Quantification of aluminium induced changes in wheat root architecture by X-ray micro-computed tomography

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Abstract

Root architectural traits are of fundamental importance for plant performance especially under unfavourable soil conditions. This study examined the effect of aluminium (Al) toxicity in different growing media (nutrient solutions and soil) on root architecture of two wheat (*Triticum aestivum* L.) cultivars with different Al tolerances.

Seedlings were grown in acid and limed soil and in two contrasting nutrient solutions. Root systems of soil grown plants were scanned using x-ray Micro Computed Tomography (µCT) while that of nutrient solution grown plants were assesses using WinRhizo, 3 and 5 days after planting (DAP), respectively.

Al caused significant reduction of all examined root traits (number of seminal roots; root length; length of the longest seminal root; root surface area; and root volume). Growth in acid soil caused significant reduction in root length, length of the longest seminal root and root surface area at 5 DAP. Soil grown plants produced larger root system compared to plants grown in nutrient solutions. Al toxicity induced differences of root traits were also found between different nutrient solutions.

Beside the well-known reduction of root length, Al toxicity had a profound effect on other root architectural traits. x-ray µCT has revealed root architectural changes under specific conditions of acid, Al toxic soil. Differences obtained in Al induced effects on root architecture between different nutrient solutions as well as between different growing systems emphasize the need for further study of root architecture especially under specific conditions of Al toxicity in acid soils.

Introduction

Aluminium (Al) is a major limiting factor of crop productivity in acid soils (Kochian 1995). Acid (pH <5.5) soils exhibiting Al toxicity comprise up to 30% – 40% of the world's

arable land, and it is estimated that over 50% of world's potentially arable land is acidic (von Uexküll and Mutert 1995). Solubilisation of Al oxides and hydroxides is enhanced by low pH, and the predominant form of Al in the acid soils (pH <5.0) is Al^{3+} (Delhaize and Ryan 1995). The most easily recognized symptom of Al^{3+} toxicity is the inhibition of root growth (Delhaize and Ryan 1995). Therefore, measurement of the root growth in solution culture assays has been used for screening Al tolerant genotypes (Samac and Tesfaye 2003). Nevertheless, in only a few cases has Al tolerance observed in solution cultures been correlated with Al tolerance in acid soils (Samac and Tesfaye 2003). Discrepancies in genotype rankings regarding Al tolerance have been attributed to different factors which affect effective Al concentration in nutrient solutions, and in addition can reduce repeatability of the results. Typically researchers used simple nutrient solutions with low ionic strength and wide range of Al concentrations. However, Gregory and Hinsinger (1999) highlighted that research on roots needs to involve complex growth medium such as soil, opposed to commonly used hydroponics, gels and sand culture. Furthermore, most research performed in nutrient solutions has focused on the root apex, which is the most sensitive site of root to Al toxicity, while the whole root architecture has gained less attention.

Although Al tolerance in wheat appears to be controlled by a single dominant gene (Delhaize et al. 1993a; Riede and Anderson 1996), many root traits are under polygenic control and expression of these genes is influenced by mutual interactions of roots with the abiotic and biotic soil environment (McCully 1999). The importance of root architecture for plant growth and performance, especially under environmental stress has recently gained more attention (e.g. Lynch 1995; López-Bucio et al. 2003). Non-invasive techniques such as x-ray Micro Computed Tomography (µCT) provide an opportunity to examine 3-D root architecture (Tracy et al. 2010) non-destructively in the opaque matrix of soil.

The aim of this study was to quantify Al induced changes in root architecture of two wheat cultivars that differ in Al tolerance (Al tolerant Sivka and Al sensitive Scout 66) grown in different growing systems (nutrient solutions and soil), and to compare the usefulness of two methods (WinRhizo and x-ray μCT) for assessing Al induced changes in the root architecture.

Materials and Methods

Plant material and growing conditions

Seeds of Al tolerant wheat cultivar Sivka were obtained from the University of Zagreb, Faculty of Agriculture, Department of Plant Breeding, Genetics, and Biometrics (Svetošimunska cesta 25, 10000 Zagreb, Croatia), and Al sensitive cultivar Scout 66 from the Crop Research Institute, Gene Bank Department (Drnovská 507, 161 06 Praha 6 – Ruzyně, Czech Republic). Seeds were surface sterilized in 2.5% sodium hypochlorite, thoroughly rinsed with distilled water and soaked for 6 h hours in distilled water. All seeds were germinated for 64 h on filter paper soaked with 0.2 mM $CaCl_2$ at 23/18 $^{\circ}$C with a 16/8 h, day/night regime.

Soil-based experiment

Soil samples (silty loam, luvisol) were collected from the Ap horizon of an arable field near Gospić, Croatia (44°32’45’’N, 15°22’28’’E). Soil samples were air dried and sieved to <2 mm diameter. Selected physical and chemical characteristics of the soil are shown in Table 1. To get soils with different pH half of the soil samples were limed using 1.0 g $CaCO_3$ kg^{-1}. Prepared soil samples were moistened to field capacity and incubated for 2 months at room temperature. After the incubation period soil pH was 5.8, and Al saturation was 3.32%. Before planting, soil samples were sieved through <1.0 mm diameter mesh and were placed

into 50 mm diameter and 100 mm high plastic columns to achieve a bulk density of 1.0 g cm^{-3}. The soil was watered and maintained at a volumetric water content of 15% and kept in growth chambers during the seed germination period (64 h). Four uniformly developed seedlings per cultivar were selected for growth (one plant per column). Germinated seeds were placed in 1 mm diameter, 2 mm deep holes drilled in the soil columns. The seeds were placed in the hole with the radical downwards before being covered with soil. Plants were grown in a growth chamber with 16/8 h, 23/18 $^{\circ}$C day/night regime and 75% relative humidity.

Table 1. Physical and chemical properties of the soil used in the study.

