Scientia Agricola
http://dx.doi.org/10.1590/0103-9016-2013-0069

Genetic variability for carotenoid content of grains in a composite maize population

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Edited by: Antonio Costa de Oliveira

Received March 27, 2013
Accepted May 05, 2014

ABSTRACT: Local maize (*Zea mays* L.) varieties are cultivated by small-scale farmers in western Santa Catarina (SC) State, in southern Brazil. These small areas frequently present many problems related to biotic and non-biotic stresses, which have limited the economic output and income of the farmers. Production from local varieties for human consumption would be an alternative way of improving income and stimulating *on farm* conservation. The genetic variability of the total carotenoid content (TCC) of kernels in a local maize population was evaluated for their economic exploitation potential as biofortified food. Two independent samples of 96 half-sib families (HSF) plus four checks were evaluated in two groups of experiments in western SC and each one was carried out in two environments. They were set out in a 10 × 10 partially balanced lattice with three replications per location; plots consisted of one row, 5.0 m long with 1.0 m between rows. TCC ranged from 11 to 23 μg g^{-1}, averaging ≈16 μg g^{-1} in the pooled analysis over the two sets. The local composite population exhibited genetic variability in order to increase the TCC of grains in the second cycle of selection by the convergent-divergent scheme.

Keywords: biofortified food, convergent-divergent selection, genetic-statistical parameters, genotype by environment interaction

Introduction

Local maize varieties (LMV) are cultivated in many Brazilian regions and frequently have been grown by small farmers in marginal agricultural environments, where the use of modern technologies and requirements for external inputs are scarce or little accessed. This scenario can be seen in a number of rural properties in western Santa Catarina (SC) State where many small farmers still maintain LMV on small farms (< 20 ha) (Kist et al., 2010). These farms are located mainly in mountain areas, with low natural soil fertility, irregular water regime, and prolonged drought periods during the growing season, which invariably compromise grain yield.

The organic farming of their own local seeds was one strategy used by farmers to enable production, which initially aims at meeting the demand for animal feeding and secondly, to generate income through the sale of grain or its products. In fact, LMV production by small-scale farmers in western SC is consumed mainly (≈90 %) on their own farms as animal and human feed. Moreover, other maize-derived products, including artisanal wares and other artifacts made with straw and grains, have also been marketed (Ogliari et al., 2013). This is particularly the case in the city of Anchieta (SC) (26°31'27" S; 53°23'14" W), where a group of farmers perceived an opportunity to improve their incomes by purchasing a small industrial mill through their association in order to process the surplus production of organic maize into flour. The sale of this special flour to specific market niches that value health foods offers an increase in income for these farmers over and above the sale of grains for animal feed.

Several studies have been performed to investigate the genetic potential of maize populations for TCC (Kurilich and Juvik, 1999; Welch, 2002; Hulshof et al., 2007; Harjes et al., 2008; Kuhnen et al., 2009), especially in elite lines and hybrids, which have been improved with a view to enhancing their economic exploitation as biofortified food. However, the literature is scarce on information concerning the chemical composition of grains of LMV, particularly where reference is made to secondary metabolites. The scarcity is higher as regards the estimates of genetic-statistical parameters for this trait in open-pollinated varieties submitted to a recurrent selection process.

Increasing the TCC of grains in LMV grown in an organic system may help small farmers to obtain a higher income and consequently contribute to their *on farm* conservation. Thus, the aim of this study was to evaluate the genetic variability for TCC of grains in a local composite population of maize formed by small-scale farmers.

Materials and Methods

The formation of the original base population, herein identified as MPA1-C0 (cycle 0), started in 1999 with technical support from SINTRAF (Small-Scale Farmers' Trade Union) and a group of small farmers from Anchieta, through their association (Association of Local Maize Variety Farmers and Processors – ASSO). The procedures used in this first stage, which resulted in the population known as MPA1-C0 (original), are described in Kist et al. (2010). Its formation started in 1999 by intercrossing 25 populations, represented by 18 commercial hybrids, four local maize varieties (LMV)

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maintained by farmers in this municipality, and three varieties obtained from local farmers from selection within the germplasm group known as Pixurum. In 2000, five other LMV of unknown origin were incorporated into the base composite. The recombination of the first 25 varieties and the incorporation of the additional five varieties were carried out using the "topcross" procedure for intercrossing. Three cycles of stratified mass selection (Gardner, 1961) on small farms at Anchieta (SC) were completed for this composite population.

The procedures used in the first cycle of half-sib family (HSF) recurrent selection (second stage) by the convergent-divergent scheme (CDS), which resulted in the MPA1-C1 (cycle 1) population, are described in Kist et al. (2010). In 2003, a sample of 186 ears was taken from MPA1-C0 to represent the HSF of the first cycle. In 2004, the 186 HSF were divided into three experiments with 62 families, each one of which (three independent samples of HSF) were evaluated in three farms or environments (divergent step of first cycle) in Anchieta (SC) with technical support from the Federal University of Santa Catarina (UFSC).

