Crop Breeding and Applied Biotechnology 7: 314-320, 2007
Brazilian Society of Plant Breeding. Printed in Brazil

Genetic variability and morphological modifications in flooding tolerance in maize, variety BRS-4154

Juliano Lino Ferreira[1*], Celso Henrique Moreira Coelho[1], Paulo César Magalhães[2], Elto Eugênio Gomes e Gama[2], and Aluízio Borém[1]

Received 04 July 2006

Accepted 28 July 2007

ABSTRACT - *Maize plants of the variety BRS 4154 - Saracura were evaluated in different cycles of recurrent selection to determine genetic gains in flooding tolerance, arranged in a random block design. The maize variety BRS 4154 in four selection cycles (1, 5, 9 and 15), together with the flooding-sensitive variety BR 107 and single cross BRS 1010 as control, were sown and evaluated. The stress caused by waterlogged soil reduced the weight of 100 seeds and grain yield but did not affect the number of kernel rows, ear length or prolificacy. Selection for yield resulted in higher yields.*

Key words: flooding, genetic variability, maize breeding.

INTRODUCTION

Approximately 6% of the earth surface is temporarily flooded. In Brazil some 33 million hectares are lowlands (alluvial or hydromorphic soils), of which around 12 million lie in the Cerrado region (Santos 1999). Waterlogged soils represent a stress condition and the cultivation of other crops than rice in flooded conditions is hardly viable.

Maize is the primary raw material for different production sectors and is tolerant to the herbicides used to control barnyardgrass (*Echinochloa spp*), the main weed on rice fields. Corn could therefore be an option for the use of lowland areas, either in succession to rice or as summer crop in less waterlogged areas. To reduce the risks for production in this agroecosystem cultivars would have to be adapted to poorly drained environments, in combination with adequate agricultural practices.

In the case of maize, a limiting factor for the development of cultivars is the lack of knowledge on tolerance or resistance mechanisms to waterlogging. The variability in tolerance, particularly in maize, was described by several authors such as Wu et al. (1987), Atwell et al. (1985), Parentoni et al. (1995), Sachs et al. (1996), Silva et al. (2005), Silva et al. (2006) and Mano et al. (2006). Moreover, Lemke-Keyes and Sachs (1989) mention line B73Ht, grown in the state of Illinois/USA, as flooding-tolerant. Besides, according to Sachs et al. (1996), this trait is controlled by few genes, and the gene action is mostly dominant.

In Brazil, a breeding program of the Centro Nacional de Pesquisa de Milho e Sorgo (CNPMS/Embrapa) targeted the formation of genetically broad-based maize composite by recombining 36 populations, in 1986. A modified stratification method of phenotypic recurrent selection was and is being used for the development of this genotype. After 12 study years, i.e., in the 12th selection cycle, this maize variety was released commercially, as BRS 4154, Saracura maize. The breeding of this cultivar focused on providing it with the capacity to support periods of occasional waterlogging.

[1] Departamento de Fitotecnia, Universidade Federal de Viçosa (UFV), 36.570-000, Viçosa, MG, Brasil. *E-mail: julianolf@yahoo.com.br
[2] Embrapa Milho e Sorgo (CNPMS), Rodovia MG 424, km 45, C.P. 151, 35.701-970, Sete Lagoas, MG, Brasil

Some papers in the literature deal with the tolerance mechanisms developed by plants of this variety, under conditions of oxygen deficit caused by temporary flooding. Melo et al. (2004) evaluated the influence of calcium application and of soil flooding on anatomical characteristics of Saracura maize leaves. Mano et al. (2006) studied morphological and anatomical factors related to aerenchyma formation in roots and the development of adventitious roots in maize and teosinte under normal conditions and temporary flooding. Romero et al. (2003) evaluated the effect of the different levels, sources and forms of calcium application in the soil, in flooded conditions, on the performance of some biophysical and morphological traits of Saracura maize plants, evaluated during flowering. In another study with cultivar Saracura, Vitorino et al. (2001) characterized flooding tolerance and alterations in pectic and hemicellulose fractions of maize mesocotyl subjected to hypoxia (control cultivar BR 107). Moreover, Lopes et al. (2005) investigated biochemical mechanisms, such as concentration of total protein oxidation and peroxidation of membrane lipids in leaves of maize plantlets of the variety BRS 4154 (Saracura), in the stages V3-V5, after the first, eighth and sixteenth selection cycle, under intermittent waterlogging.

