Heterotic parametrization for economically important traits in popcorn

Rafael Augusto Vieira¹, Israel Leite Souza Neto¹, Lucas Souto Bignotto¹, Cosme Damião Cruz², Antonio Teixeira do Amaral Júnior³ and Carlos Alberto Scapim^{1*}

¹Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brazil. ²Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. ³Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Rio de Janeiro, Brazil. *Author for correspondence. E-mail: cascapim@uem.br

ABSTRACT. Six popcorn varieties were crossed in a diallel mating scheme to obtain 15 F_1 hybrids, with the purpose of analyzing the estimates of heterotic parametrization for agronomically important traits. The genitors and hybrids were evaluated in randomized block design with three replications in Viçosa and Visconde do Rio Branco, in Minas Gerais State, Brazil, in three growing seasons. Six traits were evaluated, including grain yield and popping expansion. There were highly significant effects for treatments, varieties and mean heterosis for plant and ear heights, and for the others traits the significance extended to all sources of variation. *Amarela* and *Viçosa* revealed superiority for grain yield in relation to performance *per se*, while *Rosa Claro* expressed the highest effect of varietal heterosis. The best combinations for grain yield were *Roxa x Viçosa, Amarela x Rosa Claro, Rosa Claro x Beija-Flor* and *Branca x Beija-Flor*. For popping expansion, there were positive and negative values for specific heterosis in all growing seasons evaluated, ratifying the influence of bidirectional dominance on this trait. Simultaneous genetic gains for grain yield and popping expansion may be obtained by using *Viçosa* in intrapopulational programs, while *Amarela* and *Rosa Claro* are recommended for using in interpopulational breeding.

Key words: diallel, heterosis, popping expansion, bidirectional dominance, Zea mays L.

RESUMO. Parametrização heterótica para características de importância econômica em milho pipoca. Seis variedades de milho pipoca foram cruzadas em esquema dialélico para obtenção de 15 híbridos F1, visando à análise das estimativas da parametrização heterótica para características de interesse agronômico. Os genitores e os híbridos foram avaliados em delineamento em blocos ao acaso, com três repetições, em Viçosa e Visconde do Rio Branco, Estado de Minas Gerais, Brasil, em três épocas de plantio. Foram avaliadas seis características, incluindo rendimento de grãos e capacidade de expansão. Houve efeitos altamente significativos para tratamentos, variedades e heterose média para altura de plantas e de espigas, enquanto as demais características expressaram efeitos significativos para todas as fontes de variação. Amarela e Viçosa revelaram-se superiores para rendimento de grãos quanto ao comportamento per se, enquanto Rosa Claro expressou o maior efeito de heterose varietal. As melhores combinações para rendimento de grãos foram Roxa x Viçosa, Amarela x Rosa Claro, Rosa Claro x Beija-Flor e Branca x Beija-Flor. Para capacidade de expansão houve valores positivos e negativos para efeitos de heterose específica em todos os ambientes avaliados, ratificando a influência da dominância bidirecional na expressão da característica. Ganhos simultâneos para rendimento de grãos e capacidade de expansão poderão ser obtidos com a utilização de Viçosa em programas intrapopulacionais, enquanto Amarela e Rosa Claro são recomendadas para seleção recorrente recíproca.

Palavras-chave: dialelo, heterose, capacidade de expansão, dominância bidirecional, Zea mays L.

Introduction

The maize seed market had 237 cultivars of corn planted in Brazil during the growing season 2005/2006. However, only six were popcorn cultivars (Zélia and Jade, both three-way hybrids; IAC-112, a modified simple hybrid; and RS-20, BRS ANGELA and UFVM2-Barão-Viçosa, the open-pollinated varieties), ratifying the small amount of genotypes available to supply the demand (SCAPIM et al., 2006a; FREITAS JÚNIOR et al., 2006; SANTOS et al., 2007; PEREIRA et al., 2008; RANGEL et al., 2008; VILELA et al., 2008).

Considering the continental dimension of Brazil, with different edaphoclimatic conditions, the releasing of popcorn hybrids by public and private institutions for each region is very important to reduce dependence on seeds from the foreign market, particularly due to the cultivation of North American hybrids (for example, P608, P608 HT, P618, P621 and P625), indexed in Brazil and used by packaging companies, e.g, Yoki Alimentos S.A., which restrict the access to seed by the company's partner producers (SAWAZAKI et al., 2003; DAROS et al., 2004a; FREITAS JÚNIOR et al., 2006; RANGEL et al., 2007).

Lined up with this, the increasing of demand for the product on the domestic market, it is unquestionable that the popcorn breeding for release hybrids has a relevant importance for the Brazilian producers and consumers (ANDRADE et al., 2002; SCAPIM et al., 2006b; MIRANDA et al., 2008; SANTOS et al., 2008). At the *Zona da Mata Norte* of Minas Gerais State, Brazil, packaging companies could buy the product if the farmers would start to sow on a large scale. However, in this region of the Minas Gerais State it is very difficult to find superior popcorn seed on the market for planting.

In this context, researches involving popcorn have been increasing for several areas of scientific knowledge in Brazil, e.g., cytogenetic (RICCI et al., 2007a and b; SILVA et al., 2007), quality of seeds (MOTERLE et al., 2008), agronomic performance (VENDRUSCOLO et al., 2001; VIEIRA et al., 2009), genetic diversity (PEREIRA et al., 2006; 2008; DANDOLINI et al., 2008; VILELA et al., 2008; SILVA et al., 2009) and methods of breeding (DAROS et al., 2004a and b; FREITAS JÚNIOR et al., 2006; VIANA et al., 2007; RANGEL et al., 2008; RODOVALHO et al., 2009; FREITAS JÚNIOR et al., 2008; ARNHOLD et al., 2009; FREITAS JÚNIOR et al., 2009).

In view of the high profitability for kernel producers as well as for seed trade, *versus* the lack of hybrids, it is imperative that studies be conducted to evaluate base populations for the potentiality of hybrids, for use at an experimental level and a possible future release as superior cultivars. For this purpose, the diallel analysis lends itself as a viable alternative, even when the number of parents involved is high (CRUZ et al., 2004; FREITAS JUNIOR et al., 2006; SCAPIM et al., 2006a; MIRANDA et al., 2008).

