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Adaptability and stability of carotenoids in maize cultivars

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ABSTRACT - *The purpose of this study was to investigate the adaptability and stability of carotenoids in maize cultivars in the 2004/2005 growing season. Total carotenoids (TC), total carotenoids with provitamin A activity (Pro VA) ($\mu g\ g^{-1}$) and grain yield (kg ha^{-1}) were quantified in 10 cultivars at five locations. The chemical analyses were conducted in a laboratory of the EMBRAPA/CNPMS, in Sete Lagoas, Minas Gerais. The methodologies of Eberhart and Russell (1966), Lin and Binns (1988) and Rocha et al. (2005) were used to analyze adaptability and stability. In general, the linear regression model proposed by Eberhart and Russell (1966) failed to fit the Pro VA contents in the evaluated cultivars satisfactorily. However, with regard to the TC levels, all different analysis methodologies of adaptability and stability rated hybrid BRS 2020 as an ideal genotype with general adaptability.*

Key words: *Zea mays*, biofortification, vitamin A, genotype-by-environment.

INTRODUCTION

The lack of β - carotene is one of the major nutritional deficiencies in the world population, causing serious health problems, mainly night blindness in children. The World Health Organization estimates the annual number of blind children at over 250 thousand, due to insufficient vitamin A intake. This problem also affects Brazilians, mainly in rural areas and particularly in the semi-arid regions (Souza and Villas Boas 2002).

To deal with hypovitaminosis, breeders have targeted the increase of essential nutrient levels in staple foods, a process known as biofortification, by using specific strategies in programs of plant improvement and genetic transformation (Nestel et al. 2006, Oaim et al. 2007). Maize, a carotenogenic species is, as a grain, of extreme importance because it is a subsistence crop of consumers in Sub Saharan Africa, Latin America and northeastern Brazil, aside from a series of other places where vitamin A indices are higher.

Sandmann and Albrecht (1994) reported that environmental factors influence carotenogenesis. Albeit few, literature results indicate a genotype-environment interaction (GEI), considering carotenoid production in different plants (Cimmyt Medium-Term Plan 2008). So, a promising genotype developed in one specific environment may not be quite as successful in another. This fact affects the selection gain and the development of recommendable cultivars with broad adaptability and stability.

Literature contains a wealth of information about GEI and adaptability and stability of performance in different crops (Farias et al. 1997, Carvalho et al. 2000,

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Carvalho et al. 2003, Oliveira et al. 2007). However, most results characterize yield-related traits, while studies of GEI and adaptability and stability of carotenoid contents in maize are missing. With this background, the purpose of this study was to evaluate the adaptability and stability of carotenoids for different maize cultivars in five environments.

MATERIAL AND METHODS

Data from a trial of maize varieties conducted by Embrapa Maize and Sorghum in the 2004/2005 growing season were used. Five environments with different soil fertility levels were evaluated, three of them in the city of Sete Lagoas, Minas Gerais: the soil at the first location was fertile; at the second, high levels of nitrogen (120 kg ha^{-1}: 20 kg at planting and 100 kg as topdressing) were applied; and at the third a fertilizer with low nitrogen levels (20 kg ha^{-1} at sowing) was used. The two other locations lie in the counties of Planaltina and Goiania, Goias (Table 1). The traits of the ten cultivars evaluated are listed in Table 2.

The experimental design was a randomized block with two replications. The plots consisted of two rows of four meters, spaced 0.90 m apart, with a final stand density of approximately 55,000 plants per hectare. The data refers to grain weight in kg ha^{-1}, adjusted to 13% moisture, and the grain carotenoid content. The grains of 10 maize cultivars from five environments were physio-chemically analyzed for their carotenoid content in a laboratory for grain quality of the Brazilian research center of Embrapa Maize and Sorghum, in Sete Lagoas, Brazil.

