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Adaptability and stability of the zinc density in cowpea genotypes through GGE-Biplot method[1]

Adaptabilidade e estabilidade da densidade de zinco em genótipos de feijão-caupi via GGE-Biplot

Diêgo Sávio Vasconcelos de Oliveira[2], Luis José Duarte Franco[3], José Ângelo Nogueira de Menezes-Júnior[3], Kaesel Jackson Damasceno-Silva[3], Maurisrael de Moura Rocha[3*], Adão Cabral das Neves[3] and Francisco Mauro de Sousa[3]

ABSTRACT - Biofortification is a strategy that aims to improve the nutritional quality of foods through genetic breeding. Zinc is an important mineral for human health. It is used in various physiological processes such as immune function, antioxidant protection, growth and development. Therefore, zinc is one of the most studied minerals in the biofortification of grains in cowpea. The objective of this study was to evaluate the adaptability and stability of zinc density in the grain of 12 cowpea genotypes in four environments in the states of Piauí (PI) and Maranhão (MA), Brazil, by using the GGE-Biplot method. A randomized complete block design with four replications was used. Grain samples of each genotype were ground and the resulting flour was subjected to zinc density analysis by using an atomic flame absorption spectrophotometer. Analyses of variance were performed, and the adaptability and stability of zinc density in the grain was evaluated by the GGE-Biplot method. Genotypes showed different behavior depending on the environments tested for zinc concentration. According the GGE-Biplot method, Parnaiba-PI was the most discriminating environment for genotypes. Campo Grande do Piauí-PI and Parnaíba-PI were the most representative environments for selecting genotypes with zinc biofortification in the state of Piauí. Parnaíba-PI was the optimal environment for selection of genotypes adapted to high zinc density in grain. The cultivar BRS Xiquexique was the ideal genotype due to the high zinc density in the grain and high stability according to GGE-Biplot, followed by the lines MNC04-774F-78 and MNC04-782F-108.

Key words: *Vigna unguiculata*. Micronutrient. Biofortification. Genotype × environment interaction.

RESUMO - A biofortificação é uma estratégia que visa melhorar a qualidade nutricional dos alimentos via melhoramento genético. O zinco é um mineral importante para a saúde humana, por participar de vários processos fisiológicos, tais como função imune, defesa antioxidante, crescimento e desenvolvimento. Por isso, o zinco é um mineral bastante estudado na biofortificação do grão em feijão-caupi. Este trabalho objetivou avaliar a adaptabilidade e a estabilidade da densidade de zinco no grão de 12 genótipos de feijão-caupi em quatro ambientes nos estados do Piauí e Maranhão por meio da análise de GGE-Biplot. Adotou-se o delineamento de blocos completos casualizados com quatro repetições. Amostras de grãos de cada genótipo foram trituradas e as farinhas foram submetidas à análise da densidade de zinco em espectrofotômetro de absorção atômica de chama. Foram realizadas análises de variância e a adaptabilidade e estabilidade da densidade de zinco foram avaliadas via método GGE-Biplot. Os genótipos se comportaram diferencialmente com os ambientes de teste para a concentração de zinco. De acordo com a análise GGE-Biplot, o ambiente considerado mais discriminante em relação aos genótipos foi Parnaíba-PI. Campo Grande do Piauí-PI e Parnaíba-PI foram os ambientes mais representativos para seleção de genótipos biofortificados em zinco testado no Piauí. Parnaíba-PI foi o ambiente ideal para a seleção de genótipos adaptados à alta densidade de zinco no grão. A cultivar BRS Xiquexique foi considerada o genótipo ideal, por apresentar a maior concentração de zinco no grão e alta estabilidade conforme a análise GGE-Biplot, seguido das linhagens MNC04-774F-78 e MNC04-782F-108.

Palavras-chave: *Vigna unguiculata*. Micronutriente. Biofortificação. Interação genótipos x ambientes.

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INTRODUCTION

Zinc is an important mineral in various areas of the human body and is a cofactor in various enzymes and proteins. Zinc deficiency affects the immune system, and DNA synthesis; prevents combat of free radical formation; causes delayed growth and sexual maturation, hypogeusia, acrodermatitis enteropathica, alopecia, diarrhea, and rashes; and decreases appetite, and cognitive functions (PEREIRA *et al*., 2011).

Sufficient amounts of zinc in the diet promotes immunity, resistance to infection, adequate growth, development of the nervous system, and is essential for a healthy pregnancy. Approximately 17.3% of the world's population is at risk of zinc deficiency due to dietary inadequacy. Other estimates indicate that 25-33% of some populations in the world present inadequate zinc consumption and suffer from health problems due to the lack of zinc in their diet (HOTZ; BROWN, 2004; WESSELLS; BROWN, 2012).

Some solutions have been used to address these micronutrient deficiencies, such as fortification, enrichment, or addition of micronutrients in foods; this aims to increase nutrient concentration and to prevent nutritional deficiencies in the general population and more susceptible groups (VELLOZO; FISBERG, 2010).

