Dr. Gloria M. Coruzzi, Carroll & Milton Petrie Professor
New York University, Dept. of Biology
Center for Genomics & Systems Biology
12 Waverly Place
New York, N.Y. 10003

gloria.coruzzi@nyu.edu
Office Phone: 212-998-3963
RootScape: A landmark-based system for rapid screening of root architecture in *Arabidopsis thaliana*

Daniela Ristova¹,², Ulises Rosas¹, Gabriel Krouk¹,³, Sandrine Ruffel¹,³, Kenneth D. Birnbaum¹ and Gloria M. Coruzzi¹*¹

¹Center for Genomics and Systems Biology New York University, New York, NY 10003;
²Faculty of Agriculture, University of Goce Delcev, 2000 Stip, Republic of Macedonia;
³Institut de Biologie Intégrative des Plantes-Claude Grignon, Biochimie et Physiologie Moléculaire des Plantes, UMR 5004, CNRS/INRA/SupAgro/UM2, Montpellier, France
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* Corresponding author; e-mail: gloria.coruzzi@nyu.edu
Abstract

The architecture of plant roots affects essential functions including nutrient and water uptake, soil anchorage, and symbiotic interactions. Root architecture comprises many features that arise from the growth of the primary and lateral root. These root features are dictated by the genetic background, but are also highly responsive to the environment. Thus, Root System Architecture (RSA) represents an important and complex trait that is highly variable, affected by genotype x environment interactions, and relevant to survival/performance. Quantification of RSA in *Arabidopsis thaliana* using plate-based tissue culture is a very common and relatively rapid assay, but quantifying RSA represents an experimental bottleneck when it comes to medium or high-throughput approaches used in mutant or genotype screens. Here, we present “RootScape”, a landmark-based allometric method for rapid phenotyping of Root System Architecture using *Arabidopsis* as a case study. Using the software AAMToolbox, we created a 20-point landmark model that captures RSA as one integrated trait, and used this model to quantify changes in the RSA of *Arabidopsis* (Col-0) wild-type plants grown under different hormone treatments. Principal Component Analysis (PCA) was used to compare RootScape to conventional methods designed to measure root architecture. This analysis showed that RootScape efficiently captured nearly all the variation in root architecture detected by measuring individual root traits, and is five-to-ten times faster than conventional scoring. We validated RootScape by quantifying the plasticity of RSA in several mutant lines affected in hormone signaling. The RootScape analysis recapitulated previous results that described complex phenotypes in the mutants and identified novel gene-by-environment interactions.
INTRODUCTION

Roots have a crucial impact on plant survival because of their major functions: anchorage of the plant in the soil, water and nutrient acquisition, and symbiotic interaction with other organisms (Den Herder et al., 2010). One important characteristic of root systems is the manner in which the primary and lateral roots comprise the super structure or root architecture. Root architecture is an ideal system for studying developmental plasticity, as it continually integrates intrinsic and environmental responses (Malamy, 2005), which represents a vital and dynamic component of agricultural productivity (Lynch, 1995).

Root system architecture (RSA) is defined as the spatial configuration of the roots in their environment (Lynch, 1995). The complexity of RSA was initially appreciated several decades ago and terms like morphology, topology, distribution and architecture were often used to describe the nature of RSA (Fitter, 1987, Fitter & Stickland, 1991, Lynch, 1995). These early reports argued that simple traits like root mass are insufficient to describe roots, because they do not capture the spatial configuration of roots in the soil, which is critical to plant performance (Fitter & Stickland, 1991). Root systems are integrated organs that adopt specific architectures to maximal foraging of the heterogeneous soil environment in different ways (Fitter, 1987, Fitter & Stickland, 1991, Lynch, 1995). More recently, new approaches have incorporated measurement of many individual developmental traits that together comprise root system architecture (De Smet et al., 2012, Dubrovsky & Forde, 2012). For example, one recent report identified three fundamental components of RSA in generating complex topologies, including the contribution of lateral axes to branching, the rate and path of growth of the axis, and the increase in root surface area (Topp & Benfey, 2012). Thus, root system architecture is an important and complex trait that requires convenient measurement methods for rapid screening of diverse plant mutants and genotypes.

With increasing research in root system architecture in the genetically tractable model plant Arabidopsis, the need for high-throughput methods of root phenotyping has dramatically increased over the years. Consequently, different methods and approaches have been developed in order to address to this demand. Currently, three major
approaches for phenotyping RSA are used (for recent detailed reviews see Zhu et al., 2011, and De Smet et al., 2012). The first group of methods uses classical measures of RSA, which involve measurements of individual root traits. These methods often use software to manually draw the RSA onto digital 2-D images to quantify root length and number (Abramoff et al., 2004, Media Cybernetics, http://www.mediacy.com). These traditional methods provide most accurate measurements of the root system, but have a major disadvantage in being extremely time-consuming.

The second group of methods utilizes advanced semi-automated software for RSA measurements like EZ-Rhizo (Armengaud et al., 2009). EZ-Rhizo also uses digital 2-D images of plants grown on vertical plates (similar to the classical methods above), but is faster and produces different traits and basic statistics. The method works best when root features do not physically overlap, but we have found root overlap to be common when working with Arabidopsis plants older than 10 days. Other recent programs also provide semi-automated analysis of RSA, including RootReader2D (http://www.plantmineralnutrition.net/rootreader.htm) and SmartRoot (Lobet et al., 2011). However, while completely automated detection is potentially the highest throughput, we found that root surface detection step is frequently prone to failure when using both of these programs, even after considerable adjustment by the user, where root features are missed or background noise is incorrectly labeled as roots.

Finally, in a third group, recent developments include 3-D analysis of RSA of plants grown on transparent gel cylinders or in soil. 3-D gel-based imaging approach is reported to be suitable for high-throughput phenotyping (Iyer-Pascuzzi et al., 2010). However, this approach requires special equipment and imaging the root system of single plants can take 10 minutes (Iyer-Pascuzzi et al., 2010). X-ray computed tomography (Perret et al., 2007, Tracy et al., 2010) and magnetic resonance imaging (MRI) (Van As, 2007), also provide highly detailed 3-D RSA analysis, but require long scanning time and are extremely expensive and inaccessible. Most labs still utilize relatively convenient, inexpensive, and rapid 2-D phenotypic characterization of RSA, at least for initial screening purposes.

