FAISAL RAHIM

# IDENTIFICATION OF POPCORN INBRED LINES FOR ALUMINUM RESISTANCE

Thesis submitted to Federal University of Viçosa, in partial fulfillment of the requirements of the Genetics and Breeding Graduate Program, for the Degree of *Doctor Scientiae*.

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| Rodrigo Oliveira de Lima | Antônio Carlos Baião de Oliveira |
| Cleberson Ribeiro (Coadviser) | Rogério Faria Vieira |

José Marcelo Soriano Viana
(Adviser)

*I dedicate this humble effort to*

*my parents & brothers*

**Dedication**

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**Thanks**

## BIOGRAPHY

My name is FAISAL RAHIM. I am the son of Khurseed Begum and Rahim Hussain Khan. I was born on April 1, 1984, in Rawlakot, Pakistan held Jammu Kashmir, a beautiful but disputed territory between India and Pakistan since 1947. I graduated from intercollege Datote, Rawalakot, Jammu Kashmir in 2001. Then I attended my college and after two years I entered in University of Azad Jammu & Kashmir from which I graduated in 2008 with a degree in Plant Breeding & Molecular Genetics. After receiving my master/Mphil degree I have joined department of agriculture Azad Jammu & Kashmir as a research officer in 2011. Then I planned my doctorate program and started PhD on August 2013 in Genetics and Breeding, Federal University of Viçosa, Minas Gerais, Brazil, and submitting thesis for defense in August 2016. I lived all of my life in state of Jammu Kashmir and it was quite a new experience living hundreds of miles away from home. As hard as it was to move away from home, the experiences I have had and the friends I have made at the UFV have made my experience extremely rewarding and fruitful.

## SUMMARY

## RESUMO

RAHIM, Faisal, D.Sc., Universidade Federal de Viçosa, Agosto de 2016. **Identificação de linhagens de milho-pipoca quanto à resistência a alumínio.** Orientador: José Marcelo Soriano Viana. Coorientadores: Cleberson Ribeiro e Fabyano Fonseca e Silva.

Melhoramento genético é uma abordagem essencial para enfrentar a toxicidade de alumínio em solos ácidos. O objetivo desse trabalho foi avaliar linhagens de milho-pipoca quanto à resistência a alumínio. As linhagens foram avaliadas em três experimentos em solução nutritiva com e sem alumínio, no delineamento inteiramente casualizado com três ou quatro repetições. A concentração de alumínio na solução nutritiva foi 540 μM $AlCl_3$ (160 μM $Al_3^+$). O primeiro experimento foi uma avaliação preliminar de 18 linhagens. Então, em um segundo experimento, foram avaliadas 10 das 18 linhagens, incluindo as duas mais resistentes, as duas menos resistentes e outras seis com comportamento intermediário, escolhidas ao acaso. Finalmente, as duas linhagens mais contrastantes e um híbrido comercial resistente de milho (2B710PW) foram avaliados em um terceiro experimento. As linhagens foram avaliadas quanto às características crescimento radicular relativo (RRG), coloração por hematoxilina, conteúdo de alumínio em ápice de raiz e morfologia externa de raiz com base em análises estereoscópicas e de microscopia eletrônica. A linhagem 11-60 apresentou o menor RRG (0.02 and 0.06), a maior concentração de alumínio (1,660 μg/g), a maior intensidade de coloração e a maior degradação da epiderme. A linhagem11-133 apresentou o maior RRG (0.15 to 0.37), a menor intensidade de coloração, o menor acúmulo de alumínio (926.4 μg/g), sem danos nos ápices radiculares. A linhagem 11-133 é tão resistente quanto o híbrido 2B710PW.

## ABSTRACT

RAHIM, Faisal, D.Sc., Universidade Federal de Viçosa, August, 2016. **Identification of popcorn inbred lines for aluminum resistance**. Adviser: José Marcelo Soriano Viana. Co-advisers: Cleberson Ribeiro and Fabyano Fonseca e Silva.

Genetic improvement of crops is an essential approach to overcome the aluminum toxicity in acidic soils. The objective of the present investigation was to assess popcorn inbred lines for aluminum resistance. Eighteen tropical popcorn inbreds were assessed in three experiments in the presence and absence of aluminum, in a complete randomized design with three or five replications. The aluminum concentration in the nutrient solution was 540 μM $AlCl_3$ (160 μM $Al_3^+$). The first experiment provided an initial assessment of these inbreds. Then, in a second experiment we assessed ten of the 18 inbred lines, including the two most resistant, the two less resistant, and other six with intermediate performance, choose at random. Finally, we assessed the two most contrasting inbred lines in a third experiment, including also a resistant check (hybrid 2B710PW). The inbred lines were assessed for relative root growth (RRG), hematoxylin staining, aluminum content and external morphology of roots, based on stereoscopic and electron microscopy analyses. The inbred 11-60 showed the lowest RRG (0.02 and 0.06), the greatest aluminum accumulation (1,660 μg/g), and exhibited stronger blue staining with intense epidermal degradation. The inbred 11-133 showed the greatest RRG (0.15 to 0.37) and the lowest hematoxylin staining score, aluminum accumulation (926.4 μg/g) and root inhibition in the root tips, associated with no damages on root apices. Scanning electron micrographs showed severe cell disruption and deep ruptures of the protodermic and outer cortex layers of the root cells of 11-60. The inbred line 11-133 was as resistant as the check.

