VINICIUS COSTA ALMEIDA

INHERITANCE OF ALUMINUM TOLERANCE IN TROPICAL POPCORN USING HAYMAN'S DIALLEL ANALYSIS

Thesis submitted to the Breeding and Genetics Graduate Program of the Universidade Federal de Viçosa, in partial fulfillment of the requirements to obtain the degree on *Doctor Scientiae*.

Advisor: José Marcelo Soriano Viana

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ABSTRACT

ALMEIDA, Vinícius Costa, D.Sc., Universidade Federal de Viçosa, February, 2020. **Inheritance of aluminum tolerance in tropical popcorn using Hayman's diallel analysis.** Advisor: José Marcelo Soriano Viana.

Understanding the genetic control of root growth is essential to improve the aluminum (Al) tolerance in crops. The objective of this study was to investigate the inheritance of Al tolerance related traits in tropical popcorn. Eight inbred lines were crossed in a diallel design and jointly assessed with their F_1s and two checks in a completely randomized design with two replications. The Al tolerance related traits were Al content, relative and net total, lateral, and axial root length, and number of root tips. The diallel analysis followed the Hayman's proposal. There was dominance for all traits. Only the net axial root length had bidirectional dominance. The dominant genes are not symmetrically distributed between the parents. The dominance was negative for relative total, lateral, and axial root length and positive for the other three traits. In general, there was evidence of complete dominance. Only for the relative lateral root length and net axial root length there were overdominance. None of the assessed inbred lines reached the selection limits. Inbred lines 11-133, 11-403, and 11-383 have the higher number of recessive (favorable) genes for relative total, lateral, and axial root length, respectively. Inbred lines 11-403 and 11-383 also have the higher number of dominant (favorable) genes for reduced Al content. Regarding net total and lateral root length and number of root tips, the inbred line 11-142 has the higher number of dominant (favorable) genes. For net axial root length, the inbred lines 11-109 and 11-133 have more favorable dominant and recessive genes, respectively. Our results evidenced that dominance is an important effect determining popcorn root traits and aluminum content in roots and should be explored in hybrid breeding.

Keywords: Aluminum tolerance. Genetic control. Relative root length. Net root length.

RESUMO

ALMEIDA, Vinícius Costa, D.Sc., Universidade Federal de Viçosa, fevereiro de 2020. **Estudo de herança da tolerância ao alumínio em milho-pipoca tropical utilizando análise dialélica de Hayman.** Orientador: José Marcelo Soriano Viana.

O objetivo deste estudo foi investigar a herança de caracteres de raiz relacionados com tolerância ao Al em milho-pipoca tropical. Oito linhagens de milho-pipoca foram cruzadas em um esquema de dialelo completo e posteriormente avaliadas com os respectivos híbridos e duas testemunhas em um delineamento inteiramente casualizado com duas repetições. Os caracteres avaliados foram conteúdo de Al na raiz, comprimento líquido e relativo de raízes laterais, axiais, e total e número de ápices radiculares. Houve dominância para todos os caracteres avaliados. Apenas crescimento líquido de raízes axiais apresentou dominância bidirecional. A dominância foi negativa para crescimento relativo de raízes laterais e axiais, crescimento líquido total e positivo para os demais caracteres. Em geral houve evidencia de dominância completa para todos os caracteres. Apenas crescimento relativo de raízes laterais e crescimento líquido de raízes axiais apresentaram sobredominância. Nenhuma das linhagens avaliadas alcançaram o limite de seleção para tolerância ao Al. As linhagens 11-133, 11-403 e 11-383 apresentaram maior concentração de genes recessivos (favoráveis) para crescimento relativo de raízes laterais, axiais e total, respectivamente. Linhagens 11-403 e 11-383 também apresentaram maior concentração de genes dominantes (favoráveis) para redução do conteúdo de Al. Em relação ao crescimento líquido de raízes laterais, total e número de ápices radiculares a linhagem 11-142 apresentou maior concentração de genes dominantes (favoráveis). Para crescimento líquido de raízes axiais, as linhagens 11-109 e 1-133 apresentaram maior concentração de genes dominantes e recessivos, respectivamente. Os resultados evidenciaram que dominância é importante na herança da tolerância ao Al e pode ser explorada no desenvolvimento de híbridos de milho-pipoca.

Palavras-chave: Tolerância ao alumínio. Controle genético. Comprimento relativo da raiz. Comprimento líquido da raiz.

SUMMARY

1. Introduction

Aluminum (Al) toxicity of acid soils is one of the major factors reducing crop production in tropical and subtropical regions. In acidic soils, Al is solubilized as Al^{3+} ions, which are toxic to plants (Kochian *et al.* 2015). The primary effect of the Al toxicity in plants is the inhibition of root growth (Čiamporová 2002). Changes in the architecture and morphology of roots, such as decreased lateral root elongation, reduced number of root tips, and stunted root growth are common Al toxicity symptoms. In the leaf tissues, Al toxicity also causes damages such as cell rupture, reduced cell ion content, and decreased photosynthesis (Wang *et al.* 2015).