The aim of this work is to address the need for a simple method to measure many different aspects of root architecture for high-throughput laboratory screening of mutants.
and genotypes in Arabidopsis. Herein, we describe a landmark-based allometric (size and shape) approach called “RootScape”, a user-friendly software platform that enables rapid, comprehensive and integrative phenotyping of the RSA in Arabidopsis. Unlike recent methods that collect information on different root traits to describe the RSA, RootScape places user-defined root landmarks on a two-dimensional grid to measure root architecture as a single integrated root system. The method employs rapid manual placement of root system landmarks. This manual step avoids one of the most problematic steps in automated image analysis (recognition of the root surface), providing a simple tool that does not require image processing. This method uses simple, 2-D digital images of the root system, and a 20-point landmark model created in AAMToolbox, a freely available MATLAB plug-in. While in-depth developmental analysis of root systems will often require knowing the contribution of individual traits, RootScape is a rapid method to access the holistic contribution of many individual root traits to RSA, and to capture the overall property of the spatial configuration of roots in the soil (Fitter & Stickland, 1991). To demonstrate its utility, we used RootScape to quantify the root plasticity of Arabidopsis plants (Col-0) grown on four different media and compared the RootScape results to conventional measurements of individual root traits captured using the Optimas6 image analysis software (MediaCybernetics, Silver Spring, MD). This analysis showed that by measuring integrative root traits using RootScape, we are able to capture the vast majority of the individual trait variation, as verified by multiple regression analysis. Additionally, we tested the ability of RootScape to quantify the plasticity response in Arabidopsis mutants defective in hormone signaling. For this analysis, wild-type (Col-0) and three hormone signaling mutants (axr4, abi4 and cre1), were a treated with auxin, cytokinin or ABA vs. controls. Statistical analyses (ANOVA/MANOVA) allowed us to confirm most of the previously known interactions of genotype with these distinct environments and to potentially identify novel ones. Thus, we demonstrate that RootScape can be used as a rapid and efficient approach for quantifying the plasticity of the root system architecture in mutant (or ecotype) backgrounds of Arabidopsis and can identify new conditional root phenotypes.
RESULTS

**RootScape: Adapting software platform to measure Root System Architecture.**

Allometric methods have been previously applied to measure plant organs such as leaves (Langlade et al., 2005, Bensmihen et al., 2008). To implement an allometric method for quantifying Root System Architecture (RSA) we created a 20-point landmark template (model) in the *AAMToolbox* software, a publicly available MATLAB plugin ([http://cmpdartsvr1.cmp.uea.ac.uk/wiki/BanghamLab/index.php/Software](http://cmpdartsvr1.cmp.uea.ac.uk/wiki/BanghamLab/index.php/Software)) originally developed for face shape recognition in lip-reading (Matthews et al., 2002). This 20-point template captures the main characteristics of the RSA. The six “primary” landmarks (green points in Fig. 1A), are defined by recognizable developmental landmarks on the root. These included four of the primary landmarks, placed on the transition between shoot and root (point 1), the position of first lateral root (point 2), the position of last lateral root (point 6) and the apex of primary root (point 12) (see points on Fig. 1A). Two other primary landmarks (points 14 and 16) were placed at the apex of the lateral root that was farthest from the primary root along an axis perpendicular to the primary on each side (Fig. 1A). All primary landmark points of the template are placed manually at the defined developmental positions of each root sample (Fig. S2). Secondary landmarks or semi-landmarks (red points in Fig. 1) were defined as having a position between the primary landmarks. These secondary landmarks were also placed manually on the lateral root periphery to capture the corresponding shape, and along the length of the primary root. Following manual placement of these secondary landmarks, the *AAMToolbox* software automatically spaces these secondary landmarks evenly between the primary landmarks (Fig. S2). In a side-by-side comparison performed by the same researcher, this new, landmark-based method called “RootScape” (RS) was five-to-ten times faster than individual measurements of root traits as performed using Optimas6 (MediaCybernetics, Silver Spring, MD).

Plant root system does not have a defined shape, for instance compared to leaves, and the number of lateral roots is very variable depending on the conditions or genotype. Thus, the constructed model of 20 landmarks represents the primary root as a line and lateral roots as polygon, which is the shape that explains how the root system spans the
surface of its growth environment on the plate. Root shapes that were captured by this 
model were aligned by translation according to the root shape centroids and translated to minimize the variance between corresponding landmarks. No size normalization of the root shapes was performed in this model, since the aim is to capture the variation in both the size and shape (allometry) of the root system in response to the hormonal treatments. This RootScape model of 20 landmarks yields 40 coordinate values (two per point) that define a 40-dimensional space in which each axis represents variation in one of the coordinate values. Each root shape can be characterized as a single point in this space, where all root shapes from the same treatment together define a “cloud” of points. The center of the cloud, corresponding to the mean root shape, is defined by the means of each of the 40 coordinate values.

We used the RootScape template of 20 landmarks to quantify the RSA of *Arabidopsis* (Col-0) plants grown on four conditions. Conditions consisted of control and three hormones treatments: auxin (IAA), cytokinin (CK) and abscisic acid (ABA) with 24 to 25 plant replicates for each treatment condition. These treatment conditions were selected to create a variety of well-documented and stereotypical root architectures (Nibau et al., 2008). Plants were initially grown on a single growth media and then transferred to either a control plate, or one of the three hormone treatment conditions (see Fig. S1 and Methods for details). After five days on the different hormonal treatments or control plates, the root system was imaged using a document scanner, and the unprocessed images were used for subsequent analysis using the 20-point allometric template of RootScape (See Fig. S2). This landmark dataset was used to create the RSA morphospace, and was named the “Allometric Col-0 plasticity space”. This data was further processed by the AAMToolbox software in a Principal Component Analysis (PCA) which captures the main trends of variation in the root architecture. This analysis revealed that five PCs captured more than 95% of the root variation of the total root shape and size variance in Col-0 genotype (Fig. 2A). Figure 2 shows a plot of the range of the RootScape-derived Principal Component (PC$_{RS}$) values of the first five PCs, to identify the treatments that are driving the extreme phenotypes in RSA (Fig. 2B). PC$_{1RS}$ of the allometric Col-0 model explains 79.08% of the total variance and mostly affects size, but also affects the shape of the RSA, and thus represents a major allometric trait.
Low PC$_{1RS}$ values relate to having a longer primary root and a smaller polygon of lateral roots with a narrowed polygon base (Fig. 2A, Video S1), which corresponds to plants grown in control media or with ABA (Fig. 2B). High PC$_{1RS}$ values relate to shorter primary root and rectangular-like shape of the lateral root polygon (Fig. 2A, Video S1). This extreme value for PC$_{1RS}$ corresponds to the IAA treated plants (Fig. 2B). PC$_{2RS}$ accounts for 8.32% of the variance in RSA and affects mostly the shape of the polygon occupied by lateral roots. Low PC$_{2RS}$ values give a longer, rectangular like polygon (Fig. 2A, Video S1) and correspond to IAA-treated plants (Fig. 2B), whereas higher PC$_{2RS}$ values have a diamond shaped lateral root polygon (Fig. 2A, Video S1) and correspond to CK-treated plants (Fig. 2B). Together, PC$_{3RS}$ and PC$_{4RS}$ capture about 7% of the RSA variance and affect only the asymmetry of the lateral root polygon, which reflects the random variation in root shape among individual plants, but does not represent any trends resulting from the hormone treatment (Fig. 2B). PC$_{5RS}$ accounts for only about 2% of the variance in RSA and mainly affects the lateral root polygon width (i.e., the length of the lateral roots that make the polygon shape). Negative PC$_{5R}$ values correspond to RSA with a wider lateral root polygon, while a positive value corresponds to RSAs with a very narrow lateral root polygon (Fig. 2A, Video S1).