The tolerance of Saracura to temporary waterlogging is well-documented, and a number of papers shed light on some of the tolerance mechanisms in this variety. Still, other tolerance-related traits must be investigated more carefully, for a deeper understanding of the variations that occur in the selection cycles.

MATERIAL AND METHODS

This study was conducted in an experimental area of Embrapa Maize and Sorghum, in Sete Lagoas, state of Minas Gerais (lat 19° 28' S, long 44° 15' W, 732 m asl) in lowland soil.

The four selection cycles 1, 5, 9 and 15 of the variety of maize BRS 4154 were evaluated in a randomized block design together with two waterlogging-sensitive controls, i.e., the variety BR 107 and single hybrid BRS 1010. The experiment was arranged in a random block split plot design with six treatments and four replications in conditions with supplementary irrigation (normal cultivation) and with waterlogging stress (flooded cultivation). The soil was flooded with a water level of 20 cm above the soil surface, three times a week, initially in V6 (stage when the plant has six developed leaves) before flowering, and was maintained until physiological maturity (R6). The stage V6 was used since according to results of Zaidi et al. (2004) maize is highly susceptible to waterlogging stress before reaching stage VT (tasseling).

To make the implementation of waterlogging easier, the area was leveled and divided. The experimental plots consisted of four rows of 4 m, and a spacing of 0.90 m between rows and 0.20 m between plants in the row, with a total area of 14.4 m^2. A greater number of seeds was planted and thinned to a density of 80 plants per plot. The two center rows were used for data collection.

At harvest the following data were evaluated: plant height (from the soil level to the point of insertion of the last leaf) and of the ear insertion (from the soil level to insertion height of the first ear), prolificacy (ratio between total number of ears and number of plants), ear length, ear weight, number of kernel rows, number of grains per plant, corrected moisture content of grain weight and weight of 100 seeds.

RESULTS AND DISCUSSION

The experimental data were subjected to analysis of variance (Tables 1, 2 and 3) and the means compared by Tukey's test (5% probability). Significant differences between environments were observed for ear length, number of grains per plant, weight of 100 seeds and grain weight per hectare. This environmental effect

Table 1. Analysis of variance of plant height (PH) and ear insertion height (EIH) of plants of four selection cycles of the maize variety BRS 4154 (C1, C5, C9 and C15), variety BR 107 and single hybrid BRS 1010, evaluated in two environments

Source of variation	df	Mean square	
		PH	EIH
Blocks	3	0.077672**	0.033275
Environment	1	0.004987	0.029569
Env x block	3	0.025305	0.021194
Genotypes	5	0.089085**	0.079871**
Gen x env	5	0.049754*	0.037178*
Residue	30	0.013505	0.010761
Mean		2.33	1.29
C V (%) error A		6.83	11.25
C V (%) error B		4.99	8.02

*Significant by the F test at 5%
**Significant by the F test at 1%

Table 2. Analysis of variance of the ear length (EL), ear index (EI), number of kernel rows (NKR) of plants of four selection cycles of the maize variety BRS 4154 (C1, C5, C9 and C15), variety BR 107 and single hybrid BRS 1010, evaluated in two environments

Source of variation	df	Mean square		
		EL	EI	NKR
Blocks	3	1.12	0.018889	0.72
Environment	1	7.93**	0.053333	0.33
Env x block	3	4.00*	0.006667	0.72
Genotypes	5	3.34**	0.020333	0.63
Gen x env	5	1.16	0.020333	0.23
Residue	30	0.89	0.012778	0.86
Mean		14.74	1.09	14.33
C V (%) error A		13.56	7.49	5.93
C V (%) error B		6.40	10.36	6.45

* Significant by the F test at 5%.
** Significant by the F test at 1%.