Another way to combat these nutrient deficiencies is biofortification. This strategy consists of an increase in the concentration of nutrients by genetic breeding. Research has shown that biofortified foods are an alternative source for the low-income population to access nutritious food in a sustainable and low-cost way (GONÇALVES *et al*., 2015).

The development of biofortified cowpea cultivars in iron and zinc is one of the objectives of the cowpea breeding program (FREIRE FILHO *et al*., 2011). The development of biofortified cultivars as a strategy to combat micronutrient deficiency has several stages: germplasm screening, selection of parents, crossings, obtaining of segregant populations, advancing generations, multi-local validation of lines, and release of the cultivar.

The cowpea biofortification program had initially concentrated its efforts on the screening of existing germplasm in the work collection, and then on accessions of the active germplasm bank, with emphasis on the iron and zinc concentrations in the grain, combined with yield and adaptability to the cultivation regions (ROCHA *et al*., 2011a). The results have shown considerable variability in the zinc concentrations in the grain (DIAS-BARBOSA, 2015; MURANAKA *et al*., 2016; ROCHA *et al*., 2008; ROCHA *et al*., 2011b).

In the final stage of a breeding program, the best lines are tested and validated in various environments. This allows an investigation of the magnitude of the genotype × environment interaction (G×E) as well as the genotype's adaptability and stability (HONGYU *et al*., 2015; MURANAKA *et al*., 2016; SANTOS *et al*., 2015). These studies are important because they assist the breeder when recommending cultivars to farmers.

There are several methodologies to evaluate the adaptability and stability of genotypes; those with a multivariate approach is used the most. Among the most commonly used methodologies for cowpea is the AMMI (BARROS *et al*., 2013; DDAMULIRA *et al*., 2015) and GGE-Biplot (AKANDE; BALOGUN, 2009; CARVALHO, 2015; OLAYIWOLA; SOREMI; OKELEYE, 2015; OKORONKWO; NWOFIA, 2016; SANTOS *et al*., 2016); both are based on Biplot graphs, generated from matrices of means of genotypes obtained in each environment. These methodologies have been most used in cowpea in studies on adaptability and stability for grain yield.

Studies on the adaptability and stability of cowpea genotypes to nutrient concentrations in grain are scarce. Ddamulira *et al.* (2015) used the AMMI analysis to evaluate the adaptability and stability of genotypes for protein concentration. However, studies in this line of research—with minerals and use of GGE-Biplot method—were not found in other studies. Thus, the objective of this study was to evaluate the adaptability and stability of the zinc density in the grain of 12 cowpea genotypes in four environments in the states of Piauí (PI) and Maranhão (MA), Brazil, by using the GGE-Biplot method.

MATERIAL AND METHODS

Twelve genotypes of cowpea were evaluated—10 lines and two cultivars (controls)—from the Embrapa Mid-North Cowpea Breeding Program (Table 1). The lines were selected from a screening of 30 genotypes, based on iron and zinc concentrations in the grain (DIAS-BARBOSA, 2015).

The validation tests for grain zinc concentrations were conducted in the dry season in three locations in the state of Piauí (PI)—São João do Piauí, Campo Grande do Piauí, and Parnaíba—and in one location, Balsas, in the state of Maranhão (MA), Brazil (Table 2), during the 2014/2015 crop season. Sowing in Campo Grande do Piauí occurred in February, and in São João do Piauí, Parnaíba, and Balsas, sowing was in March.

The experiments were conducted in a randomized complete block design with 12 treatments and four replications. The treatments were represented by a plot

Table 1 - Cowpea genotypes evaluated and their genealogy, commercial subclass (CS), and growth habit (GH)

GC	Line/Cultivar	Genealogy	CS	GH
G1	MNC04-769F-55	CE-315 × TE97-304G-12	ML	SP
G2	MNC04-782F-108	(TE97-309G-24 × TE96-406-2E-28-2) × TE97-309G-24	SV	SP
G3	MNC04-774F-78	TE97-309G-18 × TE97-304G-4	ML	SP
G4	MNC04-795F-158	MNC99-518G-2 × IT92KD-279-3	SV	SP
G5	MNC04-774F-90	TE97-309G-18 × TE97-304G-4	SV	SP
G6	MNC04-769F-45	CE-315 × TE97-304G-12	SV	SP
G7	MNC04-769F-31	CE-315 × TE97-304G-12	ML	SP
G8	MNC04-769F-26	CE-315 × TE97-304G-12	SV	SP
G9	MNC04-792F-146	MNC00-553D-8-1-2-3 × TV × 5058-09C	ML	SP
G10	MNC04-762F-9	TE96-282-22G × (Te96-282-22G × Vita7)	BL	SP
G11	BRS Xiquexique (C)	TE87-108-6G × TE87-98-8G	BL	SP
G12	BRS Tumucumaque (C)	TE96-282-22G × IT87D-611-3	BL	SP