## 1. Introduction

Aluminum toxicity is one of the major limiting factors for crop production in acidic soils and limits worldwide agricultural production, particularly in developing countries of South America, Central Asia and South East Asia (Kochian et al. 2015). Approximately twenty percent of all maize grown on acidic soils and eighty percent reduction in yield has been reported (Conceição et al. 2009). At pH four, aluminum becomes soluble in the form of exchangeable $Al^{3+}$ ions that results in accumulation of aluminum in the root apices of sensitive plants. The accumulated aluminum links with some important constituents of carboxylic group of cell wall and reduces mitotic activity in root tips, ultimately resulting in stunted root growth (Yang et al. 2008; Horst et al. 2010). Aluminum increases the rigidity of cell wall and ruptures the rhizodermis and outer cortex of the meristem, which adversely affects the tip growth of the roots. Cracks can be observed in epidermis of aluminum injured stubby roots which reduces fine branching and root hairs resulting in poor water use efficiency (Jones et al. 2006; Kopittke et al. 2008). Severe effects on root growth by aluminum results in susceptibility to mineral nutrients deficiencies and consequently decreasing crop yield (Poschenrieder et al. 2008; Farias et al. 2011).

The mechanism responsible for aluminum resistance in plants is still not completely understood (Horst et al. 2010), but it is believed that two main aluminum resistance mechanisms have been used by different plants to avoid the damaging effects of aluminum toxicity: (i) apoplast or exclusion mechanism prevents aluminum to enter in the root cells, and (ii) symplast or tolerance mechanism immobilizes aluminum within cell. Aluminum exclusion mechanism involves the release of organic acid anions in the rhizosphere were they form nontoxic compounds by chelating $Al^{3+}$ ions, whereas aluminum tolerance mechanism tolerates aluminum by translocating the accumulated

aluminum from root cell wall into the root cytoplasm and then transport and hide away in the vacuole (Kochian at al. 2015). Aluminum exclusion mechanism has been reported in maize in which resistant plants accumulate less aluminum in root apices as compared to the sensitive plants. In addition, resistant plants produce specific organic acids such as citrate and oxalate. These organic acids restrict aluminum to enter in the root apices by forming complex compounds with aluminum (Piñeros et al. 2005; Liu et al. 2009; He et al. 2012). Aluminum-activated malate transporter (ALMT) and multi-drug and toxic compound extrusion (MATE) transporter are responsible for aluminum resistance based on root malate and citrate exudation in response to aluminum stress. In aluminum resistant maize plants, *Zee mays* ALMT1 (ZmALMT1) and *Zee mays* MATE1 (ZmMATE1) transporters trigger the malate and citrate exudation when exposed to aluminum and are significantly higher in aluminum resistant maize genotypes (Maron et al. 2010; Kochian at al. 2015).

Many studies assess aluminum resistance in maize (Cançado et al. 1999; Matonyei et al. 2014; Piñeros et al. 2005) and other crops (Famoso et al. 2010). These experiments assessed the genotypes in a nutrient solution with and without aluminum having $Al^{3+}$ activity ranged from 40 to 160 μM. The main measures were relative root growth (RRG), hematoxylin staining and aluminum content determination in root apices. The resistant plants showed highest RRG, less aluminum content and no blue coloration of root apices as compared to sensitive plants. Hematoxylin binds with aluminum in the symplast and cell membranes and show intense blue coloration. That's why the aluminum sensitive roots that accumulate more aluminum in the root apices turn dark blue when stained with hematoxylin. Famoso et al. (2010) compared aluminum resistance in rice, wheat, maize and sorghum genotypes in a nutrient solution with and without aluminum. These authors measured the degree of root inhibition by

RRG and based on this variable, showed that rice was significantly more tolerant than maize, wheat and sorghum.

Other studies evaluated the toxic effect of aluminum on root growth and external morphology of two contrasting maize (Souza at al. 2016) and cowpea (Kopittke et al. 2008) populations. External morphology of root apices, with and without aluminum, has been evaluated by scanning electron microscopy and stereoscopy. Energy dispersive X-ray spectrometry (EDS) and stereoscopy showed greater aluminum accumulation in root apices of aluminum sensitive genotype as compared to resistant genotype. Results of scanning electron microscopy showed intense cell disorganization, injuries, deeper raptures and separation of the rizodermis and outer cortex from inner layers of root tips of sensitive populations. Souza et al. (2016) and Kopittke et al. (2008) concluded that aluminum resistant populations showed less aluminum accumulation and avoid severe injuries and damages on the surface of root tips as compared to the sensitive populations. Noguchi et al. (2015) compared two melaleuca species to identify their distinct aluminum resistance level. Aluminum accumulation in root tips was determined by analysis of aluminum content. Root tips were stained with hematoxylin and photographed under stereomicroscope to study root tissue damages. Results showed that aluminum sensitive plants accumulate 50% more aluminum in root apices as compared to the resistant plants. High rates of aluminum penetration and tissue damages were observed in aluminum sensitive plants as compared to the resistant plants.

