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Genetic control of aluminum tolerance in tropical maize germplasm

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ABSTRACT: Aluminum (Al) toxicity is the most limiting factor to maize crop productivity in acid soils. Therefore, the understanding of inheritance of Al tolerance in maize is important for the development of more adequate procedures for Al tolerant genotypes selection. In this sense, the objectives of this study were to determine the inheritance, and the general (GCA) and specific combining ability (SCA) for Al tolerance in tropical maize. First, we evaluated diallel crosses of maize from landrace and hybrid germplasms for Al tolerance through the minimal solution methodology. The DIF data (root growth difference) were analyzed by Griffing diallel model. Later the additive-dominant genetic model proposed by Mather and Jinks (1971) was used to estimate the genetic effects. The results of the diallel analysis showed greater variability associated with the estimates of the SCA for both germplasm. The diallel crosses involving the V 06 (Dente de Ouro 2) landrace stood out by high SCA and GCA for Al tolerance. The generation mean analysis indicated quantitative inheritance of Al tolerance in this germplasm, with most of the variance explained by the additive effects. The heritability in the narrow sense varied from 47% to 71%, indicating the possibility of genetic gain with the selection of tolerant genotypes in F_2 generation. Additive gene action associated with intermediate heritability and quantitative inheritance demonstrates the possibility of genetic gains with artificial selection for Al tolerance in this maize germplasm.

Key words: combining ability, heritability, inheritance, *Zea mays* L.

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INTRODUCTION

Aluminum (Al) toxicity is one of the most limiting factor to cultivation in acid soils. In low pH (< 4.5) Al is solubilized in soil solution, favoring the absorption of the element by root plants (Ezaki et al. 2013). The root apex is the first site of Al toxic action, which interferes not only on cell division but also on root cell elongation, causing root growth reduction and consequently decline on crop production (Doncheva et al. 2005).

The maize crop, among others Poaceae, presents high sensibility to Al toxicity in soil, since most of the time elite germplasm with elevated productive potential is extremely sensitive to this element. However, high genetic variability to Al tolerance has been observed in many maize germplasms (Ninamango-Cárdenas et al. 2003; Coelho et al. 2016). In this way, breeding programs aimed at the selection of Al tolerant genotypes, making their utilization an alternative to maize crop on regions with high Al saturation. The knowledge and understanding of genetic inheritance involved on Al tolerance in maize are important to the development of adequate methodologies for selection of tolerant genotypes.

It is known that the Al tolerance in maize is determinate genetically and the majority of studies on inheritance are based on root growth. Some studies indicate qualitative inheritance involved in maize Al tolerance, defined by a major gene probably involved on citrate exudation (Garcia and Silva 1979; Jorge and Arruda 1997; Rhue et al. 1978). On the other hand, there are reports of quantitative inheritance, evidencing a greater number of tolerance alleles associated to some populations or germplasms (Magnavaca et al. 1987; Sawazaki and Furlani 1987; Kochian et al. 2004).

The great divergence of results for Al tolerance in maize may be correlated to the type of germplasm used and the different genotypic constitutions, which can generate conflicting phenotypic expressions (Boni et al. 2009). Therefore, further scientific studies are required on the genetic inheritance involved in Al tolerance in maize. In this sense, this study aimed to determine the inheritance, and the general combining ability (GCA) and specific combining ability (SCA) for Al tolerance, through analysis of diallel crosses in maize germplasm (landraces and hybrids) and generation mean analysis from contrasting crosses to Al tolerance.

MATERIAL AND METHODS

Diallel analysis

Coelho et al. (2016) screened two maize germplasms (hybrids and landraces) for Al tolerance by the minimal solution methodology, which consists of exposing maize seedlings to a solution containing only Ca+Al. From these results, it was possible identify tolerant, intermediate and sensitive genotypes to Al. The contrasting genotypes were artificially crossed in diallel design including the reciprocal crosses (Table 1). The resulting generation of diallel crosses of both germplasms and the parental lines were evaluated for Al tolerance through minimal solution methodology. The experimental design →

Table 1. List of landraces varieties and maize hybrids, with the respective Al tolerance index (ATI) and Al tolerance classification.

