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ALUMINIUM TOLERANCE OF OAT CULTIVARS UNDER HYDROPONIC AND ACID SOIL CONDITIONS

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SUMMARY

Aluminium toxicity is an important abiotic factor limiting the growth and yield of oat plants (*Avena sativa* L.) and other cultivated species. The objectives of this study were to evaluate the response of oat cultivars at the reproductive stage to aluminium under acid soil conditions and to compare with the responses observed at seedling stage under hydroponic conditions. In the soil, the damage to the above-ground part of the plant was estimated by the morphological response to aluminium, shoot length, shoot dry mass and plant height and, to the below-ground part of the plant as the length of roots and root dry mass. In hydroponics, the primary root regrowth was used to define the level of tolerance. The comparison of the results obtained in acid soil with those obtained in hydroponics demonstrated that both conditions produced essentially the same responses. The use of hydroponic solution can be a valuable tool for phenotyping large populations, especially useful for breeding programmes located in regions were aluminium is not present at toxic levels in the soil.

INTRODUCTION

Several abiotic factors negatively affect the growth and yield of oat plants and other cultivated species. One of these factors is the prevalence of soluble aluminium (Al^{3+}) ions present in acid soils (pH values at or below 5.0). The Food and Agriculture Organization of the United Nations (FAO) lists aluminium toxicity as affecting 14% of all soils worldwide (http://www.fao.org/nr/aboutnr/nrl/en/#terra-statdb). However, this proportion can be higher than 50% of potential arable land in many countries, especially in tropical and subtropical areas, where food production is considered a critical issue (Kochian *et al.*, 2004).

Plants cultivated in the presence of Al^{3+} in the soil display inhibited root growth, low water and nutrient uptake, less vigour, reduction of the photosynthetic rate and, therefore, lower yield and quality of grains (Kochian *et al.*, 2005). Furthermore, Al^{3+} affects cellular functions by modifying intracellular and extracellular interactions, such as the obstruction of anion channels, reduced uptake of Ca^{2+} and Mg^{2+}, competition with Ca^{2+} for essential binding sites in the apoplast, alteration in the cytoskeleton

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structure, interaction with DNA, disruption of signal transduction pathways and triggering the production of reactive oxygen species, which may be related to the inhibition of root growth caused by aluminium (Kochian *et al.*, 2004; Ramos-Díaz *et al.*, 2007; Ryan *et al.*, 2010; Sivaguru *et al.*, 2000).

During their evolution, plants have developed sophisticated mechanisms to manage the negative effects caused by toxic aluminium. These mechanisms can be divided into two main strategies: tolerance mechanisms (symplastic) and exclusion mechanisms (apoplastic) (Kochian *et al.*, 2004). Tolerance mechanisms allow plants to safely accommodate the Al^{3+} that reaches the symplast by chelating it in the cytosol or sequestering it in cellular organelles where it will not affect cell metabolism (de Andrade *et al.*, 2011; Illes *et al.*, 2006). In contrast, exclusion mechanisms do not allow for the accumulation of Al^{3+} in the symplast, but they are based on the exudation of organic acids through the roots that detoxify cations in the apoplast, in the transport systems of Al^{3+} from the symplast to the apoplast and in the ability to repair the damage to the cell walls caused by Al^{3+} (Delhaize *et al.*, 2012; Huang *et al.*, 2009; 2012, Taylor, 1995). Genetic variability related to aluminium tolerance can be observed between and within species. Genotypes within the same species can differ greatly in relation to their ability to tolerate toxic aluminium. This variation has been used by plant breeders in the development of cultivars that are more adapted to acid soils (Garvin and Carver, 2003).

The experimental screening of genotypes that are aluminium sensitive or tolerant can be conducted in hydroponic solution or in acid soils with a high concentration of aluminium. Evaluations of aluminium tolerance in acid soils have been described for various species, such as alfalfa, barley, sorghum, wheat and maize (Foy, 1996). However, difficulties in quantifying and homogenizing the aluminium in the soil, its interactions with other elements such as phosphates and, the effects of other toxic elements increased by low pH, namely manganese and iron, are significant limitations of this method (Kerridge *et al.*, 1971). The use of a hydroponic solution, however, can overcome the restrictions imposed by the soil in the screening of aluminium tolerance because nutritional and toxicity factors, such as pH, toxic aluminium, manganese, iron and other factors, can be controlled.

