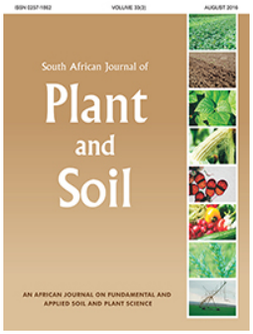

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Effects of rooting media on root growth and morphology of *Brassica rapa* seedlings

Michael O Adu[1,2,3]*, David O Yawson[4], Frederick A Armah[5], Paul A Asare[3], Malcolm J Bennett[2], Martin R Broadley[2], Philip J White[1] and Lionel X Dupuy[1]

[1] Department of Ecological Sciences, The James Hutton Institute, Invergowrie, Dundee, Scotland, UK
[2] Plant and Crop Sciences Division, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire, UK
[3] Department of Crop Science, School of Agriculture, College of Agriculture and Natural Resources, University of Cape Coast, Cape Coast, Ghana
[4] Department of Soil Science, School of Agriculture, College of Agriculture and Natural Sciences, University of Cape Coast, Cape Coast, Ghana
[5] Department of Environmental Science, School of Biological Science, College of Agriculture and Natural Sciences, University of Cape Coast, Cape Coast, Ghana
** Corresponding author, email: michael.adu@ucc.edu.gh*

Rooting media used in current root phenotyping studies can have substantial effect. In this study, the effects of three different nutrient conducting papers (Black construction paper, Anchor blue germination paper and Kimpak paper) and soil-filled boxes on root growth and root system architecture (RSA) of *Brassica rapa* (cultivars 'R500' and 'IMB211') were investigated. Seedlings of the two *B. rapa* genotypes were supplied with nutrients on the nutrient conducting papers and in the soil-filled boxes. The papers and soil-filled boxes were fixed to flatbed scanners and two-dimensional images of roots were periodically taken and analysed. Root media effects on shoot and root biomass and on topological indices (TI) were observed. For example, root branching was more pronounced on the construction paper. Mean TI of 0.82 and 0.93, recorded for R500 and IMB211, respectively, on the construction paper indicated that substrates affect the herringbone pattern of brassica roots. Whilst it was indicated that different results could be obtained for the same RSA when different germination papers are used, the results showed that Anchor blue germination paper is an ideal proxy for soil in phenotyping seedlings for RSA traits and root growth.

Keywords: germination paper, phenotyping, rooting media, root system architecture, topological index

Introduction

Root system architecture (RSA), root morphology and growth are crucial for a plant to effectively explore and efficiently use resources from the soil environment. As a result, studies of RSA characteristics and root growth are increasing. Studying RSA and root growth in a natural environment is a challenge due to the subterranean nature of plant roots. This challenge is heightened by not only the need to match phenotypic information of RSA with the plant genotype, but also to monitor growth of roots over time and understand the influence of the environment on root growth (Downie et al. 2015).

Efforts to understand interactions of roots and their environments are frequently constrained by increased root plasticity, the need for increased number of replications, high impacts of ontogenetic variation, and carbon and mineral status of plants on root growth and architecture (Oborny 2004; Adu 2014; Adu et al. 2014). Instantaneous variables, such as root length, often therefore provide only limited descriptions of the root system. Complex integrated traits, such as root growth dynamics, must therefore be measured and this will require improvements in the phenotyping process (Adu 2014; Adu et al. 2014). It is important that reproducible, methodological and technical alternatives that are also simple, economical and widely accessible are sought. Whilst it is critical to characterise dynamic responses of roots to environmental variables, it is also important that information is given about how closely data produced in controlled environments relate to that produced in soil or under field conditions. For example, Dubrovsky and Forde (2012) have stressed the importance of adequately characterising the growth conditions, especially the rooting medium, employed in the quantification processes of root features.

Reliable data on root responses to environmental conditions are only possible if a sufficient number of replicates are included in a study (Adu 2014; Adu et al. 2014; Paulus et al. 2014). Hence, root phenotyping protocols with the potential for high throughput are required. There is thus a compelling demand for low-cost and easily accessible materials for root system phenotyping platforms. However, conventional materials for laboratory-based root system studies are usually expensive and not readily accessible, particularly for resource-poor laboratories.

To tackle these challenges whilst simultaneously circumventing the limitation posed by the opaqueness of the soil

to studying RSAs, many laboratory-based techniques employing root growth media that are approximations of field conditions have been developed (López-Bucio et al. 2002; Gregory et al. 2009; Dupuy et al. 2010; Iyer-Pascuzzi et al. 2010; Dai et al. 2012; Galkovskyi et al. 2012; Nagel et al. 2012; Faget et al. 2013; Adu 2014; Adu et al. 2014, 2015). It is desirable that these laboratory-based root growth cultures are economical, readily accessible and provide repeatable root growth conditions over time (Crush et al. 2005). For root system studies employing imaging technologies, it is also desirable that root growth cultures enhance the contrast between roots and their background to ease the process of image analyses (Mairhofer et al. 2013). A common artificial rooting medium that has been used successfully to screen crop plants for variation in root system characteristics is the paper pouch or germination paper approach (Liao et al. 2001; Hund et al. 2009; Adu 2014; Adu et al. 2014, 2015; Atkinson et al. 2015).