The first cycle of recurrent selection by CDS (second stage) was completed in 2005, when a sample of 192 ears was visually selected from the best plants within the best HSF of the recombination block (convergent step of first cycle). A seed sample of all the selected ears (192 ears) was equally mixed and the seed bulk resulted in the MPA1-C1 population; on the other hand, the selected individual ears resulted in the 192 HSF used in this study.

The third stage, which corresponded to a divergent step of the second cycle of recurrent selection by CDS, started in 2007/2008. Thus, the 192 HSF were divided into two random samples (or sets) containing 96 HSF each. Each sample of HSF plus four checks formed a set of 100 treatments and each set (both Set 1 and Set 2) was planted on two farms in western SC. Thus, the two sets gave rise to a total of four different environments within this region to represent the target area. Set 1 was evaluated in Iporã D'Oeste (E_1) and Anchieta (E_2); Set 2 was evaluated in Guaraciaba (E_3) and Novo Horizonte (E_4) (Figure 1).

The four experiments were designed as a 10 × 10 partially balanced lattice, with three replications, following the model described in Cochran and Cox (1980). Plots were represented by one row 5 m long with 1 m between rows, under a plant density of 40,000 plants ha^{-1} after thinning. Plot size was based on the work of Chaves and Miranda Filho (1992). The four checks common to the two sets were the MPA1-C0, and MPA1-C1 populations, and the improved open-pollinated varieties BRS-4150 and SCS-154 Fortuna, recommended by the Brazilian Enterprise for Agricultural Research (EMBRAPA) for the southern region (Brazil) and by the Company of Farming Research and Agricultural Extension of Santa Catarina (EPAGRI) for Santa Catarina, respectively.

Two applications of fertilizers (450 kg ha^{-1} of aviary manure in each application) were used, following the re-

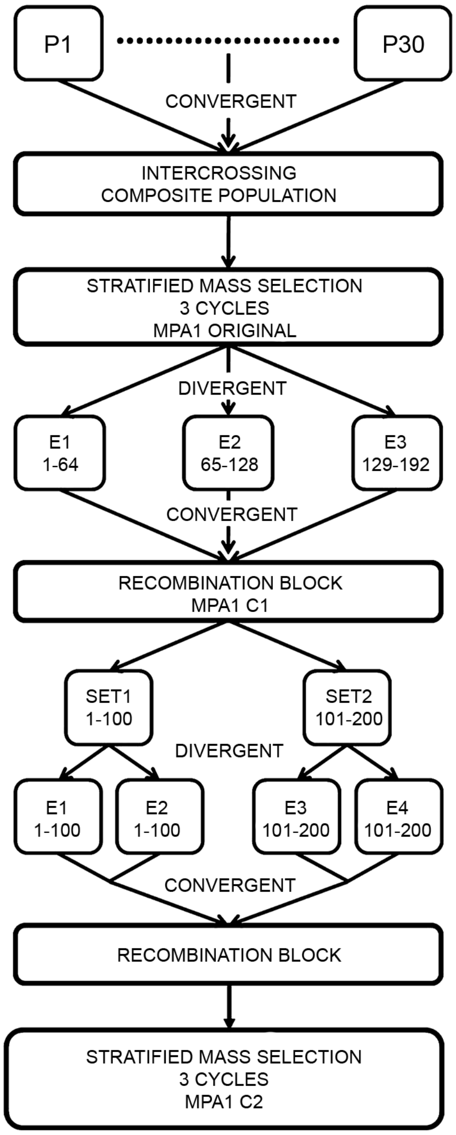

Figure 1 – Improvement in maize population through convergent-divergent selection scheme with intercrossing among 30 populations (P1 to P30), followed three cycles of stratified mass selection for formation of the original base population MPA1-C0 (convergent phase of the first stage); evaluation of three independent samples of 62 half-sib families (divergent phase of the second stage) in different environments (E_1: *São Domingos*, E_2: *São Roque*, and E_3: *Café Filho* at *Anchieta*); recombination in an isolate block represented by the three samples of constant size of all selected families for formation of population MPA1-C1 (convergent phase of the second stage); evaluation of two independent samples of 100 half-sib families (divergent phase of the third stage) in two grouped experiments (Set 1: E_1 and E_2 at *Iporã D'Oeste* and *Anchieta*, respectively; Set 2: E_3 and E_4 at *Guaraciaba* and *Novo Horizonte*, respectively) in western Santa Catarina; recombination in an isolate block represented by the two samples of constant size of all selected families (convergent phase of the third stage) followed three cycles of stratified mass selection for formation of population MPA1-C2.

sults of soil analyses (Table 1), the first being at sowing and the second at the near eight leaves stage. The E_1 and E_2 (Set 1) experiments were conducted in areas of maize crop succession, and E_3 and E_4 (Set 2) in succession with black oat (*Avena strigosa*). In all locations, minimum tillage was performed. Weed control was conducted whenever necessary, mechanically and without the use of herbicides or water supply, following the usual farm cropping conditions.

