Inheritance of tolerance to flooded soils in maize

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ABSTRACT - *Maize could be an excellent option for crop rotation in flooded areas in southern Brazil. There are, however, few studies on the genetic basis of tolerance to flooding in maize. The objective of this study was to determine the inheritance of this tolerance based on plant and root dry matter of young maize plants. Maize lines were crossed to form three populations in the F_1, F_2, and backcross generations. Tolerance to flooding was assessed over two years in a greenhouse. The performance of the generations showed the presence of heterosis. The variances in the segregating generations were higher than in the fixed generations throughout, indicating genetic variability for plant and root dry matter. The frequency distribution of F_2 suggested that tolerance to flooding is controlled by many genes of complementary effect.*

Key words: flooding, *Zea mays*, heritability, genetic effects

INTRODUCTION

Flooded soils in southern Brazil occupy an area of approximately 6,800,000 ha and are preferentially cropped with irrigated rice. Deficient drainage is the dominant characteristic of such soils (Klamt et al. 1985). Another relevant aspect that characterizes flooded soils is the naturally medium to low fertility. This is unfavorable for upland crops, causing poor development and low yield of these species. The main cause of these losses is the lack of free oxygen in the soil. However, plant species differ in their response to flooded soils. Plant tolerance can vary from only a few hours to many days or weeks, depending on the species, on the directly affected organs, the development stage, and the temperature conditions (Vartapetian and Jackson 1997).

Maize (*Zea mays* L.) presents genetic variability for tolerance to flooded soil (Lemke-Keyes and Sachs 1989,

Sachs et al. 1996); but there are few studies on the genetic basis of flooding tolerance. Studies by Lemke-Keyes and Sachs 1989 with seedlings of crosses of lines tolerant and sensitive to flooded soils showed that anoxia tolerance was dominant and presented simple segregation, indicating that one or two genes were involved in the manifestation of the trait. On the other hand, Schild et al. (1999) showed that maize was very sensitive to conditions of water-saturated soil and verified a direct relation of the damage with the duration of flooding.

Flooding-tolerant genotypes must be developed if maize is to become an option for crop rotation in flooded areas. Due to environmental variation, the trait is difficult to assess on the field. In addition, this trait is related with the coordinated action of morphological, anatomical and biochemical adaptations (Bucher and Kuhlemeier 1993). However root and plant dry matter,

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correlated with flooding tolerance, are easily measurable traits, which allows an estimate of flooding tolerance in young maize plants (Dias-Filho and Carvalho 2000, Lizaso et al. 2001, Vitorino et al. 2001, Dias-Filho 2002).

The objective of this study was to determine the inheritance and heritability values of flooding tolerance in maize based on the root and plant dry matter traits assessed in young plants tested in a greenhouse.

MATERIALS AND METHODS

Three maize lines of the breeding program for flooding tolerance of Embrapa Clima Temperado (Pelotas, RS) were used in this study. The lines were derived from a previously selected base population composed of subtropical germplasm with different performances regarding flooding tolerance, namely the tolerant line CT954330, called T2, and the susceptible lines CT966200 and CT966208, called S5 and S6, respectively.

In the growing season 1998/99 crosses were made to obtain three populations in the F_1 generation (T2 x S5, T2 x S6 and S5 x S6) on an experimental field of Embrapa Clima Temperado. In the growing season 1999/2000, the parents and F_1 generation were sown on an experimental field of the Faculdade de Agronomia of the Universidade Federal do Rio Grande do Sul to obtain the F_2 generation and backcrosses BC_1F_1 (BC_1) and BC_2F_1 (BC_2); the lines were used as female parents and F_1 as the male parent.

Flooding tolerance was assessed in experiments carried out in a greenhouse of Embrapa Clima Temperado in 2000 and 2001. A complete randomized design was used with two replications. Fifteen plants were assessed in each fixed generation (parents and F_1), 150 plants from the F_2 generation and 45 plants from each backcross. The genotypes were sown into 200 mL plastic cups with perforated bottom that were filled with corrected and fertilized soil. The cups were placed in wooden boxes lined with plastic to prevent water leakage. One day before sowing the boxes were filled with water up to a level of 4 cm. Under these conditions, one seed was placed approximately 2 cm deep in the soil of each cup. The plants were first flooded eleven days after emergence in 12 cm of water and kept submersed for four days. After this period of flooding, the water was drained from the box and the cups were maintained in 4 cm of water for 10 days. They were then flooded again. After four days of flooding, the excess water was removed and the experiment harvested after seven days.