Five hundred million hectares of land are acidic in Brazil (Vitorello et al. 2005) comprising roughly two third the territory, the largest area of acidic soils within a country. An alternative to raise pH of acid soils is lime application, which immobilizes and precipitates aluminum (Marion et al. 1976), but there are economical and technical difficulties to solve the aluminum problem solely by lime application (Fageria and

Baligar 2008). The best strategy to improve yield in acid soils is the use of aluminum resistant plants in association with pH neutralization by liming (Kosarveva 2012; Setotaw et al. 2015). Therefore, to achieve efficient agriculture practices, identification of aluminum resistant cultivars is very important, especially for soils with high aluminum toxicity. The identification and utilization of well adapted and resistant plant cultivars to acid soils will solve the problem related to acidic soils. The objective of the present investigation was to assess popcorn inbred lines for aluminum resistance.

## 2. Materials and methods

The 18 inbred lines assessed in this study represent a random sample of the inbred lines derived from the tropical popcorn populations Viçosa and Beija-Flor, developed by the Federal University of Viçosa (UFV), Minas Gerais state, Brazil. The inbred lines were developed based mainly on selection for expansion volume, a measure of popcorn quality (Viana et al. 2012). The first experiment provided an initial assessment of these inbred lines. Then, in a second experiment we assessed again ten of the 18 inbred lines, including the two most resistant, the two less resistant, and other six with intermediate performance, choose at random. Finally, in a third experiment we assessed the two most contrasting inbred lines, including also a resistant check, the hybrid 2B710PW from Dow AgroSciences.

The three experiments were performed in a greenhouse at UFV (lat $20^{0}50^{/}$S; long $42^{0}48^{/}$W; alt 640 m asl) using a complete randomized design and nutrient solution at constant temperature of 24º ± 1 C, 16 hour light and 8 hour dark photoperiod. The numbers of replications of the experiment one, two and three were three, three, and five and the experimental plot corresponded to four, four, and 16 seedlings, respectively. In these trials we assessed 432, 240, and 480 seedlings, respectively. The trials were performed from May 16, 2014 to May 23, 2014, March 01, 2015 to March 10, 2015 and April 05, 2016 to April 11, 2016. We adopted a standard design to measure aluminum resistance: the complete experiment in aluminum solution (+Al) and the same experiment in no aluminum solution (-Al).

Seeds of each inbred line were treated with the fungicide (captan-400) and germinated in rolled wetted filter papers in a dark chamber at 26º C ±1 for 5 days. Upon germination, undamaged seedlings of each inbred line with uniform root length were transferred to 8-litters tubs containing nutrient solution for 24 hours with continuous

aeration. Each tub was filled with eight litters of nutrient solution which was prepared as described by Magnavaca et al. (1987) and modified by Famoso et al. (2010). The solution contained (in mmole $L^{-1}$): 1 KCl, 1.5 $NH_4NO_3$, 1 $CaCl_2$ and (in μmole $L^{-1}$) 45 $KH_2PO_4$, 200 MgSO4, 500 $Mg(NO_3)_2$, 155 MgCl2, 11.8 $MnCl_24H_2O$, 33 $H_3BO_3$, 3.06 $ZnSO_47H_2O$, 0.8 $CuSO_45H_2O$, 1.07 $Na_2MoO_4H_2O$ and 77 Fe-EDTA. Tubs were covered with the metal plate having 24 holes at a distance of 3 cm to install the plants which were supported with foam strips with a slit cut into the foam to anchor the stem.

After 24 hours in the nutrient solution, seedlings of each inbred line were photographed and initial root growth (IRG) was measured using the WinRhizo Pro 2009a software. The roots were placed on a table with the blue background in a rectangle of 30 cm long and 20 cm wide and photographed from the uniform distance (30 cm) by using a digital camera fixed with a stand (Clark et al. 2013). All pictures were photographed with the same dimensions. Subsequently, half seedlings from each inbred line were transferred again to the nutrient solution without aluminum and half seedlings to the nutrient solution containing 540 μM $AlCl_3$ (160 μM $Al_3^+$). To prevent aluminum precipitation pH of the nutrient solution was adjusted to 7.8 with KOH before adding $AlCl_3$ and finally pH was adjusted to 4.0 with 1 N HCl (Famoso et al. 2010).

After 7 days, the seedlings were removed from the solutions and roots were scanned with EPSON LA2400 Perfection V700/V750 scanner equipped with additional light (transparency unit) at a resolution of 400 dpi. Final root growth (FRG) was measured by using WinRhizo software (Bouma et al. 2000). Net root growth (NRG) of plant seedlings, in aluminum solution (+Al) and in no aluminum solution (-Al), was calculated as follows

NRG= FRG-IRG

Finally, RRG was calculated as

RRG = NRG (+Al) / NRG (-Al).