Carotenoids were extracted from flour samples by grinding a random sample of grains obtained from each plot. For most of the samples, the extraction was usually performed immediately after grinding the flour. Alternatively, some samples were transferred to Falcon type tubes and stored at -18 °C for later extraction. The flour samples were sieved to a particle size of 0.05 mm and dried at 50 °C for 24 h. Thereafter, the samples (1.0 g dry weight) were dissolved in 20 mL of hexane:acetone (v/v) containing 100 mL of BHT (butylated hydroxytoluene) for 30 min. Subsequently, the samples were filtered through a cellulose support, the extract washed with 15 mL of ultrapure water (3x) and the solvent evaporated in an oven at 50 °C.

The concentrated extract was re-suspended in 3 mL of hexane and centrifuged for 15 min (3200 rpm). The extract was then subjected to UV-visible spectrophotometry (U-1800 Spectrophotometer) for the determination of the total carotenoid content (TCC) reading the absorbance at 450 ηm in triplicate samples (Aman et al., 2005). The TCC was determined according to Lambert-Beer law, using a molar extinction coefficient (ε) of 2,348 M^{-1} cm^{-1} (zeaxanthin), as described by Britton and Goodwin (1982). The molar extinction coefficient of zeaxanthin was used because this is the major carotenoid contained in the MPA1-C0 maize population (Kuhnen et al., 2009).

The data analysis was performed in accordance with the completely randomized block design, as recommended when there is evidence of the uniformity of the experimental area or when plots of an experiment designed as lattice are lost (Rademacher et al., 1999; Doerksen et al., 2003). Losses of experimental plots resulted in 87 treatments (83 HSF and four checks) in E_1 and E_2 (Set 1) and 71 (68 HSF and three checks) in E_3 and E_4 (Set 2). The TCC variable was tested for the normality of error effects and homogeneity of variance (Levene's Test) referring to the variance analyses for a group of experiments (Set 1: E_1-E_2 and Set 2: E_3-E_4).

Analysis of variance was performed for the two sets (Set 1 and Set 2). The appropriated random model is $Y_{ijk} = m_0 + f_i + l_j + fl_{ij} + r_{j(k)} + e_{ijk}$, where: Y_{ijk} is the experimental unit (plot total for total carotenoid content of grains), corresponding to the i-th family (i = 1, 2, ..., 83 in Set 1; i = 1, 2, ..., 68 in Set 2), j-th location (j = 1, 2 in each set) and k-th block (k = 1, 2, 3); m_0: general mean of evaluated HSF; f_i: family effect; l_j: effect of location or environment; fl_{ij}: effect of family by location interaction; $r_{j(k)}$: effect of replication within location; and e_{ijk}: pooled experimental error. Besides the family means, other parameters were estimated as direct and indirect functions of the mean squares for families (MS_F), family by location interaction (MS_{FL}) and mean error (MS_E) (Table 2). Significant differences ($p < 0.01$) were detected by the *F* test.

The second cycle of recurrent selection by CDS (third stage) was completed in 2009, when a sample of 200 ears was visually selected from the best plants within the best HSF of the recombination block (convergent step of second cycle). A seed sample of all the selected ears were equality mixed. Three cycles of stratified mass selection in small farms in western Santa Catarina (Anchieta, Guaraciaba and Novo Horizonte) were completed in 2012 and the seed bulk resulted in the population MPA1-C2 (Figure 1).

Results and Discussion

The tests for normality, independence of errors, and homogeneity of variances (Levene's) revealed no abnormality and no further correction was necessary for adjustment to stand variation. Thus, the combined variance analyses referring to the evaluation of two random

Table 1 – Coordinates, altitudes and soil properties in four environments located in Iporã D'Oeste, Anchieta, Guaraciaba, and Novo Horizonte in the western region of Santa Catarina State (southern Brazil).

