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Genetic parameters and QTL for tolerance to flooded soils in maize

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ABSTRACT - *Flooding tolerance is important for maize in southern Brazil. Polypeptides involved in glycolysis and fermentation are generally tolerance-related. Objective of this work was to associate markers for tolerance to flooding in maize. One hundred and seventeen F_3 families were phenotyped for shoot (SDM) and root (RDM) dry matter and 74 were selectively genotyped with 44 microsatellite markers. A single marker analysis was performed to detect QTL. Analysis of variance indicated the presence of significant genetic variability for both traits. Three markers were associated to SDM and two to RDM. For SDM the loci involved were glutamine synthetase (chromosome 5, R^2=0.16), zein (chromosome 4, R^2=0.15), and triosephosphate isomerase (chromosome 3, R^2=0.14). For RDM, zein (chromosome 4, R^2=0.11) and triosephosphate isomerase (chromosome 3, R^2=0.11) were associated. Multiple loci analysis indicated R^2=0.32 for SDM and R^2=0.19 for RDM, indicating that a fraction of the genetic variation present in F_2 was explained by the analyzed markers.*

Key words: microsatellite, heritability, *Zea mays*.

INTRODUCTION

Tolerance to flooded soil is related to the coordinated action of morphological, anatomical and biochemical adaptations (Bucher and Kuhlemeier 1993). Kennedy et al. (1992 indicated that anaerobic stress determined by soil flooding gave rise to changes in the protein pattern of several species, due to the dissociation of the polyribosomes, which made mRNA transaction impossible. A group of 20 anaerobic polypeptides (anps) designed by Sachs et al. (1980) was selectively synthesized in corn primary roots after six hours of anoxia. Simultaneously, aerobic protein synthesis was significantly repressed. These anaerobic polypeptides are well-documented in corn and are generally involved in glycolysis and fermentation (Lazlo and Lawrence 1983, Kennedy et al. 1992, Andrews et al. 1993). Crawford (1992), in line with the data reported by Sachs and Freeling (1978) stated that the phenomenon most associated with oxygen deficit is the induction of alcohol dehydrogenase (ADH) enzyme synthesis and activity. These authors found that the ADH enzyme was the main anaerobic polypeptide synthesized under oxygen deficit.

Anaerobic stress can significantly reduce the

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survival and growth of seedlings in flooded soils. Most corn genotypes survive up to three days under anaerobic treatment at 27 °C (Subbaiah and Sachs 2003). On the other hand, mutants whose ADH activity is null survive only for a few hours. Results from crosses between tolerant and sensitive genotypes have suggested that the trait anoxia-tolerance is dominant and presents simple segregation (Sachs et al. 1996).

Advances at the molecular level have been made by the analysis of various cDNAs and genes involved in the anaerobic response (Sachs 1993, 1994, Sachs et al. 1996). In addition, three gene systems have recently been described that seem to function outside the glycolysis pathway (Huq and Hodges 2000). In spite of this progress in knowledge, the information has not directly helped to develop new flood-tolerant corn genotypes. The selection of individuals is complex because of the difficulty of a phenotypic assessment of the trait. Some researchers, e.g. Subbaiah and Sachs (2003), are however developing anoxia-tolerant lines to analyze the genes associated with this trait. The identification of QTL associated to flooded soil-tolerance could be an alternative for tolerant genotype selection. Molecular markers have been used very successfully in this sense with corn (Taramino and Tingey 1996, Smith et al. 1997, Lubberstedt et al. 1998, Pejic et al. 1998). Objective of this study was therefore to identify QTL involved in tolerance to flooded soil of young corn plants.

MATERIAL AND METHODS

Phenotypic analysis and leaf sampling for DNA extraction were carried out in an experiment in a greenhouse at Embrapa Clima Temperado from December 2001 to January 2002. A total of 117 F_3 families were assessed. The families were derived from crosses between two corn lines, one tolerant (R2) and the other sensitive (S5) to flooded soil. The parents and two controls, AG5011 and BRS3060 hybrids, were also assessed. These two hybrids were chosen because they respond distinctly to flooding and are used as controls in the official experimental network for corn in flooded soil.

A complete randomized block design with two replications was used and 12 seeds were sown per replication for each F_3 family. They were sown in 12 cm high 200 mL plastic cups with a perforated base full of soil containing 20% sand. The cups were placed in plastic-lined wooden boxes to prevent water leakage. One day before sowing the box was filled with water to 4 cm from the bottom. Under these conditions three seeds per cup were sown embryo-up approximately 2 cm deep.