The total carotenoids (TC) were extracted following a protocol proposed by Rodriguez-Amaya and Kimura (2004), with subsequent quantification in a Cary spectrometer 50 Conc UV-Visible (VARIAN - Australia). The carotenes (α and β-carotene) and monohydroxylate (β-cryptoxanthin) were quantified by High-Performance Liquid Chromatography (HPLC) in a HPLC system (Shimadzu LC-10) equipped with a YMC C 30 column (5 μm, 4.6 x 250mm, Waters, Milford, MA, USA), coupled with UV-photodiode array detection. The elution gradient was conducted at 0.8 mL min^{-1} in a 25-min linear gradient from 80:20 to 15:85 methanol: Methyl tert-Butyl Ether, followed by 5 minutes constant at 80:20, ending with 6 minutes in equilibrium. The total carotenoids with provitamin A activity (Pro VA) was calculated as the sum of the amount of β-carotene + ½ β-cryptoxanthin + ½ α-carotene, considering 100% activity of provitamin A β-carotene and 50% of the other

Table 1. Geographic coordinates of the five evaluation environments in the growing season 2004/2005

State	County	Latitude	Longitude	Altitude (m asl)	Sowing date
MG	Sete Lagoas[1]	19°28’00"	44°15’00"	732	11/27/2004
	Sete Lagoas[2]	19°28’00"	44°15’00"	732	12/03/2004
	Sete Lagoas[3]	19°28’00"	44°15’00"	732	12/03/2004
GO	Planaltina	15°27’10"	47°36’48"	1000	11/09/2004
	Goiânia	16°28’00"	49°17’00"	823	11/26/2004

[1] Fertile soil; [2] High levels of nitrogen fertilization: 120 kg ha^{-1} (20 kg at sowing and 100 kg as topdressing); [3] Low levels of nitrogen fertilization: 20 kg ha^{-1} (at sowing)

Table 2. Origin, grain type and population of maize cultivars

Cultivars	Origin	Grain type and color	Population
BRS 2020	Embrapa	Semi-flint/orange	Double-cross hybrid
Fundacep 35	Fundacep	Semi-flint/yellow-orange	Variety
CMS 104	Embrapa	Semident/yellow	Variety
BRS Caatingueiro	Embrapa	Semi-flint/yellow	Variety
BRS 473 cIII	Embrapa	Semi-flint/yellow-orange	Variety
UFVM100	UFV	Dent/yellow-orange	Variety
CMS 102	Embrapa	Semident/yellow	Variety
CMS 101	Embrapa	Semident/yellow	Variety
BRS Missões	Embrapa	Dent/yellow	Variety
BRS São Francisco	Embrapa	Semident/yellow-orange	Variety

two variables (Rodriguez-Amaya and Kimura 2004). The results were expressed on a dry basis, by the moisture analysis in the samples in duplicate, according to the 44-15A of AACC method (2000).

Analyses of individual variance of all experiments and analysis of homogeneity of residual variance were carried out in the beginning, using the model in randomized blocks. Afterwards, joint analyses were performed considering the effects of cultivars as fixed and those of environments as random. Means were compared by Duncan Test at 5% probability. Adaptability and stability were analyzed by the methods proposed by Eberhart and Russell (1966), by Lin and Binns (1988) and the centroid method (Rocha et al. 2005)

The method proposed by Eberhart and Russell (1966) is based on linear regression analysis, where the response of each genotype to environmental variations is measured. The adaptability parameters are the overall mean of genotype i ($\hat{\beta}_{oi}$) and the coefficient of linear regression ($\hat{\beta}_{1i}$). The stability is assessed by deviations from regression (σ^2_{di}) and coefficient of determination (Ri^2).

The methodology proposed by Lin and Binns (1988) uses the mean square of the distance between the cultivar mean and the highest mean response of all environments, as a measure to estimate the stability in plants, characterizing the parameter P_i. In the analysis, the lower the P_i value, the more stable the cultivar is.