The complex maize root system comprises different root types with distinct functions (Hochholdinger et al. 2018). Hund *et al.* (2009) pointed out that axial and lateral roots are important components of the maize root system and exhibit different growth patterns. The axial roots exhibit an almost linear growth, whereas lateral root length increases exponentially at the seedling stage. Despite their morphological similarity, lateral roots play an important role in the acquisition of immobile nutrients such as phosphorus (Lambers *et al.* 2006), while axial roots are mainly responsible for water and nitrogen uptake in deeper soil layers (Chun *et al.* 2005; Hund *et al.* 2009). According to Bushamuka and Zobel (1998) and Clark *et al.* (2013), Al toxicity has different impacts on the elongation of distinct root types. Therefore, understanding how different root types respond to Al toxicity may be useful to support breeding strategies for acidic soils.

Because the roots are the primary organ affected by Al toxicity, the assessment of Al tolerance has been based on root growth indexes. The most common measures of Al tolerance are the relative root length and the net root length of hydroponically grown seedlings in nutrient solution containing Al (Magnavaca *et al.* 1987a). These Al indexes have been widely used for QTL mapping and diallel analysis (Cançado *et al.* 1999; Ninamango-Cárdenas *et al.*

2003; Conceição *et al.* 2009), generation mean analysis (Magnavaca *et al.* 1987b), maize germplasm characterization (Coelho *et al.* 2016), and morpho-physiological analysis for Al tolerance (Awasthi *et al.* 2017). The number of root tips are also an important indicator of Al tolerance. According to Piñeros *et al.* (2001), root tips are responsible for the exudation of organic acids, which transform Al^{3+} ions into nontoxic components that cannot enter the root, leading to a decreased Al content in the root tissue. Zhang *et al.* (2014) used the number of root tips to evaluate the tolerance of maize inbred lines to low phosphorus (P). In these conditions, the release of organic acids is also important to mobilize P from fixed sources in the soil (Vance *et al.* 2003). Therefore, the number of root tips can be used to infer the plant's ability to support abiotic stresses.

The inheritance of Al tolerance based on root indexes has been reported in various studies. Conceição *et al.* (2009) evaluated F_2 generations obtained from a diallel cross involving two Al sensitive and three Al tolerant maize inbred lines. They reported that the relative root length has a complex inheritance determined by additive and non-additive genetic effects. Similarly, Cançado *et al.* (1999) observed that both additive and non-additive effects influenced the relative and net root length and hematoxylin staining in maize. Khatiwada *et al.* (1996) investigated the genetic control of Al tolerance in rice using the Hayman's diallel analysis, observing positive unidirectional incomplete dominance for relative root length.

Although most inheritance studies under stress conditions have used Griffing's method (Narro *et al.* 2010; Makumbi *et al.* 2011; Mutimaamba *et al.* 2017; Ertiro *et al.* 2017), the diallel analysis proposed by Hayman (1954a, b) provides a more deep understanding on the genetic control of traits. The analysis shows how to measure additive and dominance variation, how to describe the relative dominance properties of the parental lines, and how to detect non-allelic gene interaction. Considering that modern maize hybrid breeding is also focused on improvement for abiotic stresses and given the limited knowledge on the genetic control of Al tolerance in tropical popcorn, the main objective of this study was to investigate the inheritance of Al tolerance.

2. Materials and Methods

2.1. Genetic materials and experimental procedures

Eight tropical popcorn inbred lines (11-403, 11-387, 11-383, 11-142, 11-133, 11-109, 11-60, 11-50) developed by the Federal University of Viçosa (UFV), Minas Gerais, Brazil, were assessed. The inbred lines were previously screened for Al tolerance based on relative root growth, hematoxylin staining, aluminum content, and external morphology of roots, from stereoscopic and electron microscopy analyses, by Rahim *et al.* (2019). The inbred line 11-133 was classified as Al tolerant, the inbred line 11-60 as Al sensitive, and the other inbred lines as intermediate. The inbred lines were crossed in a diallel design, and 28 F_1 hybrids were obtained. The experiment was performed at UFV (20°50′ S, 42°48′ W; alt. 640 m asl) in a greenhouse with temperature and moisture control, using a completely randomized design with two replications. Each plot consisted of eight seedlings. We also included two Al tolerant checks, the maize hybrids 2B710PW and MG580 from Dow AgroSciences.

2.2. Growth conditions and assessment of Al tolerance indexes

The seeds of the inbred lines, F_1 hybrids, and checks were first treated with the fungicide Captan-400 and germinated in rolled wetted filter papers in a dark chamber at 25° C ± 1 for seven days. After germination, undamaged seedlings with uniform root length were transferred to 8-L plastic trays containing 8 L of nutrient solution. These plastic trays were covered with an acrylic plate containing 24 holes (3 cm apart) for the seedlings. These were supported with foam strips with a slit cut into them to anchor the stem. The nutrient solution contained: 1 mM KCl, 1.5 mM NH_4NO_3, 1 mM $CaCl_2$, 45 mM KH_2PO_4, 200 mM $MgSO_4$, 500 mM $Mg(NO_3)_2$, 155 mM $MgCl_2$, 11.8 mM $MnCl_2{\cdot}4H_2O$, 33 mM H_3BO_3, 3.06 mM

$ZnSO_4 \cdot 7H_2O$, 0.8 mM $CuSO_4 \cdot 5H_2O$, 1.07 mM $Na_2MoO_4 \cdot H_2O$, and 77 mM Fe-EDTA (Magnavaca *et al*. 1987a).