But, there are few reports on diallel analysis in popcorn for Brazilian conditions. For example, Zanette (1989) analyzed diallel of seven popcorn populations and inferred the existence of intermediate heterosis for popping expansion. Later, Andrade et al. (2002) obtained low or even negative specific heterosis for this trait. In another diallel study (SCAPIM et al., 2002), nine yellow grain popcorn populations were evaluated in Paraná State, which concluded by recommending the *Beija-Flor* and RS-20 populations for a reciprocal recurrent selection program. Both are complementary regarding grain yield and popping expansion, aside from the possibility of developing composites among populations of high general combining ability.

More recently, Scapim et al. (2006a), based on results of yield and popping expansion, used eight popcorn populations in a mating crosses diallel scheme to obtain all possible pair combinations in the Northwestern region of Paraná State. With the objective of indicating the best performance strategy and select parents to begin an improvement program of white grain popcorn, the authors recommended the application of intrapopulation breeding on BRS ANGELA and SC 002. Additionally, the application of intense selection was indicated to reduce plant and ear heights.

In another study, Freitas Júnior et al. (2006) evaluated the combining ability of ten genitors and fifteen hybrids of popcorn in a partial circulant diallel crossing in two environments in Rio de Janeiro State, Brazil. The results indicated the genitors UNB2U-C1, BRS ANGELA, UNB2U-C2 and Viçosa-UFV for intrapopulation breeding, while for interpopulation breeding the best combinations were PR-Ervália x BRS ANGELA and UNB2U-C1 x BRS ANGELA.

Miranda et al. (2008) working with only five genitors in a diallel scheme crossing from advanced generations of popcorn hybrids (IAC 112 and Zélia) and three open-pollinated varieties (RS 20, Branco and SAM), concluded: i) Brazilian popcorn has reduced heterosis and genetic variability for popping expansion in relation to commercial cultivars; ii) there is genetic variability among Brazilian popcorn populations that allows the exploitation of additive and non additive effects for grain yield; and iii) it would be difficult to obtain commercial hybrids directly from local varieties because they have poor performance for popping expansion.

Admitting that the major importance of additive effects for popping expansion (PEREIRA; AMARAL JÚNIOR, 2001; SIMON et al., 2004; FREITAS JÚNIOR et al., 2006; RANGEL et al., 2007), but not the bidirectional effects could receive combinations with good expression for popping expansion exactly in the local Brazilian varieties. In this respect, Viana and Matta (2003), Freitas Júnior et al. (2006), and Rangel et al. (2008), working with Brazilian popcorn landraces, found that the specific combining ability estimates were important, but their mean values for popping expansion were very low, indicating the effect of the bidirectional dominance as well as observed by Andrade et al. (2002).

With the purpose of obtaining more precise estimates to achieve a diallel mating scheme with Brazilian popcorn populations, the present study was carried out, using landraces *Amarela*, *Roxa*, *Branca*, *Rosa Claro*, *Beija-Flor* and *Viçosa* and their 15 hybrids F_1 diallelic in two contrasting environments in three growing seasons for evaluating agronomically important traits based on analysis of heterotic parametrization.

Material and methods

Six varieties of popcorn (*Amarela, Roxa, Branca, Rosa Claro, Beija-Flor* and *Viçosa*) were used in diallelic crosses to obtain 15 F_1 hybrid combinations without reciprocals. For establishing the hybrid combinations, the varieties were grown in 6-m rows, paired in all necessary combinations, in a row-plant spacing of 1.00 m between rows and 0.40 m between plants. At flowering, approximately 90 crosses were performed so as to establish a sufficient quantity of hybrid seed to ensure the continuity of the process. Kraft paper bags were used to collect the pollen grains and for the proper pollination.

Hybrids and parents were evaluated in two contrasting environments in the State of Minas Gerais: Visconde do Rio Branco and Viçosa; during three growing seasons. Each environment, 21 treatments (the six parents and fifteen hybrids) were arranged in a randomized block design with three replications. Plots of experiments consisted of a single 10-m row (1.00 m between-row spacing and 0.20 m in-row spacing), which were fertilized with 500 kg of 4-14-8 (N-P₂O₅-K₂O) plus 40 kg of additional N, applied 40 days after sowing. The experiments had two additional surrounding border rows.

The following traits were evaluated: plant height (PH) in cm; ear height (EH) in cm; grain yield (GY) in kg ha⁻¹; weight of ears (WE); mass of one hundred seeds (MS) in g; and popping expansion (PE). PE was measured in samples with 12% humidity, from a humidity-controlled room. At a temperature of 270°C, in a machine developed by Embrapa-Instrumentação Agropecuária, the popcorn kernels were popped for 2.5 minutes and evaluated by the ratio mL g⁻¹, that is, volume compared to mass of 30 g of popped kernels, with two replications for each plot.

The analyses of variance were carried out for the data set from each place at 1% of probability by the F-test. The Scott-Knott procedure at 1% of probability was used to discriminate the treatments. According to the results from the analyses of variance, the modified statistical model of Gardner

and Eberhart (1966) was fit to each appropriated data set. The individual and joint analyses of variance were performed using GENES software (CRUZ,

Results and discussion

2006).

Considering that the maximum F test (p < 0.05) did not disclose heterogeneity for mean squares errors of the individual analyses, for all traits, it was possible the performed the jointed analysis considering the three growing seasons.

Significant differences in the mean squares for the treatments were found by F-test (p < 0.01) in joint analysis, allowing the partition of the square sum between varieties and heterosis effects (data not shown). As plant varieties are non-homogeneous populations, heterosis effects were detected in the hybrids for all traits (p < 0.01). It ratifies that there was sufficient genetic divergence among landraces genitors assessed, resulting in a favorable situation for breeding.

The further heterotic parametrization on the square sum for heterosis effects for plant and ear heights attachment revealed that only the mean squares of the mean heterosis were significant (p < 0.01). In addition, there was a large variance in the genic frequencies within varieties, probably caused by action of dominant loci on the genome of the landraces used in this study. But the non-significant effects of varietal and specific heterosis indicated no differences in the mean genic frequencies among them with a similar dispersion of the genic frequencies in the landraces evaluated for plant and ear height.

Scapim et al. (2002) working with a diallel set of crosses among nine varieties of popcorn for four traits evaluated in two crop years in Iguatemi, Paraná State, Brazil, also obtained significance for mean heterosis effects for plant and ear heights, nevertheless not for varietal and specific heterosis.