GC = Genotype code; C: Control; ML = Mulato, SV = Sempre-verde, BL= Branco Liso; SP = Semi-prostrate

Table 2 - Geographical coordinates, soil types and biomes of the locations used for the cowpea experiments, 2015

Code	Location	Altitude	Latitude	Longitude	Soil	Biome
SPPI	São João do Piauí/PI	316 m	08º20’ S	42º19’ W	QN	Caatinga
CGPI	Campo Grande do Piauí/PI	425 m	07º08’ S	41º02’ W	QN	Caatinga
PAPI	Parnaíba/PI	5 m	02°57’ S	41°43’ W	QN	Costeiro
BAMA	Balsas/MA	324 m	07º54’ S	45º96’ W	YL	Cerrado

QN = Quartzeneic Neosol; YL = Yelow Latosol. Source = Wikipédia (2016), Embrapa (1999)

of 1.5 m x 5.0 m with three 5.0 m rows spaced 0.50 m apart with 0.25 m between plants, and the evaluation area consisted of the central row. Four seeds were sown per hole. The plants were thinned 20 days after sowing, leaving two plants per hole. The experimental areas were not fertilized.

Conventional soil preparation with one plowing and one harrowing were used. Weed control was carried out with the herbicide Dual Gold (1.2 L ha^{-1}); it was applied in pre-emergence in Balsas-MA and Parnaíba-PI, and in all studied locations, two manual weeding sessions were carried out during the crop cycle. Insect pest control was carried out with two applications of the insecticides Dimetoate (20 mL c.p. 20-L $water^{-1}$) and Thiamethoxam (5 g c.p. 20-L $water^{-1}$). The genotypes were harvested at the physiological maturity stage, when the pods were completely dry and the grains had 15% moisture. The grains were sun dried to reduce their moisture to 13%—ideal moisture for storage; this was determined by a moisture analyzer (GEHAKA AGRI, G650).

Grain samples of each genotype were randomly collected, washed, ground, and 200 mg were put into a digestion tube; then, 5 ml of the digest solution (2:1 nitro-perchloric solution) was added into it. The tubes were placed in the digester block for approximately two hours until reaching 200 °C. After digestion, the extracts were transparent and clear with an approximate volume of 2 mL.

After the digestion step, extract received distilled water until reaching 20 mL. Afterwards, the extract was homogenized for readings in an atomic flame absorption spectrophotometer (GBC, B462) by using the specific wavelength of the zinc element in the software of the device. The zinc densities were obtained in parts per million (ppm) and then converted to mg 100 g^{-1}.

Individual analysis of variance were performed for the environments, and joint analysis of variance for the zinc concentration. The joint analysis of variance considered the effect of genotypes as fixed, and the effect of environments as random. The statistical model used followed the equation:

$$Y_{ijk} = \mu+g_i+e_j+ge_{ij}+\beta_{k(j)}+\varepsilon_{ijk} \quad (1)$$

wherein Y_{ijk} is the observed value of the genotype *i* in the environment *j* and block *k*; *μ* is the overall mean of the trait; g_i is the effect of the genotype i; e_j is the effect of the environment j; ge_{ij} is the effect of the interaction of the genotype i with the environment j; $\beta_{k(j)}$ is the effect of the block k within the environment j; and ε_{ijk} is the experimental error associated with the plot ijk.

The GGE-Biplot methodology (YAN; KANG, 2003; YAN, 2011) was used for the adaptability and stability analyses by applying the double entry table containing the phenotypic means of the genotypes (G) in each environment (E). In other words, GGE is the matrix of the effects of genotypes plus the effects of G×E. The matrix of adjusted means of the G and GE effects was decomposed by SVD (Singular Value Decomposition) with the objective of analyzing the multiplicative part of the GGE-Biplot model, as follows:

$$GGE = \sum_{k=1}^{n} \lambda_k \gamma_{ik} \alpha_{jk} + \rho_{ij} \quad (2)$$

wherein λ_k is the singular value of the k^{th} axis of the principal component analysis; γ_{ik} and α_{jk} are the k^{th} eigenvectors of the n principal components of genotypes and environments retained in the model; and ρ_{ij} is the residue associated with the multiplicative term GGE.

Yan and Tinker (2006) proposed an Information Ratio (IR) to evaluate the suitability of a Biplot in displaying the patterns of a double-entry table with *g* (genotypes) and *e* (environments). The IR can be calculated for each Principal Component (PC), which is the ratio of the total variance explained by each PC multiplied by *k*. The interpretation is as follows: a PC with IR>1 contains patterns—associations between environments—and a PC with IR<1 does not contain any pattern. The dimension-2 Biplot (PC1×PC2) adequately represents the patterns in the data if at least one of the first two PCs has an IR>1.

The GGE-Biplot method was performed computationally in the R environment (R Development Core Team, 2014) from a script that used the R agricolae (MENDIBURU, 2014) and GGEBiplotGUI (FRUTOS; GALINDO; LEIVA, 2014).