In the second experiment we also measured the hematoxylin staining. After seven days exposure to the aluminum, the roots of four seedlings from each replication were removed from the solution and gently shaken in 200 ml distilled water for 20 minutes. After that, water was replaced by 200 ml of aqueous hematoxylin solution (0.2% hematoxylin and 0.02% potassium iodide, w/v) and gently shaken again for 30 minutes. Finally, the first step was repeated by using 200 ml water to remove the excessive stains on the root surface. Staining was performed as described by Polle et al. (1978) and modified by Cançado (1997). Staining intensity was scored using a 0 to 5 visual scale as described by Guevara et al. (1992): 0, roots without any stain; 1, very light blue coloration; 2, light blue color in tips and above elongation zone; 3, light blue color in elongation zone; 4, more intense blue color with some lightly stained patches; and 5, dark blue staining of the whole root.

Besides determining the RRG and the hematoxylin staining score, in the third experiment we further preceded electron microscopy analysis and determined the aluminum content in the seedlings root tips. After seven days in the respective treatments, 0.5 cm root tip of two randomly selected seedlings were removed and stored in 2.5% glutaraldehyde. Before electron microscopy, the root tips were dehydrated in an ethylic series (30 to 100%; v/v) and dried with $CO_2$ at critical point (Baltec model CPD 030, Liechtenstein). The dried tips were fixed in an aluminum bracket and transferred to Metallizer to get covered with gold. The scanning of root tips was conducted using a scanning electron microscope (Leo model 1430VP, Cambridge, England) operated at 20 kV.

After seven days, 1 cm root tip of eight randomly selected seedlings was removed, oven dried and grinded to determine aluminum content in the root tips of seedlings

growing with and without aluminum. Approximately 0.1 g of grinded root tips was incubated in 2 M HCl for 48 h (Ma et al. 2004). After that, the Al content of the samples were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES) at a wavelength of 394.401 nm.

Stereoscopic analysis was performed after plant exposure to presence and absence of aluminum in a nutrient solution. Two seedlings were selected randomly and 1 cm root tips were removed and stored in 2.5% glutaraldehyde. After that, root tips were removed from 2.5% glutaraldehyde and gently shaken in 100 ml distilled water for 20 minutes. Then, water was replaced by 100 ml of aqueous hematoxylin solution and was left under slow agitation for 30 minute. Finally, the root tips were washed again with distilled water to remove the excessive stains, assessed and photographed under stereoscope.

The RRG, hematoxylin staining score, and aluminum content data were submitted to analysis of variance using the GLM procedure of the SAS system v9.2 (SAS Institute 2007) and the treatment means were compared by the Tukey's test at 5% level of significance.

## 3. Results

Analysis of variance for RRG, hematoxylin staining score and aluminum content showed highly significant differences among inbred lines in all three experiments (Table 1). The inbred line 11-133 showed the highest means for RRG and the lowest means for hematoxylin staining score and aluminum content followed by the inbred line 11-363, whereas the inbred line 11-60 showed the lowest means for RRG and the highest means for hematoxylin staining score followed by 11-423 (Table 2). The inbred line 11-60 showed 1.6 to 1.8 times more aluminum content as compared to 11-133 and 2B710PW (Table 2). Inbred lines 11-133 and 11-60 and 2B710PW showed the greater differences in root growth (Fig. 1). The coefficient of variation for RRG ranged from 14.5 to 24.8, evidencing good experimental precision. The CVs for hematoxylin staining score and aluminum content were 5.4 and 12.8, also showing good precision.

The stereoscopy and electron microscope scanning of root tips grown without aluminum of all three cultivars didn't show any abnormality on the apical region (Fig. 2 and 3). Stereoscopic analysis showed intense dark blue coloration by aluminum treated root tips of inbred line 11-60 in 2 mm apical root region when stained with hematoxylin (Fig. 2). In contrast, the inbred line 11-133 and 2B710PW showed a less blue staining to the root tips (Fig. 2). Whereas, the electron micrographs of the aluminum treated inbred line 11-60 showed severe damage and cell disorganization in the apical region (1 mm from the tip). In contrast, inbred line 11-133 and 2B710PW did not show severe damages or ruptures in the apical region of the root tip (Fig. 3). The ruptures were big and deep in 11-60 when examined with high magnification (Fig. 4).

## 4. Discussion

For all traits, the coefficients of variation were lower than those obtained by Cançado et al. (1999) and Giaveno et al. (2000), who evaluated RRG, net root growth and hematoxylin staining score. The CVs for hematoxylin staining score ranged from 12.7 to 22.6%. For RRG, Cançado et al. (1999) observed 30.5%. Thus, our CVs indicated good experimental precision.