Eight days after emergence one leaf was collected from all 24 plants of each F_3 family and a bulk was formed to represent an original F_2 plant. The DNA for molecular analysis was extracted from this bulk. Shortly after collecting the leaves, nitrogen was applied in the proportion of 120 kg ha^{-1} urea. Flooding was initiated on the 22nd day after emergence and maintained during four days (water level covering cups by 1 cm). After this flooding period, the water excess was drained from the boxes and the cups with the plants kept in 1 cm water for 7 more days after which the plants were harvested. This means that the plants were maintained in water-saturated soil up to the experiment harvest, reproducing soil conditions of lowland areas in southern Brazil. Leaves and roots of each plant were collected separately and dried in an oven at 60° C for 5 days to assess dry weight. The variables evaluated to access tolerance to flooding were root dry matter (RDM) and shoot dry matter (SDM). The SDM and RDM data for the F_3 generation were submitted to analysis of variance, using the random effect model which permitted the estimation of variances and heritability. The analysis of variance included only F_3 progenies and the model was $X_{ij} = \mu + \delta_i + \tau_j + \varepsilon_{ij}$ (X_{ij}=phenotypic value of the j^{th} F_3 line and i^{th} block for the SDM or RDM traits, μ =general mean, δ_j=random effect for the j^{th} block, τ_j = random effect for the i^{th} F_3 line, and ε_{ij} = experimental error ~ N (0, ^{2}). The variance among F_3 progenies is given by ^2_A 1/4 ^2_D (experimental error) (Hallauer and Miranda 1988). Means of both traits for the parents, the controls and F_3 generation were compared by the t test using an overall $\alpha = 0.05$.

The microsatellite analysis was carried out in the Laboratório de Biologia Molecular do Departamento de Plantas de Lavoura of the Faculdade de Agronomia at the UFRGS. The primers were chosen from the corn database (http://www.maizegdb.org) and 44 pairs were tested. The primer selection followed the criterion of sampling all chromosomes, preferring those linked to genes belonging to metabolic pathways involved with glycolysis and fermentation (Table 1).

Genotypic analysis was performed with only 74 F_3 lines selected from the most contrasting F_3 progenies for SDM and RDM. The selective genotyping method was proposed as a way to increase efficiency without increasing the number of families under analysis (Tanksley 1993). DNA was extracted according to the protocol described by Edwards et al. (1991. PCR reactions were prepared for a volume of 25 mL. Each reaction mixture

Table 1. Microsatellite primers used in 74 F_3 corn families

Primer	Gene linked to the marker	Chromosome	Bin*
phi037	*umc128*	1	1.08
umc1726	*adh1* – alcohol dehydrogenase 1	1	1.10
umc1064	*fdx3* – ferrodoxin 3	1	1.11
umc1622	*crr1* – cytokinin response regulator 1	2	2.0-2.01
umc1185	*ole1* – oleosin 1	2	2.03
phi029	*tpi4* – triose phosphate isomerase 4	3	3.04
phi046	*npi 257A*	3	3.08
umc1010	*plt2* – phospholipid transfer protein homolog 2	3	3.09
nc004	*adh2* – alcohol dehydrogenase 2	4	4.03
phi021	*adh2* – alcohol dehydrogenase 2	4	4.03
umc1550	*pdi1* – protein disulfide isomerase 1	4	4.03
phi074	*zp22.1* – zein 22.1	4	4.04
nc005	*gpc1* – glyceraldehyde-3-phosphate dehydrogenase 1	4	4.05
phi026	*gpc1* – glyceraldehyde-3-phosphate dehydrogenase 1	4	4.05
phi079	*gpc1* – glyceraldehyde-3-phosphate dehydrogenase 1	4	4.05
umc1466	*pdh1* – pyruvate dehydrogenase 1	4	4.08
umc1173	*rpd3* – RPD3 histone deacetylase homolog	4	4.09
phi006	*cat3* – catalase3	4	4.11
umc1197	*cat3* – catalase3	4	4.11
umc1610	*cpn10* – chaperonin 10	4	4.11
umc1056	*px13* – peroxidase13	5	5.03
phi008	*rab15* – responsive to abscisic acid 15	5	5.03
umc1564	*rps15* – ribosome protein 15	5	5.03
phi085	*gln4* – glutamine synthetase 4	5	5.06
umc1023	*fdx2* – ferrodoxin 2	6	6.00
umc1018	*gpc2* – glyceraldehyde-3-phosphate dehydrogenase 2	6	6.01
nc012	*pdk1* – pyruvate, orthophosphate dikinase 1	6	6.05
phi081	*pdk1* – pyruvate, orthophosphate dikinase 1	6	6.05
umc1341	*roa2* – replication origin activator 2	6	6.06
umc1545	*hsp3* – heat shock protein 3	7	7.00
umc1627	*oec23* – oxygen-evolving complex 23	8	8.03
umc1741	*rps28* – ribosome protein 28	8	8.03
umc1172	*pdc1* – pyruvate	8	8.04
phi060	*rip1* – ribosome-inactivating protein 1	8	8.04
umc1202	*rip1* – ribosome-inactivating protein 1	8	8.04
phi015	*gst1* – glutathione S transferase 1	8	8.08
phi065	*pep1* – phosphoenolpyruvate carboxylase 1	9	9.03
phi016	*sus1* – sucrose synthetase 1	9	9.04
phi032	*sus1* – sucrose synthetase 1	9	9.04
umc1094	*sod9* – superoxide dismutase 9	9	9.05
umc1733	*hb1* – hemoglobin 1	9	9.06
umc1576	*Gdcp1* – glycine decarboxylase 1	10	10.02
phi071	*hsp90* – heat shock protein, 90 kDa	10	10.04
umc1344	*crr2* – cytokinin response regulator 2	10	10.07

