Environmental and Experimental Botany 110 (2015) 11–18

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Using X-ray Computed Tomography to explore the role of abscisic acid in moderating the impact of soil compaction on root system architecture

Saoirse R. Tracy[a,*], Colin R. Black[a], Jeremy A. Roberts[a], Ian C. Dodd[b], Sacha J. Mooney[a]

[a] School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK
[b] The Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK

ARTICLE INFO

Article history:
Received 10 January 2014
Received in revised form 8 September 2014
Accepted 12 September 2014
Available online 22 September 2014

Keywords:
Abscisic acid (ABA)
Root system architecture
Soil compaction
Solanum lycopersicum
Computed TomographyROIRegion of interest

ABSTRACT

Background and Aims: Understanding how soil compaction affects root system architecture (RSA) and root deployment within soil is critical to maximise crop growth. This study examined the role of abscisic acid (ABA) in mediating root responses to soil compaction using tomato genotypes with differing endogenous ABA concentrations.
Methods: Plants of the wild-type tomato genotype (*Solanum lycopersicum* L. *cv*. Ailsa Craig) and its ABA-deficient mutant *notabilis*, of uniform developmental stage, were transplanted to columns containing a loamy sand soil at bulk densities of 1.2, 1.4 and 1.6 Mg m^{-3}. Fourteen days after transplanting (DAT), an X-ray μCT scanner acquired non-destructive 3-D images of RSA. Destructive analysis of RSA was undertaken using WinRHIZO® 2-D scanning equipment.
Key results: Increased bulk density decreased root volume, surface area, rooting depth and lateral root number ($P < 0.05$), which adversely affected RSA and the subsequent volume of soil explored. The ABA-deficient mutant *notabilis* displayed a poor rooting phenotype with reduced root volume, surface area and lateral roots at all bulk densities.
Conclusions: The response of RSA to soil compaction *in situ* differed between the ABA-deficient mutant genotypes of tomato. The differences in rooting phenotype between genotypes suggest that endogenous ABA concentration has a positive influence on RSA when roots encounter compacted soil.

1. Introduction

The ability of plants to overcome spatial and temporal variation in environmental conditions by altering their development and physiology is crucial and phenotypic plasticity is particularly important for survival under sub-optimal soil conditions (von Arx et al., 2012). Zolla et al. (2010) described the three-dimensional (3-D) deployment of roots in soil as a perfect example of developmental plasticity and suggested environmental factors such as soil type, water content and nutrient availability are vital in determining root architecture. Root system architecture (RSA) is important in determining the capacity of plants to exploit soil water and nutrient reserves (Grime et al., 1986), and the ability to alter RSA in response to environmental cues may confer a selective advantage (Malamy, 2005). Soil conditions affecting root growth are frequently dynamic; for example, as soil dries from field capacity to permanent wilting point, roots communicate with shoots to limit water loss and maximise productivity to an extent related to the prevailing soil water content (Dodd, 2005). This root-to-shoot signalling may result in little or no detectable change in shoot water or nutrient status as soil conditions change (Andrade et al., 1993). Although within-root communication is vital when different parts of the root system encounter contrasting soil conditions, the signalling mechanisms used to coordinate growth responses to spatial heterogeneity in soil remain poorly understood (Bengough et al., 2006).

Abscisic acid (ABA) is involved in controlling plant responses to soil compaction and water stress (Hussain et al., 2000). Tardieu et al. (1992) showed that ABA concentration in the root tips of maize (*Zea mays* L.) increased when seedlings were grown on compacted soil. Application of synthetic ABA also increased root diameter,

Abbreviations: ABA, abscisic acid; RSA, root system architecture; DAT, days after transplanting; DW, dry weight; 2-D, two dimensions; 3-D, three dimensions; 4-D, four dimensions; μCT, micro-computed tomography; ROI, region of interest.

* Corresponding author. Tel.:+44 0 115 9516296.
E-mail address: saoirse.tracy@nottingham.ac.uk (S.R. Tracy).

http://dx.doi.org/10.1016/j.envexpbot.2014.09.003
0098-8472/

perhaps facilitating penetration through compacted soil (Hartung et al., 1994). The observation by Mulholland et al. (1996a) that the roots of ABA-deficient mutant plants were thinner than in wildtype plants suggests that ABA may have a role in determining root diameter. The use of ABA-deficient mutants, also suggests that ABA may be involved in inducing drought-stress genes (Pilet, 1998). During periods of high transpiration, root xylem ABA concentrations and resistance to water movement from soil to roots both increase in response to soil compaction (Tardieu, 1994). Mulholland et al. (1996b) found that leaf expansion in wildtype barley plants (*Hordeum vulgaris* L.) was unaffected at a bulk density of 1.6 Mg m^{-3} relative to plants grown in uncompacted soil, but was greatly reduced in the ABA-deficient mutant, *Az34*.

Further work is needed to gain a full understanding of how complex interactions between ABA, other growth regulators and intrinsic response pathways influence RSA (Ingram and Malamy, 2010). Although increased internal [ABA] has generally been suggested to inhibit root growth (Newton, 1974; Finkelstein et al., 2002), other studies have provided conflicting evidence which indicates that ABA may promote root growth (Spollen et al., 2000) and is necessary to maintain root growth during water stress (Sharp and LeNoble, 2002). The use of ABA deficient mutants is important to elucidate the mechanisms underlying hormonal signalling and growing evidence suggests ABA has a central role in mediating developmental plasticity and lateral root formation (De Smet et al., 2006). During periods of water shortage, elevated endogenous [ABA] may maintain root growth (Saab et al., 1990) but reduce lateral root number (Guo et al., 2009), thereby altering RSA and, potentially, resource capture and plant growth. Increased exogenous ABA decreases primary root growth under well watered conditions (Hooker and Thorpe, 1998), possibly due to inhibition of cell division (Zhang et al., 2010), whereas sufficient endogenous ABA concentrations may enhance primary root growth in well watered soil conditions (Sharp et al., 2004). Full understanding of how ABA induces such contrasting effects on root growth has still to be established (Zhang et al., 2010).