Several studies have been conducted to determine the genetic components involved in the control of the tolerance to aluminium in hexaploid oat (Castilhos *et al.*, 2011; Nava *et al.*, 2006; Oliveira *et al.*, 2005; Wagner *et al.*, 2001). In all these studies, the strategy used for the identification of genotypes that are aluminium sensitive or tolerant was based on the regrowth of seedlings main root in hydroponic solution. The results provided important evidence on the genetic mechanisms involved in the control of aluminium tolerance in this species. Nevertheless, none of these reports compared the response of the evaluated genotypes in hydroponic solution with their response to aluminium in acid soil. This comparison is crucial to estimate the efficiency of the different strategies that may be used by oat-breeding programmes in the screening and selection of tolerant genotypes to toxic aluminium. The objectives of this study were to evaluate the response of oat plants at the reproductive stage to aluminium under acid soil conditions,

and to compare to the responses observed at seedling stage under hydroponic conditions.

MATERIALS AND METHODS

Plant material

A genetic population of recombinant inbred lines (RILs) developed from the cross 'UFRGS 930598–6' (sensitive to Al^{3+}) and 'UFRGS 17' (tolerant to Al^{3+}) was analysed in this study. The parental lines UFRGS 930598–6 and UFRGS 17 were obtained from the oat breeding programme at the Federal University of Rio Grande do Sul (UFRGS), Brazil. From this cross, a total of 155 RILs were developed by single-seed descent to the F_5 generation. This population was selected based on the differential response to aluminium tolerance of the parental lines observed in previously reported studies (Oliveira *et al.*, 2005; Wagner *et al.*, 2001). The response to aluminium toxicity of the parental lines and RILs was evaluated in hydroponic solution under controlled conditions. A sample of RILs that exhibited different responses to toxic aluminium tolerance in hydroponic solution was selected to be assessed under acid soil conditions.

Aluminium tolerance screening in hydroponic solution

The aluminium response of each RIL and parental line was determined as the regrowth rate of the main root after exposure to Al^{3+} as reported by Nava *et al.* (2006). Briefly, the experiment was conducted in a complete randomized design with two replicates. Each replicate consisted of a sample of about 10 seedlings and the average root regrowth of each sample was used as the replicate value. Pre-germinated seeds with approximately five mm-long radicles were selected and distributed on screens adapted to lids placed over 8.3 litre plastic pots, arranged in a water bath and kept at constant temperature of 17±1 °C. The hydroponic solution was constantly aerated and the pH was adjusted to 4.5 and maintained at this level by providing sulphuric acid (H_2SO_4) as needed. Pre-germinated seeds were first grown for 48 hours in a complete solution (free of aluminium), then transferred to the treatment solution (740 μM of $Al_2(SO_4)_3.18H_20$) for another 48 hours, and finally transferred back to the complete solution for an additional 72 hours. Root growth was reinitiated after removal from aluminium solution and root regrowth of the main root of each seedling was measured starting from the point of root thickening (callosity). The lines were ranked based on root regrowth and characterized as sensitive and tolerant depending on the similarity to the parental lines performance. The complete solution was: $Ca(NO_3)_2$. 4 H_2O – 4 mM, $MgSO_4$. 7 H_2O – 2 mM, KNO_3 – 4 mM, $(NH_4)_2SO_4$ – 0.435 mM, KH_2PO_4 – 0.5 mM, $MnSO_4$. H_2O – 2μM, $CuSO_4$ – 0.3μM, $ZnSO_4$. 7 H_2O – 0.8μM, NaCl – 30μM, Na_2MoO_4.2 H_2O – 0.10μM, H_3BO_3 – 10μM. The treatment solution was comprised of one-tenth of the complete solution except for phosphate which was omitted in order to avoid precipitation with aluminium. The availability and activity of aluminium in the solution was calculated using the software Visual Minteq 3.0.