Based on these results, it could be possibly assumed that plant and ear height traits are dispensable to be measure next study with the purpose of indicating the best performance hybrid for them. It would simplify the work in the experiments evaluated in different localities. But, Ji et al. (2006) on the evaluation of tropical maize by diallel analysis awared for the plant and ear eights had great importance for selection criteria in most maize breeding programs. For these authors, especially ear height has a great importance when it comes to root and stalk lodging. In fact, high ear position is likely to become more susceptible to root and stalk lodging. Thus, the discarding of these traits is not interesting for popcorn breeding selection, mainly because popcorn plants are more fragile when compared to the common maize. But, usually there is a high correlation between these traits in popcorn (COIMBRA et al., 2001), indicating that it is not necessary to measure both of them in field experiments.

The grain yield as well the weight and number of ears had significant effects of mean and specific heterosis (p < 0.01), rather than varietal heterosis (p < 0.05), suggesting differences in the levels of the genic frequency complementation.

The mass of one hundred seeds had significant effects of the mean, varietal and specific heterosis (p < 0.01), suggesting a large variance in genic frequencies among the varieties, and that some of them were different based on the mean or the dispersion of genic frequencies. Additionally, differences among the levels of genic frequency complementation may be present in the landraces evaluated.

Concerning popping expansion, there were significances of the mean square at p < 0.01 for varietal and specific heterosis, but rather than the mean heterosis (p < 0.05), indicating bidirectional dominance for this trait. According Vencovsky and Cruz (1989), significant mean square for heterosis and predominance of unidirectional dominance indicate importance for varietal and specific heterosis only when the mean square of the mean heterosis is also significant.

The results from the analysis of variance are important to choose the suitable model to describe each trait as a complement about the genetic structure of varieties enclosed in the diallel. Considering the mean squares of the mean heterosis of the plant and ear heights were significant (p < 0.01) in opposite of the square means of varietal and specific heterosis, making possible to reach the goodness of fit for these traits on the model II (GARDNER; EBERHART, 1966; CRUZ et al., 2004). On the other hand, the model IV was suitable to fit the other significant traits, according to Gardner and Eberhart (1966), Scapim et al. (2002), and Cruz et al. (2004).

Estimates of the mean effect due to varieties (Tables 1 and 2) revealed the higher values of \hat{v}_j for plant and ear heights from the landraces *Amarela* and *Roxa*. In opposite, *Beija-Flor* and *Branca* had the lower estimates, indicating a small performance *per se*. The effects of the other traits were due to the genitors' performance *per se*. The *Viçosa* variety, e.g., had the highest estimate of the mass of one hundred seeds with positive \hat{v}_j , value that were 4.78 times greater than the standard deviation.

The \hat{v}_j estimates for grain yield showed only two landraces with positive values: *Amarela* and *Viçosa*, with the highest magnitude for *Amarela*. This variety

field values for \hat{v}_j estimates, considering the three growing compare evaluated (Tables 1 and 2)

of ears and number of ears.

growing seasons evaluated (Tables 1 and 2). Therefore *Viçosa* has a good potential for intrapopulation breeding, to improve both grain yield and popping expansion, over all for the later trait, in consequence of the additive effect genes that had the most important influence for expansion, according to Larish and Brewbaker (1999), Pereira and Amaral Júnior (2001), Andrade et al. (2002), Scapim et al. (2002), Freitas Júnior et al. (2006), and Rangel et al. (2007) and because to demonstrate small positive value for \hat{v} , of grain yield.

also revealed the superiors estimates for the weight

Considering the two most important traits (GY and PE), only *Vicosa* had simultaneous positive

Table 1. Estimates of the mean parameters, the varieties (\hat{v}_j) effects and their standard error deviation (S.D.) between two populations for six agronomical traits assessed in six popcorn varieties crossed in a diallel scheme.

		Traits ¹				
Varieties	PH	EH	GY	WE	NE	MS
Amarela	0.257	0.259	0.492	0.525	7.042	-0.277
Roxa	0.182	0.179	-0.028	0.025	3.222	-0.637
Branca	-0.233	-0.162	-0.038	0.015	0.552	-0.877
Rosa Claro	0.060	-0.032	-0.108	-0.185	3.722	-0.087
Beija-Flor	-0.268	-0.232	-0.498	-0.565	-10.088	-0.427
Viçosa	0.003	-0.012	0.182	0.185	-4.448	2.303
S.D. $(\hat{v}_i - \hat{v}_j)$	_j)0.061	0.039	0.219	0.281	3.256	0.481
Mean	1.897	1.112	1.178	1.565	31.058	13.837

¹PH: plant height, EH: ear height, GY: grain yield, WE: weight of ears, NE: number of ears, and MS: mass of one hundred seeds.

Table 2. Estimates of the mean parameters, the varieties (\hat{V}_j) effects and their standard error deviation (S.D.) between two populations for popping expansion (PE) in three growing seasons assessed in six popcorn varieties and their F_{1s} hybrids.

-		Growing Seasons 1	
Varieties	Ι	IĪ	III
Amarela	-2.565	-2.660	-2.407
Roxa	-2.335	-2.190	-0.507
Branca	3.035	1.770	2.363
Rosa Claro	-1.865	-1.460	-0.937
Beija-Flor	0.465	3.570	0.893
Viçosa	3.265	0.970	0.593
S.D. $(\hat{v}_i - \hat{v}_j)$	1.730	1.494	1.638
Mean	15.265	10.990	12.067

¹I: grown season at the first evaluation year in Viçosa, II: growing season at the second evaluation year in Visconde do Rio Branco, and III: growing season at the second evaluation year in Viçosa.

The varieties *Branca*, *Beija-Flor* and *Viçosa* had positive \hat{v}_j values for popping expansion, in all the growing seasons evaluated. In opposite, *Amarela*, *Roxa* and *Rosa Claro* revealed negative estimates. It is very important to perceive that there were no changes in the signals of the \hat{v}_j value estimates for all landraces in all growing seasons evaluated, that is, under field conditions of the first growing season of Viçosa and Visconde do Rio Branco, and the second growing season of Viçosa, respectively. This result

favor the hypothesis that de environment influence on popping expansion is not so important to delay the conduction of breeding program in one locality for release superior material for a large edaphoclimatic conditions.