* Chromosome locations of the marker in the maize map are listed on the right (decimal part) and the chromosome on the left (complete part)

contained 60 ng of genomic DNA; 10X Buffer (Gibco BRL); 1.5 mM $MgCl_2$ (Gibco BRL); 0.2 mM dNTP (Gibco BRL); 1U Taq-DNA Polymerase (Gibco BRL); 0.2 mL of each primer and the amplifications were performed in a thermocycler (model PTC-100, MJ Research, Inc.). A touchdown-type program was used to amplify the genomic DNA consisting of 18 cycles at 94 °C for 1 min followed by a decrease at 1 °C every two cycles (64 to 55 °C) and 72 °C for 1 min and a further 30 cycles at 94 °C for 1 min, 55 °C for 1 min and 72 °C also for 1 min. The amplification products were separated in 3% agarose gel and the amplified fragments visualized with ethidium bromide under ultraviolet light. The gel images were recorded with the Kodak Digital science 1D v. 3.0.1 program.

The amplified products in each bulked F_3 progeny allowed the identification of the original F_2 plants, which were classified according to their similarity with the parents. QTL were identified by the establishment of a statistical relationship between each marker and the mean of each F_3 family. The data were submitted to analysis of regression according to the model Y_i $_0$ $_1X_i$ $_i$ (Y_i=mean phenotypic value of the ith F_3 line for the SDM or RDM traits, X_i=genotypic value of the ith original F_2 plant, $_0$=intercept parameter, $_1$=slope parameter and $_i$=experimental error ~ N (0, ^{2}). Whenever the estimates of the b_1 parameter were significant at 5% probability by the F test, the marker was considered associated to genes for tolerance to flooded soil. Each marker was considered individually, an approach supported by Goldman et al. (1994) and Barbosa Neto et al. (2001).

RESULTS AND DISCUSSION

The parents, control hybrids, and F_3 generation for SDM and RDM were compared using t tests (Table 2). Line R2, classified as flooding-tolerant, did not differ significantly from the control hybrids for the SDM variable but differed from the flooding-sensitive S5 line and from the mean of the F_3 population. The S5 line produced less RDM and differed significantly from AG5001 and R2. These results confirm the contrasting performance among the parents and support the use of this cross for genetic mapping. Regarding the performance of the lines and commercial hybrids, Fausey and McDonald (1985) indicated that lines were more flooding-tolerant than hybrids. On the other hand, Lemke-Keyes and Sachs (1989) observed a similar response of lines and hybrids to tolerance to anaerobic stress when assessed at the pre-emergence stage of the seedlings. In a previous study, Silva et al. (2001) had demonstrated the occurrence of marked heterosis and a maternal effect for tolerance to flooded soils in F_1 hybrids, disagreeing with the statements of Lemke-Keyes and Sachs (1989). The assessed combinations and the genotypes used as female parent were probably decisive for the manifestation of heterosis.