Table 3. Analysis of variance of the number of grains per plant (NGP), weight of 100 seeds (P100) in grams, and grain weight (GW) in tons per hectare; of plants of four selection cycles of the maize variety BRS 4154 (C1, C5, C9 and C15), variety BR 107 and single hybrid BRS 1010, evaluated in two environments

Source of Variation	df	Mean square		
		NGP	P100	GW
Blocks	3	8675.35	4.03	0.15
Environments	1	110620.8**	202.80**	61.43**
Env x block	3	2664.59	14.61	1.26
Genotypes	5	13982.69*	44.91**	4.26**
Gen x env	5	2615.32	3.62	0.84
Residue	30	4733.52	8.25	0.69
Mean		529.36	29.90	6.71
CV (%) error A		9.75	12.78	16.70
CV (%) error B		13.00	9.60	12.40

*Significant by the F test at 5%
**Significant by the F test at 1%

means that the genotypes respond unequally to differences in the environments. Relationships between these traits can be inferred, once they are directly related to yield. The analysis of variance for the genotypes shows that only prolificacy and the number of kernel rows did not differ significantly, suggesting that these two traits are not relevant as discriminators of genotypes in a breeding program for this environmental condition.

The genotype x environment interaction was only significant for the traits plant height and ear insertion height; in this case, the genotype classification changed according to the environment (Table 4).

The mean performances of the genotypes for plant height and ear insertion are shown in Table 4. Tukey's test (5% probability) revealed significant differences between the genotypes in both cultivation environments. Plant height and ear insertion decreased in the selection cycles 5, 9 and 15 in waterlogged soil. This demonstrates that selection effectively reduced plant height and ear insertion, which contributes to reduce lodging and to make this variety more recommendable for lowlands. Similar results in the literature were reported by Tripathi et al. (2003).

The mean values for number of kernel rows (NKR) on the ears of the genotypes, in both cultivation conditions, are presented in Table 5. No statistical differences were verified between genotypes, indicating that this trait is not an adequate discriminator in the selection of genotypes for this stress type. Besides, in this experiment the effect of the environments was non-significant, which had been expected, since the

Table 4. Means for plant height (PH) and ear insertion height (EIH), of plants of four selection cycles of the maize variety BRS 4154 (C1, C5, C9 and C15), variety BR 107 and single hybrid BRS 1010, evaluated in two environments

Genotypes	**PH** (m)		**EIH** (m)	
	Environments			
	normal	**flooded**	**normal**	**flooded**
C 1	2.30 ABa	2.45 Aa	1.25 ABb	1.43 Aa
C 5	2.40 Aa	2.40 ABa	1.39 Aa	1.35 ABa
C 9	2.45 Aa	2.43 ABa	1.35 Aa	1.33 ABa
C 15	2.40 Aa	2.18 Bb	1.30 ABa	1.13 Cb
BR 107	2.28 ABb	2.50 Aa	1.25 ABb	1.43 Aa
BRS 1010	2.08 Ba	2.18 Ba	1.08 Ba	1.15 BCa
C V (%)	4.48	5.89	7.93	9.32

Means followed by at least one same upper case letter in the columns for genotypes, or lower case letter in the rows, for environment, did not differ by the Tukey test at 5% probability.