Variety	Name	Collection site	ATI	Classification
V 6	Dente de Ouro 2	Pelotas – RS	7.5	Tolerant
V 3	Catete Amarelo	Canguçú – RS	4.2	Intermediary
V 50	Fortaleza	Muqui – ES	3.7	Intermediary
V 29	Crioulo Cunha Roxo	Veranópolis – RS	2.8	Intermediary
V 41	Caiano	Rio Azul – PR	2.1	Sensible
Hybrid	Type	Seeds company	ATI	Classification
H 44	Simple modified	Dow AgroSciences	5.0	Tolerant
H 27	Simple	Syngenta	4.8	Tolerant
H 34	Simple	Syngenta	3.3	Intermediary
H 18	Simple	Pioneer	2.5	Intermediary
H 22	Simple	Syngenta	1.0	Sensible

Data from Coelho et al. (2016).

was randomized blocks, with two blocks, been used 24 seedlings by replication/blocks.

Seedlings (crosses and parental) were evaluated for main root initial length in centimeters (FR – first reading). After that, they were placed on expanded polystyrene trays and then deposited in a tank with minimal solution composed of 4 mg·L^{-1} Al + 40 mg·L^{-1} Ca according to Coelho et al. (2015). After 48 h of exposition it was evaluated the main root final length again (SR – second reading). The difference between the measured variables (SR – FR) was denominated DIF (cm) (Coelho et al. 2015). DIF data was tested by analysis of variance and the means of the treatments subjected to grouping analysis by Scott & Knott test at 5% of probability using the package "ScottKnott" in R software (R Core Team 2013).

DIF data were subjected to diallel analysis by Griffing (1956) model method 2 (parental, hybrids and reciprocal) obtaining the estimates of general and specific combining ability. To estimate the genetic action and the number of genes involved in Al tolerance, the observed frequencies were tested in relation to expected frequencies to the hypothesis of one gene differentiating Al tolerant genotypes from sensitive by χ^2 test, through GENES program (Cruz 2013).

Generation mean analysis

From phenotypic characterization of Al tolerance in inbred lines belonging to the UEPG (State University of Ponta Grossa) breeding program were developed five families from crosses between contrasting lines for tolerance (L 99-4, L 118-8 and L 03-2) and sensitivity to Al (L 04-2, L 95-1, L 71-1 and L 23-1). The five segregating families were obtained from the crosses: (1) L 04-2 × L 99-4, (2) L 03-2 × L 95-1, (3) L 23-1 × L 99-4, (4) L 118-8 × L 71-1, and (5) L 118-8 × L 95-1.

The experiment was set up in randomized blocks design with three replications (blocks). The treatments were arranged in split plot, where, in the plot were evaluated the families and, in the subplot, the generations. An expanded polystyrene tray with 288 cells (12 columns × 24 rows) represented the plots. In the subplots the generations considered genetically uniform (P_1, P_2 and F_1) of each family, were represented by a row with 12 seedlings per replication, the segregating populations of F_2 generation by 17 rows (204 seedlings) per replication, and the backcross generations (BC_1 and BC_2) by two rows (24 seedlings) per replication. The evaluation methodology followed the same described previously.

DIF data were by analysis of variance. In the presence of significant effect of generation whitin family, it was proceeded the decomposition of generations within family. The mean values from generations were compared by Tukey's test at 5% probability, in R software (R Core Team 2013). The genetic effect was estimated through generation mean analysis by complete genetic model of Mather and Jinks (1971).

From the individual DIF data were obtained the estimates for each segregating population of the F_2 generation: the phenotypic variance $\hat{\sigma}^2_{f(F_2)} = \hat{\sigma}^2_{F_2}$, being the total phenotypic variance of the F_2 generation; the genotypic variance $\hat{\sigma}^2_{f(F_2)} = \hat{\sigma}^2_{f(F_2)} - \hat{\sigma}^2_{m(F_2)}$, being $\hat{\sigma}^2_{m(F_2)}$ the environment variance; the additive genetic variance $\hat{\sigma}^2_{a(F_2)} = 1/2a^2 = 2\hat{\sigma}^2_{g(F_2)} - (\hat{\sigma}^2_{g(BC_1)} + \hat{\sigma}^2_{g(BC_2)})$, were â is the variance from additive effects; and the dominant genetic variance $\hat{\sigma}^2_{d(F_2)} = 1/2d^2 = \hat{\sigma}^2_{g(F_2)} - \hat{\sigma}^2_a$, where $\hat{d}$ corresponds to variance from dominant deviations. The heritability in the narrow sense was estimated by $\hat{h}^2_r = (\hat{\sigma}^2_{a(F_2)}/\hat{\sigma}^2_{a(F_2)}) \times 100$. The heterosis percentage ($\hat{H}_{\%}$) was estimated from: $\hat{H}(\%) = (\hat{H} \times 100)/MP$, being $\hat{H}$ given by: $\hat{H} = \overline{F}_1 - MP$, where $\overline{F}_1$ represents the phenotypic average of F_1 generation and MP the average of parental lines (tolerant and sensitive). The minimum number of effective genes (n) for Al tolerance was estimated from: $n = [R^2(1 + 0{,}5k^2)]/(8\hat{\sigma}^2_g)$, where R is the amplitude of the F_2 generation DIF values and $k = = \sqrt{(2\hat{\sigma}^2_d)/(\hat{\sigma}^2_a)}$, being k the medium degree of dominance based on variances, where $\hat{\sigma}^2_d$ corresponds to the genetic variance of dominance deviations and $\hat{\sigma}^2_a$ is the genetic variance from additive effects of genes. The analyses were conducted on GENES program (Cruz 2013).