RRG is the most commonly used trait to assess aluminum resistance of cereal crops (Magnavaca et al. 1987; Cançado et al. 1999; Famoso et al. 2010). In the first experiment, inbred lines 11-133 and 11-363 exhibited the highest RRG that is comparable to the value reported by Famoso et al. (2010) for aluminum resistant maize inbred line B57 (0.13), while in second experiment inbred lines 11-133 and 11-363 showed higher RRG as compared to aluminum resistant maize Cateto (0.17) and B57 assessed by Famoso et al. (2010) in 160 μM $Al^{3+}$. Similarly, the inbred lines 11-133 and 11-363 were as resistant as the aluminum resistant check, 2B710PW, which showed the largest indices of aluminum resistance when compared with all other commercial hybrids from the company Dow AgroSciences (confidential information - data not published). These results demonstrated that our inbred lines are more aluminum resistant, especially 11-133 which showed 50% more RRG as compared to the aluminum resistant maize Cateto. Cateto was bred for acid soils in Brazil and it is one of the most aluminum resistant maize inbred lines (Piñeros et al. 2005). In contrast, the inbred lines 11-60 and 11-423 showed the lowest RRG that is even much lower than the value reported by Famoso et al. (2010) for aluminum sensitive inbred line B73 (0.07). Inbred line B73 is aluminum sensitive and widely used as a sensitive check in aluminum resistance studies (Moran et al. 2010). Comparison of RRG showed that the inbred lines 11-60 and 11-423 are more sensitive as compared to the maize sensitive inbred line

B73. We have observed variation for the mean values of same inbred lines for RRG among three experiments. Similarly, aluminum resistant maize inbred line Cateto also showed variation for RRG. Famoso et al. (2010) reported approximately 50% less RRG (0.45) as compared to the value obtained by Matonyei et al. (2014) for Cateto, in 27 μM $Al^{3+}$ (0.92). There is always a chance of variation for RRG of different experiments even between the same populations in the same level of aluminum concentration.

Hematoxylin has the property of turning blue when it forms a complex with aluminum (Delhaize et al. 1993; Cançado et al. 2009). Therefore, aluminum sensitive plants showed dark blue staining because of accumulated aluminum in the root apices as compared to the aluminum resistant plants (Polle et al. 1978; Castilhos et al. 2011). In a study of Piñeros et al. (2002), aluminum treated resistant "cv Cateto-Colombia" showed light blue coloration in the root tips similar to our inbred lines 11-133 and 11-363 that obtained very light blue coloration indicating less aluminum accumulation in root apices. Hematoxylin staining score of inbred lines 11-60 and 11-423 were comparable to the scores reported by Piñeros et al. (2002) for aluminum sensitive maize inbred line Mo17 which exhibited dark blue staining of the whole root because of the dramatic aluminum accumulation in root apices. Stereoscopic analysis of inbred line 11-133 and aluminum resistant check showed very light traces of blue coloration, which indicated the less aluminum accumulation in their root apices. In contrast, 11-60 showed intense dark blue coloration throughout the root tip because aluminum accumulated in root apices. Other studies also suggested the same pattern of coloration for aluminum resistant and sensitive populations. Souza et al. (2016) reported similar degree of staining for aluminum resistant maize populations UFM-200 and UFM-100. Resistant UFM-100 exhibited greater dark blue staining as compared to the sensitive UFM-200.

The degree of aluminum resistance is correlated with the root tip aluminum content because an aluminum exclusion mechanism underlies aluminum resistance in maize and aluminum sensitive plants lacking such exclusion mechanism, which results in more accumulation of aluminum in root tips as compared to resistant plants (Piñeros et al. 2005). The inbred line 11-133 showed the greater resistance by accumulating the lower concentration of aluminum content as compared to resistant Cateto (2,192 μg Al/g) examined by Famoso at al. (2010). Piñeros et al. (2005) and Matonyei et al. (2014) reported lower aluminum concentrations in root apices of resistant Cateto as compared to 11-133. This might be due to the use of lower aluminum concentration (40 μM $Al^{+3}$) as compared to 160 μM $Al^{+3}$, which was used in present experiments. Inbred line 11-133 accumulated almost 11 percent less aluminum as compared to the aluminum resistant check. This difference was not statistically different but it showed that the inbred line 11-133 has better level of resistance as compared to 2B710PW. In contrast, inbred line 11-60 tended to have approximately 1.8 times higher aluminum concentration in root apices as compared to 11-133. Similarly, Famoso et al. (2010) reported that aluminum sensitive inbred line B73 accumulated 2 times more aluminum as compared to the resistant Cateto. Mayonyei et al. (2015) reported same level of aluminum accumulation (1,614 μg Al/g) in root apices of aluminum sensitive maize B73 as compared to the inbred line 11-60.

Electron microscope scanning revealed marked similarities between the nature of the ruptures observed by the inbred line 11-60 and the ruptures found by Kopittke et al. (2008) on the root apices of sensitive cowpea seedlings. They found deep ruptures and damage to the root apices of sensitive seedlings along with severe root inhibition when exposed to higher aluminum concentration. Souza et al. (2016) found intense wider and deeper ruptures on the root apices of aluminum sensitive maize UFM-100. This pattern

of injury is in clear agreement with our observations in root apices of inbred line 11-60. They also reported negligible damages to aluminum resistant maize UFM-200, in contrast to 11-133 that did not show any visible damage to root apices. This suggested that 11-133 is more resistant to aluminum as compared to aluminum resistant maize UFM-200. Inbred line 11-133 avoids any physical damage and ruptures to the root apices and showed similar level of resistance as compared to 2B710PW. Motoda et al. (2011) observed similar effects of aluminum on external morphology of resistant and sensitive genotypes in pea. The cause of these ruptures in aluminum sensitive plants is the accumulation of more aluminum in root tips which affects root elongation (Giannakoula et al. 2008) and increases root diameter and tears external cortex and rhizodermic cells of the elongation root zone (Motoda et al. 2010).