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Aluminium tolerance screening in acid soil

A sampling of 22 RILs that showed different responses to aluminium in hydroponic solution and the parental lines UFRGS 930598–6 and UFRGS 17 were included as entries in an experiment designed to measure differences in aluminium tolerance under acid soil conditions in the field. The experiment was carried out at the National Wheat Research Center – CNPT/Embrapa, which is located in Passo Fundo, RS, Brazil (28° 15′ 46″ S; 52° 24′ 24″ W), with a mean altitude of 687 meters above sea level, a humid subtropical climate and a clayey Dystrophic Red Latosol, containing 44.1 $mmol_c\ dm^{-3}$ of toxic aluminium. Each plot was sown in five rows, three meters long, with a space of 0.20 meters between the rows, with a sowing density of 300 seeds per square meter, under a tillage system. An experimental design of randomized blocks was used, with four replicates. The basic fertilization was composed by the combination of 300 kg ha^{-1} of nitrogen–phosphorus–potassium at a 5–25–25 ratio. Plant responses to the aluminium present in the soil were measured based on the damage to the above-ground and below-ground part of the plant. The response variables measuring above-ground part of the plant included the morphological response to aluminium, shoot length, shoot dry mass and plant height. The response variables measuring below-ground part of the plant were the root system length and root dry mass.

Morphological response to aluminium

Morphological response to aluminium was estimated for each entry (RIL and parental lines) by means of a visual scale adapted from wheat (de Souza, 1998), varying from zero to nine. The scoring criteria considered the level of damage invoked by aluminium to the shoot (above-ground part of the plant). Based on this scale: 'nine' corresponded to the maximum aluminium tolerance (no visual effects); 'eight' = highly tolerant, normal plant development, absence of chlorosis and substantial number of tillers; 'seven' = aluminium tolerant, normal plants, absence of chlorosis, slightly less vigour than class eight and substantial number of tillers; 'six' = aluminium tolerant, normal plants, absence of chlorosis, less vigour and slightly reduced number of tillers, when compared to the previous class; 'five' = moderately aluminium tolerant, absence of chlorosis and fewer tillers than previous class; 'four' = moderately aluminium sensitive, presence of few tillers and vigour significantly reduced; 'three' = aluminium sensitive, reduced leaf size, no tillers and presence of chlorosis, mainly in basal leaves; 'two' = aluminium sensitive, reduced leaf size, no tillers and presence of chlorosis in all leaves, 'one' = highly aluminium sensitive, poor plant development and presence of necrosis, mainly in older leaves; 'zero' = highly aluminium sensitive, poor plant development and presence of necrosis in all leaves. The evaluation was conducted within each plot and among the different replicates of the same line during the reproductive phase of plants at stage 55th on Zadoks' growth scale, when the toxic effects of aluminium were most evident.

Shoot length, shoot dry mass, root system length and root dry mass

Ten random plants within each plot were evaluated at stage 34th on Zadoks' growth scale, always in the same position within the plots, not taking into consideration the presence or absence of damage caused by the aluminium to the shoot. The shoot length was measured from the base of the shoot to the leaf apex. The root system was accessed by carefully cutting the soil near the plant using a shovel at a depth of approximately 25 cm and washed. Roots were not observed beyond this depth. The root system length was carried out through the measurement of roots extended in a horizontal position, from root tip to the base of the root. Roots were separated from the shoot of the plants to measure shoot dry mass and root dry mass.

Plant height

Plant height was evaluated at stage 90th on Zadoks' scale, through the measurement of ten random plants of each line and in each replicate. The measurement consisted of the distance between the base of the shoot close to the ground and the tip of the panicle. The mean height of the plants in this study was also compared to the mean height of the same RILs and parental lines evaluated in an aluminium-free soil experiment. This experiment was conducted in a sandy clay loam Acrisol (Paleudult) located at the Agronomic Experimental Station of UFRGS in Eldorado do Sul, RS, Brazil (30° 06′ 12″ S; 51° 40′ 14″ W). The plots were sown in five rows, three meters long, with a space of 0.20 meters between the rows, with a sowing density of 300 seeds per square meter, under a tillage system. An experimental design of randomized blocks was used, with four replicates.