RESULTS AND DISCUSSION

Diallel analysis

The results of the analysis of variance showed significant effect ($p < 0.01$) of treatments for both maize germplasms (hybrids and landraces) for DIF. Significant differences were observed between the parents used in the crosses, as well as in the diallel crosses. Additionally, there were significant effects in the contrast parents versus crosses for both germplasms (data not shown).

The DIF mean grouping by Scott-Knott at 5% indicated the formation of six statistical groups for hybrid germplasm and seven for landrace (Table 2). For hybrid germplasm the →

DIF means demonstrated amplitude of 0.74 cm (H 22) to 2.38 cm (H18 × H 44). Crosses that involved the hybrid H 44 stood out for higher tolerance, indicating the presence of favorable alleles for Al tolerance in this hybrid. In this sense, stand out the crosses H 18 × H 44 (2.38 cm) and H 44 × H 34 (2.28 cm), which were, in average, more tolerant than the tolerant parent (1.77 cm) (Table 2).

In contrast, the landrace maize germplasm presented genotypes mean amplitude of 1.82 cm (V 06 × V 29) to 3.91 cm (V 06 × V 41), being 0.93 cm higher than hybrids. The crosses V 06 × V 41 (3.91 cm) and V 06 × V 03 (3.80 cm), highlighted with the highest root growth, being considered highly tolerant to Al (Table 2).

Table 2. DIF (difference in root growth) means for 5 parents (maize hybrids and landraces) and the respective diallel crosses.

Hybrids		Landraces	
Treatments	**DIF (cm)**	**Treatments**	**DIF (cm)**
H 18 × H 44	2.38 a*	V 06 × V 41	3.91 a*
H 44 × H 34	2.28 a	V 06 × V 03	3.80 a
H 22 × H 44	2.11 b	V 03 × V 06	3.65 b
H 27 × H 18	2.02 b	V 03 × V 29	3.30 c
H 44 × H 27	2.02 b	V 03 × V 41	2.88 d
H 34 × H 18	1.90 c	V 29 × V 50	2.86 d
H 22 × H 18	1.90 c	V 06	2.84 d
H 22 × H 27	1.86 c	V 29 × V 41	2.83 d
H 44 × H 18	1.86 c	V 03	2.77 d
H 27 × H 22	1.84 c	V 29	2.65 e
H 44	1.77 c	V 41 × V 03	2.64 e
H 18 × H 27	1.72 c	V 50 × V 03	2.60 f
H 18 × H 34	1.69 c	V 50	2.54 f
H 34 × H 22	1.69 c	V 06 × V 50	2.54 f
H 18 × H 22	1.68 c	V 29 × V 06	2.53 f
H 22 × H 34	1.66 c	V 41 × V06	2.51 f
H 27 × H 44	1.60 c	V 50 × V 29	2.50 f
H 34 × H 44	1.56 c	V 03 × V 50	2.45 f
H 34 × H 27	1.55 c	V 29 × V 03	2.44 f
H 44 × H 22	1.43 d	V 41	2.40 f
H 27 × H 34	1.28 d	V 50 × V 06	2.34 f
H 27	1.23 d	V 41 × V 29	2.30 f
H 34	1.21 d	V 41 × V 50	1.98 g
H 18	1.01 e	V 50 × V 41	1.83 g
H 22	0.74 f	V 06 × V 29	1.82 g
Mean	1.74		2.67

*Mean values with different letters in the same column are significantly different by Scott- Knott's test at 5% probability.

The analysis of variance of diallel crosses showed that the mean square from the general combining ability (GCA) was significant ($p < 0.05$) for DIF in both germplasms. In this way, the effects of specific combining ability (SCA) were significant for hybrids ($p < 0.05$) and landraces ($p < 0.01$). In the two evaluated germplasms there was no significance for the reciprocal crosses.