After 24 hours in nutrient solution with continuous aeration and temperature of 24° C under a 16:8 h light:dark photoperiod, the seedlings were photographed and the initial root length (IRL) was measured using the WinRHIZO Pro 2009 software (Regent Instruments, Quebec, Canada). The entire root system was placed on a table with a blue background in a 30 × 20 cm rectangle and photographed from a uniform distance using a digital camera fixed with a stand. All images were taken with the same dimensions and resolution (400 dpi) (Clark *et al*. 2013). The seedlings were then returned to the nutrient solution and 0.540 mM $AlCl_3$ was added into the plastic trays. To prevent Al precipitation, the pH of the nutrient solution was adjusted to 7.8 with KOH before adding the $AlCl_3$, and then to a final pH of 4.0 with 1 mM N HCl (Famoso *et al*. 2010).

After seven days in the nutrient solution, the root system was collected, washed with deionized water and placed in a solution of 25% ethanol for posterior scanning and Al content determination. The final root length (FRL) of the seedlings were measured using the WinRHIZO Pro 2009 software coupled to an EPSON Perfection 10000XL (Epson, Nagano, Japan) scanner (Bouma *et al*. 2000). Finally, the total net root length (cm) was computed as NRL = FRL − IRL and the total relative root length as NRL/IRL (Magnavaca et al. 1987a). We also evaluated the Al tolerance indexes according to different root diameter classes. Roots with a diameter below 0.5 mm were defined as lateral and those with diameter above 0.5 mm as axial (Trachsel *et al*. 2009). These measures were extracted from the root length-in-diameter class distribution obtained by WinRHIZO. We also measured the total number of root tips as suggested by Zhang *et al*. (2014). After scanning, three representative seedlings in each replication were used for Al quantification. The Al content (µg/g) was determined using atomic absorption spectrometry (AAS). For each sample, the whole root system was dried at

60° C, ground to fine powder, and 0.01 to 0.005 g of the root tissue was digested in nitro-perchloric acid. After digestion, the extract was submitted to AAS analysis (Sarruge and Haag, 1974).

2.3. Diallel analysis

The Hayman's diallel analysis is a method for investigating the genetic control of traits based on means, variances, and covariances of a diallel table (Hayman 1954b). The adequacy of the data to the hypothesis (i) diploid segregation, (iii) independent action of non-allelic genes, (iv) no multiple allelism, (v) homozygous parents, and (vi) genes independently distributed between the parents was performed testing the homogeneity of the difference Wr – Vr and if the slope of the regression of Wr on Vr is 1, where Wr is the covariance between the parent and their offspring in the rth array and Vr is the variance of the rth array. Under these hypotheses, the intercept of the regression of Wr on Vr is a constant ((1/4)(D – H_1)). After accepting the adequacy of the model, we performed the analysis of variance of the diallel table (Hayman, 1954a). The analysis provides tests for genetic variation (a), dominance (b), direction of dominance (unidirectional versus bidirectional) (b1), and symmetry for the distribution of the dominant genes in the parents (b2).

We used the least square method to estimate the genetic components of variation (D, H_1, H_2, F, and h^2) proposed by Hayman 1954b). D is the additive component, H_1 and H_2 are the dominance components, F is the covariance between additive and dominance effects, and h^2 is four times the squared difference between the diallel mean (m_{L1}) and the parents mean (m_{L0}). The genetic parameters computed were average degree of dominance ($\sqrt{H_1/D}$), gene asymmetry (the mean value of the product of allele frequencies at loci exhibiting dominance) ($H_2/4H_1$), ratio between dominant and recessive genes ($k_D/k_R = \left(\sqrt{4DH_1} + F\right)/\left(\sqrt{4DH_1} - F\right)$), and number of dominant genes with major effects (h^2/H_2) (Hayman 1954b). Hayman (1954b) emphasizes that even assuming equality of genes effects, the ratio h^2/H_2

underestimates the number of genes with major effects and provides no information about groups of genes exhibiting little or no dominance. The direction of dominance was inferred from the sign of h. The analysis of the (Wr, Vr) graph provides detailed information on the distribution of the dominant genes between the parents. The order of dominance of the parents is determined by Wr + Vr. Parents with lower and higher (Wr, Vr) graph coordinate values have a preponderance of dominant and recessive genes, respectively (Hayman 1954b). The preponderance of dominant genes can mean more dominant genes with higher effect. The limits of selection (Y_R and Y_D) (Hayman 1954b) were also computed. These values are the delimiters of the parabola and represent the completely recessive and dominant homozygous parents that can be obtained by selection, respectively.

Additionally, the Scott and Knott (1974) clustering test was used to group the inbred lines and the two checks. The ANOVA was performed using the GLM procedure on SAS System v 9.2 software (SAS Institute, 2007) and the clustering test using the R package ‘ScottKnot’.

3. Results

The analyses of variance of Wr − Vr revealed homogeneity for all traits and the regression analyses of Wr on Vr were highly significant ($P < 0.01$) with a slope equal to one, indicating the adequacy of Hayman’s five hypotheses (except hypothesis (ii) no difference between reciprocal crosses) (Table 1). It is interesting to highlight that all F-values were greater than one and the coefficients of determination of the regression analyses ranged from 66% to 95%. The analysis of variance of the diallel tables showed genetic variability, dominance, and gene asymmetry at loci exhibiting dominance for all traits (Table 2). Except for net axial root length, which exhibited bidirectional dominance, the evidence for the other traits was of unidirectional dominance. The coefficients of variation ranged from 13% for relative total root length to 33% for net lateral root length and were comparable to other Al tolerance studies.