Sand[a]	Silt[a]	Clay[a]	pH[b]	C_{org}[c]	N[d]	P[f]	ECEC[g]	Ca	Mg	K	Na	Al	Al[h]
———%———			H_2O	%	mg kg^{-1}		———cmol(+) kg^{-1}————						sat (%)
8.0	72.3	19.7	4.6	2.9	0.4	12	4.46	1.4	0.54	0.62	0.05	1.85	41.5

[a]Soil particle size distribution was determined by pipette-method with sieving and sedimentation.
[b]pH potentiometrically.
[c]Organic carbon content (C_{org}) determination after dry combustion.
[d]Total nitrogen by modified Kjeldahl method.
[f]Phosphorus by ammonium lactate method.
[g]Effective cation exchange capacity (ECEC = Ca + Mg + K + Na + Al) and base saturation level were determined in barium chloride extracts; Determination of exchangeable acidity in barium chloride extracts.
[h]Al sat – Al saturation =100 x (exchangeable Al)/(ECEC).

Nutrient solution experiment

The experiment was prepared as a randomized block design with ten replicate plants of each cultivar per treatment. Plants were grown on an opaque plastic mesh in two different nutrient solutions which were previously used in experiments related to Al toxicity. The first nutrient solution (NSR) was used previously by Rengel and Jurkić (1992; 1993) and the second nutrient solution (NSD) was used by Delhaize et al. (1993a; 1993b). Treatments were represented as control nutrient solutions, pH 4.0, without aluminium (NSR0 and NSD0, respectively), and nutrient solutions with aluminium (supplied as $AlCl_3$), pH 4.0 (NSR1,

NSD1, respectively). Ionic activities and Al speciation in nutrient solutions were calculated by GEOCHEM-EZ (Shaff et al. 2010) and are shown in Table 2. Based on the calculations, the free activities of Al^{3+} were 0.0 (in NSR0 and NSD0) and 72.0 µM L^{-1} (in NSR1 and NSD1). Nutrient solutions were continuously aerated, daily replenished and the pH was adjusted with 0.1 M HCl. Plants were grown in a growth chamber with 16/8 h, 23/18 °C day/night regime and 75% relative humidity.

Table 2. Chemical composition and ion activities of nutrient solutions calculated by GEOCHEM-EZ.

Nutrient solution	NSR0	NSR1	NSD0	NSD1
pH	4.0	4.0	4.0	4.0
Ionic strength	0.02063	0.02117	0.00283	0.00329
Nutrient	Free concentration in nutrient solution mM L^{-1}			
NO_3	10.0	10.0	1.75	1.75
NH_4	0.5	0.5	0.25	0.25
K	1.99	1.99	0.5	0.5
Ca	3.61	3.63	0.5	0.494
Mg	1.84	1.85	0.124	0.124
SO_4	1.68	1.63	0.128	0.107
PO_4	-	-	*	*
Fe	-	-	0.009 E-03	*
$B(OH)_4$	-	-	*	*
Mn	-	-	1.97 E-03	1.97 E-03
Zn	-	-	0.344 E-03	0.34 E-03
Cu	-	-	0.195 E-03	0.196 E-03
Cl	0.126	0.565	0.11	0.37
Al	-	0.072	-	0.072
Al complex with SO_4	-	0.072	-	0.00074
Al complex with OH	-	0.004	-	0.0115

Notes. * Almost the entire nutrient is in complexes. E-03 concentrations are in µM L^{-1}.

Root Imaging

For the x-ray µCT scanning, the columns with live plants were scanned on the third and fifth day after planting (DAP) using a Phoenix Nanotom® (GE Measurement & Control

Solutions, Wunstorf, Germany) x-ray µCT scanner set at 100 kV and 210 µA, with a 0.2-mm copper filter and voxel resolution was set at 50 µm. For each column, 1200 image projections were collected over a 30-min period. Image slices were reconstructed into 3D volumes using software Datos|x with beam-hardening reduction algorithms applied and then visualised and analysed in VGStudioMax® 2.0 (Volume Graphics GmbH, Heidelberg, Germany). Roots were segmented from the obtained images using the *Region Growing* selection tool following the method of Tracy et al. (2012). Segmented root systems were used for quantitative determination of number of seminal roots, root length, length of the longest seminal root, root surface area and root volume.

After the final µCT scan at 5 DAP, roots were extracted from the soil and carefully washed and scanned using Epson Perfection V700 photo scanner and WinRhizo® software (WinRhizo 2009 Reg., Regent Instruments Canada Inc.). Root measurements of the plants grown in nutrient solutions were conducted at 3 and 5 DAP, using Epson Perfection V700 photo scanner and WinRhizo software.

Statistical analysis

Data were analysed using the SAS® 9.2 statistical package (SAS Institutes, Cary, NC). For the comparison of the scanning techniques (x-ray µCT versus WinRhizo) results of the root traits (number of seminal roots, root length, the length of the longest seminal root, root surface area and root volume) of soil grown plants that were obtained at 5 DAP were compared using ANOVA, followed by the use of Tukey's honestly significant difference (HSD) test. For comparisons of different nutrient solutions (NSR0, NSR1, NSD0, and NSD1), soil treatments (acid versus limed soil), and growing systems, results of the root traits were analysed using repeated measures (Mixed Model Repeated Measures, Littell et al. 1996).

Results

Effect of aluminium toxicity and soil acidity on root traits

Root traits of Al tolerant (Sivka) and Al sensitive (Scout 66) wheat cultivars grown in different nutrient solutions with toxic concentrations of Al and in control solutions (without Al) and in acid and limed soil are shown in Figure 1,2,3,4, and 5.

The number of seminal roots was consistently larger for Scout 66 compared to Sivka across nutrient solutions ($P < 0.001$) and soil treatments ($P < 0.05$). In both nutrient solutions (NSD and NSR) and at both measurement times (3 and 5 DAP) Al treatments reduced ($P < 0.01$) the number of seminal roots (from 5.01 in Al treatment solutions to 4.61 in control solutions) (Figure 1A). In soil the number of seminal roots increased over time, from 3.5 (3 DAP) to 4.88 (5 DAP) ($P < 0.05$) (Figure 1B).