During the analysis in the R environment—generating of the Biplot graphics by the GGEBiplotGUI package—the model selected was: *no scaling*, with G+GE centered by the tester (tester-centered G+GE), which represents the GGE model; the method by singular partitioning value (SPV) type column metric preserving; and the Biplot type PC1×PC2, which shows the first against the second principal component.

RESULTS AND DISCUSSION

The joint analysis of variance of the grain zinc concentration of the cowpea genotypes is presented in Table 3. The genotypes differed significantly (p = 0.02%) in the zinc concentration, which denotes the zinc variability, and the possibility for selection and breeding. Although some genotypes show some relation in terms of genetic origin and commercial subclass (Table 1), a significant variation was observed in the grain zinc concentration.

Rocha *et al*. (2008, 2011b) and Dias-Barbosa (2015) evaluated cowpea genotypes and also reported the presence of genetic variability in this mineral. However, most of these studies were conducted in a single environment, which may have inflated the estimates of genetic variance as a result of G×E.

The environments also differed for the zinc concentration ($p < 2.2e^{-16}$) (Table 3), showing that the eco-geographic characteristics of the test locations (Table 2), combined with climatic variations, were different for this mineral. Environments that differ for this mineral were also observed by Rocha *et al*. (2011b), who evaluated the eight cowpea genotypes' adaptability and stability in three environments in the North and Northeast regions of Brazil.

Table 3 - Joint analysis of variance for grain zinc concentration of 12 cowpea genotypes evaluated in four environments of the Mid-North region of Brazil, 2015

Source of variation	DF	Mean square	p (>F)
Blocks/Environments	8	0.3392	0.0133*
Genotypes (G)	11	0.274	<2.2e-16***
Environments (E)	3	5.2307	0.0002***
G×E	33	0.1815	0.0004***
Residue	44	0.0749	
CV (%)		5.8579	

p = probability; Significance code: 0 "***" 0.001 "**" 0.01 "*" 0.05 "·" 0.1 " " 1. CV = Coefficient of variation; DF = Degrees of freedom

The G×E for zinc concentration was highly significant ($p = 0.0004$) (Table 3); this shows the different behavior of this mineral in genotypes depending on the testing environment. Rocha *et al*. (2011b) evaluated a group of six cowpea genotypes in three environments in the Northeast region of Brazil; and Muranaka et al. (2016) evaluated 20 cowpea genotypes in Nigeria during two crop seasons. Both studies found complex G×E for the zinc concentration in cowpea grains.

According to Hongyu *et al*. (2015) four main objectives for multi-environment data (MET) can be carried out by the GGE-Biplot method: 1) investigate the mega-environment to understand the target environment; 2) evaluate genotypes; 3) evaluate the testing environments within each mega-environment; and 4) understand the causes of G×E. The analysis of adaptability and stability of the zinc concentration based on this methodology is presented in Table 4 and Figures 1, 2, 3, and 4.

It was observed that the principal components analysis (PCA) decomposed the effect of genotypes + G×E (GGE) into four principal components (PC); the first two, PC1 and PC2, explained 49.73% and 28.08% of the total variation, respectively, exploring 77.81% of the GGE variation (Table 4).

According to the IR of the four PC, only the first two (PC1 and PC2) contain patterns (IR>1). Therefore, the Biplot is considered adequate to represent the data pattern. According to Yan and Tinker (2006), the dimension-2 Biplot adequately represents the patterns in the data, if only the first two PCs have an IR≥1. The existence of patterns in the first two PCs confirms the results of the F test of the joint analysis, which detected a significant difference for the effect of the G×E. This shows that the genotypes behaved differently depending on the testing environment with a magnitude that justifies the use of the GGE-Biplot method.

The variation explained by the first two PCs of 77.81% (Table 4) indicates that the singular value decomposition of the effects of the GGE matrix, showed high efficiency. Different results were found by Akande and Balogun (2009), who evaluated 10 cowpea genotypes in three locations for two years in Nigeria, Carvalho (2015), who evaluated 20 cowpea genotypes in 82 environments in the North, Northeast, and Center-West regions of Brazil. Both studies found that the first two axes of the GGE-Biplot method explained a low percentage (57.61% and 34.5%, respectively) of the GGE effects. However, Okoronkwo and Nwofia (2016), who evaluated the yield adaptability and stability of cowpea genotypes in Nigeria by using the GGE-Biplot, also found high efficiency for this method with the first two principal components explaining 100% of the total GGE variation. These differences, regarding the efficiency percentage of the methodology, in explaining the GGE variation depend on the trait, genotypes, and environments (locations and years) evaluated.

The mean zinc concentrations of the 12 cowpea genotypes obtained in the four testing environments are shown in Table 5. The zinc concentration ranged from 3.66 mg 100 g^{-1} (G10 MNC04-792F-146, BAMA) to 5.66 mg 100 g^{-1} (G11 BRS Xiquexique, PAPI). This variation was greater than that observed by Muranaka *et al*. (2016), who evaluated 240 cowpea genotypes with results ranging from 3.27 to 4.78 mg 100 g^{-1}. The overall mean found in this study (4.45 mg 100 g^{-1}) is similar to that found by Rocha *et al.* (2011b) (4.38 mg 100 g^{-1}), who evaluated 20 lines of black eye cowpea.