The phenotypic and genotypic variances and heritability of the SDM and RDM variables were estimated using among-family mean squares of the analysis of variance. Results indicated that there was genetic variability among the F_3 progenies tested for the SDM and RDM traits. The estimated heritability for SDM was 0.95 and 0.93 for RDM (Table 3). These values indicated a reduced influence of the environment on the expression of both traits; it is however important to consider that the genotype x environment interaction was not assessed, and the estimated values might be inflated by this interaction.

The polymorphism among the parents was 72.7% for the 44 tested markers. This polymorphism level was expected, because the selection of the markers had been focused on marker-linked genes involved in glycolysis and fermentation. However, several markers among the analyzed parents associated to important genes for these metabolic pathways were monomorphic, e.g., marker umcl1726, linked to the *adh1* gene, which is very important in alcohol fermentation; markers phi006 and umc1197, linked to the *cat3* genes, that codify for the catalase 3 enzyme; phi065, linked to the pep1 gene, that codifies for the phosphoenolpyruvate carboxylase 1, both enzymes involved in glycolysis; and phi016 and phi032, linked to the *sus1* gene, that codifies for the saccharin synthetase 1 enzyme. This monomorphic performance did not mean that these genes presented the same final product in each parent, since their regulation may be different in each one, resulting in different final products. Similarly the gene, even when present, may not be active or only partially activated. However, these markers were not considered important in our analysis because they did not segregate in the cross under study.

Three QTL were identified for SDM and two for RDM (Table 4). For SDM, the QTL with the largest effect was located on chromosome 5 close to the phi085 marker that showed the genetic effect of dominance for tolerance. This marker was however not significant for the RDM variable, suggesting that this region is specifically expressed in the shoot. The second-largest QTL for SDM was located close to the phi074 marker. This QTL was also significant for RDM. For both traits dominance effects were important. The last QTL identified was significant for both traits as well and was located close to the phi029 marker. Similar to

the second QTL, dominance was the main genetic effect involved. The genetic effect of dominance was important for all identified QTL suggesting the possibility of heterosis for flooding-tolerance in hybrid varieties.

The QTL identified by marker phi029 located on chromosome 3 explained 14% of the variation for shoot dry matter (SDM) and 11% of the root dry matter (RDM). This marker is linked to the triose phosphate isomerase 4 gene, an enzyme that catalyzes the isomerization of glyceraldehydes phosphate to dihydroxyacetone phosphate, to form triose phosphate in the Calvin cycle. Its action is located in the chloroplast and cytosol and is important in glycolysis (Heldt 1997). The second QTL, detected with the phi074 marker, is linked to the *zp22* gene and explained 15% of the total variation for SDM and 11% of the variation for RDM. This QTL, located on chromosome 4, is linked to a group of alpha-zein genes and is 2.5 cM from the glyceraldehyde-3-phosphate dehydrogenase 1 locus, which is a structural gene involved in the response to anaerobic stress in corn (Subbaiah and Sachs 2003). The last identified QTL, phi085, explained 16% of the variation for SDM but was not significant for RDM (Table 4). This marker is linked to gene *gln4*, which is also structural, and is considered a gene that can influence grain yield and consequently the seed size, which is very important in germination efficiency. Gene *gln4* is expressed constitutively during seed germination and in plant tissues (Sakakibara et al. 1992). A QTL located in the same region was identified for grain weight which influences the agronomic performance of corn hybrids (Limani et al. 2002).

Other polymorphic markers linked to important genes involved in glycolysis and alcohol fermentation were not significant in the QTL analysis, such as umc1622-*crr1*, phi021-*adh2*, umc1466-*pdh1*, umc1056-*px13*, umc1546-*hsp3*, umc1627-*oec23*, umc1172-*pdc1*, umc1094-*sod9*, umc1733-*hb1*, phi071-*hsp90*, and umc1344-*crr2*. This result could be associated to the regulation of these genes or to the sampling error that reduced the statistical accuracy. Simple marker analyses also have limitations related to the efficiency of QTL detection (Tanksley 1993, Lynch and Walsh 1998), but in the present experiment the chosen markers were specifically linked to important genes belonging to metabolic pathways involved with glycolysis and fermentation and there was no extensive coverage of maize chromosomes. This fact did ruled out an interval analysis.