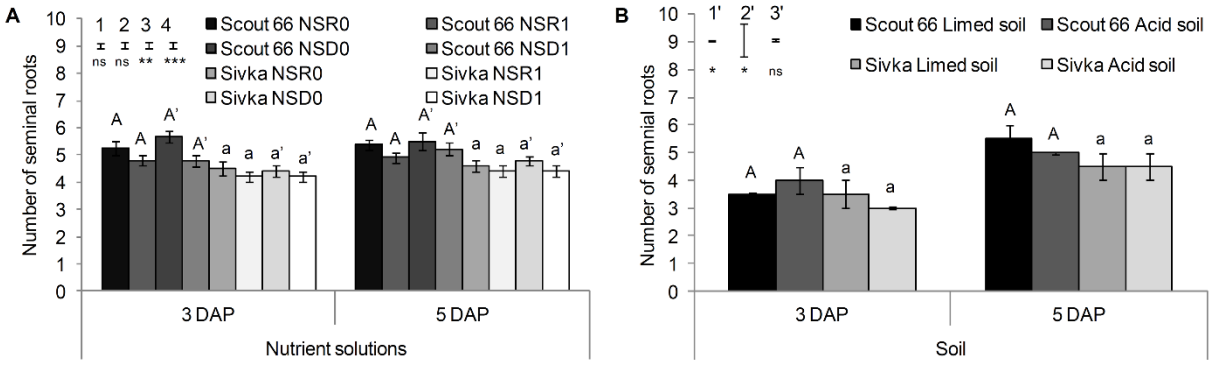

Figure 1. Comparison of the number of seminal roots of wheat cultivars Scout 66 and Sivka grown in Al treatment solutions (NSR1 and NSD1) and control nutrient solutions (NSR0 and NSD0) (A) and in acid and limed soil (B). For plants grown in soil roots were scanned by x-ray µCT and measured by (VGStudioMax), and for plants grown in nutrient solutions roots were scanned and measured by WinRhizo, at 3 DAP and 5 DAP, respectively. Error bars associated with the histograms are ±1 standard error of the mean. The vertical bars represent standard error of the difference (SED) for (1) day, (2) nutrient solution, (3) nutrient solution treatment, (4) cultivars; (1') day, (2') soil treatment, (3') cultivars. Below the vertical bars (SED) ANOVA for the main effects is presented as: *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level; and ns = not significant. For figure A: means with the same letter are not significantly different between nutrient solution treatments within each nutrient solution type at each measurement time; for Scout 66 (capital in NSD and capital with apostrophe in NSR, respectively) and for Sivka (small in NSD and small with apostrophe in NSR, respectively). For figure B: means with the same letter are not significantly different between soil treatments at each measurement time for Scout 66 (capital) and for Sivka (small).

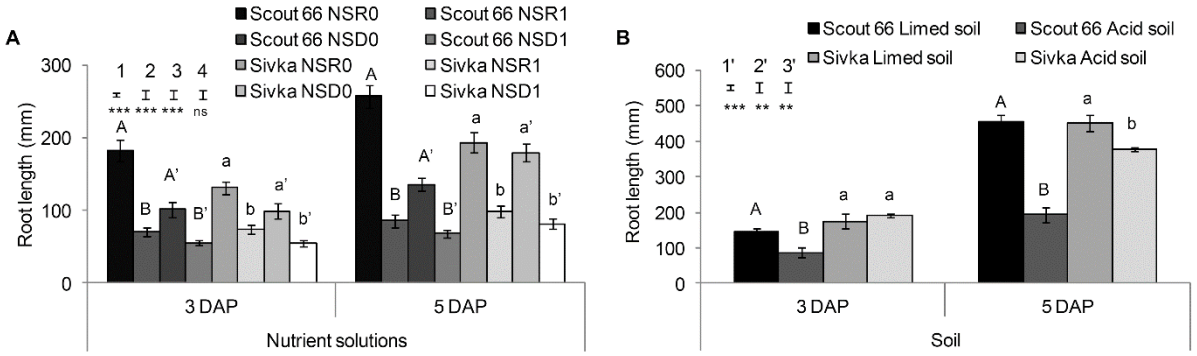

Figure 2. Comparison of mean root length of wheat cultivars Scout 66 and Sivka grown in Al treatment solutions (NSR1 and NSD1) and control nutrient solutions (NSR0 and NSD0) (A) and in acid and limed soil (B). For plants grown in soil roots were scanned by x-ray μCT and measured by (VGStudioMax), and for plants grown in nutrient solutions roots were scanned and measured by WinRhizo, at 3 DAP and 5 DAP, respectively. Error bars associated with the histograms are ±1 standard error of the mean. The vertical bars represent standard error of the difference (SED) for (1) day, (2) nutrient solution, (3) nutrient solution treatment, (4) cultivars; (1') day, (2') soil treatment, (3') cultivars. Below the vertical bars (SED) ANOVA for the main effects is presented as: *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level; and ns = not significant. For figure A: means with the same letter are not significantly different between nutrient solution treatments within each nutrient solution type at each measurement time; for Scout 66 (capital in NSD and capital with apostrophe in NSR, respectively) and for Sivka (small in NSD and small with apostrophe in NSR, respectively). For figure B: means with the same letter are not significantly different between soil treatments at each measurement time for Scout 66 (capital) and for Sivka (small).