The centroid method described by Rocha et al. (2005) is based on multivariate analysis and principal components. The cultivar response under evaluation is compared with the response of four referential ideotypes, as defined on the basis of experimental data: (I) the ideotype of maximum general adaptability, with the maximum values in all environments tested, (II) the maximum adaptability to specific favorable environments, with the highest response to favorable and lowest to unfavorable environments, (III) the maximum specific adaptability to unfavorable environments, with the maximum response to adverse and minimum to favorable environments and (IV) the minimum adaptability, with the lowest values observed in all environments. After classifying the environments, as proposed by Finlay and Wilkinson (1963), reference points are created, which are ideotypes of different responses to favorable and unfavorable environments, seeking the classification of other points of the chart considering the Cartesian distance values to each of the four ideotypes. The probability is calculated using the inverse distance between a treatment and the four ideotypes, by the equation: $Pd_{(i,j)} = \dfrac{\frac{1}{d_i}}{\sum_{i=1}^{4} \frac{1}{d_i}}$, where $Pd_{(i,j)}$: is the probability of a stability standard similar to the jth centroid; di: distance from the ith point to the jth centroid. Genetic-statistical analyses were performed using the software Genes, Cruz (2006).

RESULTS AND DISCUSSION

In view of the heteroscedasticity detected for grain yield, the degrees of freedom of the mean error and genotype-environment interaction (GEI) were adjusted according to the method of Cochran (1954). No significant GEI ($p < 0.05$) was observed for grain yield (kg ha^{-1}). This absence of GEI for grain yield is contrary to most of the results reported in the literature indicating that the evaluated genotypes and environments were not different enough to induce a different performance for this trait. For the other traits evaluated, the joint analysis of variance revealed the existence of significant genetic variance in the cultivars, significant differences between the assessed environments, as well as significance for GEI ($p < 0.01$), highlighting the need for a detailed study to identify genotypes for greater adaptability and phenotypic stability (Table 3).

The coefficients of environmental variation were low to medium (3.4, 3.5 and 15.6% for TC, Pro VA and grain yield, respectively), indicating high experimental accuracy of the estimates (Table 3) (Scapim et al. 1995).

The overall TC mean was 23.11 μg g^{-1}, with a variation from 19.32 μg g^{-1} to 26.43 μg g^{-1} among genotypes (Table 4). Harjes et al. (2008) found a mean TC of 23 mg g^{-1} in yellow maize lines, but with a greater variability (between 5.5 and 66 μg g^{-1}). In a program of biofortification however, Burt et al. (2006) developed maize lines with a mean TC between 43.6 and 88.3 μg g^{-1}, evidencing the possibility of successfully increasing the TC levels in maize grains. Moreover, it was observed that the variability for the carotenoid content and profile in grains of commercial maize varieties can be lower than in elite lines, lines and accessions of genebanks (Burt et al. 2006, Paes et al. 2006, Harjes et al. 2008).

Table 3. Results of the joint variance analysis, based on the data of 10 cultivars in five environments, for the traits Total carotenoids (TC) and carotenoids with provitamin A activity (Pro VA) and grain yield

Source of variation	**TC (μg g^{-1})**		**Pro VA (μg g^{-1})**		**Grain yield (kg ha^{-1})**	
	df	MS	df	MS	df	MS
Blocks/Environments	5	1.4215	5	0.0060	5	260,167.6319
Genotypes (G)	9	49.6227**	9	0.4308**	9	10,125,679.4410**
Environments (E)	4	36.1181**	4	0.3701**	4	104,173,748.8983**
GxE	36	7.1396**	36	0.1109**	26	957,347.9100
Residue	45	0.6255	45	0.0050	30	1,618,979.0044
Mean		23.1122		1.9581		8,146.7843
CV (%)		3.42		3.58		15.62

*, **: significant by the F test, at 1% and 5% probability, respectively

This is justified by the fact that over the years, genetic improvement programs for maize prioritized mainly yield-related agronomic traits. Also, despite the longstanding preference of poultry producers for yellow grain, the interest in finding a complementary option to the existing nutritional interventions to combat vitamin A deficiency in humans in colored maize is quite recent.