Variables	Municipalities[a]			
	Iporã D'Oeste	Anchieta	Guaraciaba	Novo Horizonte
Latitude	26°59’01”	26°31’27”	26°33’29”	26°31’15”
Longitude	53°32’40”	53°23’14”	53°36’12”	52°45’08”
Altitude (m)	540	528	520	520
Soil texture clay (%)	30	34	36	41
pH	5.4	5.4	5.0	4.8
Phosphorus (mg kg^{-1})	49.6	13.2	16.6	14.8
Potassium (mg kg^{-1})	34.0	10.0	8.0	11.0
Organic matter (%)	3.1	4.2	3.1	4.6

[a] Average annual pluviometric precipitation in western Santa Catarina from 1,800 to 2,200 mm (SPOG, 2003).

samples of HSF were performed for TCC of grains in two groups of experiments (Set 1 and Set 2) without any restriction (Table 3). The expression of genetic variance among HSF and the effect of family by location interaction (FL) were more expressive in Set 1. The values of *F* for both sources of variation were 1.76 (HSF) and 2.5 (FL) in Set 1, 2.40 (HSF) and 1.5 (FL) in Set 2, and 1.90 (HSF) and 2.2 (FL), in the pooled analysis over two sets.

Table 2 – Half-sib family means and other genetic-statistical parameters estimated by mean squares for families (MS_F), family by location interaction (MS_{FL}) and mean error (MS_E) in two grouped experiments in four environments in western Santa Catarina.

Parameters[c]	Estimate per sample[a] (Set_1 and Set_2)[b]
General mean	$m_0 = \frac{1}{IJK}\sum_{ijk} Y_{ijk}$
Genetic variance among half-sib families	$\hat{\sigma}^2_f = (MS_F - MS_{FL})/6$
Phenotypic variance among half-sib families	$\hat{\sigma}^2_{\bar{F}} = \frac{1}{6} MS_F$
Family by location interaction variance	$\hat{\sigma}^2_{fl} = \frac{1}{3}(MS_{FL} - MS_E)$
Additive genetic variance	$\hat{\sigma}^2_A = 4\,\hat{\sigma}^2_f$
Error variance	$\hat{\sigma}^2 = MS_E$
Experimental coefficient of variation	$CV_{\%} = 100.\sqrt{M_E}\,/m_0$
Genetic coefficient of variation	$CVg_{\%} = 100.\ \hat{\sigma}_f\,/m_0$
Index of variation	$Iv = CVg/CV = \hat{\sigma}_f / \hat{\sigma}$
Coefficient of heritability (narrow sense; family means)	$h^2_{f\ \%} = 100.(MS_F - MS_{FL})/MS_F$
Expected gain from selection among family means	$GS_{\%} = (i.c.\ h^2_f\ .\ \hat{\sigma}_{\bar{F}}).100/m_0$

[a]Estimates from analyses excluding checks; [b]Set 1 and Set 2 for grouped experiments containing different half-sib family samples evaluated in Iporã D'Oeste-Anchieta and Guaraciaba-Novo Horizonte, respectively. [c]For definition of parameters see Searle et al. (1992) and Hallauer and Miranda Filho (1995). In GS%, *i* = 1.097 and *i* = 1.078 is the selection differential in standard units for selection intensity of 33 % and 34 % for Set 1 and Set 2, respectively; *c* = 1 is the parental control for selection among families in both sexes.

The mean squares for the environment, families, and family by location interaction had averages of 112.93, 32.49, and 17.06, in the pooled analysis, respectively. The value of the mean squares for families was ≈90 % higher than the value obtained for FL, in the pooled analysis over two sets, thus assuring the existence of the genetic variability that is necessary for selection toward the increase of TCC in MPA1-C1. Differences for the effect of FL were also reported by Menkir and Dixon-Maziya (2004) in evaluation trials of varieties adapted to tropical environments; however, the information is scarce for TCC as regards FL and other genetic-statistical parameters for maize populations**.** The experimental coefficients of variation (CV %) ranged from 18.93 (Set 1) to 14.90 (Set 2), with a pooled average of 17.12 % over two sets (Table 3). In general, values of CV % below 20 % indicate good accuracy of the experimental results, thus assuring reasonable levels of precision of these experiments and corresponding estimates.

Biofortification involves increasing the nutrient content of a foodstuff through conventional breeding or genetic engineering (Pflffer and McClafferty, 2007). The focus on maize has been increasing because of its β-carotene content (carotenes), which is the main precursor of vitamin A in cereals (Welch, 2002). Indeed, hypovitaminosis A is a problem in some developing countries where a recurrent poverty state exists and is a cause of blindness in certain areas. This statement has been based on the dietary, biochemical data, and clinical signs. By applying rational plant breeding technologies we have the means to alleviate this nutritional deficiency. Thus, efforts by maize breeders have increased the provitamin A carotenoid content and these are nutritionally available (Howe and Tanumihardjo, 2006). However, the carotenoids that

Table 3 – Mean squares (MS), degree of freedom (df), half-sib family (HSF) and checks means, and experimental coefficient of variation ($CV_{\%}$) in the variance analysis per experimental group for evaluation of total carotenoid content of grains in half-sib families of the population MPA1-C1.