**Comparison of Principal Components captured by RootScape vs. Individual Trait measurements.** As described above, RootScape quantifies the root system architecture as a series of integrative traits, where the phenotypic output is represented by principal components that each captures different aspects of shape and size of RSA. We were interested in determining how these phenotypic outputs of RootScape are related to the classical method of RSA quantification, which measures individual traits. In order to answer this question, individual traits were first quantified using Optimas6 (MediaCybernetics, Silver Spring, MD) (see Methods and Supplemental text for details) and Principal Component Analysis (PCA) was performed on the individual traits. This allowed us to compare how Principal Components derived from the individual traits (PC$_{IT}$) relate to the Principal Components derived from RootScape (PC$_{RS}$) measurements (Table I, Supplemental text). The PCA analysis of the individual traits (Table II) revealed that several traits have a high contribution to PC$_{1IT}$ (e.g. root length ratio, root formation
zone, branching density, growth of primary root). Next, we performed Pearson correlation analysis of the first five Principal Components (PCs) of the individual traits (IT) analysis with the first five PCs derived from the RootScape analysis (Table I). PC1 from RootScape (PC1_RS) has a high and significant correlation (0.88) with PC1 from the individual trait analysis (PC1_IT) (Table I). Thus, we can conclude that individual traits such as root length ratio, root formation zone, branching density, etc., are captured by PC1 of RootScape (PC1_RS). Following this logic, PC2 from RootScape (PC2_RS) has the highest correlation (-0.7) with PC2 from the individual trait analysis (PC2_IT) (Table I), which mainly captures total root length (Table II). Note, that the direction of PC axes is arbitrary, so either correlation or anti-correlation indicates a trend between a trait and a PC. PC3_RS, PC4_RS, and PC5_RS from RootScape correlate moderately, but significantly (0.35, -0.36 and -0.5, respectively), with the same PC from the individual trait analysis, PC3_IT (Table I). The highest contribution in PC3_IT is from the average lateral root length (Table I). Therefore, RootScape captures variation in lateral root length in PC3_RS, PC4_RS and PC5_RS. This correlation analysis shows that the RootScape allometric landmark-based method captures very similar root variation as measured by many individual traits. Thus, RootScape is able to capture information contributed by many individual traits, even though it measures RSA as one integrative trait.

Furthermore, we were interested in how the results obtained by RootScape could be explained (predicted) by the main principal components identified by the individual trait analysis. To answer this question, we used multiple linear regression analysis where each of the first five PC values from RootScape were taken as a response variable, and the first five PC values from individual trait (IT) analysis taken as the explanatory variables (Table SI). We found that PC1_RS can be explained almost completely ($R^2=0.97$) by three PCs from the individual trait analysis (PC1_IT, PC2_IT, and PC4_IT). The second PC from RootScape (PC2_RS), also has a high degree of predictability ($R^2=0.82$) based on four PCs from the individual trait analysis (PC1_IT, 2_IT, 3_IT and 5_IT). The third, fourth and fifth PCs from RootScape (PC3_RS, PC4_RS, PC5_RS) can be explained to a modest extent by the first five PCs from the individual trait analysis ($R^2=0.17$, $R^2=0.22$, and $R^2=0.29$), although only the third PC (PC3_IT) from the individual trait analysis shows significance.
for prediction. These results demonstrate that RootScape phenotypic outputs can be highly predicted by classical quantification of RSA measured using individual traits.

Reciprocally, we asked how much of the RSA variation captured with the conventional individual trait analysis can be explained by RootScape allometric measurements (Table SII). The first PCIT from the individual trait analysis, is largely explained by PC1RS, PC2RS and PC5RS of RootScape (R^2=0.93). PC2IT and PC3IT from the individual trait analysis, can be also explained to a high degree (R^2=0.75 and R^2=0.69) where different PCs from RootScape have a significant contribution. Thus, this analysis confirms that the simple and rapid allometric approach of RootScape has the power to predict the first three PCs from the individual trait analysis, which accounts for about 96% of the variation using that method. More generally, the reciprocal multivariate analysis shows that RootScape and individual trait analysis largely capture the same trends in variation of root system architecture. Thus, overall, the correlation and multivariate analyses show that RootScape is a rapid method that describes the same major trends in variation of root system architecture as more detailed and time-consuming analyses of individual traits.

Using RootScape to characterize the plasticity of Root System Architecture. Different phytohormones are known to exert specific effects on root architecture, with partially overlapping effects often due to crosstalk between hormones (reviewed in Depuydt & Hardtke, 2011, Bishopp et al., 2011). We used RootScape to characterize the plasticity of the Arabidopsis root system under hormone treatments. In addition, we measured the root architecture of well-characterized Arabidopsis mutants in hormone signaling to determine if RootScape could identify known phenotypes, including insensitivity to hormone treatments, which were the basis for the mutant screens. We also characterized all of these Arabidopsis mutants with a panel of hormone treatments to explore the ability of RootScape to rapidly characterize root architecture under a variety of conditions, and to identify potential crosstalk among hormones. We analyzed three Arabidopsis mutants known to affect root development due to signaling defects in auxin, axr4-1, (Hobbie & Estelle, 1995), abscisic acid, abi4-1 (Signora et al., 2001) and cytokinin, cre1-2 (Inoue et al., 2001).
For each mutant genotype, 10 to 15 plant replicates were analyzed on each of the hormonal treatments, compared to controls. Growth conditions of the mutants were identical as for Col-0, (see Methods and Fig. S1). The same RootScape allometric template of 20 landmarks was applied to each plant. In order to compare changes in root phenotype between the mutant lines and Col-0, we projected both the root architectural plasticity in Col-0 and the three mutant lines onto PC1$_{RS}$ and PC2$_{RS}$ axes (e.g. the allometric Col-0 plasticity space defined by RootScape measurements) and obtained phenotypic scores (PC values). By carrying out this projection, we were able to visualize the distribution of phenotypes along the phenotypic Col-0 plasticity “space” (Fig. 3).