The variability of the GCA allows the inference that the parents contributed differently in the crosses in which they were involved and the variability between the SCA effects indicates the existence of combinations that have different performance than expected only based on GCA effects (Aguiar et al. 2004). The higher positive estimate of GCA effects of DIF on hybrid germplasm was observed for the tolerant hybrid H 44, while the hybrid H 34 obtained the most negative effect. For maize landrace, the highest estimate of GCA effects was verified for the tolerant variety V 06 (Dente de Ouro 2) and the lowest for V 50 (Fortaleza) (Table 3). The significance for combining ability reveals the presence of variability resulted from additive and non-additive genetic effects (Cruz et al. 2004).

The higher magnitude associated with the GCA effect will be present by the parent that have the higher frequency of favorable alleles of the target characteristic. Individually, the hybrids H 44 and H 27 presented high Al tolerance, but the reduced GCA of H 27 suggests that the utilization of this genotype on crosses probably will not result in superior genotypes to Al tolerance. On the other hand, for H 44 the inverse can be observed, being the use of this genotype recommended to obtain crosses with higher Al tolerance. In the evaluation of the GCA of five maize inbred lines, Conceição et al. (2009) observed that the favorable Al tolerance alleles are at low frequencies in crosses with low GCA.

Hybrids with higher SCA will generate potential populations for the extraction of lines, in such a way that, the lines originated from these hybrid pairs will present elevated SCA and a better exploration of the hybrid vigor (Balestre et al. 2008). For the estimates of SCA, in the hybrid germplasm, most of the parents presented negative estimates, with exception of hybrid H 44 (0.4004). The crosses that involved the hybrid H 22,H 34 × H 22 (0.3224), H 18 × H 22 (0.2934) and H 27 × H 22 (0.2844) presented the highest SCA values. On the other hand, the cross between genotypes more contrasting for Al tolerance, showed estimate of SCA →

Table 3. Estimates of general combining ability (GCA) effects of 5 parent hybrids and maize landraces, and estimates of specific combining ability (SCA) effects for parents and crosses of hybrids and landraces germplasm for DIF (difference in root growth).

Hybrids			Landraces		
Parents	**GCA**		**Parents**	**GCA**	
H 44	0.3066		V 06	0.4188	
H 27	–0.0444		V 50	–0.2972	
H 34	–0.1524		V 03	0.2768	
H 18	0.0266		V 29	–0.1512	
H 22	–0.1364		V 41	–0.2472	
SD (gi)	0.1028		SD (gi)	0.1531	
SD (gi – gj)	0.1625		SD (gi – gj)	0.2421	
Genotypes	**SCA**	**Reciprocal**	**Genotypes**	**SCA**	**Reciprocal**
H 44	0.4004		V 06	0.3852	
H 27	–0.4176		V 50	0.5172	
H 34	–0.4216		V 03	–0.4108	
H 18	–0.6796		V 29	0.1152	
H 22	–0.7036		V 41	–0.4528	
H 44 × H 27	–0.0936	0.115	V 06 × V 50	–0.4088	0.100
H 44 × H 34	–0.1606	0.170	V 06 × V 03	0.3022	0.075
H 44 × H 18	0.0504	–0.260	V 06 × V 29	–0.6548	–0.520
H 44 × H 22	–0.1966	–0.400	V 06 × V 41	0.3762	0.135
H 27 × H 34	0.0754	0.065	V 50 × V 03	–0.1818	0.075
H 27 × H18	0.1514	0.150	V 50 × V 29	0.1912	0.030
H 27 × H 22	0.2844	0.001	V 50 × V 41	–0.1178	–0.235
H 34 × H 18	0.1844	0.105	V 03 × V 29	0.2222	0.225
H 34 × H 22	0.3224	–0.080	V 03 × V 41	0.0682	0.055
H 18 × H 22	0.2934	–0.360	V 29 × V 41	0.1262	0.055
SD (sij)	0.2118		SD (sij)	0.3156	
SD (sij – sjk)	0.3447		SD (sij – sjk)	0.5135	
SD (sij – sik)	0.3250		SD (sij – sik)	0.4841	

SD (gi) = standard deviation associated with the general combining ability effect; SD (gi – gj) = standard deviation associated with the contrasting effects of general combining ability; SD (sij) = standard deviation associated with the specific combining ability effect; SD (sij – sjk)/(sij – sik) = standard deviation associated with the contrasting effects of specific combining ability.

negative with higher magnitude (Table 3). For landrace maize, the varieties V 06 (0.3852) and V 50 (0.5172) obtained the higher SCA values. The diallel crosses that demonstrated SCA superior and positive were V 06 × V 03 (0.3022), V 06 × V 41 (0.3762) and V 03 × V 29 (0.2222). While the cross V 06 × V 50 (–0.4088) presented SCA negative with higher magnitude (Table 3).