According to Singh and Chaudary (1985), the ranges of variation among the genitors and between two genitors differ when their amplitude is greater than twice the respective standard deviation. Based on Tables 1 and 2, there were variability among the landraces using as genitors for all traits with greater and smaller \hat{v}_j effects: PH (8.60), EH (12.59), GY (4.52), WE (3.88), NE (5.26), MS (6.61), PE for the grown season at the first evaluation year in Viçosa (3.33), PE for the growing season at the second evaluation year in Visconde do Rio Branco (4.17), and PE for the growing season at the second evaluation year in Viçosa (2.91).

Estimates of hybrid heterosis, in comparison to the mean parental population and the standard deviation among two hybrids without including identical genitors are showed in Tables 3 and 4. Hybrid heterosis is related of the square differences from the genic frequencies between two populations and the level of dominance. Then, heterosis appears when genetic divergence between populations exists in addition to some level of dominance. Even when heterosis does not exist it is possible to have genetic diversity among populations or absence of dominance because the sum of the dominance deviation can be zero when positive and negative dominants estimates of loci are found (VENCOVSKY; CRUZ, 1989; SCAPIM et al., 2002; CRUZ et al., 2004; CRUZ, 2005).

Positive \hat{h}_{ij} values were expressed for plant height, ear height, grain yield and weight of ears, indicating that deviation from the dominance had higher influence on the performance of these traits. Only one hybrid (*Amarela x Viçosa*) showed negative value of the \hat{h}_{ij} for number of ears, indicating a tendency for unidirectional dominance, but with moderate influence of the dominance effect. On the other hand, positive and negative \hat{h}_{ij} values in joint analysis occurred for mass of one hundred seeds, that indicates bidirectional dominance on the expression of this trait, what it means of that some genes contribute to increase the mass of the seeds while other genes favoring the reducing of this trait (Table 3).

Similarly to mass of one hundred seeds, for the popping expansion, there were positive and negative values of \hat{h}_{ij} in all growing seasons evaluated demonstrating that bidirectional dominance it influences the expression for both traits (Tables 3 and 4).

415

Table 3. Estimates of the total hybrids heterosis in comparison to the mean parental population and the standard deviation (S.D.) between two hybrids without include identical genitors for six agronomical traits assessed in fifteen $F_{1's}$ from six popcorn varieties crossed in a diallel scheme.

	Traits ²					
Hybrids ¹	PH	EH	GY	WE	NE	MS
1 x 2	0.085	0.025	0.175	0.325	3.085	-0.480
1 x 3	0.150	0.075	0.450	0.540	0.490	0.775
1 x 4	0.155	0.040	0.915	1.025	3.235	1.175
1 x 5	0.225	0.145	0.545	0.730	3.080	1.245
1 x 6	0.050	0.010	0.480	0.565	-0.690	2.045
2 x 3	0.015	0.000	0.525	0.615	2.660	0.925
2 x 4	0.175	0.080	0.590	0.690	3.730	-0.115
2 x 5	0.125	0.005	0.515	0.605	3.175	0.830
2 x 6	0.105	0.005	1.005	1.425	7.415	1.715
3 x 4	0.250	0.150	0.720	0.860	2.575	1.015
3 x 5	0.280	0.165	0.635	0.795	9.990	-0.095
3 x 6	0.060	0.050	0.320	0.435	0.665	1.185
4 x 5	0.335	0.200	0.990	1.165	6.490	2.565
4 x 6	0.185	0.120	0.765	0.875	0.900	2.490
5 x 6	0.140	0.075	0.210	0.200	3.565	0.005
S.D. $(\hat{h}_{ij} - \hat{h}_{kl})$	0.011	0.005	0.048	0.079	10.600	0.231
S.D. $(\hat{h}_{ij} - \hat{h}_{jk})$	0.009	0.003	0.036	0.059	7.950	0.173

¹1: Amarela, 2: Roxa, 3: Branca, 4: Rosa Claro, 5: Beija-Flor, and 6: Viçosa. ² PH: plant height, EH: ear height, GY: grain yield, WE: weight of ears, NE: number of ears, and MS: mass of one hundred seeds.

Table 4. Estimates of the total hybrids heterosis and the standard deviation (S.D.) between two hybrids without include identical genitors for popping expansion (PE) in three growing seasons assessed in fifteen F_{1s} from six popcorn varieties crossed in a diallel scheme.

I I-shaiidal		Growing season	
nyonus	Ι	II	III
1 x 2	0.615	2.330	-0.110
1 x 3	0.180	-0.130	-0.215
1 x 4	-1.185	1.565	-0.350
1 x 5	-0.470	-0.550	0.070
1 x 6	0.030	-0.650	-2.450
2 x 3	-0.535	0.285	-2.030
2 x 4	-0.985	2.000	0.485
2 x 5	4.665	2.765	2.020
2 x 6	-1.435	2.465	0.005
3 x 4	-1.335	-1.665	-1.400
3 x 5	-2.635	-4.880	-1.300
3 x 6	-2.120	-1.915	-0.645
4 x 5	-2.835	0.320	-0.065
4 x 6	-2.935	-0.800	0.170
5 x 6	-0.020	-0.750	1.805
S.D. $(\hat{h}_{ij} - \hat{h}_{kl})$	2.994	2.232	2.683
S.D. $(\hat{h}_{ij} - \hat{h}_{ik})$	2.246	1.674	2.012

¹1: Amarela, 2: Roxa, 3: Branca, 4: Rosa Claro, 5: Beija-Flor, and 6: Viçosa. ²1: grown season at the first evaluation year in Viçosa, II: growing season at the second evaluation year in Visconde do Rio Branco, and III: growing season at the second evaluation year in Viçosa.

This result had also been reported by Andrade et al. (2002), Scapim et al. (2002) and Viana and Matta (2003). If there was a link between grain size and capacity of expansion, it is feasible to assume that minor grains have a proportionally capable to optimize the capacity of to expand in relation to the bigger grains. In fact some authors demonstrated the occurrence of negative correlation between grain size and popping expansion (SAWAZAKI et al., 2003; DAROS et al., 2004b).

Consequently not only grain yield and popping

expansion are the most important agronomical traits in popcorn, but also size of grains. In reason of this, high importance must be directed for grain size in breeding programs, e.g., recurrent selections. In this respect although some breeding programs announcing success on release of new cultivars based mainly grain yield and popping expansion, the caution needs be dispensable for the selection of grain sizes too, because of the possible occurrence of the higher difference between the upper and the lower limit for popping expansion in the progenies under advanced selection cycles. This can be occurred even though in recurrent selection breeding programs with value of mean for popping expansion of the 30 mL g⁻¹ or superior mean magnitudes.