In general, the regression intercept was not significantly different from zero evidencing complete dominance (Table 1). The evidence for Al content, relative axial root length and net lateral root length was overdominance. However, all estimates of average degree of dominance were greater than one evidencing overestimation (Table 3). Based on the sign of h, there is on average negative dominance for relative total, lateral and axial root length (recessive favorable genes) and positive dominance for net total and lateral root length and number of root tips (dominant favorable genes) (Table 3). Regarding Al content, the negative sign of h indicates dominant favorable genes toward a reduced Al content in the root. The inferred asymmetry for all traits is confirmed by the $H_2/4H_1$ values lower than 0.25. Only for the Al content and number of root tips there was evidence of a greater proportion of favorable (dominant) genes, showing that none of the assessed inbred lines reached the selection limits. Without exception, no superior inbred line was greater than the completely recessive or dominant parent (Table 4). Except for the Al content, net total and axial root length, there is also evidence for at least one dominant major gene controlling number of root tips and net and relative lateral root length and at least three dominant major genes determining the relative total and axial root length (Table 3).

The (Wr, Vr) graph coordinates provide detailed information on the order of dominance of the parents and the identification of the superior inbred lines. The inbred lines 11-133, 11-403, and 11-383 have the highest number of recessive (favorable) genes or more recessive genes with large effects for relative total, lateral, and axial root length, respectively (Fig 1B, C, and D). These inbred lines also have a high number of dominant (favorable) genes or more dominant genes with large effects towards a reduced Al content (Fig 1A). Regarding net total and lateral root length and number of root tips, the inbred line 11-142 has the highest number of dominant (favorable) genes or more dominant genes with large effects (Figure 1E, F, and

H). Because net axial root length is determined by favorable dominant and recessive genes (bidirectional dominance), the inbred lines 11-403 and 11-60 have the maximum number of dominant and recessive genes (favorable and unfavorable) or more recessive and dominant genes with large effects, respectively (Figure 1G), but the superior inbred lines are 11-109 and 11-133 (Table 4). Based on the order of dominance, we can infer that the former has more favorable dominant genes and the latter has more favorable recessive genes.

Comparing the superior inbred lines with the tolerant maize hybrids, we can state that several inbred lines are equivalent to the checks for relative total and axial root length and net total and axial root length (11-133, 11-383, among others) and some are superior to the checks concerning relative lateral root length (11-403), net lateral root length (11-109 and 11-133), and Al content and number of root tips (11-133).

4. Discussion

Modern maize hybrid breeding should be also focused on stress tolerance. Tollenaar and Lee (2002) consider that the improvement in maize yield has been and will be mainly due to increased stress tolerance and due to heterosis. Thus, the development of stress tolerant single crosses requires the assessment of genetic control of the many traits related with stress tolerance. Our results evidenced that dominance plays the major role in the inheritance of Al content, relative and net total, lateral, and axial root length as well as the number of root tips. These findings are consistent with previous results obtained by Cançado *et al.* (1999), Conceição *et al.* (2009), and Mutimaamba *et al.* (2017) who evidenced preponderance of dominant effects in the genetic control of net root length in maize. Based on hematoxylin staining and net root length, Singh *et al.* (2010) observed that Al tolerance is governed by dominant effects in pea. Khatiwada *et al.* (1996) also reported that dominant effects contributed for increasing the relative root length in rice.

According to Lynch *et al.* (2015), an Al tolerant genotype needs to exhibit greater lateral and axial root development, which ensures a better efficiency in water and nutrient uptake in acid soils. The ability to release organic acids from the root tips is also an important mechanism of Al tolerance and allows the root exploration of acid subsoils (Kochian *et al.* 2015). Therefore, plants with a higher number of root tips are better adapted to avoid Al toxicity and increase the uptake of nutrients in deep soil layers (Zhang *et al.* 2014; Lynch *et al.* 2015). The present study evidenced that the relative total, lateral and axial root length have similar genetic control governed by favorable recessive genes. Thus, Al tolerant single crosses should be derived from crossing superior recessive and complementary inbred lines (no or reduced heterosis). For Al content, net total and lateral root length and number of root tips, controlled by favorable dominant genes, Al tolerant single crosses can be obtained by crossing superior dominant and complementary inbred lines (no or reduced heterosis) or contrasting inbreds (high heterosis). When there is bidirectional dominance, as for net axial root length, the breeder should allow heterosis only for the favorable dominant genes.

Although we did not observe major dominant genes for net root length, Ninamango-Cárdenas *et al.* (2003) found five QTLs explaining a high proportion (60%) of the phenotypic variation at seedling stage. They also reported that these QTLs showed dominant effects and were co-located with genes related with organic acid exudation from the root tips. Thus, net root length and the number of root tips are efficient traits for improving Al tolerance. Concerning the Al content, Matonyei *et al.* (2014) reported a weak functional relationship between Al content and relative root growth ($r^2 = 0.19$) and citrate exudation ($r^2 = 0.38$) in maize, which may suggest that these traits possess different inheritance patterns. Guimarães *et al.* (2014) reported five QTLs explaining 63% of the relative root growth in maize. Traschel *et al.* (2009) and Cai *et al.* (2012) identified several QTLs for axial and lateral root length in maize. In both studies, the QTLs showed additive effects with a phenotypic variation from 4 to 13%.