Data analysis

The results were evaluated using analysis of variance and the F test, with a probability of 5 and 1%, respectively, to verify differences between the different lines and variables. In the analysis of variance, the sum of squares of the lines was partitioned into groups of sensitive and tolerant to toxic aluminium and evaluated by the F test, with a probability of 1%. The mean value of the response variables measured in acid soil were compared within genotype groups, using Tukey's test at $\alpha = 0.05$. The association between primary root regrowth (independent variable) and morphological response to aluminium (dependent variable) was performed by linear and non-linear regressions. The best fit was reached through a bi-segmented linear regression, which was estimated by the software SegReg. The segmentation of the linear regression is achieved by the estimation of a break point able to maximize the coefficient of determination (R^2) and to increase the significance of the regression model, as compared to the non-segmented linear regression. In this case, both segmented and non-segmented linear regressions were significant, but the segmented regression had higher significance and higher coefficient of determination.

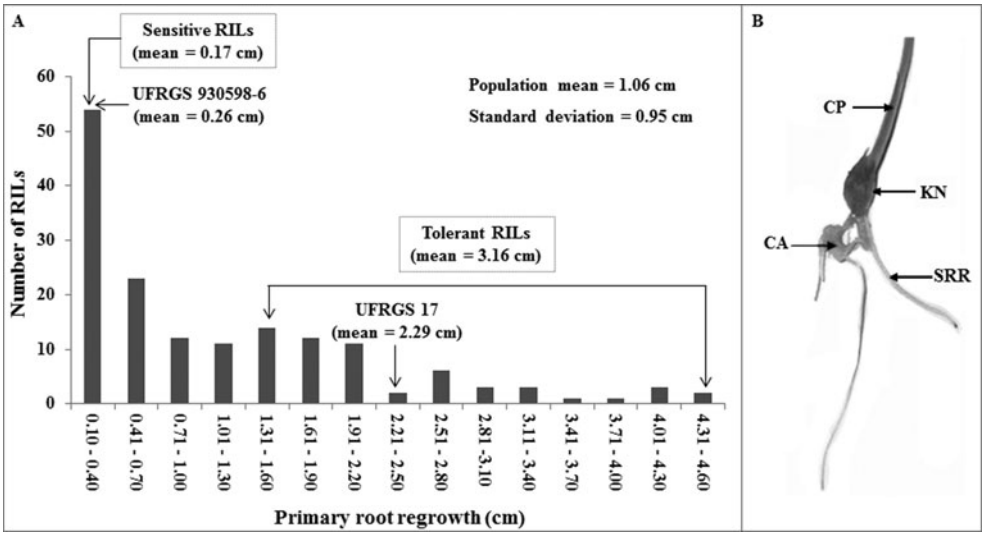

Figure 1. Aluminium tolerance screening at hydroponic conditions. (A) Primary root regrowth distribution of contrasting oat recombinant inbred lines (RILs) in relation to their tolerance to toxic aluminium in hydroponic solution. The mean primary root regrowth of the parental lines UFRGS 930598–6 and UFRGS 17 and the RILs were estimated from 10 seedlings, replicated twice. Analysis of variance demonstrate that the RILs differed among sensitive and tolerant to toxic aluminium (F test = 189.25; $p \leq 0.0001$). (B) Growth patterns of the primary root of a tolerant oat plant after exposure to aluminium in hydroponic solution. CP = coleoptile, KN = oat kernel, CA = callosity, SRR = secondary root regrowth and PRR = primary root regrowth. During the exposure to aluminium, the growth and development of the root system are completely inhibited. Root growth is reinitiated after removal from the aluminium solution and root regrowth starts at the root callous.

RESULTS AND DISCUSSION

The mean primary root regrowth distribution of the RILs evaluated in hydroponic solution is presented in Figure 1A. The parental lines UFRGS 930598–6 (sensitive to Al^{3+}) and UFRGS 17 (tolerant to Al^{3+}) showed mean primary root regrowth of 0.26 and 2.29 cm, respectively (Figure 1A). Among the RILs assessed at acid soil conditions in the field, 11 RILs exhibited mean primary root regrowth of 0.17 cm (varying from 0.12 to 0.22 cm), which is similar to the parental line UFRGS 930598–6 and were classified as sensitive to aluminium. Five lines were classified as tolerant to aluminium and showed mean primary root regrowth of 2.04 cm (varying from 1.5 to 2.5 cm), similar to the parental line UFRGS 17. Six lines were classified as highly tolerant to aluminium with mean primary root regrowth of 4.0 cm (varying from 3.3 to 4.6 cm); above to the parental line UFRGS 17 (Figure 1A).