Table 5. Means for number of kernel rows (NKR) and ear length (EL), of plants of four selection cycles of the maize variety BRS 4154 (C1, C5, C9 and C15), variety BR 107 and single hybrid BRS 1010, evaluated in two environments

Genotypes	**NKR**		**EL** (cm)	
	Environments			
	normal	**flooded**	**normal**	**flooded**
C 1	16 Aa	14 Aa	15 ABa	14 Aa
C 5	16 Aa	15 Aa	14 Ba	15 Aa
C 9	14 Aa	14 Aa	15 ABa	15 Aa
C15	16 Aa	16 Aa	15 ABaa	15 Aa
BR 107	14 Aa	14 Aa	15 Ba	13 Ab
BRS 1010	14 Aa	16 Aa	17 Aa	15 Ab
C V (%)	5.48	5.53	4.76	8.01

Means followed by at least one same upper case letter in the columns for genotypes, or lower case letter in the rows, for environment, did not differ by the Tukey test at 5% probability

environmental variance for number of kernel rows documented in the literature is small, practically insignificant (Daniel 1963).

For the trait ear length (EL), the genotypes did not differ significantly in the waterlogged environment (Table 5). Additionally, the performance of the selection cycles of BRS 4154 was not affected by the environment. In the controls the values of this trait decreased significantly, in agreement with the study of Lizaso and Ritchie (1997), who report that stress caused by excess of water reduces ear length. This yield component therefore expresses the tolerance of the selection cycles of BRS 4154 under this stress condition.

For the ear index (EI) or prolificacy, the means of the genotypes in the two cultivation conditions are given in Table 6. The amplitude of variation in the genotypes was 1 to 1.23 ears plant^{-1} in normal cultivation and 1 to 1.33 ears plant^{-1} in waterlogged soil. It is noteworthy that this variation was not significant for any source of variation in the analysis of variance. Similarly, Parentoni et al. (1995) observed a variation of 0.90 to 1.19 in lowland cultivation in the state of Minas Gerais. In the selection cycles of Saracura, these authors observed an increase of 12% in prolificacy in the waterlogged environment and further reported a reduction of this yield component in the waterlogged compared to the normal environment. The non-significant gains of prolificacy in the selection cycles in the waterlogged environment may be due to physiological processes in the presently used maize types, which in the past had undergone selection for plants with only one ear (Hallauer 1974).

The environmental effect on the trait number of grains per plant (NG/plant) was highly significant (Table 6). The values of the genotypes within each environment did not differ, although there was a clear range of variation. According to Lizaso and Ritchie (1997), waterlogging in the initial growth stages delays the receptiveness of the style/stigma of the ear more than tasseling, which can contribute to a reduction in the NG/plant, due to the loss of synchronism between the emission of the pollen grains

Table 6. Means for prolificacy (EI) and number of grains (NG) per plant, of plants of four selection cycles of the maize variety BRS 4154 (C1, C5, C9 and C15), variety BR 107 and single hybrid BRS 1010, evaluated in two environments

Genotypes	EI		NG plant $^{-1}$	
	Environments			
	normal	flooded	normal	flooded
C 1	1.00 Aa	1.03 Aa	500 Aa	465 Aa
C 5	1.23 Aa	1.00 Aa	646 Aa	503 Ab
C 9	1.23 Aa	1.10 Aa	613 Aa	503 Ab
C15	1.13 Aa	1.08 Aa	608 Aa	533 Ab
BR 107	1.10 Aa	1.03 Aa	534 Aa	422 Ab
BRS 1010	1.08 Aa	1.33 Aa	564 Aa	481 Aa
C V (%)	9.78	10.96	13.87	10.78

Means followed by at least one same upper case letter in the columns for genotypes, or lower case letter in the rows, for environment, did not differ by the Tukey test at 5% probability

and the receptivity of the style/stigma, which is confirmed in the growth of ears without grains in the tips.

For weight of 100 seeds (Table 7), the effect of genotypes differed significantly in cultivation under normal conditions; the highest value was observed for hybrid BRS 1010 and the lowest for variety BR 107. Amplitude of variation of 28.12 to 36.69 grams and of 26.37 to 31.6 grams in the normal and waterlogged environment was verified here, respectively. In the waterlogged environment the seed weight was 13% lower than in the normal environment.