This analysis showed that auxin treatment mapped to one extreme of PC1$_{RS}$, showing its strong effect on a suite of traits that varied dramatically among the treatments (Fig. 3A). We did not find any specific treatment grouping in the second PC (Fig. 3B). We next used PC values for the first five PCs to identify any genotype by treatment interactions. For this analysis, we performed a 2-way Analysis of Variance (ANOVA) for each of the first five PCs, following the model $PC_{RS} = \alpha_{genotype} + \beta_{treatment} + \gamma_{genotype*treatment} + \epsilon$. There were significant differences due to genotype (PC1$_{RS}$ F=70.84, p<0.0001; PC2$_{RS}$ F=23.31, p<0.0001; PC4$_{RS}$ F=11.26, p<0.0001; and PC5$_{RS}$ F=10.25, p<0.0001), treatment (PC1$_{RS}$ F=343.37, p<0.0001; PC2$_{RS}$ F=54.15, p<0.0001; PC3$_{RS}$ F=3.06, p=0.0288; PC5$_{RS}$ F=16.52, p<0.0001), and the interaction between genotype and treatment (PC1$_{COL}$ F=16.04, p<0.0001; PC2$_{COL}$ F=2.82, p=0.0036; PC3$_{RS}$ F=5.63, p<0.0001; PC4$_{RS}$ F=6.16, p<0.0001; and PC5$_{RS}$ F=3.09, p=0.0015). Significant differences between the genotypes and treatments, when compared to wild-type (Col-0) genotype and control treatment are shown in Table III. RootScape measurements of the mutants and wild-type, were able to recapitulate most of the known genotype by hormone interactions. For example, $axr4$, which was originally isolated as an auxin resistant mutant (Hobbie & Estelle, 1995), displayed an interaction with auxin (IAA) in PC1$_{RS}$ and PC4$_{RS}$. In addition, $axr4$ showed an interaction with abscisic acid (ABA) in PC1$_{RS}$, PC3$_{RS}$ and PC4$_{RS}$ (Table III). These results support the variable resistance of $axr4$ to ABA, which was reported previously (Hobbie & Estelle, 1995). Additionally, another study reported crosstalk between ABA and auxin signaling ($axr4$) (Rock & Sun, 2005). The interaction of $axr4$ with cytokinin was present in all five PC$_{RS}$ (Table III), and fits with
previous observations on variation in relative root elongation reported for *axr4-1* at particular CK concentrations (Hobbie & Estelle, 1995). Thus, RootScape can identify many of the conditional phenotypes identified in the literature for the *axr4* mutant.

The RootScape analysis also uncovered an interaction of *cre1-2* with all three hormonal treatments in at least one PC$_{RS}$ (Table III). First, the *cre1-2* interaction with CK captured in PC$_{1RS}$ and PC$_{5RS}$, is consistent with previously published phenotypes for this mutant, fitting with its role as CK receptor (Inoue et al., 2001). This analysis also identified an interaction of the *cre1-2* mutation with ABA. This is reminiscent of another *crel* allele (*crel-1*), where *crel-1* was shown to have lower root growth in response to low levels of ABA (Inoue et al., 2001). The *abi4-1* mutant had only one interaction with CK in PC1 (Table III), which may be related to interactions found by another study in which CK treatment increases the transcript level of *ABI4* (Shkolnik-Inbar & Bar-Zvi, 2010). Thus, RootScape is a sensitive and robust tool to characterize root architectural phenotypes using a rapid and simple protocol.

**A visual representation of phenotypic plasticity space.** We have shown that RootScape can capture many of the individual traits that determine overall root system architecture (RSA). Furthermore, the principle component analysis (PCA) showed that suites of co-varying traits can be summarized in single axes or principle components, capturing similar trends compared to the parallel analysis of individual traits. Thus, RootScape, in combination with PCA, offers a way to rapidly summarize the complex characteristics of root systems architecture in a given genotype or background, as has been done for other traits (Adams, 2010). The first two principal components from RootScape capture about 87% of the total root shape variation for the allometric Col-0 plasticity space (Fig. 2). These PCs represent suites of individual root traits (Table I, Table SI). The RootScape PC space therefore provides the opportunity to quantify and visualize trends in root architecture in two dimensions providing a visual overview of phenotypic space.

To compare the root phenotypes of the various Arabidopsis hormone signaling mutants, we plotted PC$_{1RS}$ against PC$_{2RS}$ values of root shapes for each genotype (Col-0, *axr4*, *abi4* and *crel*) on four treatment media (Control, IAA, CK and ABA) (Fig. 4). By plotting PC$_{1RS}$ against PC$_{2RS}$ values one can visually assess how the plasticity of the root
system architecture is affected in different genotypes (Fig. 4). For example, as previously reported (Depuydt & Hardtke, 2011, Hobbie & Estelle, 1995) and shown by ANOVA analysis above, the change of root plasticity of axr4 (Fig. 4B) compared to wild-type (Fig. 4A), is visually apparent in the first two PCs of RootScape (87% of variation). When wild-type (Col-0) root plasticity space is compared with abi4 (Fig. 4A and 3C), it is evident that the overall trend is maintained. In this case, the appropriate analysis to test whether root architecture is significantly different in the two-dimensional PC space is Multivariate Analysis of Variance (MANOVA, Pillai test). We used MANOVA, and corrected for multiple pair-wise testing by applying a Bonferroni adjustment (alpha=0.008). This MANOVA analysis detects differences in the plasticity space occupied by all the different treatments applied to abi4. Mapping the individual samples on the two dimensional PC space, shows that the variability within treatments is much reduced in abi4, without reducing the differences among treatments. This leads to the observation that the abi4 mutant appears to constrain variability within all treatments; such a trait might not have been a part of quantitative measurements in the screening process, but becomes apparent with visualization. This shows how this visual representation of RSA space can help screen for complex phenotypes that could be followed up with subsequent quantitative analysis.

DISCUSSION

This manuscript presents RootScape, a rapid method for the allometric and integrative quantification of Root System Architecture (RSA) applied to Arabidopsis thaliana. The RootScape method uses a 20-point landmark template created in AAMToolbox (a MATLAB plugin), which can rapidly and accurately characterize RSA variation in different genetic backgrounds or treatments. RSA variation was generated experimentally by supplying Arabidopsis plants grown in full MS media with three different hormones known to affect different aspects of root development (auxin, cytokinin and abscisic acid). The landmark data from the root templates applied to this dataset was then used in a Principal Component Analysis (PCA). These PC values were
then used for correlation and multiple regression analysis, in order to compare how the RootScape method is related to standard measurements of individual root traits. This analysis showed that RootScape is able to recapitulate very similar trends of variation and is able to capture trends exhibited by nearly all individual traits. Additionally, we tested three mutant lines in hormone signaling known to affect root development, and validated the RootScape technique by recapitulating previously characterized phenotypes and potentially describing new aspects of mutant phenotypes.

In a typical analysis with RootScape, a user could apply simple criteria for comparing root architectures, such as a significant difference in one or several principal components, for example, between wild type and mutant samples. Examination of components can identify the root system properties that are changing along a principal component.