The overall mean of Pro VA variation in the cultivars was between 1.73 and 2.36 μg g^{-1}. These values are similar to those found by Ewool et al. (2006) for the genotypes GH9866SR and GH120DYFP (between 2.9 μg g^{-1} and 3.5 μg g^{-1}, respectively), in the growing season 2004, in Ghana, Africa (Table 4).

The grain yield varied between 5.4 and 11.1 ton ha^{-1}, with a mean of 8.1 ton ha^{-1}, which exceeds the national mean of 4 ton ha^{-1} (Conab 2008). The mean was highest in environment 5 (11 ton ha^{-1}) and lowest in environment 4 (5.4 ton ha^{-1}) (Table 5). The highest mean yields were observed for hybrid BRS 2020 and the varieties Fundacep 35 and CMS 102 (9.48, 9.03 and 8.86 ton ha^{-1}, respectively), with statistically higher means than for BRS Caatingueiro (6.17 ton ha^{-1}, respectively) (Table 5). The mean yield of the cultivars evaluated here exceeded the national mean, indicating another aspect of success in the maize biofortification programs.

The data evaluated did not fit satisfactorily to the linear regression model proposed by Eberhart and

Table 4. Estimates of the adaptability and stability parameters ($\hat{\beta}_{0i}$, $\hat{\beta}_{1i}$ and σ^2_{di}), by the methodology of Eberhart and Russell (1966), for the Total carotenoids and carotenoids with provitamin A activity (Pro VA) traits in μg g^{-1}

Cultivars	**Total carotenoids (μg g^{-1})**						**Pro VA**	
	Mean $^{(1)}$($\hat{\beta}_{0i}$)	$\hat{\beta}_{1i}$	σ^2_{di}	R_i^2 (%)	Mean $^{(1)}$($\hat{\beta}_{0i}$)	$\hat{\beta}_{1i}$	σ^2_{di}	R_i^2 (%)
BRS 2020	26.43a	0.77	0.45	65.00	2.36a	2.94**	0.18"	54.54
Fundacep 35	21.65de	1.17	7.84"	28.71	1.88cdef	1.33	0.00	90.13
CMS 104	19.32f	1.02	4.83"	32.81	1.73f	1.53**	0.01"	81.78
BRS Caatingueiro	24.92ab	0.65	1.01’	43.38	2.24a	2.11**	0.01’	92.41
BRS 473 cIII	22.61cd	0.25	0.84’	11.22	1.78def	0.88	0.04"	32.76
UFVM100	22.46cd	0.12*	0.04	9.49	2.07b	-0.02**	0.05"	0.02
CMS 102	23.87bc	0.33	3.16"	7.22	1.91cde	0.13**	0.01"	2.55
CMS 101	20.51ef	2.74**	0.22	97.12	1.75ef	0.44**	0.09"	5.05
BRS Missões	23.96bc	0.99	3.94"	35.65	1.92cd	-0.15**	0.03"	1.77
BRS São Francisco	25.38ab	1.96**	3.08"	73.16	1.95bc	0.82	0.01"	52.24
Mean	23.11				1.96			

** and *: significantly different from one, by the t test, at 1 and 5% probability, respectively

“ and ‘: significantly different from zero, by the F test, at 1 and 5% probability, respectively

^{1} Means followed by the same lower case letter in a column are not significantly different in Duncan test, at 5% probability

Table 5. Yield means (kg ha^{-1}) in maize cultivars, Growing season 2004/2005

Cultivars	**Environments**					
	1	**2**	**3**	**4**	**5**	**Mean**[1]
BRS 2020	10481	10697	7431	6258	12552	9484 a
Fundacep 35	8955	10316	7143	6147	12596	9031 a
CMS 104	8340	9833	6178	5544	11513	8282 ab
BRS Caatingueiro	6529	6968	4597	4429	8305	6166 b
BRS 473 cIII	8516	8401	5513	3876	8818	7025 ab
UFVM100	8672	8860	7027	5448	12013	8404 ab
CMS 102	9529	8930	6583	6851	12408	8860 a
CMS 101	9699	8938	6677	5638	11710	8532 ab
BRS Missões	9214	9724	6283	4907	11405	8307 ab
BRS São Francisco	8399	8595	5776	4834	9282	7377 ab
Means	8833.40	9126.20	6320.80	5393.20	11060.20	
CV (%)	16.26	8.96	21.97	13.55	4.08	