With respect to the grain weight (GW) or yield (Table 7), the mean yield of the genotypes in the non-flooded environment was 7.91 t ha^{-1}, and 5.56 t ha^{-1} in the waterlogged environment, which represents an average reduction of 30%. In the waterlogged environment, cultivar BR 107 (4.22 t ha^{-1}) was the least productive, while the highest-yielding genotype was cycle 15 (6.45 t ha^{-1}). The selection gain in the waterlogged environment, from cycle 1 to cycle 15, in t ha^{-1}, was 19%. It is noteworthy that the grain yield of all genotypes was lower in the waterlogged than in the normal environment. This result is similar to that reported by Joshi and Dastane (1966), who affirm that excess soil moisture during ear formation interferes with ovule fertilization, and later, with the accumulation of grain reserves.

It was concluded that in the waterlogged condition, stress caused by excess soil moisture reduced the grain yield but had no effect on the number of kernel rows, ear length or prolificacy, while selection for number of grains per plant results in yield increase.

ACKNOWLEDGEMENTS

To CNPMS/EMBRAPA for the experimental field and lab facilities, to CNPq for the scholarship of the first and second authors, and FAPEMIG for supporting the project.

Table 7. Means for weight of 100 seeds (P100) and grain weight (GW) of plants of four selection cycles of the maize variety BRS 4154 (C1. C5. C9 and C15), variety BR 107 and single hybrid BRS 1010. evaluated in two environments

Genotypes	**P100** (g)		**GW** (t ha^{-1})	
	Environments			
	normal	flooded	normal	flooded
C 1	32.46 ABa	27.14 Ab	7.45 Aba	5.19 ABb
C 5	28.12 Ba	26.37 Aa	7.25 Ba	5.91 ABb
C 9	32.04 ABa	27.22 Ab	7.85 Aba	5.54 ABb
C 15	32.51 ABa	28.22 Aa	8.00 Aba	6.45 Ab
BR 107	29.94 Ba	26.55 Aa	6.96 Ba	4.22 Bb
BRS 1010	36.69 Aa	31.60 Ab	10.00 Aa	6.06 ABb
C V (%)	7.14	12.07	10.88	15.02

Means followed by at least one same upper case letter in the columns for genotypes, or lower case letter in the rows, for environment, did not differ by the Tukey test at 5% probability

Avaliação da variabilidade genética e modificações morfológicas para tolerância ao encharcamento do solo na variedade de milho BRS-4154

RESUMO - *Este trabalho objetivou avaliar plantas de milho dos diferentes ciclos de seleção recorrente da variedade de milho BRS 4154 – Saracura quanto aos ganhos genéticos obtidos ao longo dos ciclos de seleção sob encharcamento intermitente do solo. Quatro ciclos de seleção da variedade de milho BRS 4154 foram plantados sob delineamento em blocos casualizados nos quais foram avaliados os ciclos 1, 5, 9 e 15, incluindo a variedade BR 107 e o híbrido simples BRS 1010 como testemunhas, por serem sensíveis ao encharcamento. O estresse causado pelo excesso de água no solo diminuiu o peso de 100 sementes e o rendimento de grãos e, não houve efeito para número de fileiras de grãos, comprimento da espiga e índice de espiga. E a seleção para rendimento de graos por planta implicou em aumento de produtividade.*

Palavras-chaves: encharcamento, variabilidade genética, melhoramento de milho.

REFERENCES

Atwell BJ, Thomson CJ, Greenway H, Ward G and Waters I (1985) A study of the impaired growth of roots of *Zea mays* seedlings at low oxygen concentrations. **Plant Cell and environment 8**: 178-188.

Daniel L (1963) Analysis of inheritance of the number of kernel rows in maize. **Theoretical and Applied Genetics 33**: 290-301.