The RootScape template of 20 landmarks is meant to quantify RSA as a comprehensive or holistic phenotype (see above results). However, it might be argued that the template can have a different (or more detailed) set of landmarks to enable a more detailed quantification of RSA. In such a case, a new template can easily be created that is designed to record specific aspects of root architecture. The current 20-landmark model design captures intuitive and well-established features of the RSA, with a minimal number of landmarks, permitting high-throughput phenotyping. The secondary landmarks add sensitivity to detect a range of phenotypes, such as bending of the root in agravitropic mutants. However, we note that the primary intention of RootScape is rapid screening of RSA of Arabidopsis seedlings grown vertically on agar plates. It may not capture all possible defects in root architecture. For example, the RootScape method does not measure the diameter of roots and subtle bending or waving phenotypes may be missed.

We show that the first PC captured by RootScape, PC$_{RS}^{1}$ has highest correlation with first PC derived from measurements of individual traits PC$_{IT}^{1}$, that mainly captures traits that are related or function of primary root length (e.g. root length ratio, growth of the primary root, root formation zone and branching density). The second PC$_{RS}^{2}$ is significantly correlated with PC$_{IT}^{2}$, in which total root length and lateral root number have major contributions. We note that RootScape does not explicitly measure traits like
lateral root number or branching density, but it appears these traits correlate with other root features that RootScape does effectively capture. RootScape also effectively captures other traits that are based on ratios of two important root traits, for example, length ratio (total lateral root length/primary root length). The important point is that RootScape captures trends in the variation of root architecture that are described by standard measurements, even if they were not directly measured. The first and second PCs captured by RootScape significantly correlate with more than one PC of individual traits, signifying the integrative nature of RSA captured by RootScape.

One interesting finding is that lateral root length is captured by three different RootScape PCs (PC3<sub>RS</sub>, 4<sub>RS</sub> and 5<sub>RS</sub>). The RootScape template was designed to capture the set of lateral roots that grew farthest away from the primary root axes. In addition, RootScape measures the extent of lateral root outgrowths along the primary root. This design appeared to effectively capture both the length of the lateral roots, and much of the shape of the space covered by lateral roots on the two dimensional plate. This enabled us to measure different ways in which lateral root length influences root architecture, as multivariate analysis showed that this trait contributed to three different RootScape derived PCs. These findings are consistent with earlier opinions that simple traits like the length of roots, and total root mass, are unable to explain the RSA variation and complexity, while spatial configuration and topology have more crucial impacts on RSA (Fitter & Stickland, 1991). Correlation and multiple regression analysis indicate that the first two PCs of RootScape are correlated with more than one PC from the individual traits. This shows that PCA analysis on RootScape groups the co-variation among traits somewhat differently than PCA derived from an individual trait analysis, as can be visualized in PC “walks” (Video S1) that represent the variation in root forms along a PC. Thus, RootScape measures the spatial configuration of roots to provide a new view of RSA – one that adheres to the concept that roots are integrated organs (Fitter, 1987, Fitter & Stickland, 1991, Lynch, 1995).

We generated experimental RSA variation by supplementing wild-type Arabidopsis (Col) with three different hormones, all known to affect root development. One of the hormones auxin, has a dramatic effect on the root phenotype, inhibiting new outgrowth of the primary root and stimulating initiation of new lateral roots, while
inhibiting their elongation. It is possible that because of this strong effect, the RSA variation created in our experimental design will be driven by auxin treatment. This appears to be the case, since the first principal component (PC1_RS) captures mainly variation in primary root length or overall size. However, this trend of capturing size effect in the first PC is observed in separate landmark-based quantification of leaf shape (Bensmihen et al., 2008, Langlade et al., 2005). We have also used the RootScape method for RSA quantification on 69 Arabidopsis ecotypes (Rosas et al., in preparation) on one environment, and found that first PC is also driven by the size effect, even if no auxin treatment was applied.

Testing the RSA plasticity space in hormone signaling mutants with RootScape followed by 2-way ANOVA, confirmed most of previously reported phenotypes and hormone interactions in the mutant lines. For example, axr4 was isolated for its resistance to auxin treatment, and also shows variable resistance to abscisic acid (Hobbie & Estelle, 1995). In the same study (Hobbie & Estelle, 1995), axr4 was reported to be sensitive to a particular kinetin concentration. Here, we able to use RootScape to confirm the interaction of axr4 with auxin (PC1_RS and PC4_RS), with abscisic acid (PC1_RS, PC3_RS & PC4_RS), and with cytokinin (PC1_RS-PC5_RS). In addition, cre1 was originally isolated as having reduced sensitivity to cytokinin in the inhibitory effect on primary root growth (Inoue et al., 2001). We confirmed this interaction of cre1 with cytokinin (PC1_RS and PC5_RS). Additionally, our 2-way ANOVA results indicate interaction of cre1 with auxin (PC1_RS) and abscisic acid (PC3_RS), and abi4 with cytokinin (PC1_RS). These findings support the results from the correlation and multiple regression analysis, that RootScape is able to recapitulate very similar trends in RSA variation as classical measurements of individual root traits. In addition, RootScape can potentially identify new interactions of genotypes with treatments. We identified two new genotype x environment interactions (cre1*IAA, abi4*CK), as well as three previously identified interactions (axr4*ABA, axr4*CK, cre1*ABA) (Hobbie & Estelle, 1995, Inoue et al., 2001). This result demonstrates that RootScape can be used as a sensitive screening tool to explore RSA plasticity space of mutant lines (alleles or ecotypes) exposed to different environments in Arabidopsis thaliana. Furthermore, with ANOVA or/and MANOVA analysis, this plasticity of RSA and sensitivity can be visualized and quantified.
The experimental design presented herein provides quantification of RSA at single time point, at 12 days after germination. However, there is need to observe the dynamics of root development, as recently advised (De Smet et al., 2012, Wells et al., 2012). In a pilot experiment, we tested if RootScape is able to distinguish RSA changes in different developmental stages of Col-0 genotype grown on a single media, 1 mM KNO₃. In this analysis PC1₁₀₁₄ showed a significant difference between day 10 vs. day 14 (Fig.S3, Supplemental text), confirming that RootScape can also be used for quantification of RSA dynamics.

Root system architecture differs between species by enormous degree. We have developed RootScape, and its current template, using Arabidopsis thaliana as a model system. To be able to apply the RootScape to other species, it will be necessary to adapt an optimal landmark template. This could potentially be a complex task, since the diversity of RSA is vast among ecotypes of the same species, and even more diverse amongst different species. The current 20-landmark template developed for Arabidopsis, is suitable for quantification of any root system that has a dominant primary root. We therefore tested this 20 point template on a pilot set of Medicago trunculata ecotypes. Five Medicago ecotypes (Longi, A17, 2HA, Gaerta and Tribu) were grown on 1 mM KNO₃ for 14 days, and RSA quantified with RootScape using the current template. This pilot study on a small number of ecotypes and replicates, reveals a set of PC that capture differences in RSA between the ecotypes captured mainly in the first five PCs (Fig. S4, Supplemental text).