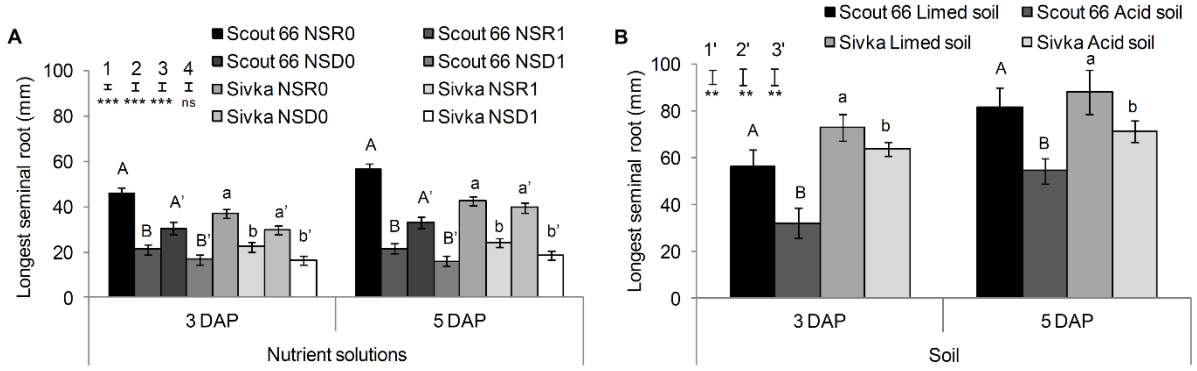

Figure 3. Comparison of length of the longest seminal root of wheat cultivars Scout 66 and Sivka grown in Al treatment solutions (NSR1 and NSD1) and control nutrient solutions (NSR0 and NSD0) (A) and in acid and limed soil (B). For plants grown in soil roots were scanned by x-ray μCT and measured by (VGStudioMax), and for plants grown in nutrient solutions roots were scanned and measured by WinRhizo, at 3 DAP and 5 DAP, respectively. Error bars associated with the histograms are ±1 standard error of the mean. The vertical bars represent standard error of the difference (SED) for (1) day, (2) nutrient solution, (3) nutrient solution treatment, (4) cultivars; (1') day, (2') soil treatment, (3') cultivars. Below the vertical bars (SED) ANOVA for the main effects is presented as: *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level; and ns = not significant. For figure A: means with the same letter are not significantly different between nutrient solution treatments within each nutrient solution type at each measurement time; for Scout 66 (capital in NSD and capital with apostrophe in NSR, respectively) and for Sivka (small in NSD and small with apostrophe in NSR, respectively). For figure B: means with the same letter are not significantly different between soil treatments at each measurement time for Scout 66 (capital) and for Sivka (small).

In nutrient solutions Al treatments reduced ($P < 0.001$) root length of both cultivars (Scout 66 and Sivka), grown in both nutrient solutions (NSD and NSR) and at both measurement times (3 DAP and 5 DAP). In addition, for all cultivar × nutrient solution × treatment combinations, root length increased with time ($P < 0.05$) except for Scout 66 grown in NSR1 ($P > 0.05$) (70.64 mm and 85.65 mm, 3 and 5 DAP, respectively) and in NSD1 ($P > 0.05$) (55.6 mm and 68.47 mm, 3 DAP and 5 DAP, respectively) (Figure 2A). Root length of plants grown in soil was affected by cultivar × treatment × measurement time interaction ($P < 0.05$). Reduction of root length of Scout 66 grown in acid soil was evident at both measurement time (i.e. 87.5 mm vs. 146.23 mm at 3 DAP, $P < 0.05$ and 454.37, mm vs. 194.7 mm 5 DAP, $P < 0.001$, in acid vs. limed soil, respectively). A significant reduction in root length of Sivka grown in acid soil was recorded at 5 DAP (376.86 mm in acid vs. 453.24 mm in limed soil, $P < 0.05$) (Figure 2B).

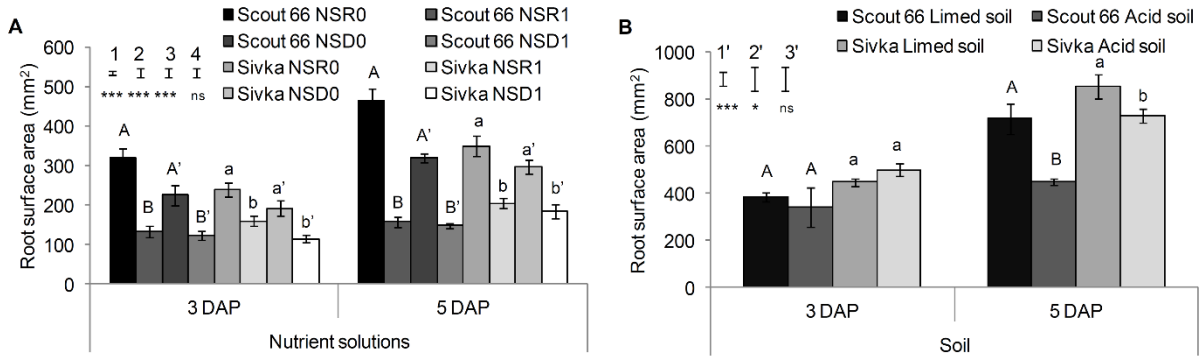

Figure 4. Comparison of root surface area of wheat cultivars Scout 66 and Sivka grown in Al treatment solutions (NSR1 and NSD1) and control nutrient solutions (NSR0 and NSD0) (A) and in acid and limed soil (B). For plants grown in soil roots were scanned by x-ray µCT and measured by (VGStudioMax), and for plants grown in nutrient solutions roots were scanned and measured by WinRhizo, at 3 DAP and 5 DAP, respectively. Error bars associated with the histograms are ±1 standard error of the mean. The vertical bars represent standard error of the difference (SED) for (1) day, (2) nutrient solution, (3) nutrient solution treatment, (4) cultivars; (1') day, (2') soil treatment, (3') cultivars. Below the vertical bars (SED) ANOVA for the main effects is presented as: *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level; and ns = not significant. For figure A: means with the same letter are not significantly different between nutrient solution treatments within each nutrient solution type at each measurement time; for Scout 66 (capital in NSD and capital with apostrophe in NSR, respectively) and for Sivka (small in NSD and small with apostrophe in NSR, respectively).For figure B: means with the same letter are not significantly different between soil treatments at each measurement time for Scout 66 (capital) and for Sivka (small).