The superiority of zinc concentration in the cultivar BRS Xiquexique was also found by Rocha *et al.* (2008), who evaluated 42 cowpea genotypes in Teresina, Piauí, and found a mean of 5.36 mg 100 g^{-1}, which is higher than that found in this study. The environments Campo Grande do Piauí-PI (CGPI) and Parnaíba-PI (PAPI) showed the highest zinc concentrations in the grain and the environment São João do Piauí-PI (SJPI) had the lowest concentrations; the later was an unfavorable environment for the grain zinc concentration.

Soil analyses of SJPI, CGPI, PAPI, and Balsas-MA (BAMA) showed zinc concentration in mg dm^{-3} of 2.29; 0.49; 0.63; and 0.62 respectively, denoting that even in environments with lower zinc content in the soil, some

Table 4 - Explained and accumulated ratio, and Information Ratio (IR) of the four Principal Components (PC) resulted from the Principal Component analysis, for the grain zinc concentration in 12 cowpea genotypes in four environments of the Mid-North region of Brazil, 2015

PC	Explained variation (%)	Accumulated variation (%)	IR
1	49.73	49.73	1.99
2	28.08	77.81	1.12
3	17.41	95.22	0.70
4	4.78	100.00	0.19

Table 5 - Averages zinc concentrations in the grains of 12 cowpea genotypes evaluated in four environments of the Mid-North region of Brazil, 2015

Line/Cultivar	Genotype	SJPI (mg 100 g^{-1})	Environment			
			CGPI (mg 100 g^{-1})	PAPI (mg 100 g^{-1})	BAMA (mg 100 g^{-1})	Overall mean
MNC04-769F-55	G1	3.89	4.41	4.90	4.19	4.35
MNC04-782F-108	G2	3.93	4.30	5.26	4.17	4.42
MNC04-774F-78	G3	3.94	4.53	5.25	4.22	4.49
MNC04-795F-158	G4	3.79	4.37	4.88	4.30	4.34
MNC04-774F-90	G5	4.57	4.74	4.77	3.96	4.51
MNC04-769F-45	G6	4.19	4.38	4.94	4.02	4.38
MNC04-769F-31	G7	4.46	4.44	4.63	4.03	4.39
MNC04-769F-26	G8	3.95	4.65	4.91	3.99	4.37
MNC04-792F-146	G9	3.95	4.52	4.72	3.66	4.21
MNC04-762F-9	G10	4.07	4.24	4.47	4.42	4.30
BRS Xiquexique(1)	G11	4.11	5.02	5.66	4.51	4.82
BRS Tumucumaque(1)	G12	4.16	4.48	4.76	4.59	4.50
Overall mean		4.08	4.51	4.93	4.26	4.45

[1]Control. SJPI = São João do Piauí, CGPI = Campo Grande do Piauí and PAPI = Parnaíba, PI, Brazil; BAMA = Balsas, MA, Brazil

genotypes had the ability to extract and concentrate zinc in the grains. The nutrient uptake by the plant is complex, and are affected by the availability of nutrients and water, climate, genotype, cropping systems, and yield level.

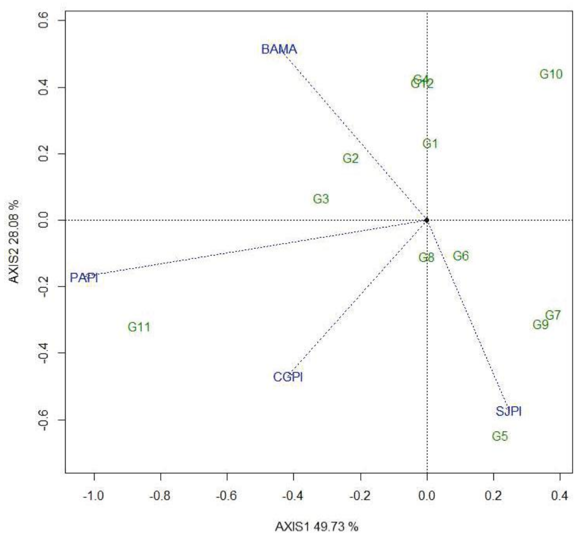
The classification of genotypes based on performance in the environments, and of environments on the genotype performances is shown in Figure 1. Genotypes G1, G4, G10 and G12 formed obtuse angles with all of the environments except BAMA; these genotypes had lower average zinc concentration in most environments. This information is confirmed in Table 3.

Genotypes G2, G3, and G8 formed an acute angle with all the environments, except SJPI (G2 and G3) and BAMA (G8); therefore, this group of genotypes concentrated more zinc in the grain than average in most of the testing environments. The genotype G11 (BRS Xiquexique) formed an acute angle with all environments, also concentrating more zinc in the grain than the average in all environments.