The assessed traits were root dry matter (RDM) and plant dry matter (PDM). At harvest, the roots and leaves of each plant of each replication were collected separately. After washing, the material was oven-dried at 60 °C for 5 days and then weighed. The data were submitted to analysis of variance to verify the effect of replication and generation. Means were separated by Duncan's test. The means, variances and heritability were estimated based on the frequency distribution of the traits in the different generations (Allard 1999). The genetic parameters mean. [m], additivity [a], and dominance [d] were also estimated for each cross by analysis of generation means, using the expected least square method (Mather and Jinks 1982). These estimates were followed by the joint scaling test, where the observed and expected means were compared by the Chi-square test with 5 df (number of generations minus one).

RESULTS AND DISCUSSION

Plant performance varied depending on the year when the experiment was performed. Plant growth was greater in 2000, resulting in increased root and plant dry matter yields (Table 1), whereas in 2001, less development was observed and the variables analyzed presented lower values. This different performance can be explained by the environmental variations, especially temperature, which was higher in the experiment carried out in 2001. Temperatures over 27 °C are harmful to plants under flooding stress (Lemke-Keyes and Sachs 1989, Lizaso et al. 2001). According to Van Toai et al. (1985), the causes of increased susceptibility of plants suffering flooding stress under high temperatures may be the reduced solubility of oxygen in water, the higher metabolic indices in the plant, the accumulation of toxic metabolic products, the high expiration indices, or increases in pathogen activity. Another factor that may have influenced the reduction in these variables in 2001 was the use of soil without fertilization that may have resulted in plants with less vigor and higher stress sensitivity.

A joint analysis of the years was not possible due to the genotype x environment interaction although the ranking of performance of the parents remained stable over the course of the two years. Line T2 was the genotype with the greatest dry matter production, both in roots and leaves, confirming its stress tolerance, while line S6 presented intermediate performance and line S5 presented the worst performance for flooding tolerance, demonstrating high sensitivity to this factor (Table 1).

The average performance of the F_1 generation for the plant dry matter trait was greater than the parents in

Table 1. Means for the traits plant dry matter (PDM) and root dry matter (RDM) and the general mean of the experiment carried out in a greenhouse in 2000 and 2001

Genotype	PDM (mg plant[-1])		RDM (mg plant[-1])	
	2000	**2001**	**2000**	**2001**
T2	869.23 a[1]	657.31 a[1]	333.08 a[1]	285.77 a[1]
S5	406.15 c	224.12 c	180.38 c	81.37 c
S6	680.37 b	382.57 b	264.81 b	158.86 b
General mean	825.24 A	609.24 B	341.82 A	264.28 B

[1] Means followed by the same lowercase letter in the column, or the same uppercase letter in the row did not differ significantly by Duncan's test at 5% probability

Table 2. Mean (mg plant[-1]) and variance of plant dry matter for the parents and F_1, F_2, BC_1, and BC_2 generations of three maize populations evaluated in 2000 and 2001

Generation	T2 x S5		T2 x S6		S5 x S6	
	2000	**2001**	**2000**	**2001**	**2000**	**2001**
			Mean			
P_1	885.8	685.7	855.0	624.2	435.4	222.6
P_2	376.9	225.8	657.7	483.3	701.4	330.0
F_1	1,007.3	718.8	1,023.0	1,630.4	782.4	621.7
F_2	875.8	657.0	822.8	722.7	747.0	550.0
BC_1	1,205.4	1,221.2	1,202.3	628.9	727.1	454.0
BC_2	763.1	683.1	1,091.4	480.6	855.7	530.5
			Variance			
P_1	4,026.5	2,641.8	4,826.9	7,299.2	2,626.9	5,073.8
P_2	4,706.4	4,042.8	2,135.9	4,551.5	4,167.0	6,454.5
F_1	5,078.1	7,518.3	5,201.1	7,529.4	4,199.0	7,571.0
F_2	30,701.3	68,386.9	55,942.9	56,896.9	34,494.2	38,379.1
BC_1	11,026.9	32,144.3	16,552.6	31,047.5	15,745.1	27,093.9
BC_2	23,973.1	62,009.2	17,490.1	23,680.9	19,118.7	17,154.0

the three studied populations, suggesting the presence of heterosis for tolerance in the trait control (Table 2). The variances of the segregating generations were higher than those observed in the fixed generations in all cases (Table 2), indicating the presence of genetic variability for plant dry matter in the analyzed crosses. Generally, lower variances were observed in the parents and the F_1 generation, suggesting that the environments had a similar influence on all generations. The results of means and variances obtained for the variable root dry matter were similar (Table 3) and also indicated the presence of genetic variability and heterosis in the three crosses.