In relation to grain yield, the highest estimate of heterosis was revealed by the hybrid Roxa x Vicosa, whereas the combination Amarela x Roxa and Beija-Flor x Viçosa had the lower estimates. The higher values of $\hat{h}_{ij}\text{'s}$ were hierarquically expressed by combinations Roxa x Viçosa, Rosa Claro x Beija-Flor, Amarela x Rosa Claro, Rosa Claro x Viçosa and Branca x Beija-Flor (Table 3). As result, these hybrids had importance for using in experimental plantation or to be used in breeding programs, as recurrent selections, to obtain superior cultivar for one the most important economically trait in popcorn. Moreover, it is important to emphasize that the differences among heteroses were small in comparison to the standard deviation for grain yield.

Regarding the popping expansion (Table 4), the hybrids *Branca* x *Rosa Claro*, *Branca* x *Beija-Flor* and *Branca* x *Viçosa* showed negative values of heterosis for all growing seasons evaluated (Table 4), revealing that all hybrids derived from *Branca* expressed expected results, based on the estimates of \hat{v}_j values (Table 2), which ratifies the poor performance *per se* of this variety, resulting in inferior genic accumulation on the hybrid combinations in which it participates. There was only one combination (*Roxa* x *Beija-Flor*) with positive heterosis estimates in all environmental conditions studied (Table 4).

The better hybrids for grain yield not presented the better-quality performance for popping expansion. But, the pair *Roxa* x *Beija-Flor* should not be disregarded in future breeding programs for obtained genetic gains for both traits, because it did not had negative heterosis for grain yield, although it was allocated in the tenth placed in the rank for effect of total heterosis (Table 3) amongst the fifteen hybrids achieved.

Moreover, even though negative values for heterosis were found in popping expansion (Table 4), the exploitation of heterosis for this trait can be obtained in view of the fact that crossing among varieties whose dominance deviations predominantly act in the direction to increase this trait. This situation occurs when they have negative \hat{S}_{ij} .

The mean heterosis for grain yield and weight of ears were 0.589 ± 0.069 kg ha⁻¹ and 0.723 ± 0.089 kg ha⁻¹ higher than the means of the varieties, respectively (Table 5). These expressive values are eight times superior to the standard deviation, indicating that hybrids had high variability for the both traits to be exploited in the future breeding programs. *Rosa Claro* expressed de highest effect of varietal heterosis.

In spite of no significance for the mean squares of the varietal heterosis for number of ears (p < 0.01), the varieties *Roxa*, *Rosa Claro* and *Beija-Flor* showed positive values of varietal heterosis (\hat{h}_i) indicating existence of genetic divergence among the varieties for this trait. In relation to the mass of one hundred seeds, the amplitude between two more discrepant genitors for \hat{h}_i estimate was superior on 3.88 times in relation the respective standard deviation, making possible the discrimination the varieties al least two groups, as following: i) the first, including the varieties with positive estimates values for \hat{h}_i , which are Viçosa and Rosa Claro; and ii) the second group, formed by the varieties Amarela, Roxa, Branca and Beija-Flor. On this respect, the Viçosa and Rosa Claro were the most divergent varieties among the landraces used in this study. Thus, the variation for grain sizes in the varieties evaluated were sufficiently higher to require attempt of the breeders on improvement of this trait for more uniformity in size of grains, but associated with high popping expansion.

Table 5. Estimates of the effects of mean (\hat{h}) and varietal (\hat{h}_j) heterosis and its standard deviation (S.D.) for four agronomical traits in six popcorn varieties crossed in a diallel scheme.

	Traits ¹			
Varieties	GY	WE	NE	MS
Amarela	-0.095	-0.108	-1.897	-0.083
Roxa	-0.034	0.011	0.819	-0.555
Branca	-0.074	-0.093	-0.102	-0.322
Rosa Claro	0.258	0.250	0.035	0.509
Beija-Flor	-0.013	-0.030	2.378	-0.136
Viçosa	-0.042	-0.029	-1.233	0.587
S.D. $(\hat{h}_i - \hat{h}_j)$	0.134	0.172	1.994	0.294
$\hat{\overline{h}}$	0.589	0.723	3.358	1.019
S.D. $(\hat{\overline{h}})$	0.069	0.089	1.030	0.152

 $^1\mbox{GY}$ grain yield, WE: weight of ears, NE: number of ears, and MS: mass of one hundred seeds.

Concerning the popping expansion, the estimates of mean heterosis were negative in two growing seasons (first and second evaluation years in Viçosa). Besides, on the third growing season (second evaluation year in Visconde do Rio Branco) this trait revealed small value of the mean heterosis,

with magnitude eighteen times inferior to the respectively standard deviation (Table 6). It evidences the existence of genetic differences among the varieties and their hybrid combinations as well the effect of dominance on genic complementation aren't important for to produce popcorn with desirable capacity to popper. Considering that the dominance effect is the component on expression of heterosis that less impact has for popping expansion BREWBAKER, 1999; (LARISH; PEREIRA; AMARAL JÚNIOR, 2001; SCAPIM et al., 2002; FREITAS JÚNIOR et al., 2006; RANGEL et al., 2008), then the genetic distance assume great importance on expression of popping expansion, but only when the additive gene effects is loaded in the materials for crosses.

Table 6. Estimates of the effects of mean (\hat{h}) and varietal (\hat{h}_j) heterosis and its standard deviation (S.D.) between two populations for popping expansion (PE) in three growing seasons assessed in six popcorn varieties and their F_{1s} hybrids.

		Growing Seasons	1
Varieties	Ι	II	III
Amarela	0.709	0.609	-0.430
Roxa	1.498	2.429	0.427
Branca	-0.695	-2.109	-1.063
Rosa Claro	-1.402	0.322	0.044
Beija-Flor	0.593	-0.806	0.967
Viçosa	-0.703	-0.445	0.055
S.D. $(\hat{h}_i - \hat{h}_j)$	1.060	0.915	1.003
$\hat{\overline{h}}$	-0.733	0.026	-0.267
S.D. $(\hat{\overline{h}})$	0.540	0.472	0.518

¹I: grown season at the first evaluation year in Viçosa, II: growing season at the second evaluation year in Visconde do Rio Branco, and III: growing season at the second evaluation year in Viçosa.