Based on the dominance order and F_1 performance, the inbred lines 11-133, 11-403, and 11-383 are not complementary regarding relative total, lateral, and axial root length. Inbred lines 11-133 and 11-109 are not complementary regarding net axial root length. Inbred lines 11-142 and 11-133 are not complementary for number of root tips. Thus, to achieve the selection limits for these traits, the other favorable recessive and dominant genes should come from other Al tolerant inbred lines. However, 11-142 and 11-133 are complementary regarding net axial root length and inbreds 11-403 and 11-133 are complementary regarding Al content. Betrán *et al.* (2003) and Kagoda *et al.* (2011) were also able to identify inbred lines with high number of dominant genes for grain yield under low nitrogen and drought stress and for nematode resistance in maize, respectively. They stated that divergent inbred lines with a high frequency of desirable genes are good candidates for recycling of inbred lines and for producing high-performance hybrids. Finally, the best source of dominant and recessive favorable genes for the assessed Al tolerance traits is the inbred 11-133.

5. Conclusion

There were genetic variability and dominance for all Al tolerance traits. None of the assessed inbred lines reached the selection limits. The inbred lines 11-133, 11-403, and 11-383 have the highest number of recessive (favorable) genes for relative total, lateral, and axial root length, respectively. Inbred lines 11-403 and 11-383 also have the highest number of dominant (favorable) genes for reduced Al content. Regarding net total and lateral root length and number of root tips, the inbred line 11-142 has the highest number of dominant (favorable) genes. We concluded that dominance is an important effect determining popcorn root traits and aluminum content in roots and should be explored in hybrid breeding.

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Table 1. Analyses of variance of Wr – Vr and regression analyses of Wr on Vr

Intercept and regression coefficient estimates and significance levels for the hypotheses $H_0: \beta_0 = 0$ and $H_0: \beta_1 = 1$, and coefficient of determination (r^2) concerning aluminum content (Al, µg/g), relative total, lateral (lat), and axial (axi) root length (RRL), net total, lateral (lat, cm), and axial (axi, cm) root length (NRL, cm), and number of root tips (RT, count). ns: non-significant at P = 0.05; * and **: significant at P < 0.05 and P < 0.01, respectively.

Source of variation	df	Al	RRL	RRL$_{lat}$	RRL$_{axi}$	NRL	NRL$_{lat}$	NRL$_{axi}$	RT
Arrays	7	$8.74\text{x}10^{9\ \text{ns}}$	$8.40\text{x}10^{-6\ \text{ns}}$	4.280^{ns}	$5.06\text{x}10^{-5\ \text{ns}}$	121.390^{ns}	440.130^{ns}	18.152^{ns}	$5.53\text{x}10^{5\ \text{ns}}$
Error	8	$4.22\text{x}10^{9}$	$2.92\text{x}10^{-6}$	1.487	$3.15\text{x}10^{-5}$	76.321	402.680	6.638	$4.64\text{x}10^{5}$
Regression	1	$4.80\text{x}10^{11**}$	$1.48\text{x}10^{-4**}$	26.119^{**}	$3.61\text{x}10^{-4**}$	1499.474^{**}	1597.560^{**}	186.338^{**}	$4.25\text{x}10^{6**}$
Error	6	$4.20\text{x}10^{9}$	$2.33\text{x}10^{-6}$	2.199	$2.11\text{x}10^{-5}$	58.461	158.882	10.814	$2.56\text{x}10^{6}$
Intercept		$-1.59\text{x}10^{5**}$	-0.001^{ns}	-1.446^{ns}	-0.010^{*}	-8.440^{ns}	-36.831^{*}	-8.022^{ns}	-550.603^{ns}
Coefficient		1.107^{ns}	0.883^{ns}	0.811^{ns}	1.034^{ns}	0.729^{ns}	0.720^{ns}	1.494^{ns}	0.646^{ns}
r^2		0.950	0.915	0.664	0.740	0.813	0.655	0.742	0.734

Table 2. Hayman's diallel analyses of variance and coefficients of variation

Aluminum content (Al, μg/g), relative total, lateral (lat), and axial (axi) root length (RRL), net total, lateral (lat, cm), and axial (axi, cm) root length (NRL, cm), and number of root tips (RT, count). Degrees of freedom (df), genetic variation (a), dominance (b), direction of dominance (b1), and symmetry of genes (b2). ns: non-significant at P = 0.05; * and **: significant at P < 0.05 and P < 0.01, respectively.