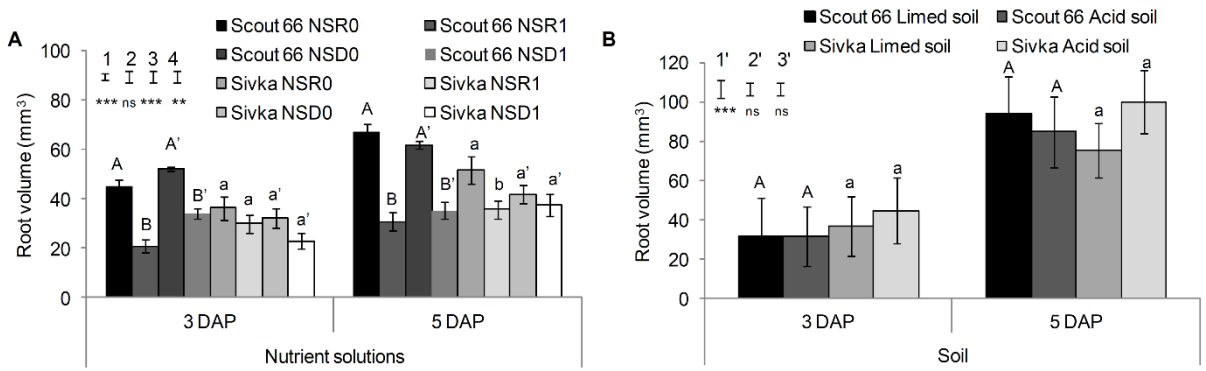

Figure 5. Comparison of root volume of wheat cultivars Scout 66 and Sivka grown in Al treatment solutions (NSR1 and NSD1) and control nutrient solutions (NSR0 and NSD0) (A) and in acid and limed soil (B). For plants grown in soil roots were scanned by x-ray μCT and measured by (VGStudioMax), and for plants grown in nutrient solutions roots were scanned and measured by WinRhizo, at 3 DAP and 5 DAP, respectively. Error bars associated with the histograms are ±1 standard error of the mean. The vertical bars represent standard error of the difference (SED) for (1) day, (2) nutrient solution, (3) nutrient solution treatment, (4) cultivars; (1') day, (2') soil treatment, (3') cultivars. Below the vertical bars (SED) ANOVA for the main effects is presented as: *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level; and ns = not significant. For figure A: means with the same letter are not significantly different between nutrient solution treatments within each nutrient solution type at each measurement time; for Scout 66 (capital in NSD and capital with apostrophe in NSR, respectively) and for Sivka (small in NSD and small with apostrophe in NSR, respectively). For figure B: means with the same letter are not significantly different between soil treatments at each measurement time for Scout 66 (capital) and for Sivka (small).

In nutrient solutions Al treatments reduced ($P < 0.001$) length of the longest seminal root of both cultivars (Scout 66 and Sivka), grown in both nutrient solutions (NSD and NSR) and at both measurement times (3 DAP and 5 DAP). In addition, there was a significant interaction of cultivar × nutrient solution × treatment ($P < 0.01$). No significant difference in length of the longest seminal root was obtained for Sivka grown in NSR0 ($P > 0.05$) (39.77 mm) and NSD0 (34.68 mm), while cultivar Scout 66 produced longer seminal root in NSR0 ($P < 0.001$) (51.25 mm) compared to NSD0 (31.78 mm). The opposite was obtained in Al treatment solutions where no significant difference ($P > 0.05$) was found between NSR1 (21.40 mm) and NSD1 (16.37 mm) grown Scout 66, while significantly ($P < 0.05$) longer seminal roots were obtained for NSR1 (23.24 mm) compared to NSD1 (17.55 mm) grown Sivka (Figure 3A). In soil, length of the longest seminal root was affected by measurement time ($P < 0.01$), by cultivar ($P < 0.01$) with average length of 55.97 mm for Scout 66

281 compared to 74.08 mm for Sivka, and by treatment ($P < 0.01$) with average length 55.35 mm
282 in acid soil compared to 74.71 mm in limed soil (Figure 3B).

283 In nutrient solutions, root surface area was affected by nutrient solution × treatment ($P <$
284 0.01) and cultivar × treatment ($P < 0.001$) interaction. Al treatments reduced root surface area
285 in both nutrient solutions, as well as for both cultivars. However, this reduction was more
286 pronounced in NSR (344.51 mm^2 in NSR0 vs. 165.35 mm^2 in NSR1) compared to NSD
287 (258.65 mm^2 in NSD0 vs. 142.57 mm^2 in NSD1) and for cultivar Scout 66 (332.99 mm^2 in
288 control solutions vs. 142.1 mm^2 in Al-treatment solutions) compared to Sivka (270.17 mm^2
289 and in control solutions vs. 165.82 mm^2 in Al-treatment solutions) (Figure 4A). When grown
290 in soil, the largest mean root surface area was obtained for Sivka (634.41 mm^2) compared to
291 Scout 66 (475.14 mm^2) ($P < 0.05$), and the interaction of treatment × measurement time was
292 significant ($P < 0.05$). No significant differences ($P > 0.05$) in root surface area were found
293 between plants grown in acid (422.1 mm^2) and limed (417.27 mm^2) soil at 3 DAP, while at 5
294 DAP plants grown in limed soil produced root systems with bigger ($P < 0.01$) surface area
295 (788.59 mm^2) compared to those grown in acid soil (591.16 mm^2) (Figure 4B)

296 In nutrient solutions, root volume was affected by interactions of cultivar × treatment ($P <$
297 0.001) and cultivar × nutrient solution × measurement time ($P < 0.05$). Al treatments reduced
298 root volume of cv. Scout 66 at both measurements and in both nutrient solutions. On the other
299 hand, significant reduction of root volume of cv. Sivka was found only at 5 DAP in NSR ($P <$
300 0.01) (35.9 mm^3 in NSR1 compared to 51.6 mm^3 in NSR0) (Figure 5A). In soil, the
301 interaction of cultivar × treatment ($P < 0.05$) for root volume was significant. Root volume of
302 cultivar Sivka was greater ($P < 0.05$) in acid (74.22 mm^3) compared to limed soil (56.11
303 mm^3), while no significant differences ($P > 0.05$) were found for Scout 66 grown in acid
304 (58.14 mm^3) and limed soil (62.63 mm^3) (Figure 5B).