Inbred line 11-133 showed minimum visual score for hematoxylin staining and highest RRG in all experiments. It also showed less aluminum accumulation followed by no physical damages to the external morphology of the root apices. Based on these results we suggested 11-133 as aluminum resistant popcorn inbred line. In contrast, 11-60 recorded the lowest RRG, highest aluminum staining scores and highest aluminum content and severe damages to the root apices. It indicated that this inbred line is the most sensitive one that accumulated more aluminum in root apices and could not resist the toxic effects of aluminum, hence considered as the aluminum sensitive popcorn inbred line. Our results suggested that the resistant popcorn inbred lines used aluminum exclusion mechanism which releases organic acids, such as citrate and oxalate, from the root apices when exposed to aluminum. These organic acids form complex compounds with aluminum and restrict aluminum to enter in root tips (Kochian at al. 2015).

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**Table 1** Analyses of variance, mean, and coefficient of variation (%) for relative root growth (RRG), hematoxylin staining score (HS), and aluminum content of root tips (Al; μg/g) measured in two to 18 inbred lines and one resistant check, in three experiments

| SV | Experiment 1 | | Experiment 2 | | | Experiment 3 | | |
|---|---|---|---|---|---|---|---|---|
| | df | MS | df | MS | | df | MS | |
| | | RRG | | RRG | HS | | RRG | Al |
| Treatments | 17 | 0.05* | 9 | 0.35* | 2.70* | 2 | 0.12* | 472,014* |
| Error | 36 | 0.01 | 20 | 0.03 | 0.04 | 12 | 0.01 | 23,960 |
| Mean | | 0.06 | | 0.16 | 3.48 | | 0.19 | 1,206.40 |
| CV | | 20.7 | | 24.8 | 5.4 | | 14.5 | 12.8 |

*Significant at a P value < 0.01.

**Table 2** Average relative root growth (RRG), hematoxylin staining score (HS), and aluminum content of root tips (Al; μg/g) of inbred lines and one resistant check, in three experiments

| Experiment 1 | | Experiment 2 | | | Experiment 3 | | |
|---|---|---|---|---|---|---|---|
| Inbred | RRG | Inbred | RRG | HS | Treat. | RRG | Al |
| 11-133 | 0.15$^{A}$ | 11-133 | 0.37$^{A}$ | 1.5$^{D}$ | 11-133 | 0.26$^{A}$ | 926.4$^{B}$ |
| 11-363 | 0.11$^{B}$ | 11-363 | 0.29$^{A}$ | 3.0$^{C}$ | 2B710PW | 0.24$^{A}$ | 1,032.5$^{B}$ |
| 11-52 | 0.09$^{BC}$ | 11-209 | 0.28$^{A}$ | 3.0$^{C}$ | 11-60 | 0.06$^{B}$ | 1,660.3$^{A}$ |
| 11-209 | 0.08$^{BCD}$ | 11-383 | 0.14$^{B}$ | 4.0$^{B}$ | | | |
| 11-109 | 0.07$^{CDE}$ | 11-566 | 0.10$^{B}$ | 3.4$^{C}$ | | | |
| 11-140 | 0.07$^{CDEF}$ | 11-403 | 0.08$^{B}$ | 3.4$^{C}$ | | | |
| 11-50 | 0.06$^{CDEFG}$ | 11-15 | 0.07$^{B}$ | 3.2$^{C}$ | | | |
| 11-391 | 0.06$^{CDEFG}$ | 11-142 | 0.06$^{B}$ | 4.1$^{B}$ | | | |
| 11-15 | 0.05$^{CDEFG}$ | 11-423 | 0.06$^{B}$ | 4.3$^{B}$ | | | |
| 11-59 | 0.05$^{EFGHD}$ | 11-60 | 0.05$^{B}$ | 5.0$^{A}$ | | | |
| 11-403 | 0.05$^{EFGH}$ | | | | | | |
| 11-142 | 0.04$^{EFGH}$ | | | | | | |
| 11-566 | 0.04$^{GFH}$ | | | | | | |
| 11-383 | 0.04$^{GH}$ | | | | | | |
| 11-387 | 0.04$^{GH}$ | | | | | | |
| 11-141 | 0.03 $^{GH}$ | | | | | | |
| 11-423 | 0.03 $^{GH}$ | | | | | | |
| 11-60 | 0.02 $^{H}$ | | | | | | |

Means followed by the same letters do not differ by the Tukey's test at 5%.