These results demonstrate the genetic variability observed among RILs for tolerance to toxic aluminium, when evaluated in hydroponic solution. Despite the great genetic variability observed in oat germplasm for tolerance to toxic aluminium, the main mechanisms underlying the tolerance are still unknown (Nava *et al.*, 2006; Wight *et al.*, 2006). Differences in oxidative stress (Castilhos *et al.*, 2011) and malate secretion in response to aluminium (Radmer *et al.*, 2012) have been reported among oat genotypes.

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Table 1. Comparative response to toxic aluminium among different genotype groups of oat RILs grown at acid soil conditions in the field.

	Genotype groups		
Response variable	Sensitive	Tolerant	Highly tolerant
Morphological response to aluminium	3.4 b*	6.6 a	6.8 a
Shoot length (cm)	22.3 b	25.3 a	25.7 a
Shoot dry mass (g)	7.8 a	8.44 a	7.91 a
Root system length (cm)	9.9 a	10.6 a	10.1 a
Root dry mass (g)	1.80 a	1.78 a	1.70 a
Plant height (cm)	70.3 b	80.8 a	83.1 a

*Within rows and in different genotype groups (sensitive, tolerant, and highly tolerant lines) means followed by the same letter are not different according to Tukey's test at $\alpha = 0.05$.

The growth patterns of the primary root of a tolerant oat plant after exposure to aluminium in hydroponic solution are presented in Figure 1B. The regrowth of the primary and secondary roots demonstrated the capacity of the root apical meristem to either cope or avoid the toxic effects of aluminium. In fact, this trait measures the ability of the root to recover cell division and elongation after aluminium removal. It is expected to correlate well with aluminium tolerance per se in the whole plant in the presence of aluminium, besides the fact that it has also been used to identify genotypes tolerant to aluminium (Castilhos *et al.*, 2011; Nava *et al.*, 2006; Oliveira *et al.*, 2005; Wagner *et al.*, 2001).

When the parental lines and RILs were evaluated at acid soil conditions in the field, significant statistical differences were observed for morphological response to aluminium, shoot length and plant height. Conversely, no significant differences among the lines were observed for the variables of root system length, root dry mass and shoot dry mass (Table 1). These results demonstrate that the evaluated lines differed in relation to the above-ground variables, whereas there was no significant variation for the below-ground variables. Similar results have been reported for rice, barley, wheat and maize crops (Furlani and Furlani, 1991; Howeler and Cadavid, 1976), when evaluated in soil containing toxic aluminium.

The lack of phenotypic differentiation among the assessed lines for root variables might be associated with several factors present in the soil, which could not have been controlled during the experiment. Moreover, our procedure of always sampling from the same, previously defined plot position independently of the visual plant condition associated with the variation in aluminium concentration in the soil may have affected the results. The heterogeneous aluminium distribution, both within and between the plots, and possibly the presence of other toxic elements in the soil (especially manganese and iron) might also have contributed to mask the effects of toxic aluminium on the plants.

The results above reinforce the difficulty in analysing aluminium tolerance in field conditions. The difficulty in removing the plants from the soil without damaging the roots made it challenging to observe both the variability and the toxic effects

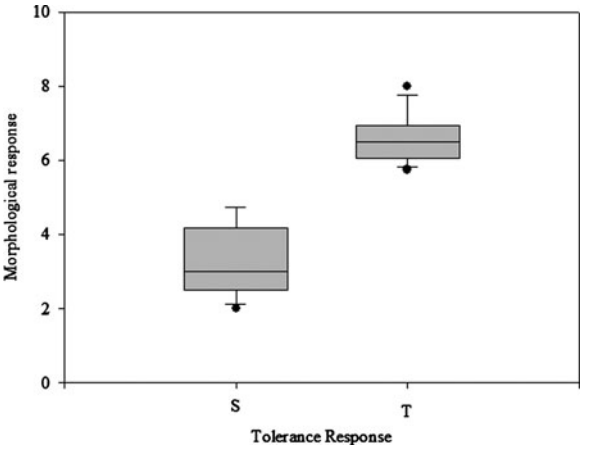

Figure 2. Morphological response to aluminium in acid soil among Al-sensitive (S) and Al-tolerant (T) oat RILs.

