 and UENF 14 did not have negative effects on the expression of PE. However, lines can be selected directly based their 'per se' value due to the predominance of additive genetic effects on the PE trait (Amaral Júnior et al., 2013). However, it should be noted that tester UENF 14 is the population from which the S$_3$ progenies were derived, and the short genetic distance (Lima et al., 2016; Pena et al., 2016) between this tester and the progenies may have interfered with the estimation of genotypic effects by BLUP. Thus, it was concluded that tester IAC 125 is the most suitable for use in the discrimination of partially endogenous progenies in both studied environments.

The mean BLUP estimates of the testers (i.e., the estimated genotypic value of each tester) obtained herein allowed for the simple and direct identification and selection of the best tester for each trait studied to be achieved. Thus, this method is highly reliable for use in this type of study, and this conclusion is reinforced by the fact that BLUP is a procedure that maximizes the selection accuracy and, consequently, improves the genetic gain and the efficiency of breeding programs (Viana & Resende, 2014).

Strong correlations were found between the overall combining ability and the genotypic effect estimated by BLUP for each tester. In this sense, only one of these parameters needs to be adopted for the selection of testers. Due to the considerable flexibility of this type of analysis, the mixed model approach (BLUP) used herein may be more useful in selective breeding than other methods (Cancellier et al., 2016).

Therefore, it is clear that this method circumvents the difficulties faced by researchers in cultivar development, and represents a method of statistical analysis that permits the reliable identification of the best testers to use to discriminate the best combinations of lines and traits in hybrid progenies. Unsurprisingly, the results we obtained by using the BLUP procedure are clearer than those obtained by Lima et al. (2016) in an evaluation of the same testers in Campos dos Goytacazes using only the conventional statistical analysis methodology based on the Fasoulas index (1983). As also, the results of the present study are clearer than those obtained by Pena et al. (2016), who only used the Fasoulas index (1983) to evaluate the same testers in the environment of Itaocara. Consequently, estimating the genotypic effects of testers by BLUP is a promising method to use to identify testers that best meet the requirement of good testers, optimize the efficiency of the discrimination of genotypes for selection purposes, are easy to manipulate, allow for the better classification of the relative merits of different lines, and maximize the genetic

gain achieved through hybrid breeding (Hallauer & Lopes Perez, 1979).

Conclusions

The prediction of genotypic values of testers using the REML/BLUP approach is an efficient method for estimating the combining abilities and discrimination of S$_3$ testers and progenies. We suggest that this analytical procedure should be incorporated into related breeding programs.

Tester IAC 125 was the one that best discriminated the relative merits of the lines in both North and Northwestern regions of the State of Rio de Janeiro. There was a significant effect of environment on the estimates of the combining abilities of the four testers for different traits; in Campos dos Goytacazes, RJ, the highest BLUP estimates were found for tester BRS-Angela, which thus produced the best hybrids there; whereas in Itaocara, RJ, tester P2 performed the best. However, the genotypic merit was the greatest in general for combinations performed with tester BRS-Angela, with which high and positive effects were estimated by BLUP for grain yield, popping expansion, and expanded popcorn volume per hectare at both locations.

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