In the two germplasms (hybrids and landraces) the squared compounds demonstrated greater variability associated with SCA estimates, indicating association to the non-additive genetic effects (epistasis and dominance), being interpreted as the deviation of a cross from the parents (Cruz et al. 2004). These results differed from that obtained by Paterniani and Furlani (2002), who verified, from a complete diallel with 10 maize inbred lines, major portion of the Al tolerance variability due to GCA. The authors concluded that the Al tolerance expression in that germplasm is mainly related to the additive gene effects.

The negatives estimate of SCA, for the most of the parents, considering the root growth, indicates that the heterosis that will be manifested on filial generation, obtained from crosses between individuals, will be, on average, positive, which is desirable in order to increase →

the Al tolerance. For the crosses in which the estimates were superior and positive, the SCA effects suggest the importance of the genes with non-additive effects. Highlighted that in landraces diallel, the combinations were the most promising to increase the Al tolerance, given the highest DIF observed.

Inheritance of Al tolerance in tropical maize

Owing to the statistical significance of generations within families, it was performed the decomposition of generations within each family for Al tolerance. The decomposition of generations confirmed the presence of phenotypic contrast for Al tolerance between the family parental lines. The root growth of tolerant lines exposed to minimal solution with Al (L_T 99-4, L_T 03-2, and L_T 118-8) varied from 1.25 cm to 1.57 cm. On the other hand, L_S 04-2, L_S 95-1, L_S 23-1, and L_S 71-1 the Al sensitive lines showed DIF averages of 0.59 cm to 0.80 cm (Table 4).

The average root growth of F_1 generations of the five families, showed a trend to higher Al tolerance, with DIF amplitude of 1.19 cm (family 5) to 1.35 cm (family 1). The Al tolerance characterization of the F_2 generations showed trend to intermediate performance in relation to parental lines used in the respective crosses. For four families, the results of average, demonstrate that the F_2 generation do not differ statistically from parental lines used as tolerance source (L_T 99-4, L_T 03-2 and L_T 118-8) (Table 4).

The phenotypic DIF average of the five BC_1 (F_1 x L_T) generations showed, in all families, DIF averages statistically similar to Al tolerant parental lines (Table 4). In contrast, the BC_2 generations from Al sensitive parental lines presented reduced root growth, evidencing a phenotypic pattern with tendency to higher sensibility to Al.

The frequency distribution of the F_2 segregation can be observed per family on Fig. 1. The segregation pattern for the five families, showed a tendency to symmetric distribution between the DIF phenotypic classes, with DIF classes varying from 0.1 cm to 4.9 cm. In families 1 (L_T 99-4 × L_S 04-2) and 3 (L_T 99-4 × Ls 23-1), on what the tolerant line L 99-4 was one of the parents, were observed more symmetric distribution frequency of DIF. →

Table 4. Decomposition effects of generations (L_T, L_S, F_1, F_2, BC_1, and BC_2) and estimates of genetic parameters narrow sense heritability ($\hat{h}^2_r$), heterosis percentage ($\hat{H}$), and number of genes (NG) in the respective families for DIF variable (difference in root growth) and percent of DIF variation explained by additive ($\hat{a}$), dominant ($\hat{d}$), and epistatics interactions ($\hat{aa}$, $\hat{ad}$, $\hat{dd}$) for each family.

Generations	Family 1	Family 2	Family 3	Family 4	Family 5
	DIF (cm)				
L_T	1.57ab*	1.25ab*	1.44ab*	1.31a*	1.39a*
L_S	0.80d	0.66d	0.61c	0.59c	0.80c
F_1	1.35bc	1.33ab	1.23b	1.24a	1.19ab
F_2	1.48bc	1.09bc	1.41ab	1.07ab	0.89c
BC_1	1.82a	1.47a	1.63a	1.20a	1.17ab
BC_2	1.23c	0.82cd	1.23b	0.83bc	1.00bc
Generations			**Estimates**		
$\hat{h}^2_r$ (%)	71.02	51.87	68.71	66.21	46.65
$\hat{H}$(%)	23.72	21.43	27.58	13.52	9.02
NG	7.4	9.8	9.4	5.6	11.1
Effect			**Variation percentage (%)**		
m	15.62	8.54	24.09	42.05	0.35
â	71.85	75.17	57.29	39.63	40.90
$\hat{d}$	4.10	3.70	6.27	4.89	14.53
$\hat{aa}$	0.37	2.72	0.73	5.06	35.70
$\hat{ad}$	2.86	8.84	0.54	1.11	3.37
$\hat{dd}$	5.19	1.02	11.09	7.26	5.16

Family 1 = L_T 99-4 × L_S 04-2; Family 2 = L_T 03-2 × L_S 95-1; Family 3 = L_T 99-4 × L_S 23-1; Family 4 = L_T 118-8 × L_S 71-1; Family 5 = L_T 118-8 × L_S 95-1. m = average effects.*Means followed by different letters in the same column are significantly different by Tukey's test at 5% of probability.