of aluminium on the roots of sensitive and tolerant plants. In general, the longer and thinner roots are lost during this process (Kroon and Visser, 2003). Moreover, differences in architecture may not be observed after removal from soil, since shallower (wider angle) and deeper roots cannot be easily distinguished, then the root system lengths look similar. The root system of sensitive plants tends to be more superficial and branched as a result of main root determination and hormonal induction of lateral roots (Poschenrieder *et al.*, 2009). In aluminium tolerant plants, the root system has a tendency of being deeper and less branched, however, in grasses, differences in branching are complicated to quantify. Additionally, in hydroponic conditions, seedlings are evaluated during a short period of time, so small changes can be measured. Over long periods plants try to acclimate to stress, and considering that carbon partition favours root development under soil stress (Angela, 2009), the aluminium toxicity effect on individual root growth may not be perceived, since shallow roots compensate for deeper ones reducing differences in mass. It may be even less accentuated under fertilized soils due to aluminium–phosphorus interaction in the soil top layers.

Based on the morphological response to toxic aluminium in the field, the parental lines UFRGS 930598–6 and UFRGS 17 exhibited mean response of 2.4 and 6.8, respectively, according to the zero to nine scale. Among the oat RILs screened for aluminium tolerance by primary root regrowth those classified as sensitive, tolerant and highly tolerant showed mean responses of 3.4, 6.6 and 6.8, respectively (Table 1). The tolerant and highly tolerant lines did not show statistical differences in the field, forming only two distinct groups; 'sensitive' and 'tolerant' to toxic aluminium (Figure 2). If the tolerant and highly tolerant lines are pulled together in the hydroponic analysis, a genetic hypothesis of a major gene involved in the control of aluminium tolerance in oat plants cannot be rejected (Nava *et al.*, 2006; Sánches-Chacón *et al.*, 2000). According to this hypothesis, tolerant genotypes may possess dominant alleles (Al_aAl_a), while the sensitive genotypes possess recessive alleles (al_aal_a) for this characteristic. Although the

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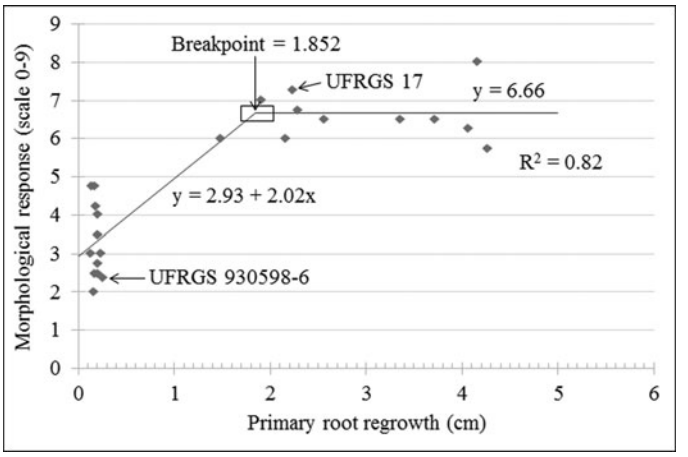

Figure 3. Association between primary root regrowth (independent variable) and morphological response to aluminium (dependent variable) performed by linear and non-linear regressions.

differences in the expression level of aluminium tolerance among tolerant and highly tolerant lines in hydroponic solution are not clear, several factors might be involved, such as the presence of minor genes, the incomplete expression of dominant alleles and loci with residual heterozygosis among lines. These differences could not be detected in the soil evaluation.