	Grouped analysis[d]					
	Set 1		Set 2		Pooled[e]	
Source	df	MS	df	MS	MS	
Environment / Location (L)	1	0.486 [ns]	1	225.373 **	112.929	
Family (F)	82	42.207 **	67	20.599 **	32.491	
Family × Location Interaction (FL)	82	23.983 **	67	8.599 **	17.065	
Error	328	9.509	268	5.535	7.722	
Half-sib family mean (µg g^{-1})		16.29		15.79	16.06	
Half-sib family mean (g ha^{-1})		98.42		93.43	96.14	
CV%		18.93		14.90	17.12	
Checks means	µg g^{-1}	g ha^{-1}	µg g^{-1}	g ha^{-1}	µg g^{-1}	g ha^{-1}
SCS154	15.41	85.41	15.48	78.22	15.44	81.80
MPA1 C0	15.45	87.61	-	-	-	-
MPA1 C1	15.54	85.75	14.78	96.02	15.20	91.31
BRS4150	16.92	87.80	17.59	96.63	17.22	91.98

**Significant ($p < 0.01$) and [ns]not significant differences by *F* test. [d]Set 1 and Set 2 for experiment groups containing different half-sib family samples evaluated in *Iporã D'Oeste-Anchieta* and *Guaraciaba-Novo Horizonte*, respectively. [e]Weighted by number of degrees of freedom (df); df: 1, 82, 82 and 328 for Environment, Family, Family × Location Interaction, and error, respectively in Set 1, and 1, 67, 67 and 268, respectively, for the same sources of variation in Set 2. Expected mean squares E(MS) in grouped analyses for Set 1 and Set 2: $E(MS_F) = \hat{\sigma}^2 + 3\hat{\sigma}^2_{fl} + 6\hat{\sigma}^2_f$; $E(MS_{FL}) = \hat{\sigma}^2 + 3\hat{\sigma}^2_{fl}$; and $E(MS_E) = \hat{\sigma}^2$.

are found in the largest quantities in maize kernel are the xanthophylls lutein and zeaxanthin (Janick-Buckner et al., 1999; Kuhnen et al., 2009), which are well known antioxidants, but non-precursors of vitamin A.

Interest is growing in these xanthophylls, since several benefits to human health have also been attributed to them. There is an inverse relationship between the consumption of diets rich in carotenoids and the incidence of some kinds of cancer (lung, colon, and prostate) and the induction of skin deterioration by ultraviolet rays, cardiovascular diseases, cataracts, and macular degeneration (Beatty et al., 1999; Handelmen, 2001; Demmig-Adams and Adams, 2002; Johnson, 2002; Fraser and Bramley, 2004; Stahl and Sies, 2005; Darvin et al., 2011; Agarwal et al., 2012; Tanaka et al., 2012).

Kuhnen et al. (2011) have shown a fairly good content of these xanthophylls (lutein and zeaxanthin) in the kernel of MPA1-C0 compared to other local varieties maintained by farmers from the same region, besides its high-yielding potential and resistance to *Exserohilum turcicum* (Kist et al., 2010; Ogliari et al., 2013). Referring to grain yield, MPA1-C0 proved to be outstanding in two trials carried out in 2002/2003 and 2003/2004 in Canoinhas (SC), Brazil (26°10'37"; 50°23'43"). The results showed a variation in grain yield for 22 LMV (3.1 to 6.9 t ha^{-1}), in 2002/2003, with MPA1-C0 showing the best performance. In the subsequent year, 28 LMV had grain yields varying from 2.1 to 8.0 t ha^{-1} and again MPA1-C0 was among the outstanding varieties with 7.4 t ha^{-1}. When compared with the average maize yield in SC over the two respective years (2002/2003 and 2003/2004), the LMV showed a mean yield of 61.5 % and 58.3 % above the state average (ICEPA, 2005), respectively.

The high-yielding potential and TCC of grains (≈20 µg g^{-1}) make MPA1-C0 economically viable (Kuhnen et al., 2009; Kist et al., 2010; Kuhnen et al., 2010; 2011), and due to this attribute it provides a potential way to improve the income of these farmers. When comparing the means of HSF obtained from the population evaluated in this study (MPA1-C1) with the value reported by Kuhnen et al. (2011) in the original population (MPA1-C0), these authors observed an average that was slightly higher (18.8 µg g^{-1}), although it is within the range of variation (11 to 23 µg g^{-1}) found in this study.