The analysis of frequency distributions of the T2 x S5 population showed the difference in the performances of the parents for the plant dry matter variable (Figure 1), indicating differences for flooding tolerance. The individuals in the F_1 generation were grouped in classes equal or superior to the tolerant parent, suggesting the presence of dominance of the

trait. Continuous variation and transgressive segregation was observed in the F_2 generation. These results indicated that many genes, which act in a complementary way among the parents, determine the trait. An increased dry matter content was detected in this generation, confirming the action of genetic dominance.

There was a small overlap of the parent curves in the T2 x S6 population, suggesting that line S6, with intermediate performance, also has genes for flooding tolerance (Figure 1). The F_1 generation confirmed the presence of dominance towards tolerance and the F_2 generation performed similarly to the T2 x S5 population. The F_1 generation presented much higher values in 2001 compared to the other generations, reaching values that outmatched the best individual in the F_2 generation, which can be attributed to the effects of the genotype x environment interaction. The S5 x S6 population represented the cross between two flooding-sensitive

Table 3. Mean (mg plant^{-1}) and variance of root dry matter for the parents and F_1, F_2, BC_1, and BC_2 generations of three maize populations evaluated in 2000 and 2001

Generation	T2 x S5		T2 x S6		S5 x S6	
	2000	2001	2000	2001	2000	2001
			Mean			
P_1	304.2	285.0	357.9	286.7	227.7	90.7
P_2	133.1	70.8	264.6	150.8	265.0	163.0
F_1	328.0	362.5	376.5	501.7	356.2	295.0
F_2	401.3	284.6	316.1	303.7	265.5	261.7
BC_1	641.5	411.2	606.9	318.4	332.1	208.0
BC_2	453.8	227.6	445.4	224.9	414.3	262.7
			Variance			
P_1	1,972.0	1,580.8	1,556.6	3,224.2	1,019.2	1,122.5
P_2	1,023.1	999.3	1,426.9	1,226.5	1,242.3	1,767.6
F_1	1,788.6	3.673.3	2,360.8	4,524.2	1,314.8	1,756.5
F_2	8,882.1	13.486.4	11,397.9	13,743.2	7,713.5	9,406.8
BC_1	7,197.4	11,369.3	9,973.1	8,446.2	3,741.2	6,987.8
BC_2	3,175.6	5,368.9	3,124.9	7,300.5	6,226.4	4,752.1

lines and the performance of all generations confirmed the previous results and showed that the lines had different genes for the trait (Figure 1).

For the root dry matter variable, the frequency distributions of the three populations presented similar performance to those observed for plant dry matter (Figure 2), indicating the presence of genetic variability among the parents and dominance effects. The correlation between both variables was r=83%; however, it is important to point out that this similar performance observed in the two measured variables does not necessarily mean that the same genes are involved in its expression. In this sense, Ellis et al. (1999) demonstrated the existence of distinct adaptive mechanisms for survival under hypoxia in roots and leaves, indicating that the alcohol fermentation process is essential to the roots, but not the leaves. These authors therefore suggested that the ABA hormone induces tolerance in roots only.

The present results indicated that the genotypes under study presented more than a single gene for flooding tolerance and that the dominant gene action and the complementary action of the genes were important aspects in the trait expression. There are no data in the literature regarding genetic control of the plant or root dry matter traits in plants cultivated under conditions of anaerobic stress. However, Sachs et al. (1996) assessed maize genotypes for tolerance to anaerobic stress, based on plant survival, and inferred that the predominant type of gene action was dominance, with few genes involved in the manifestation of the trait, in agreement with Boru et al. (2001) and Setter et al. (1997).

The estimates of variance showed that the genetic variances were higher for all crosses under study (Table 4), reflecting a high degree of genetic determination for both study traits, in agreement with the broad-sense heritability estimates. Additive variance, estimated by the relationship between the F_2 and the backcross generation variances was also high in all crosses, and was overestimated for the PDM variable in the T2 x S6 cross (Table 4). The narrow-sense heritability estimates were high as well, but these values may have been inflated because the genotype x environment interaction was not assessed (Table 4). In the case of the T2 x S6 cross, the narrow sense heritability was higher than one, which might be due to the genotype x environment interaction that may have caused a different response of the F_2 generation and the backcross generations, by non-allele interactions or by sampling errors (Ketata et al. 1976). There is little data in the literature for the estimate of heritability for plant and root dry matter under stress conditions.