Joshi MS and Dastane NG (1966) Studies in excess water tolerance of crop plants: II. Effect of different durations of flooding at different stages of growth under different layouts on growth. **Indian Journal of Agronomy 11**: 70-79.

Hallauer AR (1974) Heritability of prolificacy in maize. **Journal of Heredity 65**: 163-168.

Lemke-Keyes CA and Sachs MM (1989) Genetic variation for seedling tolerance to anaerobic stress in maize.germplasm. **Maydica 34**: 329-337.

Lizaso JI and Ritchie JT (1997) Maize shoot and root response to root zone saturation during vegetative growth. **Agronomy Journal 89**: 125-134.

Lopes MJC, Souza IRP, Magalhães PC, Gama EEG, Alves JD and Magalhães MM (2005) Oxidação protéica e peroxidação lipídica em plantas de diferentes ciclos de seleção do milho ‘Saracura´, sob encharcamento contínuo. **Revista Brasileira de Milho e Sorgo 4**: 362-373.

Mano Y, Omori F, Takamizo T, Kindiger B, Bird R Mck and Loaisiga CH (2006) Variation for root aerenchyma formation in flooded and non-flooded maize and teosinte seedlings. **Plant and Soil 281**: 269-279.

Melo HC, Castro EM, Romero JL, Alves JD, Fries DD, Melo LA and Magalhães PC (2004) Influência da aplicação de cálcio e alagamento do solo sobre características anatômicas das folhas de milho (*Zea mays* l.) “Saracura” BRS-4154. **Revista Brasileira de Milho e Sorgo 3**: 333-342.

Parentoni SN, Gama EEG, Magnavaca R and Magalhães PC (1995) Selection for tolerance to waterlogging in maize (*Zea mays L.*). In: **Simpósio Internacional sobre Estresse Abiótico**. EMBRAPA/CNPMS, Belo Horizonte, p. 434-449.

Romero JL, Magalhães PC, Alves JD, Durães FOM and Vasconcellos CA (2003) Efeito do cálcio sobre algumas características biofísicas e morfológicas de plantas de milho BRS-4154 submetidas ao alagamento do solo. **Revista Brasileira de Milho e Sorgo 2**: 21-33.

Sachs MM, Subbaiah CC and Saab IN (1996) Anaerobic gene expression and flooding tolerance in maize. **Journal of Experimental Botany 47**: 1-15.

Santos AB (1999) Aproveitamento da soca. In: Vieira NRA, Santos AB and Sant’ Ana EP (eds.) **A cultura do arroz no Brasil**. Editora EMBRAPA, Santo Antônio de Goiás, p. 463-492.

Silva SDA, Sereno MJCM, Silva CFL and Barbosa Neto JF (2006) Capacidade combinatória de genótipos de milho para tolerância ao encharcamento do solo. **Ciencia Rural 36**: 391-396.

Silva SDA, Sereno MJCM, Silva CFL, Oliveira AC and Barbosa Neto JF (2005) Genetic parameters and QTL for tolerance to flooded soils in maize. **Crop Breeding and Applied Biotechnology 5**: 287-293.

Tripathi S, Warsi MZK and Verma SS (2003) Water logging tolerance in inbred lines of maize (Zea mays L.). **Cereal research Communications 31**: 221-226.

Vitorino PG, Alves JD, Magalhães PC, Magalhães MM, Lima LCO and Oliveira LEM (2001) Flooding tolerance and cell wall alterations in maize mesocotyl during hypoxia. **Pesquisa Agropecuaria Brasileira 36**: 1027-1035.

Wu MS, Jiang CG and Song ZW (1987) Control of waterlogging in summer maize and its effect on yield. **Jiangsu - Mongye - Kexue 4**: 13-15.

Zaidi PH, Rafique S, Rai PK, Singh NN and Srinivasan G (2004) Tolerance to excess moisture in maize (*Zea mays* L.): susceptible crop stages and identification of tolerant genotypes. **Field Crops Research 90**: 189-202.