In these families, the genotypes frequency considered sensitive (until 0.9 cm) were 17% and 16%, respectively, indicating a higher pattern of Al tolerance (Figs. 1a,c). Inversely, for families 2 (L_T 03-2 × L_S 95-1) and 5 (L_T 118-8 × L_S 95-1), in which the sensitive parental was the L 95-1, we observed frequency distributions with lower symmetry for DIF. This fact can be proven by the higher frequency of sensitive individuals in these F_2 generations, with 39% and 53% for families 2 and 5, respectively (Figs. 1b, e).

The pattern of frequency distribution close to a normal and unimodal curve of F_2 individuals provides evidence that the inheritance of tolerance in this germplasm is quantitative, possibly by the presence of the tolerance alleles in the respective parental lines, source of Al tolerance. Additionally, the distribution of individuals in the phenotypic classes of DIF close to symmetry is visualized for most of the families, which indicates a predominance of additive effect on genetic control of Al tolerance (Bernardo 2010). Possibly, the absence of perfect symmetry in the frequency distribution graphs could be associated to the environmental effect on tolerance expression of the individuals, the possibility of evaluators' errors in phenotyping, size of the F_2 populations evaluated, and in some families the presence of the dominance effect of Al tolerance.

Prioli et al. (2000) and Boni et al. (2009) reported a pattern of bimodal frequency distribution for the F_2 generation individuals from the cross of contrasting lines for tolerance. The authors emphasize that this type of distribution is an indicative of monogenic inheritance with complete dominance to Al tolerance. Garcia and Silva (1979) also observed monogenic inheritance to Al tolerance. On the other hand, quantitative inheritance pattern for Al tolerance is frequently reported on literature (Sibov et al. 1999; Ninamango-Cárdenas et al. 2003; Conceição et al. 2009; Krill et al. 2010).

For Magnavaca et al. (1987), the type of observed asymmetry may be an evidence of preponderance of sensitivity genes in relation to tolerant genes. Thus, the frequency of tolerance alleles would be high at low Al concentrations, whereas in high concentrations, the sensitive alleles would be more frequent. In this way, it can be admitted that there are more than one locus participating in the expression of Al tolerance in maize. Sawazaki and Furlani (1987) confirmed these results