The other genotypes presented acute and obtuse angles, respectively, in two environments each, with zinc concentrations higher than the average in two environments and less than the average in the other two environments. Therefore, this presents specific adaptability to these environments.

According to the average × stability of zinc concentration (Figure 2), the genotypes presented the following zinc concentration in decreasing order:

Figure 1 - GGE-Biplot for the zinc concentrations of 12 cowpea genotypes evaluated in four environments of the Mid-North region of Brazil, 2015



G11>overall mean>G3>G2>G5>G8>G6>G1>G4 = G12>G9>G7>G10. The genotype G11 (BRS Xiquexique) and the line G3 (MNC04-762F-9) stood out with closer means to the average environment. Rocha *et al*. (2011a) evaluated the adaptability and stability of six cowpea genotypes for the grain iron and zinc concentrations, and also found superiority of BRS Xiquexique for zinc concentration.

Figure 2 - Average × stability of zinc concentration with the mean environmental axis (MEA), resulted from the GGE2 Biplot analysis of 12 cowpea genotypes evaluated in four environments of the Mid-North region of Brazil, 2015

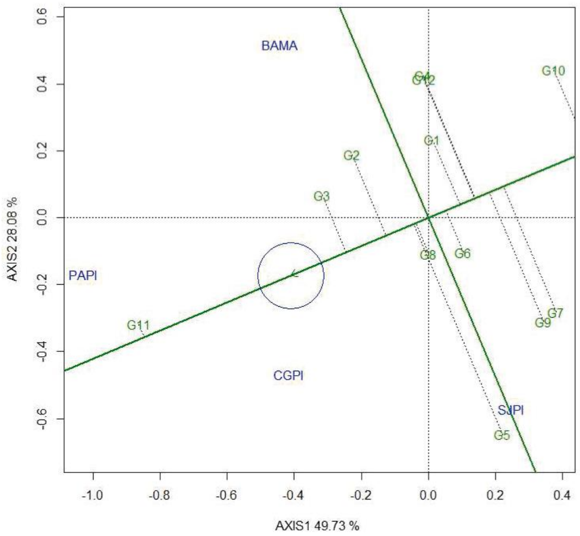
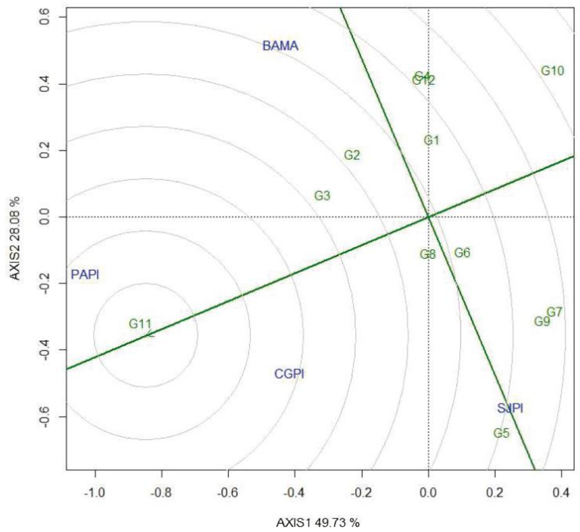


Figure 3 - Zinc concentration of 12 cowpea genotypes evaluated in four environments of the Mid-North region of Brazil in relation to an ideal hypothetical genotype resulting from GGE2 Biplot analysis, 2015



The genotypes that had zinc concentration above the average were more stable (line with two arrows perpendicular to the MEA), namely in descending order: G11>G8>G3>G2>G5 (Figure 2). G5 was highly unstable (greater projection in relation to the MEA - dotted green line), presenting a lower zinc concentration than expected in the SJPI environment, and higher iron concentrations in the PAPI and CGPI environments.

The genotype G11 (BRS Xiquexique) had the highest zinc concentration and was the most stable. Therefore, G11 (control) was the ideal genotype for selection and recommendation as a biofortified genotype with zinc, which was expected for this trait, followed by the G3 line (MNC04-774F-78). Rocha *et al*. (2011a) evaluated the stability of six cowpea genotypes in three environments and observed a similar result regarding the stability of the cultivar BRS Xiquexique, but also identified high stability of the cultivar BRS Tumucumaque; however, in the present study, this mineral was unstable in this cultivar.

Genotypes located closest to the center of circumscribed circles are the most desirable (Figure 3).