The good potential was maintained in the local composite population focused on in this study, herein named MPA1-C1, after the first recurrent selection cycle towards the increase in grain yield and decrease in plant height. Even though the MAP1-C0 population has not been selected in the first cycle towards the increase of TCC, the concentration of these secondary metabolites in MPA1-C1 may be considered relatively high and slightly superior to the average of the checks. The observed means for the two independent samples of HSF, expressed in µg g^{-1}, showed to be consistent among the two sets (16.29 and 15.79), ranging from 11.11 to 22.67 in Set 1 and 11.14 to 19.47 in Set 2 (Table 3). In the pooled analysis over two sets, this variable averaged 16.06 µg g^{-1} and was ≈1.5 % above the mean of the four checks (15.83 µg g^{-1}). The superiority is more notable when expressed in g ha^{-1}, averaging ≈9.0 % above the mean of the checks (88.18 g ha^{-1}), in the pooled analysis over the two sets. When expressed in g ha^{-1}, the observed means of TCC of grains for the two sets were 98.42 (Set 1) and 93.43 (Set 2), and in the pooled analysis over sets was 96.14 (Table 3).

Although the information concerning carotenoid accumulation in open-pollinated population is scarce, elite breeding lines and commercial populations of maize are investigated frequently in this regard for their usage as biofortified foods. Rios et al. (2009) reported TCC averaging ≈23 µg g^{-1} and a range of 14 to 28 µg g^{-1} in a study performed with 10 cultivars of yellow-orange maize evaluated in five environments in the southeastern (state of Minas Gerais) and central-western (state of Goiás) regions of Brazil. A similar average (≈23 µg g^{-1}) was reported by Harjes et al. (2008) in an evaluation of 204 strains of yellow maize grains, but in a higher range (5-66 µg g^{-1}). Higher estimates were also reported by Senete et al. (2011), in a range of 21-29 and a mean of 25 µg g^{-1}, for 21 hybrids obtained from crosses of seven lines with high provitamin A contents. Other reported values, in the range of 0.4 to 88 µg g^{-1}, taken as a reference for comparisons (Weber, 1987; Kurilich and Juvik, 1999; Hulshof et al., 2007), were above and below the values found in the present work.

The results may vary for the same sample depending on the extraction methodology, for instance. Rivera and Canela (2012) showed a variation of 25 % for TCC from the same maize sample extracted using five solvent solutions. Other aspects related to the time of exposition to the solvent, inclusion or not of agitation practices during extraction reaction, and usage of the whole (seed coat, embryo and endosperm) or parts (endosperm) of the grains may affect the final TCC.

Even though the observed mean for TCC in the present study is lower (≈16 µg g^{-1}) than the averages reported in other commercial varieties and elite lines from breeding programs aimed at the production of biofortified foods, the MPA1-C1 population may still be considered suitable for commercial exploitation. Besides the nutritional properties, the ecological and social aspects may also be incorporated into economic exploitation due to the increasing demand of Brazilian society for healthy foods that are produced in a socially correct way by farmers. On the other hand, the small-scale farmers in western SC State tend to pay more attention to other factors associated with the good quality of the food. Thus, besides focusing on high nutritional properties, other adaptive attributes (resistance to low fertility and acid soils, insects and diseases, low water supply by changes in rainfall regime) may be considered an interesting alternative for agricultural ecosystems producing with scarce financial resources, under low-input energy, and high-risk or organic growing productions.

Estimates of the components of variance for TCC of grains in the two sets are shown in Table 4. The estimates of $\sigma^2_{\bar{F}}$ (phenotypic variance among family means), σ^2_f (genetic variance among families), σ^2_A (additive genetic variance), and σ^2_{fl} (family by location interaction variance) are related to the h^2_f (coefficient of heritability for family means) and GS (expected gain from the selection). All the estimates of variance for TCC, expressed in (µg g^{-1})^2, were inconsistent among the sets, being higher in Set 1. The estimates of $\sigma^2_{\bar{F}}$, σ^2_f, σ^2_A, and σ^2_{fl} averaged 5.40, 2.57, 10.27, and 3.10, respectively, in the pooled analysis over two sets; the variation of these estimates around the pooled average was + 30 % (Set 1) to −36 % (Set 2), + 18 % (Set 1) to −22 % (Set 2), and + 55 % (Set 1) to −68 % (Set 2) for $\sigma^2_{\bar{F}}$, σ^2_f (or σ^2_A), and σ^2_{fl}, respectively, thus indicating the higher range of variation among sets for the variance component referring to FL.

When considering σ^2_f or σ^2_A per location, it must be considered that these values are biased upward by an amount proportional to the variance due to genotype by location interaction (Kist et al., 2010). In fact, the existence of significant differences for FL in each set and the high $\hat{\sigma}^2_{fl} / \hat{\sigma}^2_f$ ratio (0.30), in the pooled analysis over two sets, justifies the grouped analyses for estimating the genetic-statistical parameters with higher precision. As a result, variations in TCC are expected to depend on the environmental conditions under which MPA1-C1 is cultivated. Thus, particular attention is required during the manufacture of products for human consumption, as their composition must be standardized according to local regulations.