In summary, RootScape - a rapid, cost-effective method to capture root system architecture allometry - is appropriate for studying and quantifying a wide range of questions in root biology in the model Arabidopsis, and can be adapted to other species. We demonstrate that RootScape is able to quantify and recapitulate known root phenotypes from the literature. Characterization of RSA plasticity among different genotypes (e.g. mutants, alleles or ecotypes) on range of treatments could also be investigated by the same analysis. These examples support the notion of potentially wide application of RootScape in different areas of high-throughput root studies in plants.
MATERIALS AND METHODS

Plant Material. Arabidopsis thaliana genotypes used in this study were Columbia (Col-0), axr4-1, (Hobbie & Estelle, 1995), abi4-1 (Signora et al., 2001) and cre1-2 (Inoue et al., 2001). Medicago ecotypes used were 2HA (Medicago truncatula 2HA), Gaerta (Medicago truncatula Gaertner), Longi (Medicago truncatula var.longispina), Tribu (Medicago truncatula ssp.tribuloides, and A17 (Medicago truncatula A17).

Experimental design and treatments. To generate variation in the root system architecture (RSA), we first grew 15 seeds per plate of Col-0 wild type on full Murashige and Skoog (MS) basal media (M 5524, Sigma) with 0.1% sucrose as a carbon source, 0.05% MES sodium salt (Sigma), pH=5.7, and 1% Bacto Agar (BD), for 7 days. Seeds were first surface sterilized and plated at 10 x 10 cm square plates (without tape or parafilm), then stratified for 3-5 days in the dark at 4°C. Plates were set up vertically in a Percival growth incubator (Intellus PERCIVAL) at 22 °C with a 16 hr/8 hr light/dark cycle and a light intensity of 50 μmol m⁻² s⁻¹. After 7 days on each plate, 5 out of the 15 most uniform plants were chosen and transferred onto a new plate with different media as described in Sup. Fig. 1: full MS-control, or full MS media supplemented with 500 nmol 3-Indolacetic acid, Sigma (auxin-IAA media), 500 nmol Kinetin, Sigma (cytokinin-CK media), 1 μmol Abscisic acid, Sigma (ABA media). Each experiment was performed twice, with a total of 24 to 25 plants per treatment. The same procedure was applied for the mutant genotypes, where we carried out two experimental replicates and used a total of 10 to 15 plant replicates per genotype per treatment. Five days after the transfer of the 7-day old plants to different media, plates were scanned (Epson Perfection V350 Photo) at 300 dpi and images obtained.

Individual trait analysis of root system architecture. We scored 12 individual root traits quantified as previously described (Remans et al., 2006, Ruffel et al., 2011, Dubrovsky & Forde, 2012). Briefly, we used Optimas6 software that allows one to completely draw the root system and export the main numerical values. We measured primary root length on the transfer day (P1), primary root length growth after five days
from the transfer (P2), lateral root numbers (LR#), lateral root numbers in P2 (LR# in P2), root branching zone (BZ), root formation zone (FZ), and average lateral root length (LRl). Other traits are obtained from these main traits by applying appropriate calculations (see supplemental text and Fig. S1 for details). Using this analysis we quantified Col-0 plants (24-25 plants per treatment) in four different treatments (Control, IAA, CK, and ABA) and later performed PCA in order to compare with RootScape.

**RootScape, integrative and allometric quantification of RSA.** The same plants quantified by the individual trait analysis were also measured by RootScape. Three insertion mutations (*axr4-1*, *cre1-2*, and *abi4-1*; 10-15 plants per treatment, per genotype) were quantified by this method. Twenty landmarks (6 primary and 14 secondary) were fitted along the length of the primary (main) and lateral (secondary) roots, by placing the landmarks at key and recognizable positions of RSA: the as start and end of primary root, first and last lateral root on the main root, and widest points of lateral roots on each side of the main root (Fig. 1). Principal component analysis model of root shape and size was created from the 20 point model in wild-type Col-0 treatment dataset. In this wild-type plasticity space we projected the model shapes for the three mutant lines and obtained PCs values for later statistical analysis. Models were generated using version one of the AAMToolbox MATLAB plugin, available free from Coen’s and Bangham’s laboratories (http://cmpdartsvr1.cmp.uea.ac.uk/wiki/BanghamLab/index.php/Software). The user manual can be downloaded at the following link: http://lemur.cmp.uea.ac.uk/Research/cbg/Documents/Bangham-Coen-Group/AAMToolbox/AAMToolbox.htm.

**Procrustes alignment, normalization and representation of shapes.** For each analyzed root, root outlines were normalized using the Procrustes method, where each root shape is transformed to a mean shape using iterative translation, and rotation of the landmark data generating superimposition that minimize the aberrations from the overall mean root shape (Bensmihen et al., 2008).

**Statistical Analysis.** One-way ANOVA for treatment in wild-type Col-0 dataset for the 12 individual traits was performed in JMP 9 (student license to DR). In JMP we also performed two-way ANOVA, PCA of individual traits, correlation and multiple
Individual Trait measurements and Principal Component Analysis.

In order to evaluate which individual traits are driving each principal component, we analyzed the same response of the root system to hormonal treatments in Col-0, by scoring root system architecture (RSA) by 12 root traits. This quantification of RSA includes numeric values of the traits and was scored as previously described by using Optimas image analysis software (MediaCybernetics, Silver Spring, MD) (Remans et al., 2006, Ruffel et al., 2011) (Fig. S1). Briefly, root length was measured by manually drawing each lateral and primary root and root number was measured by counting visible lateral roots along the primary root. We refer to these traits as “individual traits” through the paper even if some of them are ratios and multiplication of the main traits. For this analysis, we measured 12 individual traits (ITs): seven main traits: primary root length on transfer (P1), growth of primary root (P2), lateral root numbers (LR#), lateral root numbers in P2 (LR# in P2), average lateral root length (LRI), branching zone (BZ) (Dubrovsky & Forde, 2012) and root formation zone (RFZ) (Dubrovsky & Forde, 2012); and five derivate traits: primary root length (P=P1+P2), branching density (Bd=LR#/BZ), length ratio (LR=(LRI*LR#)/P), total lateral root length (TLRL=LR#*LRI), total root length (TRL=LR#*LRI*P) (Fig. S1). We performed a one-way Analysis of Variance (ANOVA) to test whether or not the treatments have a significant effect on any of the ITs. We observed that all ITs displayed a significant response to the hormonal treatments (p<0.001) except P1 (data not shown). As expected, P1 trait is not affected by the treatments (length of the primary root at the beginning of the treatment) and, thus, has been excluded from the ITs.

Next, we were interested in determining how the variation is driven by the individual traits, thus we performed PCA on the twelve ITs. The first five PCs explain 99% of the variation in RSA (Table II). The first PCIT represents 63.1% of the variability,
where many traits have high contribution, but length ratio, root formation zone, branching density and growth of primary root have major contributions. PC2\(_{RT}\) captures 22.3% of the ITs variation where total root length has greatest influence. PC3\(_{RT}\), PC4\(_{RT}\) and PC5\(_{RT}\) additionally explain with 10.53, 2.22 and 1.08%, respectively of the observed RSA variability. These three components account mainly for lateral root length, lateral root number in P2 and branching density (Table II).