[1] Means followed by the same lower case letter in a column are not significantly different in Duncan test, at 5% probability. Environments: 1) Sete Lagoas (MG) - fertile; 2) Goiânia (GO); 3) Sete Lagoas (MG); 4) Sete Lagoas (MG); 5) Planaltina (GO). Results were expressed on a dry basis

Russell (1966), based on the coefficient of determination (R^2). The R^2 of only 10% of the cultivars was higher than 80%, considering the TC levels in the grains and 30%, considering the Pro VA levels (Table 4). The mean TC (β_{0i}) of the double-cross hybrid BRS 2020 was the highest (μg g^{-1}), with 26.43 μg g^{-1}c, and a coefficient of linear regression ($\hat{\beta}_{1i}$) equal to the unit, which, according to proposed methodology, characterizes the adaptability as wide or general. Furthermore, the deviation from regression (σ^2_{di}) is equal to zero, i.e., the predictability and / or stability of performance for this trait is high (Table 4), classifying the cultivar as ideal according to the methodology proposed by Eberhart and Russell (1966). The Pro VA levels (μg g^{-1}) in the BRS Caatingueiro variety were higher than the overall mean, the predictability was low, but the fitting to the model was good (92%). The variety has specific adaptability to favorable environments making it a promising source of provitamin A carotenoids, among the cultivars evaluated (Table 4).

By the methodology proposed by Lin and Binns (1988) the cultivars BRS 2020 and BRS Caatingueiro were classified as promising in terms of TC and Pro VA levels in maize grains (Table 6). The TC means of the cultivars BRS 2020, BRS Caatingueiro, CMS 102, BRS Missões and BRS São Francisco were higher than the general mean and the P_i values were low, indicating wide adaptability and performance stability. Considering the levels of Pro VA carotenoids, the means of the cultivars BRS 2020, BRS Caatingueiro and UFVM 100 were high and the P_i values low.

For the cultivar classification into one of the four groups represented by ideotypes, according to the method of principal components of the Centroid method, values higher than or equal to 40% probability were used. Considering the TC levels in maize grains, hybrid BRS 2020 (1) and the BRS Caatingueiro (4) and BRS São Francisco (10) varieties were similar to ideotype I and are, consequently, generally adaptable. Besides, the TC means were higher than the overall mean (Table 7). The Pro VA means of the hybrid BRS 2020 (1), BRS Caatingueiro (4) and UFV 100 (6) variety were higher than the overall mean. But in terms of probability associated with the cultivar classification into one of the four centroids, general adaptability was only observed in hybrid BRS 2020 (1) and BRS Caatingueiro (4) variety.

Generally speaking, these results demonstrate the need for further research on the adaptability and stability of cultivars, with regard to the carotenoid levels, prior to the recommendation as commercial products, particularly in regions where soil-climate variations and differences in cultivation techniques are considerable, as in Brazil. It should be noted that the results presented in this paper refer to data from a single year of evaluation therefore the conclusions must be drawn carefully. It should also be recognized that there are very few published works on this area for carotenoids, which makes this work more relevant to the understanding of GEI for carotenoids. Moreover, the worldwide extent of the problems of vitamin A deficiency in different regions calls for further studies of the GEI with its consequences, with a view to spread and / or exchange plant material and technologies of cultivation and processing.