The estimates of the genetic effects using the three-parameter model (Mather and Jinks 1982) adequately fitted to the S5 x S6 cross. The estimates of the genetic effects using the three-parameter model (Mather and Jinks 1982) adequately matched the data obtained with theS5 x S6 cross in the two years of
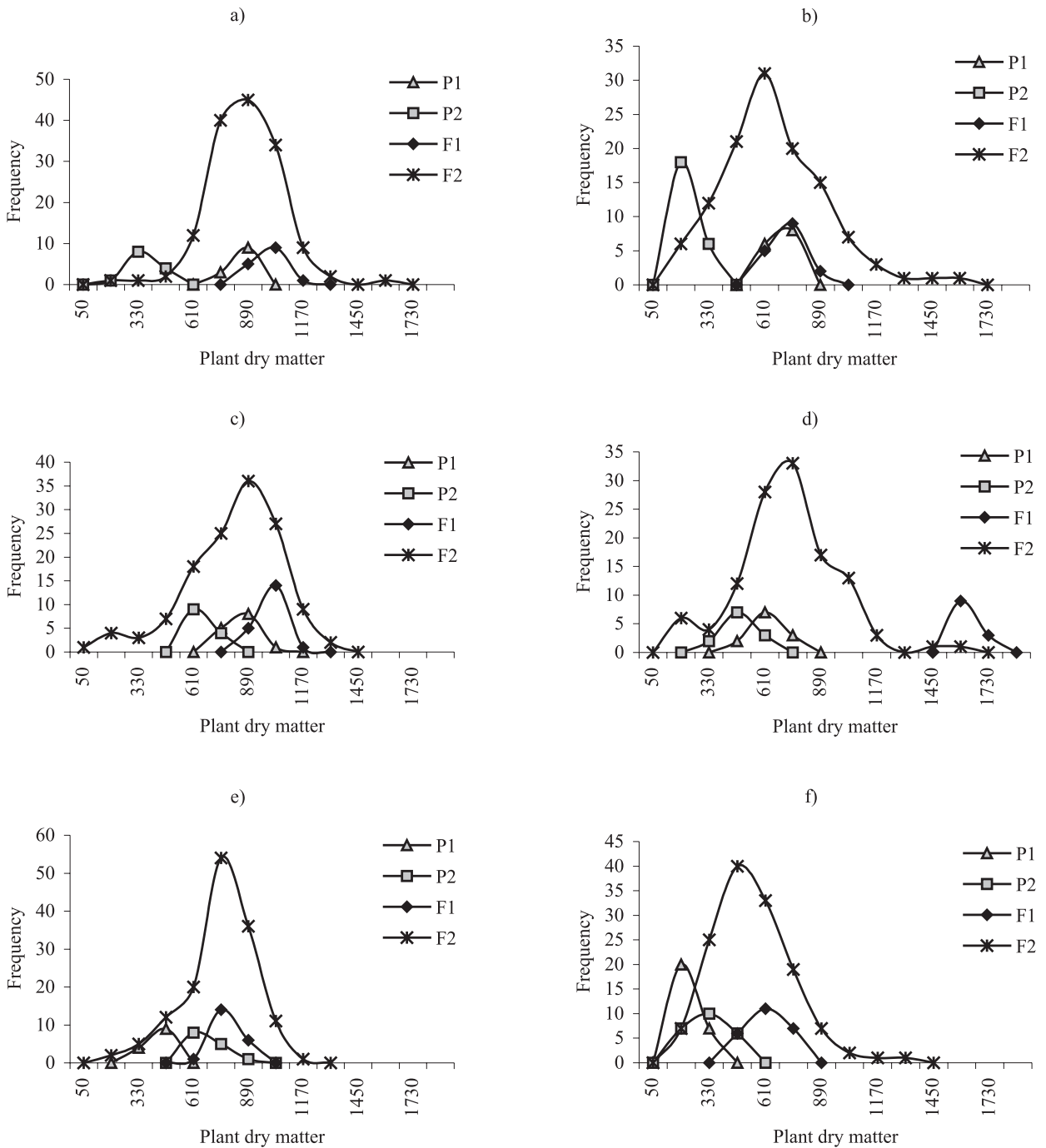
Figure 1. Frequency distributions for plant dry matter in the P_1, P_2, F_1, and F_2 generations of the T2 x S5 (a and b), T2 x S6 (c and d) and S5 x S6 (e and f) in 2000 (a, c and e) and 2001 (b, d and f)