**Quantification of developmental stages with RootScape.** To determine whether RootScape is suitable for quantifying and distinguishing RSA of plants at different developmental stages, we performed pilot experiment where we grew *Arabidopsis thaliana* Col-0 ecotype on 1 mM KNO\(_3\) for two weeks, with 16 plant replicates. Seeds preparation and sterilization was done as previously described. We scanned the same set of plant replicates on day 10 and day 14 after germination, and measured RSA with RootScape using the 20 landmark template. This data set was used to create the RSA morphospace, and was named “10vs14”. Similarly, as previously described, this data was analyzed by the AAMToolbox software to perform Principal Component Analysis (PCA) in order to capture the main trends of variation of this dataset (Fig. S3A). The first PC (PC1\(_{10vs14}\)) that represents about 81% of variation, shows a significant difference between the two developmental stages (Fig. S3B). The two developmental stages are also clearly separated, by plotting PC1\(_{10vs14}\) against PC2\(_{10vs14}\) (Fig. S3C).

**Quantification of *Medicago trunculata* with RootScape.** To test whether RootScape can be used to quantify RSA in another plant species, we performed a pilot experiment on *Medicago trunculata*. Five ecotypes of *Medicago trunculata* were grown on 1 mM KNO\(_3\) for two weeks on large (12 x 12 cm) square plates. Seed preparation and sterilization was performed as for *Arabidopsis*, as previously described. Seeds were placed in water for four hours prior to plating. The five ecotypes used were: 2HA (*Medicago truncatula* 2HA), Gaerta (*Medicago truncatula* Gaertner), Longi (*Medicago truncatula* var.longispina), Tribu (*Medicago truncatula* ssp.tribuloides, and A17 (*Medicago truncatula* A17). We applied the RootScape template of 20-landmarks tested inititally on Arabidopsis, to quantify the RSA in Medicago. This landmark data set was
then used to create the RSA morphospace for Medicago that was called “Med”. As previously described, this data was used by the AAMToolbox software to perform Principal Component Analysis (PCA) in order to capture the main trends of variation of this dataset (Fig. S4A). Despite the small number of ecotypes and limited number of replicates (4-11) used in this pilot study, RootScape was able to distinguish the ecotypes according to the main PCs (Fig. S4B and S4C).

Acknowledgements

The authors acknowledge Lawrence Hobbie for providing axr4-1 seeds, Thomas Schmülling and Michael Riefler for providing crel-2 seeds. The authors acknowledge Miriam L. Gifford for providing seeds of Medicago ecotypes. The authors acknowledge Daniel Tranchina for guidance on multiple regression analysis, and Miriam L. Gifford and Amy Marshall Colon for helpful comments and suggestions. This work was funded in part by National Institutes of Health Grant R01 GM032877 (to G.M.C.), National Institutes of Health Grant R01 GM078279 (to K.D.B.), and National Science Foundation Arabidopsis 2010 Genome Grant MCB-0929338 (to G.M.C., and S.R). D.R.’s research was supported by an International Fulbright Science and Technology Doctorate Award. G.K.’s research was supported by European-FP7-International Outgoing Fellowships (Marie Curie) (AtSYSTM-BIOL; People Outgoing International Fellowships-2008-220157), and by a Human Frontier Postdoctoral Fellowship to UR.

Literature Cited


system architecture. Plant J 57: 945-56.


define a gene important for root gravitropism and lateral root initiation. Plant J 7: 211-20.


FIGURE LEGENDS

**Figure 1.** RootScape: Landmark-based allometric model for integrative quantification of Root System Architecture (RSA). Twenty landmarks were used to describe the RSA, including six primary landmarks (green, 1, 2, 6, 12, 14, 16) placed at recognizable positions of the RSA, and fourteen secondary landmarks (red) spaced evenly between the primary landmarks. A) RootScape template of 20 landmarks; B) RSA of 12-day old seedling; C) RootScape applied on the same seedling.

**Figure 2.** Variability of the root system shape and size of Arabidopsis Col-0 quantified with RootScape, as described by the five main principal components (PC) from the the allometric Col-0 model (RS). A) For each PC the mean root shape outline is showed in black (middle), blue (left) and red (right) shapes are shown by varying the PC value minus or plus one standard deviation (-SD and +SD). The percentage of the variance of each PCs is shown next of the shapes; B) PC values for the first five principal components are plotted on the x-axis against the four different conditions. The mean of each of the four treatments is represented by a diamond and the error bars display the variation observed for each group.

**Figure 3.** Range of PC values obtained for Col-0 (diamond) and three mutants, *abi4-1* (square), *axr4-1* (circle) and *cre1-2* (triangle) on the 4 different media (control (C)-gray, auxin (IAA)-red, cytokinin (CK)-green, and abcisic acid (ABA)-blue) for the allometric Col-0 model (RS). Mean of each genotype based on the four treatments is represented by different shape and the bars represent the standard error, within the observed group. A)
PC1; B) PC2. Extreme root shapes of minus or plus one standard deviation are shown on each side below the x-axis.

**Figure 4.** Visualization and MANOVA quantification of RSA phenotypic plasticity in genotypes. Distribution of principal component 1 (PC1) and principal component 2 (PC2) values of *wt* (Col-0), *axr4-1*, *abi4-1*, *cre1-2* on four different treatments for the allometric Col-0 model quantified by RootScape. The mean of each treatment is shown by a colored diamond, and ellipsoids in the same color represent clouds around the replicates. A) wild-type; B) *axr4-1*; C) *abi4-1*; and D) *cre1-2*. MANOVA summary table for pair-wise comparison is shown bellow each plot (Pillai test with Bonferroni adjustment 0.008*).

**Table I.** Correlation matrix of first five principal components (PC) of RootScape (RS) and first five principal components of individual traits (IT), followed by variability contribution for each. Sample size (N) is represented by 24-25 plant replicates per treatment.

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Bold values show highest significant correlation

P < ***0.0001, **0.001, *0.01

**Table II.** Loading matrix of principal component analysis, scored by 10 individual traits (IT).

Bold values show the most highest contribution(s) to the component.

**Table III.** Significant differences of first five RootScape (RS) Principal Component (PC) values comparing hormone treatments and mutant genotypes to control treatment and wild-type genotype. Only significant P values are shown in the table (P < 0.05).

*indicative interaction

**Figure S1.** Experimental design of the study. Hormone treatments were used to create phenotypic plasticity in the root system. Two methods are used to measure the root system architecture: individual trait (IT) analysis measured in Optimas6 and integrative allometric landmark analysis (RootScape) measured in AAMToolbox, plugin in
MATLAB. Col-0 plants are grown on full MS media for 7 days, followed by transfer to the same media (Control), full MS media supplemented with 3-Indolacetic acid 500 nmol (auxin-IAA), full MS media supplemented with Kinetin 500 nmol (cytokinin-CK), full MS media supplemented with abscisic acid 1000 nmol (ABA). After 5 days on different treatments plates were scanned and root system architecture quantified.