Source of variation	df	Al	RRL	RRL_{lat}	RRL_{axi}	NRL	NRL_{lat}	NRL_{axi}	RT
a	7	$3.34x10^{5}$ **	0.017 **	1.954 **	0.027 **	207.700 **	58.973 **	25.324 **	2694.010 **
b	(28)	$3.76x10^{5}$ **	0.009 **	4.652 **	0.026 **	111.699 **	61.948 **	17.515 **	3300.089 **
b1	1	$3.63x10^{4}$ **	0.127 **	24.250 **	0.170 **	246.972 **	589.126 **	10.186 ns	15227.713 **
b2	7	$1.05x10^{6}$ **	0.005 **	7.786 **	0.037 **	26.441 *	24.134 **	9.063 *	1761.267 **
b3	20	$1.59x10^{5}$ **	0.004 **	2.575 **	0.016 **	134.775 **	48.824 **	20.840 **	3242.295 **
Error	36	$5.33x10^{4}$	0.001	0.253	0.002	12.090	6.659	2.945	398.642
CV (%)		13.7	17.0	21.9	25.5	25.0	24.6	32.7	24.2

Table 3. Estimates of Hayman's genetic parameters

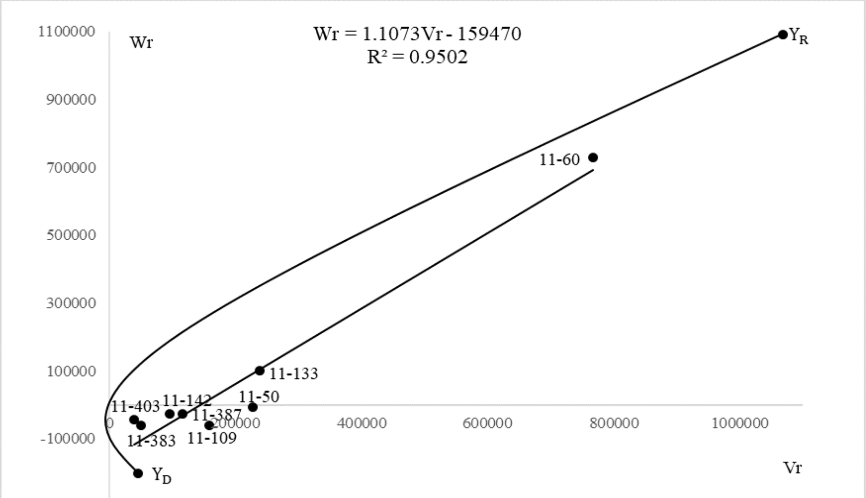
Aluminum content (Al, µg/g), relative total, lateral (lat), and axial (axi) root length (RRL), net total, lateral (lat, cm), and axial (axi, cm) root length (NRL, cm), and number of root tips (RT, count).

Parameters	Al	RRL	RRL_{lat}	RRL_{axi}	NRL	NRL_{lat}	NRL_{axi}	RT
D	$8.92x10^5$	0.014	7.261	0.039	52.027	21.495	17.647	2488.300
H_1	$1.88x10^6$	0.050	18.771	0.115	531.726	298.820	97.198	11471.348
H_2	$6.33x10^5$	0.016	8.798	0.049	270.413	225.473	39.345	5964.087
F	$1.57x10^6$	0.013	12.484	0.059	-81.603	11.019	-1.112	2715.507
h^2	$1.80x10^5$	0.054	10.498	0.138	71.203	162.890	16.439	5630.007
h	-450.689	-0.235	-3.275	-0.373	8.746	12.876	-4.210	76.186
$\sqrt{H_1/D}$	1.451	1.877	1.608	1.712	3.196	3.728	2.346	2.147
$H_2/4H_1$	0.084	0.078	0.117	0.107	0.127	0.189	0.101	0.130
k_D/k_R	4.062	1.671	3.298	2.560	0.606	1.147	0.973	1.681
h^2/H_2	0.084	3.469	1.193	2.783	0.263	0.722	0.418	0.943

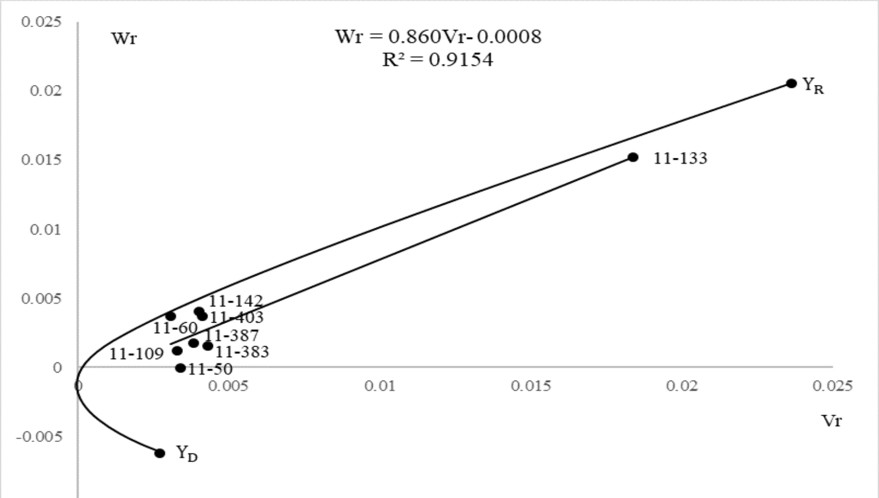
Table 4. Means of the tolerant checks, the inbred lines, and the completely recessive (Y_R) and completely dominant (Y_D) parents (estimated from theory), and the average values of the F_1 popcorn hybrids

Aluminum content (Al, µg/g), relative total, lateral (lat), and axial (axi) root length (RRL), net total, lateral (lat, cm), and axial (axi, cm) root length (NRL, cm), and number of root tips (RT, count). Means followed by the same letter in the columns are clustered in the same group by the Scott and Knott test at 0.05.