Figure 2. Frequency distributions for root dry matter in the P_1, P_2, F_1, and F_2 generations of the T2 x S5 (a and b), T2 x S6 (c and d) and S5 x S6 (e and f) in 2000 (a, c and e) and 2001 (b, d and f)

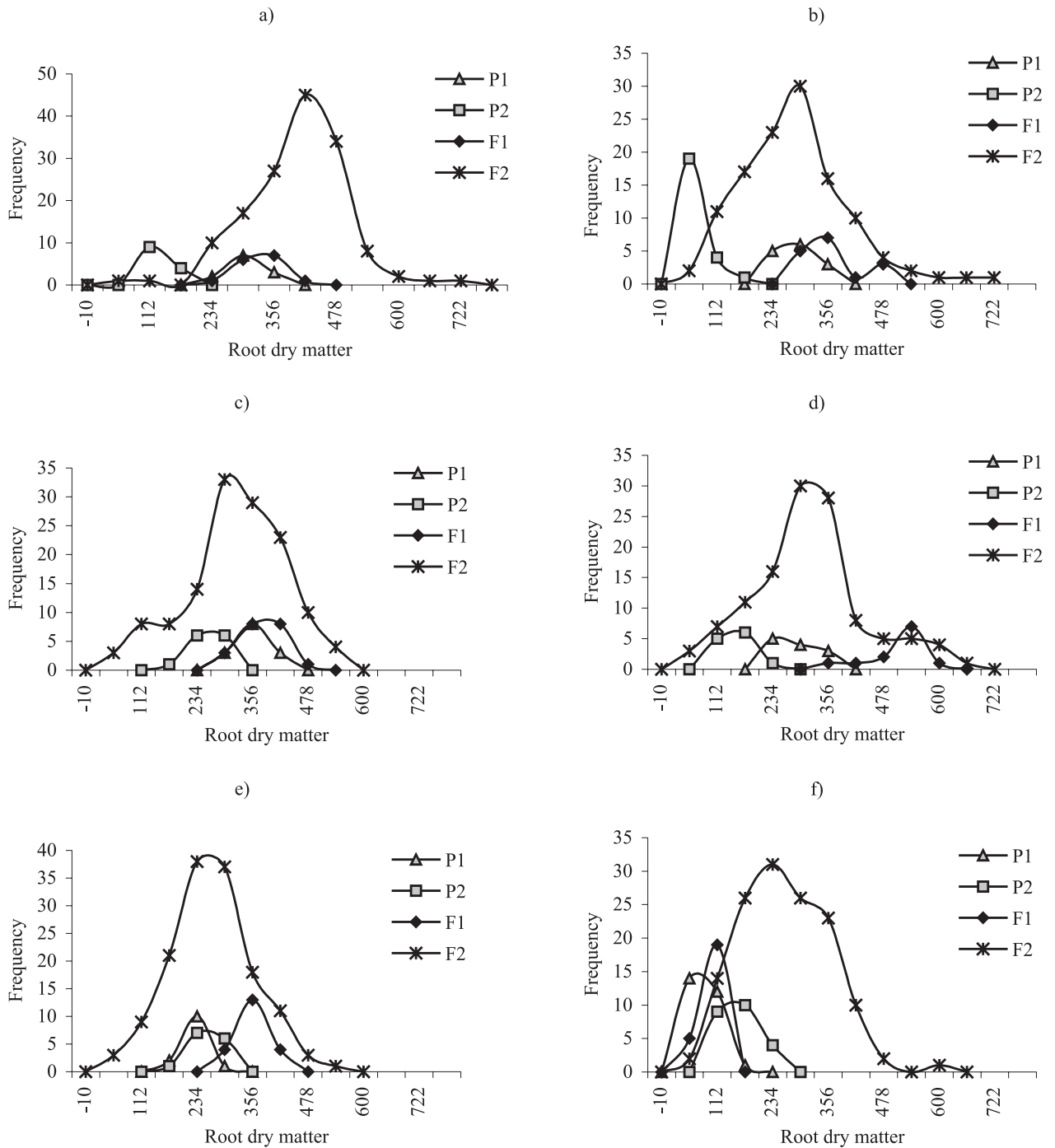**Table 4**. Phenotypic (PV), genetic (GV), and additive variance (AV), broad-sense heritability (h^2), and narrow-sense heredity (h^2_0) for the plant and root dry matter traits estimated in three maize crosses evaluated in 2000 and 2001