Knowledge about the effects of the FL and the genetic basis involved in the control of this character may help breeders and farmers to adopt satisfactory strategies for selection and production. Changes in soil and climate over the cultivation cycles and locations may favor or disfavor the production of bioactive compounds in this population. Thus, further studies are required to identify geographic areas for obtaining higher TCC of grains in MPA1-C1 aiming at the production of biofortified foods.

The values of the additive variance affect the estimates of the coefficient of heritability, which may be considered intermediate for breeding purposes. The estimates of coefficient of heritability in Set 1 (43.18 %) was lower than Set 2 (58.30 %), averaging ≈50 % in the pooled analysis over the two environments (Table 4). The index of variation (Iv) is the ratio between the genetic and environmental indices of variation, or simply the ratio between the genetic and environmental standard deviations. Both parameters (h^2_f and Iv) are related to the efficiency of selection within the base population (Kist et al., 2010). The Iv in two sets also indicated the intermediate level of variability available for selection. Similar magnitudes of Iv were estimated for the two sets (0.57 in Set 1; 0.60 in Set 2), averaging 0.58 in the pooled analysis. This estimate is slightly higher (Iv = 0.49) than that reported by Senete et al. (2011).

The observed means of checks (m_C) and HSF of the evaluated (m_0) and selected (m_S) population as well as the expected means relative to gain from selection (GS and GS$_{\%}$) and improvement population (m_I) are shown in Table 5. The estimated means (m_0) of the two independent samples of HSF were shown to be consistent among the two sets (16.29 and 15.79 µg g^{-1}). In the pooled analysis

Table 4 – Estimates of additive genetic variance ($\hat{\sigma}^2_A$), genetic variance among half-sib families ($\hat{\sigma}^2_f$), phenotypic variance among family means ($\hat{\sigma}^2_{\bar{F}}$), family by location interaction variance ($\hat{\sigma}^2_{fl}$), coefficient of heritability, narrow sense, based on family means ($h^2_{f\,\%}$), genetic coefficients of variation (CVg$_{\%}$), and index of variation (Iv), in the population MPA1-C1 for total carotenoid content of grains based on variance analysis performed per experiment group.

Grouped Analysis[f]	Estimates of parameters						
	$\hat{\sigma}^2_A$	$\hat{\sigma}^2_f$	$\hat{\sigma}^2_{\bar{F}}$	$\hat{\sigma}^2_{fl}$	h^2_f	CVg	Iv
	(µg g^{-1})^2				%		
E_1-E_2 Set 1	12.16	3.04	7.03	4.82	43.18	10.70	0.57
E_3-E_4 Set 2	7.96	1.99	3.41	1.00	58.30	8.94	0.60
Pooled[g]	10.27	2.57	5.40	3.10	49.98	9.91	0.58

[f]Set 1 and Set 2 for experiment groups containing different half-sib family samples evaluated in Iporã D'Oeste-Anchieta and Guaraciaba-Novo Horizonte, respectively; [g]Weighted mean by number of degrees of freedom.

Table 5 – Observed means (m_C: checks; m_0: evaluated sample; m_S: selected sample) and expected means (GS and GS$_{\%}$: gain from selection; m_I: improved population MPA1-C2) for total carotenoid content of grains in the population MPA1-C1.

Grouped analysis[h]	Observed Means			Ratio		Expected Means		
	m_C	m_0	m_S	m_0/m_C	m_S/m_C	GS	GS	m_I[j]
	µg g^{-1}					%	µg g^{-1}	
Set 1	15.83	16.29	19.35	1.03	1.22	7.71	1.26	17.55
Set 2	15.95	15.79	17.93	0.99	1.12	7.36	1.16	16.95
Pooled[i]	15.83	16.06	18.71	1.01	1.18	7.55	1.22	17.28

[h]Set 1 and Set 2 for experiment groups containing different half-sib family samples evaluated in *Iporã D'Oeste-Anchieta* and *Guaraciaba-Novo Horizonte*, respectively; [i]Weighted mean by number of degrees of freedom; [j]Expected mean of improved population MPA1-C2 (m_I) was calculated by adding m_0 and GS.

over two sets, this variable averaged 16.06 µg g^{-1} and was 1.45 % above the mean of the checks (m_C = 15.83 µg g^{-1}). Estimates of the expected gains from the selection were of similar magnitudes in the two sets. The means of the selected HSF were ≈18 % higher than the mean of the checks and ≈16 % higher than the mean of the evaluated HSF, averaging 18.71 (m_S), 16.06 (m_0), and 15.83 (m_C) in the pooled analysis. However, the expected mean of the improved population ($m_I = m_0 + GS$ = 17.28 µg g^{-1}) in this second cycle at a selection intensity of ≈33 % was only ≈7.6 % above the mean of the evaluated HSF, which corresponds to an increase of 1.22 µg g^{-1} (GS).