**Figure S2.** Application of 20 landmark template of RootScape. Three views are shown; before manually placing the landmarks at the relevant homologous position of the root system, the template shape of previously scored plant is overlaying (unmodified template, left view). At this point the user is able to increase/decrease the width and length of the whole template or to rotate the template for faster adjustment on the next root replicate. Next, the user will manually place all landmarks at the relevant positions, see text for details (applied template, middle view). After placing the landmarks the user will select ‘Smooth Secondary’ and the AAMToolbox software will automatically and evenly space the secondary landmarks between the primary (after smoothing, right view).

**Figure S3.** Quantification of developmental stages with RootScape. A) Variability of the root system shape and size of Arabidopsis Col-0 as described by the five main principal components (PC) from the allometric 10vs14 day model. For each PC the mean root shape outline is showed in black (middle), blue (left) and red (right) shapes are shown by varying the PC value minus or plus two standard deviation (-2SD and +2SD). The percentage of the variance of each PCs is shown next of the shapes; B) PC values for the first principal component are plotted on the x-axis against the two developmental stages (10 and 14 days). Bars indicate standard error. C) PC values of first two Principal Components plotted against each other of the two developmental stages (10 and 14 days).

**Figure S4.** Quantification of Medicago with RootScape. A) Variability of the root system shape and size of five Medicago truncatula ecotypes as described by the five main principal components (PC) from the allometric Medicago (Med) model. For each PC the mean root shape outline is showed in black (middle), blue (left) and red (right) shapes are shown by varying the PC value minus or plus two standard deviation (-2SD and +2SD).
The percentage of the variance of each PCs is shown next of the shapes; B) PC values for the first principal component are plotted on the x-axis against the five genotypes. Bars indicate standard error. C) PC values of three Principal Components (PC1, PC3, and PC4) plotted against each other for the five Medicago ecotypes. Ecotypes: 2HA (Medicago truncatula 2HA), Gaerta (Medicago truncatula Gaertner), Longi (Medicago truncatula var.longispina), Tribu (Medicago truncatula ssp.tribuloides, and A17 (Medicago truncatula A17).

**Table SI.** Multiple regression of each of the first 5 principal components (PC) of RootScape (RS), modeled by first 5 principal components of individual traits (IT). Bold values show $P < 0.01$

**Table SII.** Multiple regression of each of the first 5 principal components (PC) of individual trait (IT) analysis, modeled by first 5 principal components of RootScape (RS) measurements. Bold values show $P < 0.01$

**Supplemental file 1:** RootScape template.

**Supplemental file 2: Video 1.** Variability of the root system shape and size of Arabidopsis Col-0 quantified with RootScape, as described by the PC walks of 1SD (standard deviation) of the first five main principal components (PC) from the allometric Col-0 model (RS).

**Supplemental file 3:** Supplemental Figures and Tables.

**Supplemental file 4:** ICON file.
**Table I.** Correlation matrix of first five principal components (PC) of RootScape (RS) and first five principal components of individual traits (IT), followed by variability contribution for each. Sample size (N) is represented by 24-25 plant replicates per treatment.

<table>
<thead>
<tr>
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<th>PC3&lt;sub&gt;RS&lt;/sub&gt; 4%</th>
<th>PC4&lt;sub&gt;RS&lt;/sub&gt; 3%</th>
<th>PC5&lt;sub&gt;RS&lt;/sub&gt; 2%</th>
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<td>-0.147</td>
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<td><strong>-0.669</strong>*</td>
<td>0.197</td>
<td>-0.284*</td>
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<td><strong>-0.497</strong>*</td>
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<td>PC5&lt;sub&gt;IT&lt;/sub&gt; 1%</td>
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Bold values show highest significant correlation

P < ***0.0001, **0.001, *0.01
Table II. Loading matrix of principal component analysis, scored by 10 individual traits (IT)

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<th>Individual Trait</th>
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<td>Lateral Root number (LR#)</td>
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<td>85.40</td>
<td>95.94</td>
<td>98.16</td>
<td>99.24</td>
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Bold values show the most highest contribution(s) to the component.
Table III. Significant differences of first five RootScape (RS) Principal Component (PC) values comparing hormone treatments and mutant genotypes to control treatment and wild-type genotype. Only significant P values are shown in the table (P < 0.05).

<table>
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<tr>
<th>Groups</th>
<th>PC1&lt;sub&gt;RS&lt;/sub&gt; P value</th>
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*indicates interaction
Figure 1. RootScape: Landmark-based allometric model for integrative quantification of Root System Architecture (RSA). Twenty landmarks were used to describe the RSA, including six primary landmarks (green, 1, 2, 6, 12, 14, 16) placed at recognizable positions of the RSA, and fourteen secondary landmarks (red) spaced evenly between the primary landmarks. A) RootScape template of 20 landmarks; B) RSA of 12-day old seedling; C) RootScape applied on the same seedling.
Figure 2. Variability of the root system shape and size of Arabidopsis Col-0 quantified with RootScape, as described by the five main principal components (PC) from the allometric Col-0 model (RS). A) For each PC the mean root shape outline is showed in black (middle), blue (left) and red (right) shapes are shown by varying the PC value minus or plus one standard deviation (-SD and +SD). The percentage of the variance of each PC is shown next of the shapes; B) PC values for the first five principal components are plotted on the x-axis against the four different conditions. The mean of each of the four treatments is represented by a diamond and the error bars display the variation observed for each group.

C – control
IAA – auxin
CK – cytokinin
ABA – abscisic acid
Figure 3. Range of PC values obtained for Col-0 (diamond) and three mutants, *abi4-1* (square), *axr4-1* (circle) and *cre1-2* (triangle) on the 4 different media (control (C)-gray, auxin (IAA)-red, cytokinin (CK)-green, and abscisic acid (ABA)-blue) for the allometric Col-0 model (RS). Mean of each genotype based on the four treatments is represented by different shape and the bars represent the standard error, within the observed group. A) PC1; B) PC2. Extreme root shapes of minus or plus one standard deviation are shown on each side below the x-axis.
Figure 4. Visualization and MANOVA quantification of RSA phenotypic plasticity in genotypes. Distribution of principal component 1 (PC1) and principal component 2 (PC2) values of wt (Col-0), axr4-1, abi4-1, cre1-2 on four different treatments for the allometric Col-0 model quantified by RootScape. The mean of each treatment is shown by a colored diamond and ellipsoids in the same color represent clouds around the replicates. A) wild-type; B) axr4-1; C) abi4-1; and D) cre1-2. MANOVA summary table for pair-wise comparison is shown below each plot (Pillai test with Bonferroni adjustment 0.008*).