Parameter	T2 x S5		T2 x S6		S5 x S6	
	2000	2001	2000	2001	2000	2001
	Plant dry matter					
PV	30,701.3	68,386.9	55,942.9	56,896.9	34,494.2	38,379.1
GV	26,097.6	63,652.6	51,888.2	50,436.8	30,829.9	32,012.7
AV	26,402.5	42,620.2	77,843.1	59,065.3	34,124.7	32,510.4
h^2	0.85	0.93	0.93	0.89	0.89	0.83
h^2_0	0.86	0.62	1.39	1.04	0.99	0.85
	Root dry matter					
PV	8,882.1	13,486.4	11,397.9	13,743.2	7,713.5	9,406.8
GV	7,287.6	11,401.9	9,616.5	10,751.6	6,521.4	7,857.9
AV	7,391.2	10,234.6	9,697.9	11,739.7	5,459.4	7,073.7
h^2	0.82	0.85	0.84	0.78	0.85	0.84
h^2_0	0.83	0.76	0.85	0.85	0.71	0.75

assessment, both for PDM and RDM (Table 5). In the other crosses the model did not fit the PDM data in 2001 and RDM data in 2000. These performances were associated to effects of epitasis and to the genotype x environment interaction under more pronounced stress conditions. The results of this analysis confirmed the considerations on the frequency distributions, showing the significance of the dominance effects (Table 5).

The lower magnitude of the additive effects in all populations should be interpreted as a sign of dispersion of genes related to the manifestation of PDM and RDM in the parents, reducing the estimates of the additive effect (Table 5). This hypothesis of gene dispersion in the parents is supported by the high additive variance values observed in the crosses, which was not affected by the presence of genes that increased or decreased the trait. The sign associated to the additivity and dominance estimates indicates the parent that concentrates the greatest number of genes to increase the trait, and the convention is the positive sign for P1 and the negative sign for P2. Consequently, the positive sign of additivity in the T2 x S5 and T2 x S6 indicated that the tolerant T2 line presented the highest number of genes for flooding tolerance.

Table 5. Mean [m], additivity [a], dominance [d], and χ^2 value for plant and root dry matter estimated in three maize crosses and evaluated in 2000 and 2001

Parameter	T2 x S5		T2 x S6		S5 x S6	
	2000	2001	2000	2001	2000	2001
	Plant dry matter					
[m]	653.4±45.1	468.7±40.47	77.1±40.6	500.2±52.9	579.0±40.1	284.1±51.8
[a]	271.7±45.2	236.8±40.5	107.3±40.5	68.3±53.013	4.3±40.2	56.9±51.9
[d]	408.6±83.6	317.7±93.3	307.0±81.2	987.7±100.4	230.3±75.5	357.5±99.8
χ^2	5.1	8.3 [*]	6.7	19.8 [*]	1.4	0.4
	Root dry matter					
[m]	252.8±26.4	180.9±24.9	327.5±26.4	209.9±32.1	251.6±23.0	131.3±25.9
[a]	91.1±26.4	109.7±24.9	44.4±26.4	66.0±32.1	20.9±23.1	38.5±26.0
[d]	161.3±49.0	196.1±61.0	100.9±53.6	250.9±70.41	16.4±42.6	173.7±48.8
χ^2	24.5 [*]	0.6	8.7 [*]	1.6	2.1	0.4

* Significant values for the chi-square test at 5% probability

Herança da tolerância ao encharcamento do solo em milho

Resumo - *O milho poderá ser uma excelente alternativa para rotação de culturas em áreas de terras baixas no sul do Brasil. Entretanto, existem poucos estudos a respeito da herança da tolerância ao encharcamento nessa espécie. O objetivo do trabalho foi o de determinar essa herança baseado na matéria seca da raiz e da parte aérea de plantas jovens de milho. Linhagens de milho foram cruzadas para formar três populações nas gerações F_1, F_2 e retrocruzamentos. A tolerância foi avaliada durante dois anos em casa-de-vegetação. O comportamento das gerações demonstrou a presença de heterose. As variâncias das gerações segregantes foram superiores às das gerações fixas, indicando variabilidade genética para ambos os caracteres avaliados. A distribuição da F_2 permitiu inferir que a tolerância ao encharcamento é governada por vários genes que atuam de forma complementar.*

Palavras-chave: encharcamento, *Zea mays*, herdabilidade, efeitos genéticos.

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