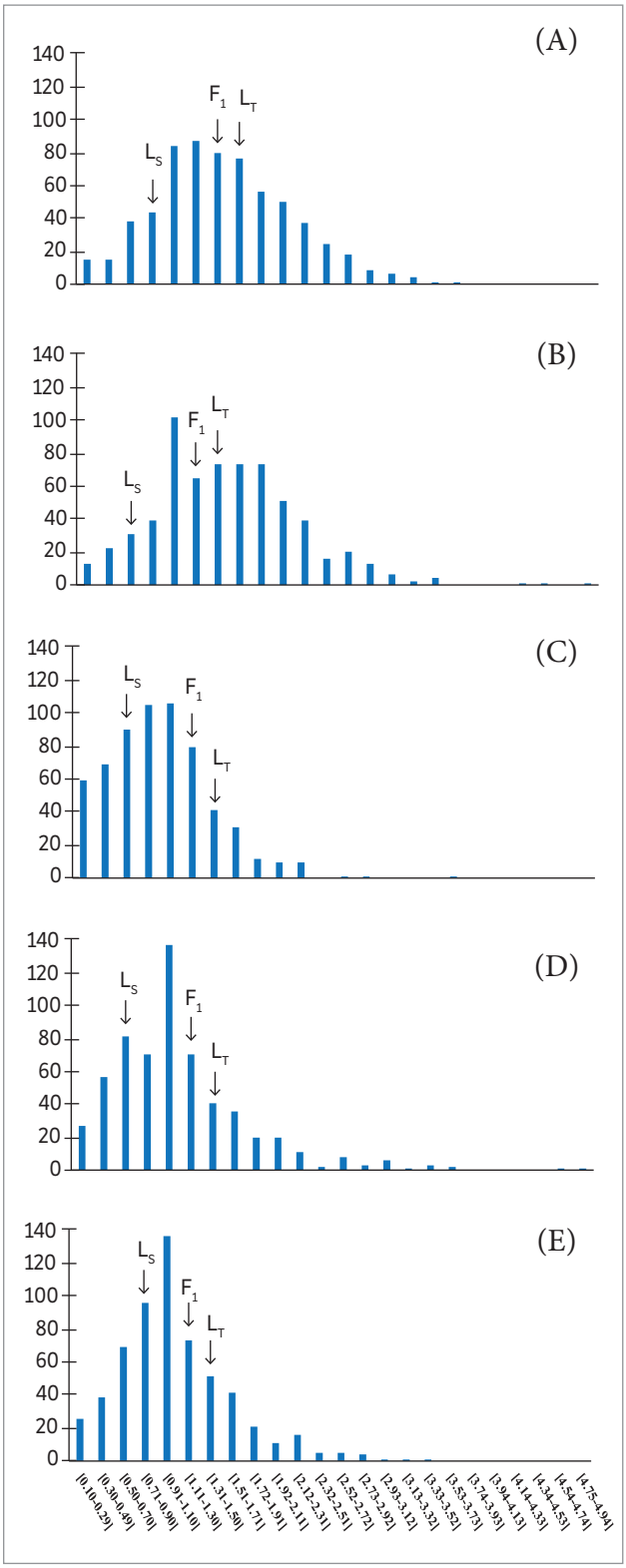

Figure 1. Frequency distribution of F_2 generation individuals in each DIF (difference in root growth) class on segregating families: (a) L_T 99-4 × L_S 04-2; (b) L_T 03-2 × L_S 95-1; (c) L_T 99-4 × L_S 23-1; (d) L_T 118-8 × L_S 71-1; and (e) L_T 118-8 × L_S 95-1. L_T = tolerant maize line, L_S = sensitive maize line, $F_1 = L_T \times L_S$.

when evaluated the Al tolerance in generations resulted from crosses between Cateto maize inbred lines. The authors observed that the distribution of F_2 generation was continuous and unimodal with negative asymmetry, presenting only the classes containing the F_1 and tolerant parent mean. Although it is not an indicative of the presence of additive effect genes, complementarily, it was verified that, through estimates of genetic parameters, the additive effects explained most genetic variation for Al tolerance in Cateto maize. Through the low estimate of the dominance degree, the abovementioned authors also indicated a tendency to Al sensitivity be partially dominant.

The narrow sense heritability ($\hat{h}^2_r$) varies from 46.7% (L_T 118-8 × L_S 95-1) to 71.0% (L_T 99-4 × L_S 04-2) (Table 4). The estimation of heterosis percentage was positive and varying from 9.0% to 27.6% (Table 4). The estimation of effective gene numbers in the five families, showed for the majority a great number of tolerance genes. The lower number was observed to the family 4 (L_T 118-8 × L_S 71-1) with 5.6, while family 5 (L_T 118-8 × L_S 95-1) evidenced 11.1 genes (Table 4).

The results of genetic analysis for the DIF data, showed the major contribution of additive genetic effects at Al tolerance genetic control in maize, with percentage values varying from 39.6% (L_T 118-8 × L_S 71-1) to 75.2% (L_T 03-2 × L_S 95-1) (Table 4). Only for the family 5 (L_T 118-8 × L_S 95-1) it was observed in addition to additive genetic effect (40.9%), a significant contribution of the epistatic interaction additive × additive (), with 35.7% of the genetic variance attributed to this interaction (Table 4).

The genetic parameter estimates associated with Al tolerance inheritance in segregating populations is important for breeding programs. These parameters allow direct the efforts to incorporate tolerance genes in the germplasm, as well as aid in the choice of the selection method to be used. The heritability in the narrow sense estimates were considered elevated when compared to others studies (Sawazaki and Furlani 1987; Prioli et al. 2000). This suggests that much of the genetic proportion is additive, evidencing possibilities of tolerant genotypes selection on F_2 generation, based on these experimental conditions. The positive heterosis percentage indicates that the variance of genic frequencies between the parents is sufficiently high, the positive values being desirable, since it is intended to obtain plants with higher root growth in the presence of the stressing factor.

The estimates of number of genes indicate quantitative inheritance for Al tolerance in maize to the evaluated germplasm. Through RFLP markers studies, Sibov et al. (1999) observed evidences of involvement of two genomic regions located on chromosomes 6 and 10, associated to Al in cateto maize populations. This same technic allowed Brondani and Paiva (1996) to associate the Al tolerance with a genomic region on maize chromosome 2, while Torres et al. (1997), located a region in chromosome 8. Maron et al. (2010) identified two genes, *ZmMATE1* and *ZmMATE2*, as major Al tolerance genes in maize. These genes are MATE (Multidrug and Toxic Compound Extrusion) family members, being the *ZmMATE1* mapped on chromosome 6 and the *ZmMATE2* on chromosome 5 of maize.