The *Roxa* variety showed positive estimates of varietal heterosis in all growing seasons evaluated what suggests that it has the highest genetic divergence among the varieties used as genitors (Table 6). On the other hand, *Branca* revealed the negative values at the same environments (Table 6) that corroborate the explanation done previously based on Tables 2 and 4 for this variety in to produce inferior hybrids.

According to the specific heterosis estimates (Table 7), for grain yield, the hybrids *Roxa* x *Viçosa*, *Amarela* x *Rosa Claro*, *Rosa Claro* x *Beija-Flor* and *Branca* x *Beija-Flor* had the higher positive values for \hat{s}_{ij} . Although not on the same order, these hybrids were the same that showed the favorable effects of \hat{s}_{ij} for weight of ears and among of them, three hybrids (*Branca* x *Beija-Flor*, *Roxa* x *Viçosa* and *Amarela* x *Rosa Claro*) performed may contribute genetically to increasing the number of ears. Regarding the MS trait, in opposite of the hybrids *Beija-Flor* x *Viçosa*, *Roxa* x *Rosa Claro* and *Amarela* x *Roxa*, the crosses *Rosa Claro* x *Beija-Flor*, *Roxa* x *Viçosa*, and *Roxa* x *Viçosa* could be indicated for programs to obtain plants with more accentuated grain sizes because they

have positive \hat{S}_{ii} values.

Amarela x Branca, Amarela x Rosa Claro, Roxa x Beija-Flor, Branca x Viçosa and Beija-Flor x Viçosa showed positive estimates of \hat{s}_{ij} values in all growing seasons evaluated for popping expansion (Table 8). Amongst these, only Amarela x Rosa Claro may be included into group of the combinations that had high estimates with regard to specific heterosis for grain yield.

Table 7. Estimates of specific heterosis (\hat{s}_{ij}) and standard deviations (S.D.) for four agronomical traits assessed in fifteen hybrids from six popcorn varieties crossed in a diallel scheme.

	Traits ²			
Hybrids ¹	GY	WE	NE	MS
1 x 2	-0.285	-0.301	0.805	-0.861
1 x 3	0.030	0.018	-0.869	0.162
1 x 4	0.163	0.160	1.739	-0.270
1 x 5	0.064	0.145	-0.759	0.446
1 x 6	0.028	-0.021	-0.917	0.523
2 x 3	0.044	-0.026	-1.415	0.783
2 x 4	-0.224	-0.294	-0.482	-1.088
2 x 5	-0.027	-0.099	-3.380	0.502
2 x 6	0.492	0.720	4.472	0.664
3 x 4	-0.054	-0.020	-0.716	-0.191
3 x 5	0.133	0.195	4.357	-0.656
3 x 6	-0.154	-0.166	-1.357	-0.098
4 x 5	0.155	0.223	0.719	1.173
4 x 6	-0.041	-0.069	-1.260	0.376
5 x 6	-0.325	-0.464	-0.937	-1.465
S.D. $(\hat{s}_{ij} - \hat{s}_{ik})$	0.134	0.172	1.994	0.294
S.D. $(\hat{s}_{ij} - \hat{s}_{kl})$	0.110	0.141	1.628	0.240

¹1: Amarela, 2: Roxa, 3: Branca, 4: Rosa Claro, 5: Beija-Flor and 6: Viçosa. ² GY: grain yield, WE: weight of ears, NE: number of ears, and MS: mass of one hundred seeds.

Table 8. Estimates of specific heterosis (\hat{s}_{ij}) and standard deviations (S.D.) between two hybrids, of popping expansion assessed in fifteen hybrids from six popcorn varieties crossed in a diallel scheme.

		Growing Seasons ²	
Hybrids ¹	I	II	III
1 x 2	-0.859	-0.734	0.160
1 x 3	0.899	1.344	1.545
1 x 4	0.241	0.608	0.303
1 x 5	-1.039	-0.379	-0.200
1 x 6	0.758	-0.840	-1.809
2 x 3	-0.605	-0.061	-1.126
2 x 4	-0.348	-0.777	0.282
2 x 5	3.308	1.117	0.894
2 x 6	-1.496	0.455	-0.210
3 x 4	1.495	0.095	-0.114
3 x 5	-1.800	-1.991	-0.936
3 x 6	0.011	0.613	0.630
4 x 5	-1.292	0.778	-0.809
4 x 6	-0.096	-0.704	0.338
5 x 6	0.824	0.475	1.050
S.D. $(\hat{s}_{ij} - \hat{s}_{ik})$	1.060	0.915	1.003
S.D. $(\hat{s}_{i;} - \hat{s}_{i+1})$	0.865	0.747	0.819

¹1: Amarela, 2: Roxa, 3: Branca, 4: Rosa Clato, 5: Beija-Flor and 6: Vigosa. ²1: grown season at the first evaluation year in Viçosa, II: growing season at the second evaluation year in Visconde do Rio Branco, and III: growing season at the second evaluation year in Viçosa.

Therefore, the hybrid *Amarela* x *Rosa Claro* had potential to be exploited in recurrent selection for obtained successive genetic gains for grain yield and popping expansion, as result of increasing the favorable alleles on consecutive cycles of selection. This is the first report in Brazil the jointed potential for grain yield and popping expansion on intercrossing of landraces, that indicates the possibility to exploit this advantage to initiate a reciprocal recurrent selection on the populations *Amarela* and *Rosa Claro*, at time its are complementary in relation to grain yield and popping expansion. Considering these varieties are prolific, the strategy proposed by Hallauer and Eberhart (1970) is a good option, because it provides the improve of the landraces by obtained full-sib progenies, that permit to obtained genetic gains *per se* and in intercrossing besides to derived lines that may be evaluated by testcrosses and obtained superior simple, simple modified or three-way hybrids.

Conclusion

For the first time in Brazil the jointed potential for grain yield and popping expansion on intercrossing of landraces is reported, by using *Amarela* and *Rosa Claro* to initiate a reciprocal recurrent selection.

The mean heterosis was the main component describing effects in plant and ear heights.

The estimates of heterosis of grain yield were positive and popping expansion had negative and positive heterosis estimates derived from bidirectional dominance due to the predominant performance of the deviation from dominance for each variety.