A comparison of the morphological response to toxic aluminium in the soil versus the hydroponic solution (expressed by the regrowth of the primary root) demonstrated that none of the lines classified as sensitive to aluminium in the hydroponic solution were found to be tolerant in the soil. Similarly, none of the tolerant and highly tolerant lines evaluated in hydroponic solution were sensitive to aluminium in the soil (Figure 3). Even though primary root regrowth at hydroponic conditions was linearly related to the visual morphological response of the above ground part of the oat plants at field conditions, as shown by the bi-segmented linear regression in Figure 3, it is possible to detect two groups of genotypes (Figure 2). The first group is formed by the genotypes with very little mean primary root regrowth, between 0.12 and 0.26 cm, and morphological response between 2 and 4.75. The second group is formed by genotypes able to regrow the primary root between 1.89 and 4.6 cm, in average, and showed morphological response of the above ground part of the plant from 5.75 to 8. There is just one genotype between these two groups with mean primary root regrowth of 1.48 and morphological response of 6. This genotype is outside the confidence block for the breakpoint, which was 1.85 cm, for the root regrowth (Figure 3).

Therefore, from the results presented in Figure 3, it is clear that genotypes with little primary root regrowth, being classified as sensitive to aluminium, show a range of visual effects on the above ground part of the plant, from 2 to 5, in a scale of zero to nine. While the genotypes able to regrow the primary root about 2 cm or more show a range of morphological effects on the above ground part of the plant, from 6.75 to 8. These results indicate that there may be other factor(s) affecting the morphological

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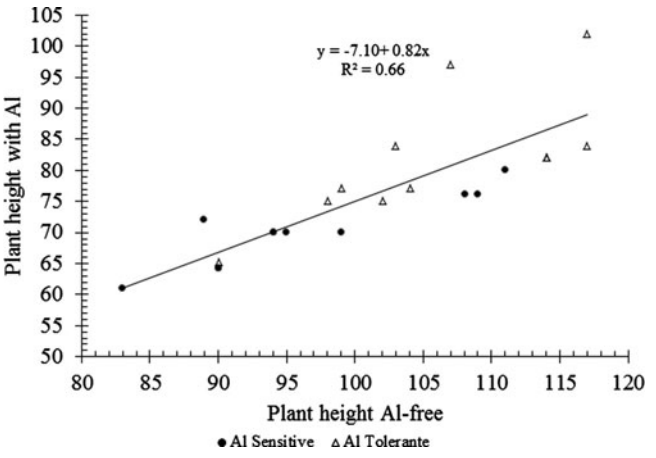

Figure 4. Association between plant heights of oat RILs grown in acid soil and in an environment free of aluminium in the soil.

response of oat plants than their tolerance to aluminium, as measured by primary root regrowth.

The absence of an absolute correlation among the RILs evaluated in the soil and their classification in hydroponics can be explained by the nature of the observed variables in the different screening strategies of aluminium tolerance. In a hydroponic solution, the primary root regrowth measures the plant's ability to resume root growth after being exposed to high concentration of the toxic element, and does not consider other effects on plant growth and development. Thus, a greater growth of the primary root suggests that the plant possesses more efficient mechanisms of tolerance to toxic aluminium. In contrast, the plant response to aluminium in the soil depends on the effects of aluminium on the roots, as well as on the interaction of the plant with various genetic and environmental factors.

Among the observed variables at field conditions, plant height was the most affected trait by toxic aluminium in the soil, even among the tolerant lines. The parental lines UFRGS 930598–6 (sensitive to Al^{3+}) and UFRGS 17 (tolerant to Al^{3+}) showed mean plant height of 67 and 82 cm when grown in a soil containing toxic aluminium and 96 and 105 cm when grown in a soil free of aluminium, respectively. Sensitive and tolerant RILs exhibited mean plant height of 70 and 82 cm when grown in a soil containing aluminium, and 96 and 106 cm when grown in a soil free of aluminium, respectively, indicating that plant height was reduced by 27% and 23% on average among sensitive and tolerant RILs, respectively (Figure 4). These results suggest that toxic aluminium in the soil interfered with vital processes of plant growth and development. The interaction of aluminium with phosphorus for example is known (Liao *et al.*, 2006) and may affect plant growth. Nevertheless the associated effect of soil acidity on nutrient availability needs to be considered.