The present study examined the role of ABA in mediating root responses to a range of soil bulk densities using tomato genotypes differing in endogenous ABA concentration. The null hypothesis was that intrinsic differences in root and shoot ABA concentrations between genotypes do not affect RSA when plants are grown in loamy sand soil with contrasting bulk densities at field capacity.

2. Materials and methods

2.1. Sample preparation and μCT scanning procedures

A Newport series loamy sand (brown soil, Eutric Arenosol) with a textural content of 83% sand, 13% clay and 4% silt, from the University of Nottingham farm at Bunny, Nottinghamshire, UK (52.52°N, 1.07°W) was air-dried and sieved to <2 mm. Columns (70 mm height × 30 mm diameter) were uniformly packed to provide bulk densities describing a wide range of compaction levels typically experienced in the field; these were 1.2 Mg m^{-3} (uncompacted), 1.4 Mg m^{-3} (slightly compacted) and 1.6 Mg m^{-3} (compacted). Mean values for soil pH, nitrate content and phosphorus were respectively 7.1, 5.48 mg L^{-1} and 29.65 mg kg^{-1}. The columns were carefully packed with air-dry sieved soil in *c.* 1 cm deep increments. The soil was swirled to distribute the different sized soil particles evenly before pouring it in small quantities into the columns. After compacting each soil layer, its surface was scarified to roughen its surface; this was essential to bind the layers together and ensure homogeneous packing (Lewis and Sjostrom, 2010). The soil columns were saturated slowly by wetting from the base for 12 h and allowed to drain freely for 48 h. All columns were weighed and maintained at this weight throughout the experiment by adding the required volume of water daily to the top of the column to ensure soil moisture content remained near a notional field capacity.

The genotypes of tomato (*Solanum lycopersicum* L.) chosen for study were the wildtype Ailsa Craig and the isogenic ABA-deficient mutant *notabilis* (Burbidge et al., 1999). Seeds of both genotypes were imbibed for 48 h so that radicle lengths were similar before planting a single seed 5 mm below the soil surface. The columns were placed in a growth room under 28/22 °C day/night conditions with a 12 h photoperiod and a photosynthetic photon flux density (PPFD) at plant level of 265 μmol m^{-2} s^{-1}. Relative humidity was set to 50%. Six replicates were used for each genotype and bulk density combination, giving a total of 36 columns. Ten biological replicates of each genotype were used for ABA analysis.

All columns were imaged 14 days after transplanting (DAT) using a Phoenix Nanotom® (GE Measurement and Control Solutions, Wunstorf, Germany) X-ray μCT scanner set at 110 kV and 180 μA, with a 0.1 mm copper filter and an image averaging of 1. Pixel/voxel resolution was set at 24 μm and each scan took 20 min to complete. The distance from sample to source was 16 cm. Twelve hundred image projections were captured for each column and each stack of images had a file size of *c.* 15 GB. The columns were scanned during the photoperiod in a fixed randomised order to ensure all treatment combinations were equally exposed to any diurnal variation in root growth to avoid systematic error. The total X-ray dose per sample was estimated to be <1 Gy.

2.2. Image processing and analysis

Root systems were non-destructively extracted from the greyscale μCT images using a segmentation method based on a combination of the *Region Growing* selection tool and erosion and dilation processes in VGStudioMAX® 2.1 software. Region Growing classifies voxels in a certain grey-value range from a starting voxel, which is initially chosen on the probability of being most likely to be plant material. In this instance this can be done most easily by highlighting the plant stem material at the top of the column. Tolerance values were adjusted to ensure that only root material was included in the root region of interest (ROI) from the original seed points due to variations in grey-value resulting from slight variation in the density of the root material. Erosion and dilation processes were used to enclose regions within the ROI. The root system models segmented from the μCT image data were used to quantify root length, volume, surface area, mean diameter, root tip diameter and vertical rooting depth. Tortuosity of the root path (the ratio of actual path length compared to the shortest possible path) was measured by comparing the length of the primary root measured using the *Polyline* tool (a distance tool that allows users to change the contour of the line to track the true root path) in VGStudioMAX® 2.1 with the vertical depth of the root system. The lengths of individual lateral roots were measured using the *Polyline* tool in VGStudioMAX®.

Several other root characteristics derived by image analysis were determined using the μCT segmented root systems, including convex hull volume, centroid and minimum enclosing circle; these are described in detail by Tracy et al. (2012). The convex hull of the root system was obtained using the QuickHull algorithm (Barber et al., 1996) and its volume was estimated using the Monte Carlo Integration (Rubinstein, 1981). The centroid is the geometric centre of an object and corresponds to its centre of mass, provided mass per unit volume is constant throughout the object. This was achieved by computing the mean *x, y* and *z* coordinates for all extracted root data voxels. Welzl's (1991) algorithm for the minimum enclosing circle was used to determine the maximum width of root systems *i.e.* the maximum horizontal distance. Maximum rooting depth was calculated by counting