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Table 6. Adaptability and stability parameters for the means of Total carotenoids and carotenoids with provitamin A activity (Pro VA) for favorable and unfavorable environments (P_i), by the method proposed by Lin and Binns (1988)

Cultivars	**Mean**	**Overall P_i**	**Mean**	**Overall P_i**
	Total carotenoids (μg g^{-1})		**Pro VA (μg g^{-1})**	
BRS 2020	26.43	0.27	2.360	0.043
Fundacep 35	21.65	17.06	1.875	0.217
CMS 104	19.32	30.17	1.732	0.317
BRS Caatingueiro	24.92	2.12	2.238	0.041
BRS 473 cIII	22.61	9.62	1.781	0.291
UFVM100	22.46	10.09	2.067	0.192
CMS 102	23.87	6.15	1.907	0.238
CMS 101	20.51	22.93	1.754	0.369
BRS Missões	23.96	5.52	1.916	0.263
BRS São Francisco	25.38	2.99	1.950	0.198
Mean	23.11		1.96	

Table 7. Classification of maize varieties in one of the four groups characterized by centroids and the probability associated to the classification, for the Total carotenoids and carotenoids with pro-vitamin A activity (Pro VA) traits in maize grains

Cult	**Mean**	**Gr**	**Probabilities**				**Mean**	**Gr**	**Probabilities**			
			I	II	III	IV			I	II	III	IV
	Total carotenoids (mg g^{-1})						Pro VA (mg g^{-1})					
1	26.43	I	0.75	0.08	0.10	0.06	2.36	I	0.45	0.21	0.19	0.15
2	21.65	III	0.22	0.21	0.30	0.28	1.88	IV	0.18	0.20	0.25	0.37
3	19.32	IV	0.13	0.19	0.18	0.50	1.73	IV	0.14	0.19	0.19	0.48
4	24.92	I	0.48	0.17	0.22	0.14	2.24	I	0.40	0.24	0.20	0.17
5	22.62	III	0.26	0.19	0.34	0.21	1.78	IV	0.14	0.16	0.25	0.44
6	22.46	III	0.24	0.18	0.36	0.22	2.07	III	0.23	0.21	0.30	0.26
7	23.87	I	0.33	0.19	0.30	0.18	1.91	III	0.16	0.15	0.36	0.33
8	20.51	IV	0.16	0.27	0.18	0.39	1.75	IV	0.16	0.19	0.23	0.43
9	23.96	I	0.35	0.20	0.27	0.18	1.92	IV	0.18	0.19	0.30	0.34
10	25.38	I	0.46	0.20	0.20	0.15	1.95	IV	0.19	0.20	0.30	0.31
Mean	23.11						1.96					

Gr = group of classification; Ideotype I = General adaptabiliity; Ideotype II = Specific adaptability to favorable environments; Ideotype III = Specific adaptability to unfavorable environments; Ideotype IV = Little adapted. 1 = BRS 2020; 2 = Fundacep 35; 3 = CMS 104; 4 = BRS Caatingueiro; 5 = BRS 473 cIII; 6 = UFVM100; 7 = CMS 102; 8 = CMS 101; 9 = BRS Missões; 10 = BRS São Francisco

Adaptabilidade e estabilidade de carotenoides em cultivares de milho

RESUMO - *O objetivo deste trabalho foi avaliar a adaptabilidade e estabilidade para carotenoides em cultivares de milho, no ano agrícola de 2004/2005. Foram avaliados 10 cultivares em cinco locais quanto ao teor de carotenoides totais (CT), total de carotenoides com atividade pró-vitamínica A (Pro VA) (μg g^{-1}) e produtividade de grãos (kg ha^{-1}). As análises* *químicas foram conduzidas no Laboratório de Qualidade de Grãos da EMBRAPA/CNPMS, em Sete Lagoas, MG. Para o estudo de adaptabilidade e estabilidade utilizaram-se as metodologias propostas por Eberhart e Russell (1966), Lin e Binns (1988) e Rocha et al. (2005). Em geral, o modelo de regressão linear proposto por Eberhart e Russell (1966), não proporcionou ajuste satisfatório considerando-se os teores de Pro VA para os cultivares avaliados. Porém, para CT, as diferentes metodologias de estudo de adaptabilidade e estabilidade classificaram o híbrido BRS 2020 como genótipo de adaptabilidade geral.*

Palavras-chave: *Zea mays*, biofortificação, vitamina A, genótipos x ambientes.

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