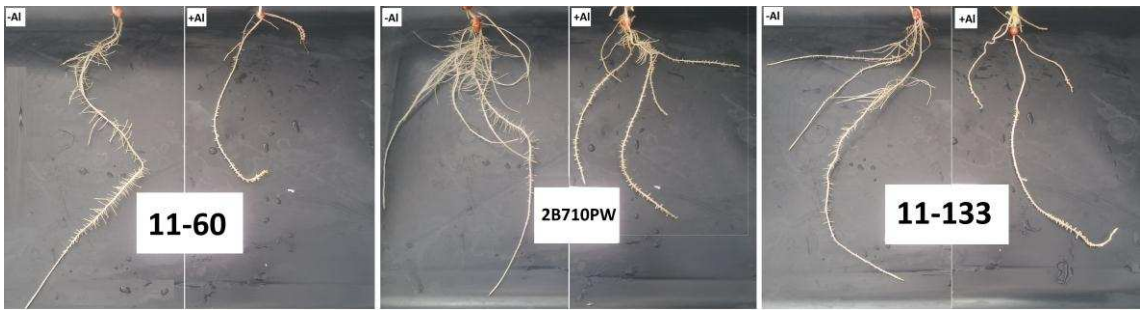


**Fig. 1** Growth of two contrasting inbred lines and aluminum resistant check after 7 days of exposure to nutrient solution in 0 and 540 μM $AlCl_3$. All pictures have the same dimensions (30 x 20 cm).

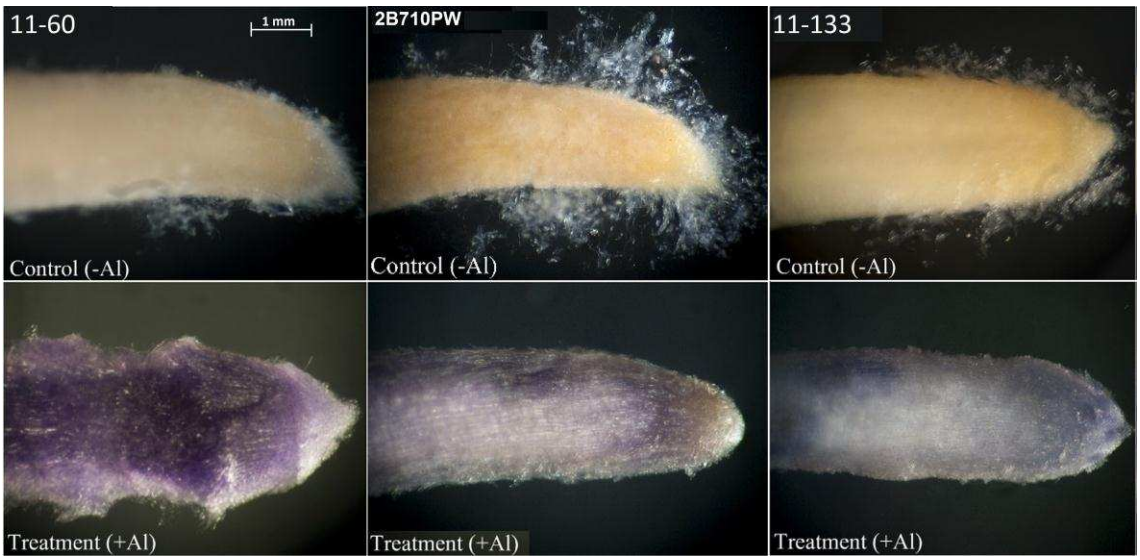


**Fig. 2** Stereoscopic view of root apices stained with hematoxylin after 7 days of exposure to nutrient solution in 0 and 540 μM $AlCl_3$.