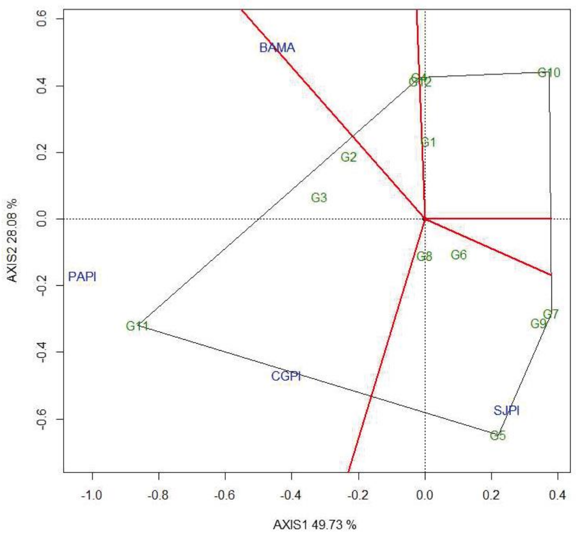
Thus, G11 was the ideal genotype regarding grain zinc concentrations in this dataset, followed by G3, G2, and G8. Although G3 and G2 had zinc concentrations well above G8, they were less stable than G8 according to Figures 2 and 3. The least recommended genotype as a biofortified zinc genotype was G10, with a greater distance from the center of the circumscribed circles, i.e., it is less able to concentrate the zinc in the grain. According to Yan and Tinker (2006) and Yan (2011), a stable genotype is desired only when it presents high average performance for the characteristics being studied.

Among all genotypes evaluated, G11 (BRS Xiquexique) is the only one that combines genes for stability and adaptability at the same time. Genotypes with these characteristics can be grown in a wide range of environments maintaining a high zinc concentration in the grain. Rocha *et al*. (2011b) also found high stability in this cultivar for grain zinc concentration in a study conducted with six genotypes in three environments.

Figure 4 shows that the vertices of the polygon are formed by genotypes G11, G5, G7, G10, and G4. The four environments were cut in 2 mega-environments by the lines that left the biplot origin and classified in two groups of environments: (I) CGPI, PAPI, BAMA, and (II) SJPI. The genotype G11 (BRS Xiquexique) is the vertex of the mega-environment I in which the environments PAPI, CGPI, BAMA were placed; therefore, it is the genotype that had better performance in this mega-environment and the most adapted in this group, followed by the genotypes G2 and G3.

G5 and G7 are vertices of the mega-environment II in which the SJPI environment was placed; therefore, these genotypes are more adapted to this group (Figure 4). In the sectors with G1, G4, G10, and G12, which do not

Figure 4 - Mega-environment (which-won-where) of the zinc concentration resulting from GGE2 Biplot analysis of 12 cowpea genotypes evaluated in four environments of the Mid-North region of Brazil, 2015



contain environments, the genotypes do not present high zinc concentration in any of the testing environments, i.e., these genotypes are the worst genotypes, considering all environments. The number of mega-environments depends on the number and characteristics of the genotypes and environments and the nature of the trait studied.

CONCLUSIONS

1. The environment Parnaíba PI, Brazil, is the most suitable for the selection of adapted genotypes for grain zinc concentration;
2. The cultivar BRS Xiquexique and the lines MNC04-774F-78, MNC04-782F-108, MNC04-769F-26 presented the highest grain zinc concentrations, combined with good adaptability and stability; therefore, these lines are promising as zinc biofortified cultivars for the states of Piauí and Maranhão, Brazil.

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REFERENCES

AKANDE, S. R.; BALOGUN, M. O. Multi-locational evaluation of cowpea grain yield and other reproductive characters in the forest and Southern Guinea Savanna agro-ecologies of Nigeria. **Electronic Journal of Environmental, Agricultural and Food Chemistry**, v. 8, n. 7, p. 526-533, 2009.

BARROS, M. A. *et al*. Adaptabilidade e estabilidade produtiva de feijão-caupi de porte semiprostrado. **Pesquisa Agropecuária Brasileira**, v. 48, n. 4, p. 403-410, 2013.

CARVALHO, L. C. B. **Interpretação da interação genótipos x ambientes em feijão-caupi usando modelos multivariados, mistos e covariáveis ambientais**. 2015. 115 f. Tese (Doutorado em Genética e Melhoramento de Plantas) - Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 2015.

DDAMULIRA, G. *et al*. Grain yield and protein content of Brazilian cowpea genotypes under diverse Ugandan environments. **American Journal of Plant Science**, v. 6, p. 2074-2084, 2015.

DIAS-BARBOSA, C. Z. M. C. **Seleção de linhagens elite de feijão-caupi (*Vigna unguiculata* (L.) Walp.) para biofortificação de ferro e zinco e características físico-químicas do grão**. 2015. 72 f. Dissertação (Mestrado em Alimentos e Nutrição) - Universidade Federal do Piauí, Teresina, 2015.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. Centro Nacional de Pesquisa de Solos. **Sistema brasileiro de classificação de solos**. Rio de Janeiro: Embrapa Solos, 1999. 412 p.

FREIRE FILHO, F. R. *et al*. **Feijão-caupi no Brasil**: produção, melhoramento genético, avanços e desafios. Teresina: Embrapa Meio Norte, 2011. 84 p.

FRUTOS, E.; GALINDO, M. P.; LEIVA, V. An interactive biplot implementation in R for modeling genotype-by-environment interaction. **Stochastic Environmental Research and Risk Assessment**, v. 28, n. 7, p. 1629-1641, 2014.