305

Comparison of the scanning techniques: X-ray µCT versus WinRhizo

A comparison of the root traits (root length, length of the longest seminal root, root surface area, and root volume) measured by VGStudioMax after x-ray µCT scanning and by WinRhizo (after washing soil from roots) at 5 DAP are shown in Figure 6. Although all measured root traits were slightly larger when measured by WinRhizo compared to VGStudioMax, there were no significant difference in root length ($P > 0.05$), root surface area ($P > 0.05$), and the length of the longest seminal root ($P > 0.05$) when these two techniques were compared. However, a significantly larger ($P < 0.05$) root volume was obtained by WinRhizo (117.96 mm^3) compared to VGStudioMax (89.44 mm^3) which can be attributed to the former capturing more of the finer roots (Figure 7).

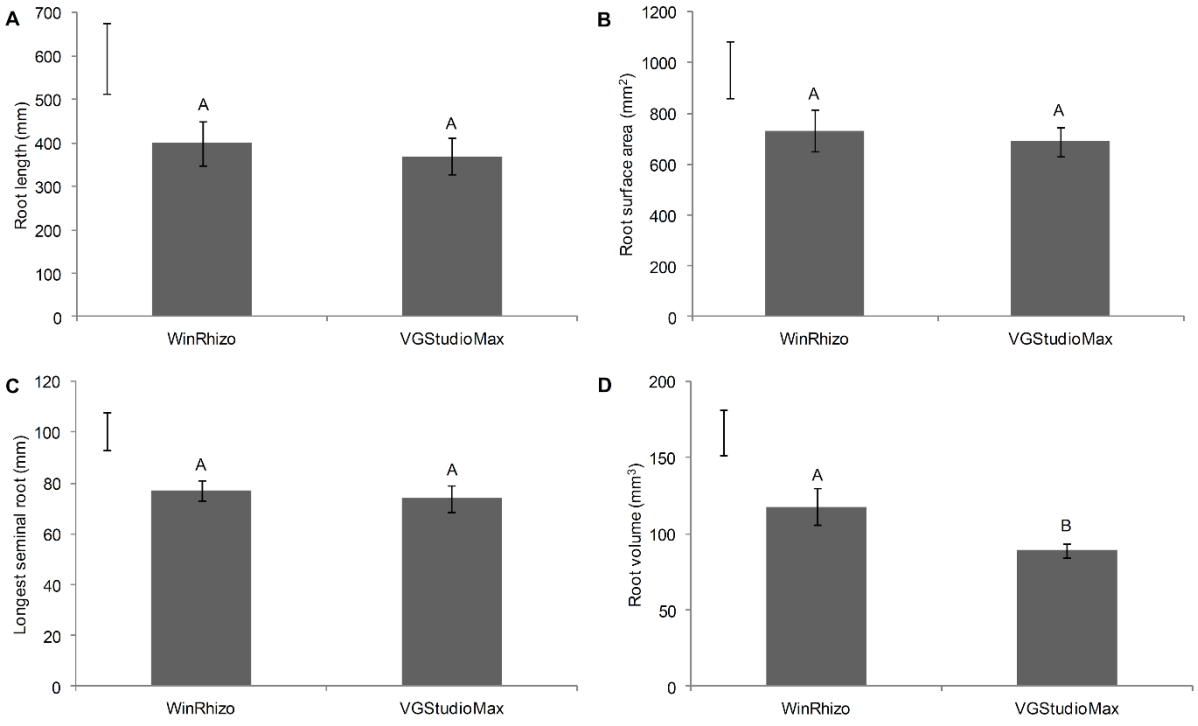

Figure 6. Comparison of the root traits of wheat cultivars Scout 66 and Sivka obtained by different scanning techniques, µCT (VGStudioMax) and WinRhizo at 5 DAP: mean root length (A), root surface area (B), length of the longest seminal root (C), and root volume (D). Error bars associated with the histograms are ±1 standard error of the mean. The vertical bars represent minimum significant difference (Tukey's HSD test, p=0.05) for comparing the mean values between scanning techniques; means with the same letter are not significantly different.