Simultaneous genetic gains for grain yield and popping expansion may be obtained by using *Viçosa* in intrapopulation recurrent selection programs.

References

ANDRADE, R. A.; CRUZ, C. D.; SCAPIM, C. A.; SILVÉRIO, L.; PINTO, R. J. B.; TONET, A. Análise dialélica da capacidade combinatória de variedades de milho pipoca. **Acta Scientiarum. Agronomy**, v. 24, n. 1, p. 1197-1204, 2002.

ARNHOLD, E.; VIANA, J. M. S.; SILVA, R. G.; MORA, F. Eficiências relativas de métodos de seleção de famílias endogâmicas em milho-pipoca. **Acta Scientiarum. Agronomy**, v. 31, n. 2, p. 203-207, 2009.

COIMBRA, R. R.; MIRANDA, G. V.; CRUZ, C. D.; VIANA, J. M. S. Correlações entre caracteres na população de milho-pipoca DFT-1 Ribeirão. **Revista Ceres**, v. 48, n. 278, p. 427-435, 2001.

CRUZ, C. D.; REGAZZI, A. J.; CARNEIRO, P. C. S. **Modelos biométricos aplicados ao melhoramento genético**. Viçosa: UFV, 2004.

CRUZ, C. D. **Princípios de genética quantitativa**. Viçosa: UFV, 2005.

CRUZ, C. D. **Programa genes**: versão windows: aplicativo computacional em genética e estatística. Viçosa: UFV, 2006.

DANDOLINI, T. S.; SCAPIM, C. A.; AMARAL JÚNIOR, A. T.; MANGOLIN, C. A.; MACHADO, M. F. P. S.; MOTT, A. S.; LOPES, A. D. Genetic divergence in popcorn lines detected by microsatellite markers. **Crop Breeding and Applied Biotechnology**, v. 8, n. 4, p. 313-320, 2008.

DAROS, M.; AMARAL JÚNIOR, A. T.; PEREIRA, M. G.; SANTOS, F. S.; GABRIEL, A. P. C.; SCAPIM, C. A.; FREITAS JÚNIOR, S. P.; SILVÉRIO, L. Recurrent selection in inbred popcorn families. **Scientia Agricola**, v. 61, n. 6, p. 609-614, 2004a.

DAROS, M.; AMARAL JÚNIOR, A. T.; PEREIRA, M. G.; SANTOS, F. S.; SCAPIM, C. A.; FREITAS JÚNIOR, S. P.; DAHER, R. F.; ÁVILA, M. R. Correlações entre caracteres agronômicos em dois ciclos de seleção recorrente em milho-pipoca. **Ciência Rural**, v. 34, n. 5, p. 1389-1394, 2004b.

FREITAS JÚNIOR, S. P.; AMARAL JÚNIOR, A. T.; PEREIRA, M. G.; CRUZ, C. D.; SCAPIM. C. A. Capacidade combinatória em milho-pipoca por meio de dialelo circulante. **Pesquisa Agropecuária Brasileira**, v. 41, n. 11, p. 1599-1607, 2006.

FREITAS JÚNIOR, S. P.; AMARAL JÚNIOR, A. T.; RANGEL, R. M.; VIANA, A. P. Genetic gains in popcorn by full-sib recurrent selection. **Crop Breeding and Applied Biotechnology**, v. 9, n. 1, p. 1-7, 2009.

GARDNER, C. O.; EBERHART, S. A. Analysis and interpretation of the variety cross diallel and related populations. **Biometrics**, v. 22, n. 3, p. 439-452, 1966.

HALLAUER, A. R.; EBERHART, S. A. Reciprocal fullsib selection. **Crop Science**, v. 10, n. 3, p. 315-316, 1970.

JI, H. C.; CHO, J. W.; YAMAKAWA, T. Diallel analysis of plant and ear heights in tropical maize (*Zea mays* L.). **Journal of the Faculty of Agriculture**, v. 51, n. 2, p. 233-238, 2006.

LARISH, L. L. B.; BREWBAKER, J. L. Diallel analyses of temperate and tropical popcorns. **Maydica**, v. 44, n. 1, p. 279-384, 1999.

MIRANDA, G. V.; SOUZA, L. V.; GALVÃO, J. C. C.; GUIMARÃES, L. J. M.; MELO, A. V.; SANTOS, I. C. Genetic variability and heterotic groups of Brazilian popcorn populations. **Euphytica**, v. 162, n. 3, p. 123-132, 2008.

MOTERLE, L. M.; SCAPIM, C. A.; BRACCINI, A. L. RODOVALHO, M. A.; BARRETO, R. R. Influência do estresse hídrico sobre o desempenho de sementes de híbridos de milho-pipoca. **Ciência e Agrotecnologia**, v. 32, n. 6, p. 1810-1817, 2008.

PEREIRA, M. G.; AMARAL JÚNIOR, A. T. Estimation of genetic components in popcorn based on the nested design. **Crop Breeding and Applied Biotechnology**, v. 1, n. 1, p. 3-10, 2001.

PEREIRA, L. K.; MANGOLIN, C. A.; SCAPIM, C. A.; PACHECO, C. A. P.; BONATO, C. M.; MACHADO, M. F. P. S. Malate dehydrogenase isozyme patterns in four cicles of half-sib selection from CMS-43 popcorn variety (*Zea mays* L.). **Maydica**, v. 51, n. 3, p. 561-566, 2006.

PEREIRA, L. K.; SCAPIM, C. A.; MANGOLIN, C. A.; MACHADO, M. F. P. S.; PACHECO, C. A. P.; MORA, F. Heterozygosity following half-sib recurrent selection in popcorn using isoenzyme markers. **Electronic Journal**

Acta Scientiarum. Agronomy

of Biotechnology, v. 11, n. 1, p. 108-115, 2008.

RANGEL, R. M.; AMARAL JÚNIOR, A. T.; VIANA, A. P.; FREITAS JÚNIOR, S. P. Prediction of popcorn hybrid and composite means. **Crop Breeding and Applied Biotechnology**, v. 7, n. 3, p. 287-295, 2007.

RANGEL, R. M.; AMARAL JÚNIOR, A. T.; SCAPIM, C. A.; FREITAS JÚNIOR, S. P.; PEREIRA, M. G. Genetic parameters in parents and hybrids of circulant diallel in popcorn. **Genetics and Molecular Research**, v. 7, n. 4, p. 1020-1030, 2008.