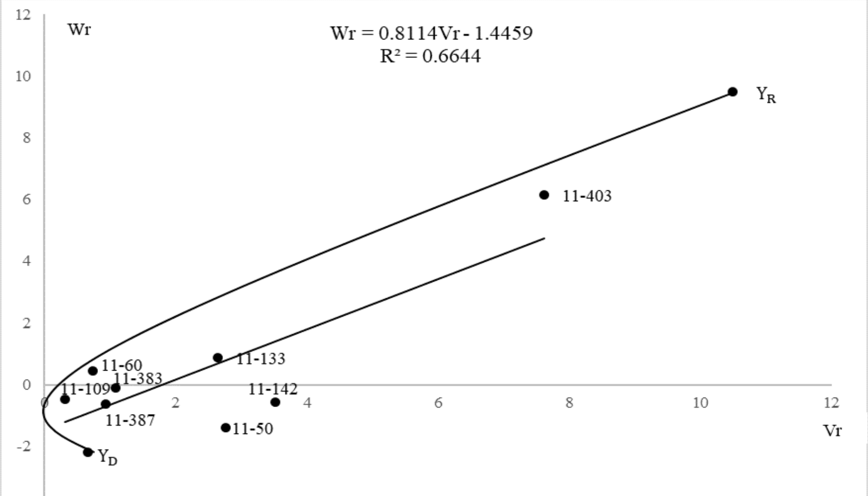
Inbreds/F_1/checks	Al	RRL	RRL$_{lat}$	RRL$_{axi}$	NRL	NRL$_{lat}$	NRL$_{axi}$	RT
MG580	2984^{A}	0.922^{A}	5.175^{C}	0.638^{A}	26.416^{A}	6.532^{B}	16.591^{A}	128^{B}
2B710PW	3444^{A}	0.825^{A}	2.068^{C}	1.167^{A}	18.544^{A}	3.659^{B}	12.672^{A}	83^{C}
11-133	1583^{C}	0.731^{A}	7.263^{B}	0.447^{A}	22.102^{A}	11.380^{A}	12.446^{A}	182^{A}
11-383	2006^{B}	0.440^{B}	4.418^{C}	0.624^{A}	9.962^{B}	3.479^{B}	6.958^{A}	41^{C}
11-403	2449^{A}	0.435^{B}	9.953^{A}	0.541^{A}	13.750^{A}	5.126^{B}	8.520^{A}	55^{C}
11-50	2335^{A}	0.434^{B}	3.168^{C}	0.592^{A}	12.120^{A}	4.606^{B}	7.519^{A}	58^{C}
11-109	2921^{A}	0.402^{B}	3.274^{C}	0.519^{A}	18.403^{A}	11.644^{A}	10.537^{A}	84^{C}
11-387	2770^{A}	0.385^{B}	3.595^{C}	0.389^{A}	14.762^{A}	8.672^{A}	8.325^{A}	78^{C}
11-60	3121^{A}	0.370^{B}	2.293^{C}	0.297^{B}	12.753^{B}	5.322^{B}	6.025^{B}	74^{C}
11-142	2412^{A}	0.327^{B}	2.941^{C}	0.242^{B}	14.665^{A}	10.337^{A}	4.351^{B}	108^{B}
F_1 hybrids	2361	0.306	2.659	0.221	21.267	16.765	7.976	143
Y_R	3259	0.837	13.247	1.189	8.207	3.559	14.71	34
Y_D	1166	0.300	2.313	0.223	24.090	12.141	3.907	183


Wr = 1.1073Vr - 159470
R² = 0.9502
Wr
Vr
1100000
900000
700000
500000
300000
100000
-100000
-300000
0
200000
400000
600000
800000
1000000
Y_R
11-60
11-133
11-50
11-403
11-142
11-387
11-383
11-109
Y_D

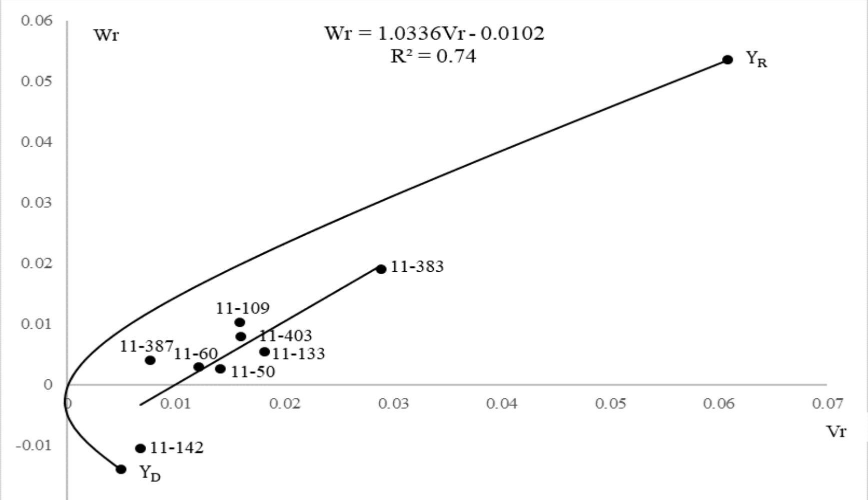
(A)