DNA molecular markers are phenotypically neutral; the R^2 of the multiple regression model can therefore be considered as a proportion of the explained genetic variance in relation to the total observed phenotypic variance, even knowing that QTL detection is influenced by the accuracy of trait phenotyping. Anderson et al. (1993) used an estimate of the proportion of the explained genetic variance compared to the total genetic variance obtained from the heritability (R^2/h^2). This statistic is important from the plant breeder's point of view because it indicates the reliability of the multiple loci model for genotypic selection. According to Barbosa Neto et al. (2001), effective multiple loci models for marker-assisted selection should have high R^2/h^2values. In this study, the R^2 for multiple regression was 31.7% for SDM and 18.6% for RDM. Based on these values it was estimated that SDM accounted for 33.3% and RDM for 19.9% of the genetic variance explained by the three identified QTL. As the heritability was high, the proportion of genetic variance explained by the QTL was relatively low, especially for RDM. This indicates that other QTL of lower value may also be involved in the determination of flooding-tolerance. It is also important to highlight that the two measured variables evaluate the trait tolerance to flooding under different criteria and their correlation was estimated at 0.63. This mean correlation value could indicate that the genes for tolerance to soil flooding have distinct expressions in shoot and root.

Table 2. Mean and standard deviation of shoot dry matter (SDM) and root dry matter (RDM) for parents, F_3 population and controls (commercial hybrids)

Genotype	**SDM** (mg)		**RDM** (mg)	
	Mean[1]	**Standard deviation**	**Mean**[1]	**Standard deviation**
R2	338.12 ab	0.884	251.25a	1.768
S5	207.27 c	0.598	97.67 b	2.203
F_3	232.17 c	54.951	160.47 ab	48.740
AG5011	423.00 a	0.661	224.15 a	0.874
BRS3060	286.98 bc	18.470	218.52 a	4.018

[1]Means followed by the same letter do not differ significantly by the t test at 5% probability

Table 3. Phenotypic variance estimates ($^{2}_{P}$), genetic variance ($^{2}_{G}$), and heritability (h^2) for shoot dry matter (SDM) and root dry matter (RDM) in F_3 families

Parameter	SDM	RDM
$^{2}_{P}$	0.408	0.177
$^{2}_{G}$	0.389	0.166
h^2	0.95	0.93

Table 4. Chromosomal location of three markers related to QTL for leaf dry matter (SDM) and root dry matter (RDM), additive (A) and dominance (D) effects, estimated mean genotypic values for R2, S5, and R2XS5 hybrid, value of probability and coefficient of determination obtained in the simple regression

Cr/Bin	Marker	Trait	A	D	Tolerant parent	Hybrid	Sensitive parent	P-value	R^2
3.04	Phi029	SDM	21.9	-24.0	257	210	213	0.01	0.14
		RDM	20.6	-12.3	180	146	138	0.03	0.11
4.04	Phi074	SDM	25.4	-19.9	263	217	212	0.01	0.15
		RDM	11.6	-25.5	184	147	161	0.04	0.11
5.06	Phi085	SDM	30.1	38.6	228	238	167	0.01	0.16
		RDM	8.4	11.1	158	161	140	0.60	0.02

R^2 = proportion of the phenotypic variance explained by the marker

Parâmetros genéticos e QTL para tolerância ao encharcamento do solo em milho

RESUMO - *Tolerância ao encharcamento em milho é importante no sul do Brasil. Geralmente, polipeptídios envolvidos na glicólise e fermentação estão relacionados à tolerância. O objetivo deste trabalho foi associar marcadores à tolerância ao encharcamento em milho. Cento e dezessete famílias F_3 foram fenotipadas para matéria seca da parte aérea (SDM) e da raiz (RDM) e 74 genotipadas com 44 microsatélites. Uma análise com marcas simples foi realizada para a detecção de QTL. A análise de variância indicou variabilidade genética para ambos os caracteres. Três microsatélites foram associados à SDM e dois à RDM. Para SDM os locos envolvidos foram glutamina sintetase (cromossomo 5, R^2=0,16), zeína (cromossomo 4, R^2=0,15) e triosefosfato isomerase (cromossomo 3, R^2=0,14). Para RDM, zeína (cromossomo 4, R^2=0,11) e triosefosfato isomerase (cromossomo 3, R^2=0,11) foram associadas. A análise múltipla apresentou R^2=0,32 para SDM e R^2=0,19 para RDM, indicando que uma fração da variância genética presente na F_2 foi explicada pelos marcadores analisados.*

Palavras-chave: : microsatélite, herdabilidade, *Zea mays*

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