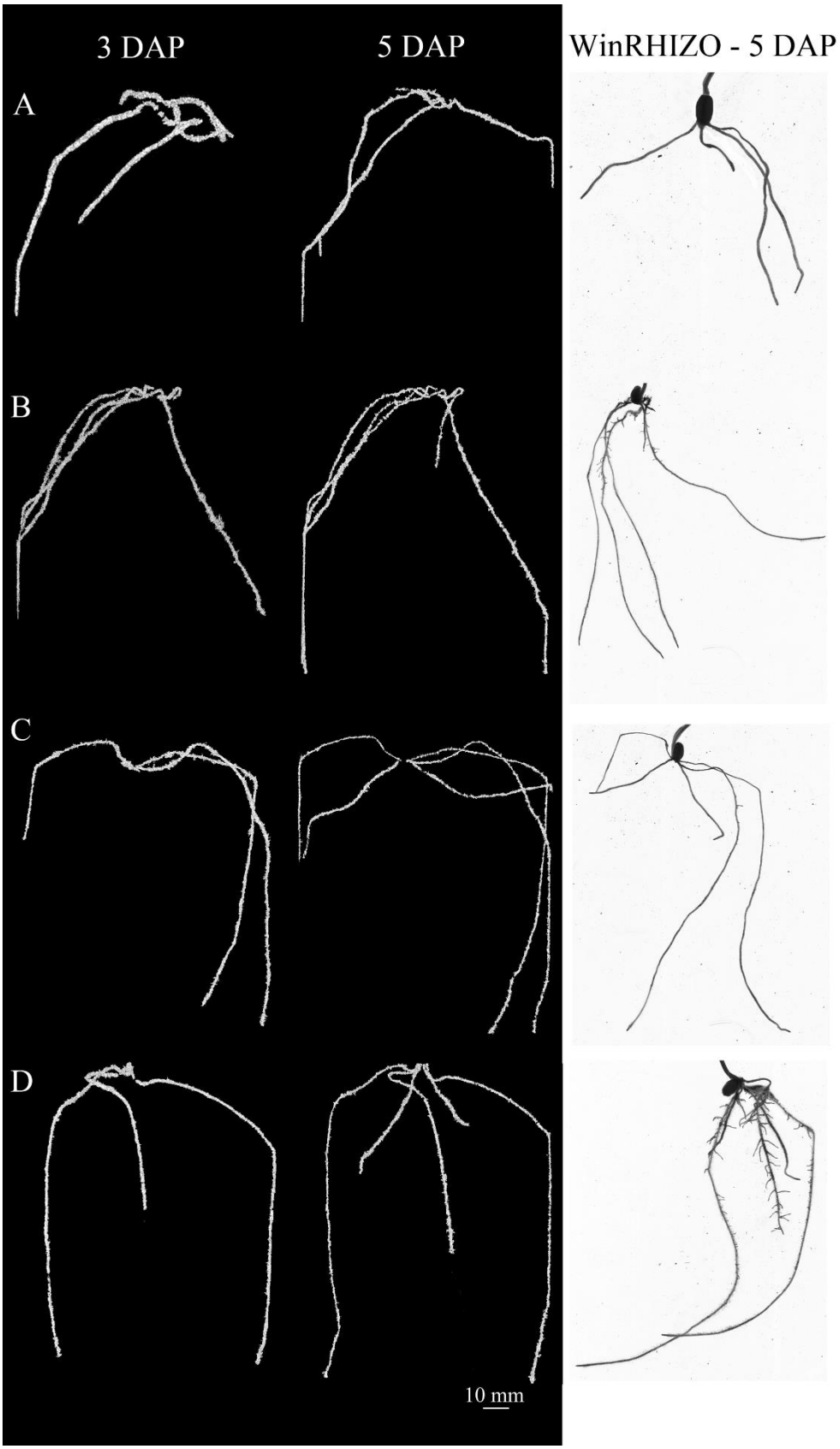

Figure 7. Root system images of wheat cultivars Scout 66 (A and C) and Sivka (B and D) at 3 DAP and 5 DAP, grown in acid (A and B) and in limed soil (C and D), scanned by μCT (left) and WinRhizo (right).

Discussion

Although all measured root traits were larger when measured by WinRhizo compared to VGStudioMax, comparison of the results of root traits obtained by these two scanning techniques showed that they did not differ significantly, except for root volume. Relatively poor correlation between root volumes measured destructively by WinRhizo and non-destructively, after x-ray µCT scan, was already described by Tracy et al. (2012). Tracy et al. (2012) have attributed these discrepancies to the better contrast between roots and their surroundings which can be obtained using WinRhizo and on the other hand to the image resolution limitation which were gained by x-ray µCT. This could also be the truth for our results (Figure 7). Additional technical disadvantage of the x-ray µCT scanning technique is the limited soil volume that can be used for growing plants which disables this technique to study older plants with more complex root architecture. Namely, all roots of the plants used in this study reached bottom and/or side walls of the columns by the 5 DAP (Figure 7). However, results of this study showed that x-ray µCT scanning technique provide reliable and good quality 3-D scans of roots in the soil, and despite its current limitations, new developments of this technique, such as automated root segmentation, and bigger, faster and more precise x-ray CT scanners with greater resolution would give the opportunity to study older more complex root systems (for the review see Mooney et al. 2012).

Aluminum toxicity reduced all examined root traits in the experiment with nutrient solutions while in soil based experiments it caused reduction of root length, length of the longest seminal root and root surface area. Al induced reduction of root size is most likely the primary cause of commonly described symptoms of Al toxicity, such as impairment of nutrient and water acquisition. Al toxicity, both in acid soil and in Al treatment nutrient solutions, caused a more pronounced reduction of all examined root traits for Al sensitive cv. Scout 66 compared to Al tolerant cv. Sivka (Figure 1, 2, 3, 4, and 5). Differences in root traits

355 determined between cv. Scout 66 and cv. Sivka are in accordance to their tolerance to
356 aluminium. It is well known that there is significant genetic variability in Al tolerance among
357 wheat cultivars and cv. Scout 66 was used as a model of an Al sensitive cultivar in previous
358 studies related to Al toxicity (e.g. Rengel and Jurkić 1992; Ryan et al. 1992), on the other
359 hand cv. Sivka was evaluated as moderately tolerant cultivar in a screening for Al tolerance
360 among Yugoslavian wheat cultivars (Rengel and Jurkić 1992).

