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

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**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.

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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.

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**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.

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