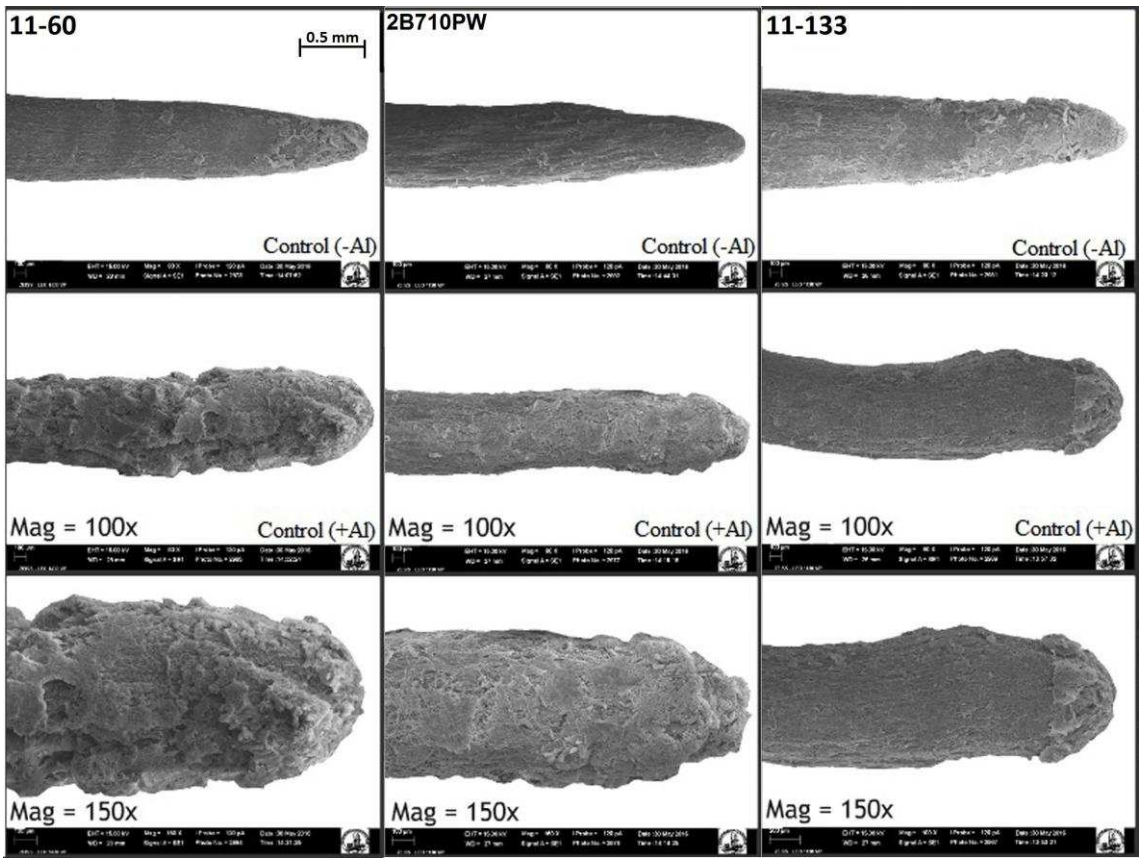


**Fig. 3** Scanning electron micrographs of the root tips of two popcorn inbred lines and a resistant hybrid, after plant treatment in 0 and 540 μM $AlCl_3$.

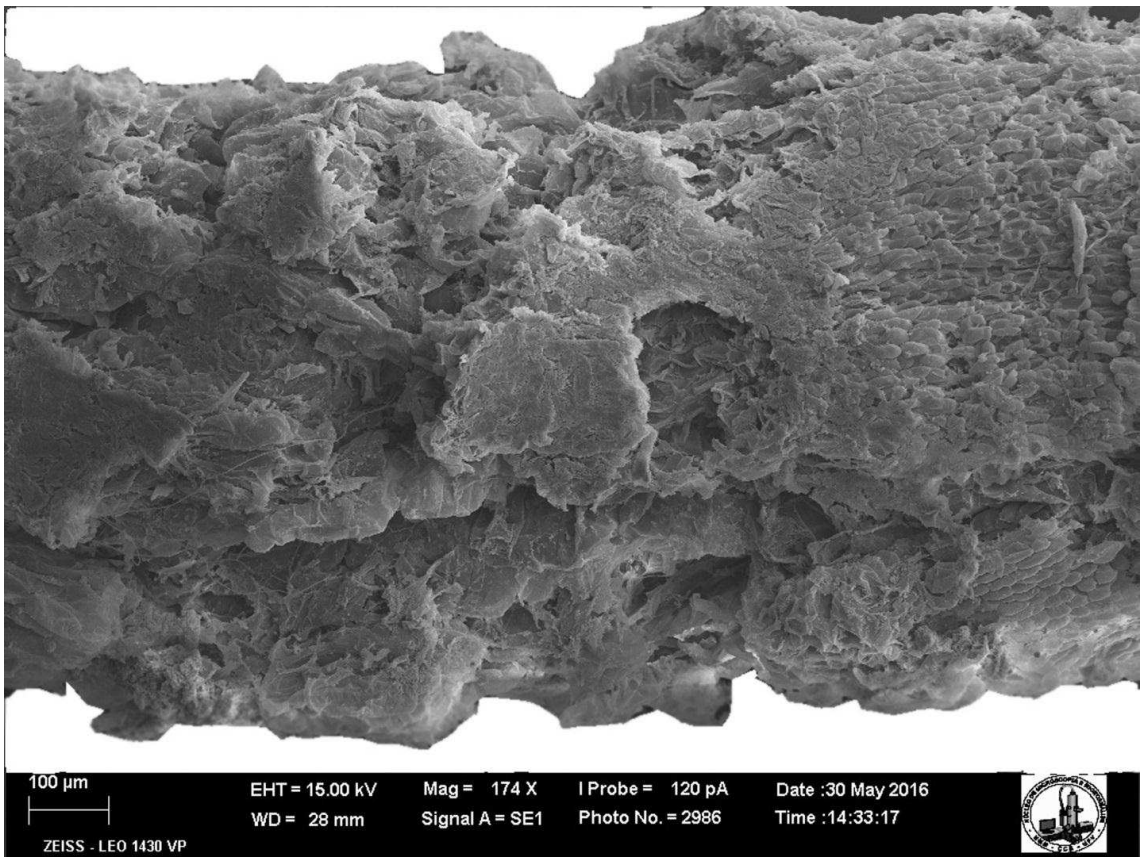


**Fig. 4** Scanning electron micrograph showing the ruptures in the root tips of the 11-60 inbred line.