Morphological response of the above ground part of the plants was linearly associated with the height of the adult plant cultivated in soil free of Al^{+3}, as can

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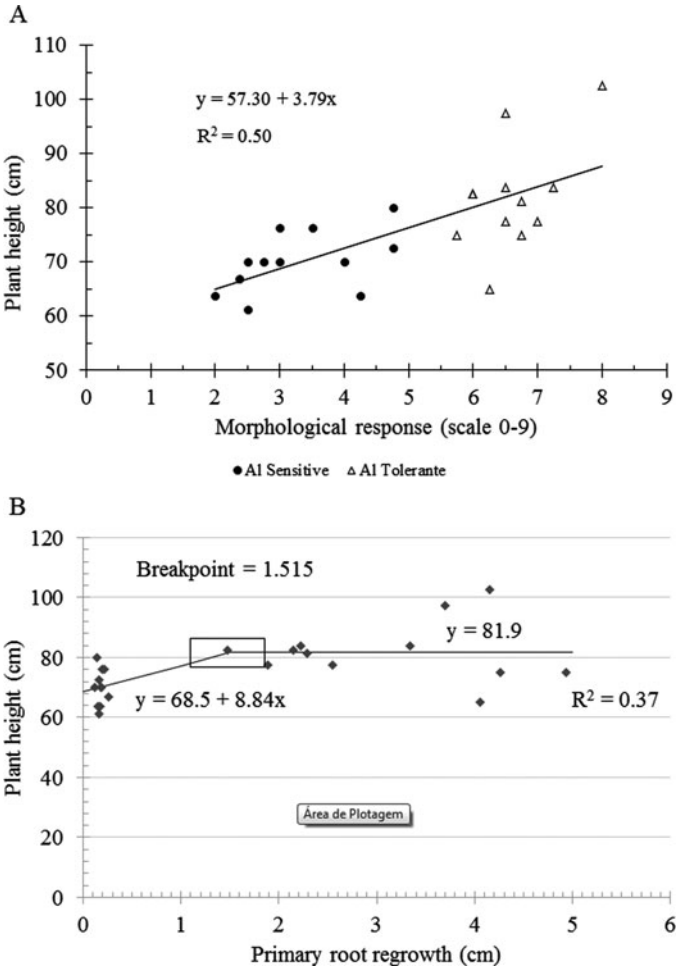

Figure 5. Association between morphological response to aluminium and plant height Al-free (A), and association between primary root regrowth and plant height Al-free (B), performed by linear and non-linear regressions.

be seen in Figure 5A. Primary root regrowth, measured in hydroponic solution with Al^{+3} available at a toxic level, was also associated with plant height in field environment free of Al^{+3} in the soil (Figure 5B). Oat genotypes with lower primary root regrowth tended to have smaller stature, while those with primary root regrowth equal or greater than 1.5 cm showed taller plants, according to bi-segmented linear regression. However, this association was not strong ($R^2 = 0.37$) and lines with smaller primary root regrowth had average plant heights between 60 and 80 cm, which were also seen in genotypes with greater primary root regrowth. Nonetheless, average plant heights between 80 and 102 were only seen in genotypes with higher primary root regrowth (Figure 5B).

Taking together the results shown in Figures 3 and 5 it is possible to conclude that visual evaluation of the above ground plant part is able to separate the oat genotypes

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in aluminium sensitive and aluminium tolerant ones. The minor factors conducting to the identification of highly tolerant lines in hydroponic solution might be more environment dependent, therefore not detectable in the field condition.

The comparison of the results obtained in acid soil with those obtained in a hydroponic solution demonstrated that both conditions produced essentially the same responses. Thus, both methods represent efficient tools in the identification of genotypes that are tolerant to aluminium in breeding programmes. However, soils with homogeneous concentration of toxic aluminium are not easily found under natural growing conditions, limiting its use in the screening of tolerant genotypes in a large number of segregating populations. Under these conditions, the use of hydroponic solution provides a precise, fast and economical alternative, especially useful for programmes located in regions were aluminium is not present at toxic forms in the soil. Moreover, the use of hydroponic solution can be a valuable tool for phenotyping large populations derived from lines with different levels of aluminium tolerance. This method can also be applied to molecular mapping of genes and quantitative trait loci associated with this feature in oats, contributing to a better understanding of the tolerance mechanisms to toxic aluminium.

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