RICCI, G. C. L.; SILVA, N.; PAGLIARINI, M. S.; SCAPIM, C. A. B chromosomes in popcorn (*Zea mays* L.). **Genetics and Molecular Research**, v. 6, n. 1, p. 137-143, 2007a.

RICCI, G. C. L.; SILVA, N.; PAGLIARINI, M. S.; SCAPIM, C. A. Microsporogenesis in inbred line of popcorn (*Zea mays* L.). Genetics and Molecular Research, v. 6, n. 4, p. 1013-1018, 2007b.

RODOVALHO, M. A.; MORA, F.; SANTOS, E. S.; SCAPIM, C. A.; ARNHOLD, E. Survival heritability in 169 families of white grain popcorn: A Bayesian approach. **Ciencia e Investigación Agraria**, v. 35, n. 3, p. 255-260, 2008.

SANTOS, F. S.; AMARAL JÚNIOR, A. T.; FREITAS JÚNIOR, S. P.; RANGEL, R. M.; PEREIRA, M. G. Predição de ganhos genéticos por índices de seleção na população de milho pipoca UNB 2U sob seleção recorrente. **Bragantia**, v. 66, n. 3, p. 391-298, 2007.

SANTOS, F. S.; AMARAL JÚNIOR, A. T; FREITAS JÚNIOR, S. P.; RANGEL, R. M.; SCAPIM, C. A.; MORA, F. Genetic gain prediction of the third recurrent selection cycle in a popcorn population. **Acta Scientiarum. Agronomy**, v. 30, n. 5, p. 651-658, 2008.

SAWAZAKI, E.; CASTRO, J. L.; GALLO, P. B.; PATERNIAN, M. E. A. G. Z.; SILVA, R. M.; LUDERS, R. R. Potencial de híbridos temperados de milho pipoca em cruzamentos com o testador semitropical IAC 12. **Revista Brasileira de Milho e Sorgo**, v. 2, n. 2, p. 61-70, 2003.

SCAPIM, C. A.; PACHECO, C. A. P.; TONET, A.; BRACCINI, A. L.; PINTO, R. J. B. Análise dialélica e heterose de populações de milho-pipoca. **Bragantia**, v. 61, n. 3, p. 219-230, 2002.

SCAPIM, C. A.; PINTO, R. J. B.; AMARAL JÚNIOR, A. T.; DANDOLINI, T. S. Combining ability of white grain popcorn populations. **Crop Breeding and Applied Biotechnology**, v. 6, n. 1, p. 136-143, 2006a.

SCAPIM, C. A.; BRACCINI, A. L.; PINTO, R. J. B.; AMARAL JÚNIOR, A. T.; RODOVALHO, M. A.; SILVA, R. M.; MOTERLE, L. M. Componentes genéticos de médias e depressão por endogamia em populações de milho-pipoca. **Ciência Rural**, v. 36, n. 1, p. 36-41, 2006b.

SILVA, M. F. P. T. B.; LOPES, E. F.; PAGLIARINI, M. S.; SCAPIM, C. A. Effects of endogamy on the microsporogenesis in popcorn. **Crop Breeding and Applied Biotechnology**, v. 7, n. 3, p. 321-326, 2007.

SILVA, T. A.; PINTO, R. B. J.; SCAPIM, C. A.; MANGOLIN, C. A.; MACHADO, M. F. P. S.; CARVALHO, M. S. N. Genetic diversity in popcorn genotypes using microsatellites in bulk genomic DNA. **Crop Breeding and Applied Biotechnology**, v. 9, n. 1, p. 31-36, 2009.

SIMON, A. G.; SCAPIM, C. A.; PACHECO, C. A. P.; PINTO, R. J. B.; BRACCINI, A. L.; TONET, A. Depressão por endogamia em populações de milhopipoca. **Bragantia**, v. 63, n. 1, p. 55-62, 2004.

SINGH, R. K.; CHAUDHARY, B. D. **Biometrical methods in quantitative genetic analysis**. New Delhi: Kalyany Publishers, 1985.

VENCOVSKY, R.; CRUZ, C. D. Comparação de alguns métodos de análise dialélica. **Revista Brasileira de Genética**, v. 12, n. 2, p. 425-438, 1989.

VENDRUSCOLO, E. C. G.; SCAPIM, C. A.; PACHECO, C. A. P.; OLIVEIRA, V. R.; BRACCINI, A. L.; GONCALVES-VIDIGAL, M. C. Adaptabilidade e estabilidade de cultivares de milho-pipoca na região centro-sul do Brasil. **Pesquisa Agropecuária Brasileira**, v. 36, n. 1, p. 123-130, 2001.

VIANA, J. M. S.; MATTA, F. P. Analysis of general and specific combining abilities of popcorn populations, including selfed parents. **Genetics and Molecular Biology**, v. 26, n. 4, p. 465-471, 2003.

VIANA, J. M. S.; TEIXEIRA, A. B.; ALMEIDA, R. V.; SCAPIM, C. A.; VALENTINI, L. Relative importance of per se and topcross performance in the selection of popcorn S3 families. **Crop Breeding and Applied Biotechonolgy**, v. 7, n. 1, p. 74-81, 2007.

VIEIRA, R. A.; RODOVALHO, M. A.; SCAPIM, C. A.; TESSMANN, D. J.; AMARAL JÚNIOR, A. T.; BIGNOTTO, L. S. Desempenho agronômico de novos híbridos de milho-pipoca no Noroeste do Estado do Paraná, Brasil. **Acta Scientiarum. Agronomy**, v. 31, n. 1, p. 29-36, 2009.

VILELA, F. O.; AMARAL JÚNIOR, A. T.; PEREIRA, M. G.; SCAPIM, C. A.; VIANA, A. P.; FREITAS JÚNIOR, S. P. Effect of recurrent selection on the genetic variability of the UNB-2U popcorn population using RAPD markers. Acta Scientiarum. Agronomy, v. 30, n. 1, p. 25-30, 2008.

ZANETTE, V. A. Análise da variabilidade genética em populações de milho pipoca (*Zea mays* L.): heterose da capacidade de expansão do grão. **Agronomia Sulriograndense**, v. 25, n. 2, p. 173-181, 1989.

Received on August 6, 2008. Accepted on January 19, 2009.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.