361 The first and most easily recognized symptom of Al toxicity is the inhibition of root
362 growth (Delhaize and Ryan 1995). Barceló and Poschenrieder (2002) stated that sensitive
363 plants exhibit statistically significant inhibition of root elongation after approximately 30 min
364 to 2 h exposure. Our results show that Al toxicity caused slower reduction of root growth in
365 acid soil compared to those that were obtained in experiments with nutrient solutions. For
366 example, reduction of root length and root surface area for plants grown in Al treatment
367 solutions was evident at 3 DAP while reduction of root length for acid soil grown cv. Sivka
368 and reduction of root surface area for both acid soil grown cultivars was evident only at 5
369 DAP. These delayed response to Al toxicity observed for acid soil grown plants could be
370 explained as a lag phase. Barceló and Poschenrieder (2002) described the lag phase as the
371 time or concentration required for Al to interfere with key processes in root growth. It was
372 estimated (Delhaize et al. 1993a) that significant Al inhibition of root growth in wheat occurs
373 at root tip Al concentrations around 1000 μg Al g^{-1}. Therefore, these results indicate that acid
374 soil grown plants, especially cv. Sivka, can tolerate a longer period of exposure to toxic Al
375 concentrations.

376 Although there are some reports about the Al induced inhibition of lateral roots in
377 sensitive genotypes of rice (*Oryza sativa* L.) (Famoso et al. 2011), soybean (*Glycine max* L.).
378 (Vilagarcia et al. 2001; Silva et al. 2001) maize (*Zea mays* L.) (Clark et al. 2013), there is lack
379 of data about the effect of Al toxicity on other root traits, especially under real acid soil

conditions. Villagarcia et al. (2001) developed a sand based screening technique which simulated growth in acid soil. In their experiments, they made comparison between hydroponic and sand based experiments by measurements of different root traits of soybean. These authors reported Al toxicity (eighteen days of exposure to 450 μM Al L^{-1}) in sand based experiments did not greatly affect the tap root length, while it caused significant reduction of root surface area (by 58%) compared to control, probably due to reduction in length of basal roots and branches. In our experiments Al toxicity induced reduction of early stage root volume for both cultivars grown in Al treatment nutrient solutions (Figure 5A), while soil acidity did not affect root volume of cv. Scout 66 and that of cv. Sivka was greater when grown in acid compared to limed soil (Figure 5B). Aluminium-injured roots are often described as stubby and brittle, with thickened lateral roots (Foy et al. 1978). Possible explanations of equal root volume (limed and acid soil grown cv. Scout 66) or increased root volume in acid soil grown cv. Sivka could be the Al induced increase in viscous and elastic extensibility of cell wall of the root apices (Ma et al. 2004) or Al induced reduction of cell length accompanied by radial cell expansion which was found on Al treated rice roots (Alvarez et al. 2012).

Plants grown in acid soil produced larger root system (root length, length of the longest seminal root, root surface area and root volume) compared to plants grown in Al treatment solutions. These results could be explained by higher activities of toxic Al in Al treatment solutions (Table 2), as well as possible mitigating effect of soil compounds like plant nutrients and organic matter on Al toxicity. Despite the high Al saturation percentage of soil used in this experiment (Table 1), Delhaize and Ryan (1995) found that exchangeable Al in soil is a poor indicator of Al toxicity. In sand based experiments, Villagarcia et al. (2001) reported that an approximate 100-fold increase in Al concentration was required to inhibit root growth to a comparable degree to hydroponic based experiments. However, high concentrations of toxic

Al are not the only reason for decreased root size in nutrient solutions. This statement is supported by the fact that acid soil grown plants produced a larger root system compared to plants grown in the control nutrient solutions. Reduced root growth of plants grown in nutrient solutions could be explained by stress caused by transfer of young seedlings to hydroponics (Tamas et al. 2006). Another possible explanation could be the more efficient detoxification of Al in soil due to slower diffusion rates of organic acids (malate) away from root surface and Al toward root surface. Kinraide et al. (2005) proposed biphasic diffusion hypothesis of Al detoxification, which suggests that majority of Al detoxification occurs just beneath the root epidermis. Our observed increase in root volume in acid soil grown plants possibly caused by radial expansion of epidermal and cortex cells may represent the evidence for such detoxification.

Despite equal concentrations of free Al in both NSD1 and NSR1 solutions (Table 2), Al toxicity caused more pronounced reduction of root growth in NSD compared to NSR. Possible explanation may lay in the different concentration of nutrients in these two nutrient solutions, especially those of calcium and magnesium, and differences in ionic strength of the solutions (Table 2). With the increasing ionic strength of the nutrient solution increases the competition between Al^{3+} and other cations for negatively charged sites within the root cell wall and plasma membrane. Due to complex chemistry of Al and its multiple interactions with different nutrients in solution, in previous studies of Al toxicity researchers used simple nutrient solutions with low ionic strength and wide range of Al concentrations (from 5 to 200 $\mu M\ L^{-1}$) (Wang et al. 2006), often avoiding usage of different plant nutrients, such as sulphur and phosphorus (Samac and Tesfaye 2003). However, it has been well documented that different concentrations nutrients such as nitrate, phosphate, sulphate and iron can lead to alterations in root growth and architecture (for review see López-Bucio et al. 2003).

Results of this study indicate that beside the well-known reduction of root length Al toxicity also has a profound effect on other root traits, e.g. in nutrient solutions Al toxicity reduced the number of seminal roots, the length of the longest seminal root, the root surface area, root volume. In addition, differences obtained in Al induced effects on root architecture between different nutrient solutions (NSD and NSR) and even more profound differences found between two growing systems (soil and nutrient solutions) emphasize the need for further investigation of wheat root architecture under specific conditions of Al toxicity. In previous experiments Al toxicity was studied under simplified conditions. X-ray μCT provides the opportunity to non-destructively study 3-D root system development in their natural environment of soil. With the further development of this technique, it will be possible to examine larger number of samples and to monitor root development over a more prolonged period across the growth cycle of a plant and to include different environmental factors or plant microbial interactions that could have significant effect on Al toxicity. For example, it would be useful to investigate Al induced root architecture changes across specific soil pH ranges (pH 4.0 – 6.0) in which Al toxicity occurs in arable soils. Furthermore, considering that in many arable soils Al toxicity occurs in acid subsoil layer, further research should focus on larger number of genotypes and on root architectures of mature more established plants.

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