Wr = 0.860Vr - 0.0008
R² = 0.9154
Wr
Vr
0.025
0.02
0.015
0.01
0.005
0
-0.005
-0.01
0
0.005
0.01
0.015
0.02
0.025
Y_R
11-133
11-142
11-403
11-60
11-387
11-109
11-383
11-50
Y_D

(B)


Wr = 0.8114Vr - 1.4459
R² = 0.6644
Wr
Vr
12
10
8
6
4
2
0
-2
-4
0
2
4
6
8
10
12
Y_R
11-403
11-60
11-133
11-383
11-109
11-142
11-387
11-50
Y_D

(C)


Wr = 1.0336Vr - 0.0102
R² = 0.74
Wr
Vr
0.06
0.05
0.04
0.03
0.02
0.01
0
-0.01
-0.02
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
Y_R
11-383
11-109
11-403
11-387
11-60
11-133
11-50
11-142
Y_D

(D)

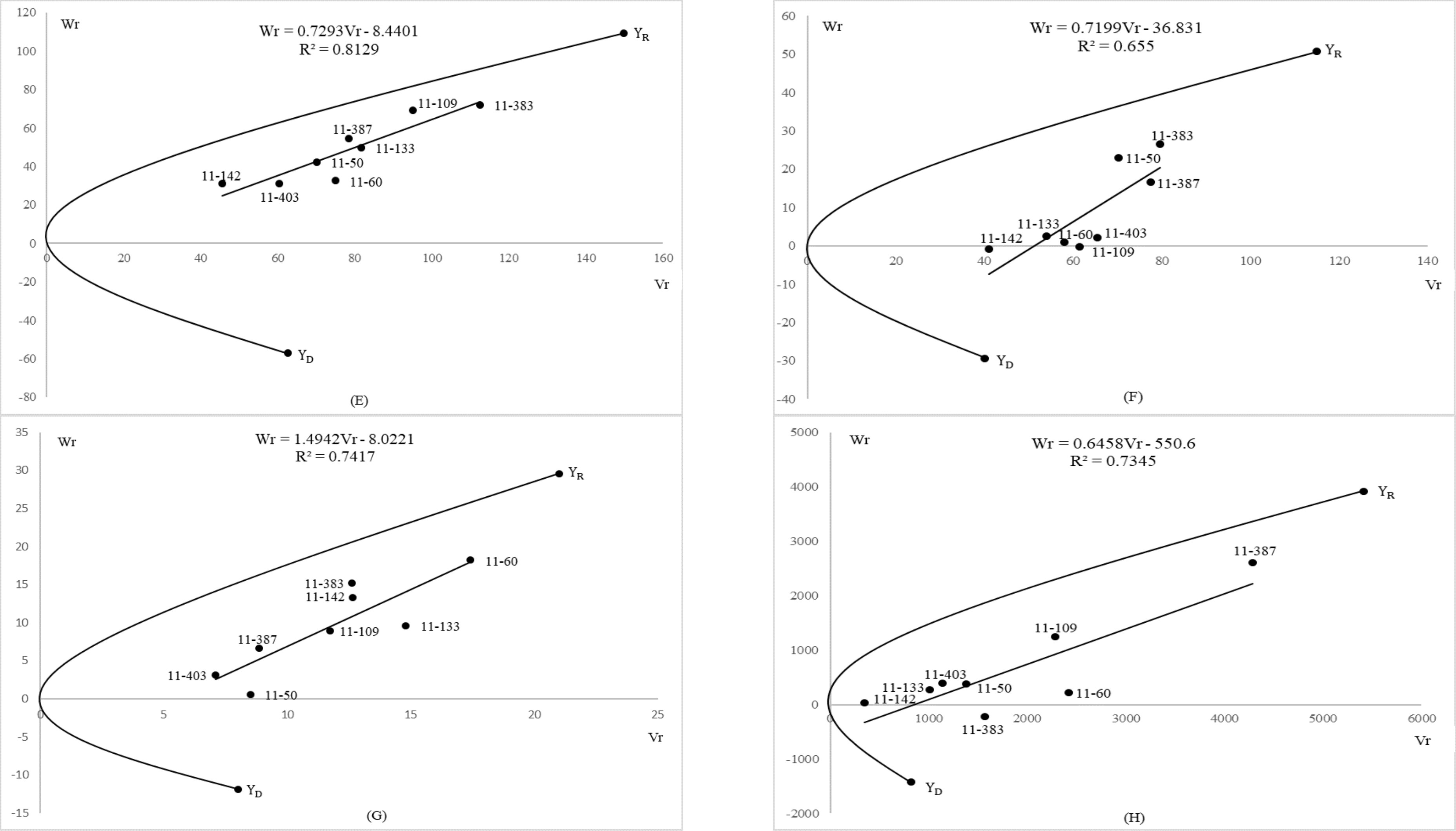


Figure 1. (Wr, Vr) graph for aluminum content (A, µg/g) relative total (B), lateral (C), and axial (D) root length, net total (E, cm), lateral (F, cm), and axial (G, cm) root length, and number of root tips (H, count).