GONÇALVES, A. S. F. *et al*. Uso da biofortificação vegetal: uma revisão. **Revista do Centro Universitário de Patos de Minas**, v. 6, p. 75-87, 2015.

HONGYU, K. *et al*. Comparison between AMMI models and GGE biplot for multi-environment trials. **Revista Brasileira de Biometria**, v. 33, n. 2, p. 139-155, 2015.

HOTZ, C.; BROWN, K. H. **Assessment of the risk of zinc deficiency in populations and options for its control**. International Zinc Nutrition Consultative Group. (Technical Document, 1). 2004. Disponível em: <http://izincg.org/files/izincgtechdocfnb2004.pdf>. Acesso em: 13 maio 2016.

MENDIBURU, F. **Agricolae**: statistical procedures for agricultural research. R package, version 1.2-1, 2014.

MURANAKA, S. *et al*. Genetic diversity of physical, nutritional and functional properties of cowpea grain and relationships among the traits. **Plant Genetics Resouces: Characterization and Utilization**, v. 14, n. 1, p. 67-76, 2016.

OKORONKWO, C. M.; NWOFIA, G. E. Yield stability and inter relationships between seed yield and associated traits of 25 cowpea (*Vigna unguiculata* [L.] Walp.) genotypes. **African Journal of Agricultural Science and Technology**, v. 4, n. 5, p. 728-734, 2016.

OLAYIWOLA, M. O.; SOREMI, P. A. S.; OKELEYE, K. A. Evaluation of some cowpea (*Vigna unguiculata* [L.] Walp.) genotypes for stability of performance over 4 years. **Current Research in Agricultural Sciences**, v. 2, n. 1, p. 22- 30, 2015. Parnaíba>; <https://pt.wikipedia.org/wiki/São_João_do_Piauí>. Acesso em: 18 nov. 2016.

PEREIRA, T. C. *et al*. Research on zinc blood levels and nutritional status in adolescents with autoimmune hepatitis. **Arquivos de Gastroenterologia**, v. 48, n. 1, p. 62-65, 2011.

R DEVELOPMENT CORE TEAM. **R**: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2014. Disponível em: <http://www. R-project.org>. Acesso em: 21 maio 2016.

ROCHA, M. M. *et al*. **Avaliação dos conteúdos de proteína, ferro e zinco em germoplasma elite de feijão-caupi**. Teresina: Embrapa Meio-Norte, 2008. 4 p. (Embrapa Meio-Norte. Comunicado Técnico, 212).

ROCHA, M. M. *et al*. **Avaliação dos teores de ferro, zinco e proteína em linhagens de feijão-caupi da classe comercial branca, subclasse fradinho**. Teresina: Embrapa Meio-Norte, 2011b. 4 p. (Embrapa Meio-Norte. Comunicado Técnico, 226).

ROCHA, M. M. *et al*. Adaptabilidade e estabilidade de genótipos de feijão-caupi para teores de ferro e zinco nos grãos. *In*: REUNIÃO DE BIOFORTIFICAÇÃO NO BRASIL, 4., 2011, Teresina. **Anais...** Rio de Janeiro: Embrapa Agroindústria de Alimentos; Teresina: Embrapa Meio-Norte, 2011a.

SANTOS, A. *et al*. Adaptabilidade e estabilidade de genótipos de feijão-caupi ereto via REML/BLUP e GGE Biplot. **Bragantia**, v. 75, n. 3, p. 55-62, 2016.

SANTOS, A. *et al*. Adaptability and stability of cowpea genotypes to Brazil Midwest. **African Journal of Agricultural Research**, v. 10, n. 41, p. 3901-3908, 2015.

VELLOZO, E. P.; FISBERG, M. A. Contribuição dos alimentos fortificados na prevenção da anemia ferropriva. **Revista Brasileira de Hematologia e Hemoterapia**, v. 32, n. 2, p. 140-147, 2010.

WESSELLS, K. R.; BROWN, K. H. Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. **PLoS ONE**, v. 7, n. 11, p. 1-11, 2012.

WIKIPÉDIA, a enciclopédia livre. Disponível em: <https:// pt.wikipedia.org/wiki/Campo_ Grande_do_Piauí>; <https:// pt.wikipedia.org/wiki/Balsas>; <https://pt.wikipedia.org/wiki/

YAN, W. GGE Biplot vs. AMMI graphs for genotype-by-environment data analysis. **Journal of the Indian Society of Agricultural Statistics**, v. 65, n. 2, p. 181-193, 2011.

YAN, W.; KANG, M. S. **GGE Biplot Analysis**: a graphical tool for breeders, geneticists, and agronomists. Boca Raton: CRC Press, 2003. 271 p.

YAN, W.; TINKER, A. Biplot analysis of multienvironment trial data: principles and applications. **Canadian Journal of Plant Science**, v. 86, n. 3, p. 623-645, 2006.