The main conclusion related to the present study is that the amount of genetic variability existing in the population MAP1-C1, such as that expressed by the additive variance and other parameters related to genetic variability in the two sets, assure an effective gain from the selection towards the increase in TCC. Thus, it seems to be adequate for the development of outstanding varieties related to nutritional properties by recurrent convergent-divergent selection. In addition, choosing grains with an intense yellow or orange color also helps to increase the carotenoid content (Kurilich and Juvic, 1999). This is important because it allows farmers to participate in the selection process of their varieties. These grain colors (yellow or orange) reflect the concentration of xanthophylls, especially lutein and zeaxanthin (Pflffer and McClafferty, 2007; Rivera and Canela, 2012). Although they do not show any pro-vitamin A activity, the effects of these chemical constituents on human health have been widely reported in the literature, mostly concerning their preventive effects on age-related macular degeneration (Beatty et al., 1999; Handelmen, 2001; Demmig-Adams and Adams, 2002; Fraser and Bramley, 2004; Stahl and Sies, 2005; Kuhnen et al., 2009; Darvin et al., 2011; Agarwal et al., 2012; Rivera and Canela, 2012).

Another important point to consider in this recurrent selection program is related to selection intensity. When considering the selection intensity of the ≈33 % applied in this second cycle, the average expected gain from the selection toward the increase in TCC may be considered significant. In general, this selection intensity is considered a quite moderate selection pressure. This procedure was adopted because MPA1-C1 is a wide base population from local germplasm sources adapted to broad areas in western SC and is only in the second cycle of recurrent selection.

The selection in the first cycle was directed to grain yield and plant height only and sufficient genetic variability was available to assure substantial gains (Kist et al., 2010). Therefore, a higher effective population size (Ne) should be retained in the initial cycles of recurrent selection to continue extending genetic gains over a long time for those and also other important agronomic, adaptive, and nutritional traits (Kist et al., 2010). In this phase of the breeding program, low selection pressure was considered appropriate for achieving significant minimization of the loss of desirable alleles and the fixation of undesirable alleles because of genetic drift and for assuring the expression of genetic variability in the next cycles of recurrent selection (Sprague and Eberhart, 1977). This procedure may undoubtedly contribute to minimizing the risks of severe attacks by pests and diseases and to maintaining the stability of grain production when cultivated under the stresses of several natures as such is commonly found in agricultural communities in western SC.

When considering the wide genetic base of MPA1-C1 to be exploited by selection, the main conclusion is that the application of moderate selection pressures is an important strategy to consider in recurrent selection, although the expected gains are not so spectacular initially. In this phase of the ongoing project, the identification, quantification, and direction of the genetic correlations established between TCC and other important characteristics should be investigated because the increase in TCC from selection can result in positive or negative changes in other important correlated characteristics. In this way, care must be taken in relation to the expected indirect undesirable correlation between TCC and characteristics of interest, such as grain yield, plant height, or any adaptive traits that are important for the maize crop in target agricultural ecosystems, so that simultaneous selection for these traits should be weighed adequately in subsequent cycles.

If there is a desirable genetic correlation between TCC and some other attributes of interest, carotenoid concentration may be indirectly increased by selecting in favor of the traits correlated with TCC, especially for those traits that are highly correlated in the desirable direction with TCC and have higher heritability coefficients. This knowledge is very important because of the difficulty of quantifying large numbers of samples for TCC or any other secondary metabolite over a short time such as is required in recurrent selection. For the same reason, the mapping of QTL associated with TCC and the high effect on carotenoid biosynthesis may be used in a marker-assisted selection program to facilitate the increasing levels of carotenoids in maize grains, in addition to enabling an understanding of the genetic basis of this trait in kernels.

Acknowledgements

We would like to thank the Brazilian Ministry of Agrarian Development for the financial support, the Coordination for Improvement of Higher Education (CAPES) for the scholarship granted to Volmir Kist, and the farmer Névio Alceu Folgiarini for providing the MPA1-C0 population. We would also like to thank ASSO, SINTRAF, and EPAGRI (*Anchieta*-SC), Microbacias 2 Program and MPA for supporting the experiments performed in the fields of the Adriano Freydag, Léo Chenet, Vunibaldo Kist, and Vilmar Dalzochio families through the support of the following technicians: Adriano Canci, Ivan José Canci, and Olavo Ghedini.

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