Recently, Guimarães et al. (2014) mapped a genomic region associated to Al tolerance adjacent to the *ZmMATE2* gene (chromosome 5). This *ZmNrat1* gene is a homologous to *OsNrat1* gene that encodes a specific Al transporter previously involved in rice tolerance. From the combination of the linkage analysis and associative map, Krill et al. (2010) identified four genes associated to Al tolerance in F_2 maize populations. The candidates *ZmASL*, *ZmALMT2*, *ME,* and *SAHH*, identified from the sequences deposited on MAGI (Maize Assembled Genomic Islands) project, are located on chromosomes 1, 10, 6, and 4, respectively. To date, the greater number of genomic regions involved in Al tolerance in maize was reported by Ninamango-Cárdenas et al. (2003), who mapped from molecular markers five tolerance QTL, explaining 60% of phenotypic variance associated with the net root growth.

In the present study, the genetic analysis evidenced the additive effect explaining most of Al tolerance genetic variation. Nevertheless, in family 5 (L_T 118-8 × L_S 95-1), it was observed a significant contribution of epistatic interaction additive × additive. According to Holland (2001) it is attributed to epistasis, the reason for the continuous success of the selection gain obtained in some breeding programs. The component additive × additive of epistatic variance is one of the mechanisms by which it is maintained in species with narrow genetic base. Magnavaca et al. (1987) analyzed the generations of six crosses, four between Al tolerance contrasting lines and two between sensitive lines. Similarly, the authors verified →

additive genetic effects explaining most of the genetic variance for the sets from contrasting lines. However, it also was observed significant contributions of the dominant deviations and, in lower magnitude, epistatic effects of dominance × dominance were verified in one of the families. For the sets from sensitive lines, were observed dominant genetic effects and of epistatic interactions of dominance × dominance, explaining the most part of phenotypic variation. The authors emphasized that the high number of Al tolerance mechanisms described for maize crop supports the concept of complex inheritance, that is, a major number of genes could be involved in the genetic control of this trait. However, the possibility of some mechanisms acting specifically more than others in function of determine stage of plant development could support the hypothesis of monogenic inheritance for Al tolerance in some cases.

On the genetics of Cateto maize Al tolerance studies, Sawazaki and Furlani (1987) verified that only additive effects were significant. These authors concluded that the high Al tolerance of Cateto maize are conditioned mainly by additive effects genes, which are concentrated, probably, on the origin variety, considering that the cultivation of this maize was done by indigenous and ancient farmers, in areas of soil with high Al content. The results obtained by Sawazaki and Furlani (1987) corroborate with the hypothesis that the Cateto maize race, of ancient origin, is considered an important Al tolerant source (Prioli and Silva 1984; Sibov et al. 1999; Boni et al. 2009). According Prioli and Silva (1984), the tolerant lines are in the most of flint endosperm, originated from Cateto race, while the most sensitive, are dent, type Tuxpeño. The authors observed that the lines from Cateto race do not develop long radicles as the lines from Tuxpeño type. Thus, in the presence of Al in toxic concentrations the harmful effect is more intense in Tuxpeño lines.

The inbred lines from UEPG breeding program have as genetic basis, maize landraces collected in different regions of Southern Brazil (States of Rio Grande do Sul and Paraná). This germplasm comes from agricultural regions with low technological level; therefore, these landraces were selected naturally for the adaptation of agricultural environments with high Al saturation. By the ancient origin of these landraces, it is believed that the most of the germplasm is composed by Cateto races, which may explain the higher tolerance of these landraces varieties in relation to the commercial/pre-commercial hybrids used in this study.

For Boni et al. (2009) the great divergence of results regarding the genetics of Al tolerance in maize can be explained by the different types of germplasms used in the evaluations. In this way, the origin of the inbred lines used on this study also confirm the results obtained by Sawazaki and Furlani (1987), supporting the hypothesis that the Al tolerance inheritance in Cateto maize is controlled by many genes, with predominance of additive genetic effects in the Al tolerance phenotypic expression.

CONCLUSION

The additive genetic action was predominant for Al tolerance genetic control in tropical maize germplasm and the narrow sense heritability coefficients confirmed the major contributions of genetic effects for Al tolerance in maize.

The Al tolerance inheritance in the set of segregating families evaluated is mainly oligogenic, with an average estimate of 8.7 genes.

The genotypes H 44, H 18, V 06, and V 03 presented positive estimates of general combining ability for Al tolerance, being promises for the generation of segregating populations with high potential to obtain Al tolerant inbred lines.

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