Despite the ease and tractability of image analysis of root systems grown on germination paper, there are concerns regarding the effect these microenvironments might have on root development (Mairhofer et al. 2013; Adu et al. 2014). It is possible that the nutrient concentrations, ionic potentials and water-holding properties for germination papers are at variance with those found in typical arable soils (Foehse and Jungk 1983; Jain et al. 2009; Gruber et al. 2013). For example, it has been shown that gelling agents used in screening plants could cause variations in plant growth responses on otherwise identical nutrient media (Jain et al. 2009). This has been attributed to the variable physiochemical characteristics, such as nutrient diffusion rate, elemental and organic impurities, and gel strength of the gelling agents (Nowak and Asiedu 1992; Scholten and Pierik 1998a, 1998b; Beruto et al. 1999; Jain et al. 2009). Differences between sand and solution culture in expressing root morphological and architectural parameters for *Trifolium repens* L. inbreds in a glasshouse experiment were reported by Crush et al. (2005). These authors recommend sand for screening white clover for root parameters when the physical effects of a solid medium on roots are important.

While germination papers and rhizoboxes have been used as rooting media to study root growth and RSA, there has been no comparison of the effects of these rooting media on RSA. It is also not certain if commonly available and cheap papers can equally be used to screen plants for root traits. The objective of this study was therefore to compare the effects of three germination papers and rhizobox-filled-soil as rooting media on root growth and RSA of *Brassica rapa* L. This study formed part of a broader study to develop a scanner-based root phenotyping system (Adu 2014; Adu et al. 2014, 2015). For the broader study, different paper cultures and soil growth media were evaluated to determine their suitability for the phenotyping platform being developed. This also enabled the response of the early phase of root growth in brassica to environmental factors, in this case, the rooting medium, to be examined. In the present study, two of the papers used are commercially available germination papers that have been treated as inert for screening plants for root traits, and the other is a cheap construction paper normally used for craft work. The cheap construction paper was included to assess the likelihood of using a low-cost non-conventional germination paper in root system phenotyping.

Materials and methods

Genetic material

Two *Brassica rapa* genotypes were selected for contrasting root morphology, namely 'IMB211' and 'R500', which are the parents of the BraIRRI mapping population (Iniguez-Luy et al. 2009; Hammond et al. 2011). Genotype IMB211 is a highly inbred rapid-cycling Chinese cabbage *B. rapa* subsp. *pekinensis* and R500 is a highly inbred annual yellow sarson *B. rapa* subsp. *trilocularis* (Iniguez-Luy et al. 2009; Xu et al. 2010).

Growth media

The germination papers used in this study were as follows. (1) Black construction paper (http://shop.hobbylobby.com): Construction paper (aka sugar paper, poster paper or craft paper) is a tough, coarse, coloured paper that is lightly sized and composed of approximately 50–70% ground wood. It also contains unbleached sulphite pulp, softwood fibres, and small amounts of cotton and blast fibres. The source material of construction papers is mainly wood pulp (Irving 1997). Construction papers are normally dyed with synthetic dyes, including basic, acid and direct aniline dyes, which provide it with a wide range of colours (Britt 1964; Irving 1997). The construction paper was cut to a dimension of 30 × 42 cm for this study. (2) Steel blue seed germination blotter (Anchor Paper Company, St Paul, MN, USA; http://www.anchorpaper.com/): this 30 × 42 cm paper standard seed germination paper is produced from 100% recycled cellulose fibre. It is a nontoxic paper with an open and porous structure free from mechanical pulp content, bacteria and other impurities and has burst resistance strength (Mullen) of 45. The steel blue seed germination paper has 5 and 0% moisture and ash content, respectively, and provides a good contrast for root growth. It also has good water retention properties and can store water 14–16 times its dry weight. The minimum capillary rise above water surface after immersion for 5 min is approximately 4 cm (Dutt et al. 2005; http://www.anchorpaper.com/). (3) Versapack or Kimpak paper (Anchor Paper Company, St Paul, MN, USA): this 30 × 42 cm paper is a lightweight cushioning material made of 100% recycled fibre with a neutral pH. It has a 10 s maximum rate of absorbency and a liquid capacity of 12 times its own weight (http://www.anchorpaper.com/). For brevity, the three paper types described above hereafter will be called construction, blue and brown papers, respectively.

The other growth medium was soil contained in rhizoboxes. The rhizoboxes were constructed after the design of Bengough et al. (2004) from two Perspex plates, one of which was opaque and the other transparent. Each plate measured 30 × 21.5 × 0.3 cm and had thin strips of Perspex as spacers around each plate. The rhizoboxes were filled with arable soil typical of the region. Soils were collected from 0 to 10 cm depth in a cultivated field at Tayport (56.45° N, 2.88° W), Scotland. Soil was air-dried, mixed and passed through a 2 mm sieve to remove coarse material and vegetative matter. Sieved soil was loosely packed into

each rhizobox at a dry bulk density of approximately 1.0 g cm^{-3} and occupied a volume of approximately 300 cm^3.

Growth conditions

Plants were grown on the paper growth media using a pouch-and-wick system as described by Adu 2014 and Adu et al. (2014, 2015) and on soil-filled rhizoboxes (Bengough et al. 2004). Seeds were pre-germinated on small germination papers (Anchor Paper, St Paul, MN, USA), which were placed in 12 × 12 cm square petri dishes. The seeds were sprayed with deionised water and the petri dishes were covered with aluminium film and placed vertically in a Sanyo MIR153 incubator at 20 °C for 3 d. Following germination, seedlings of similar size were transferred to the paper rooting media (30 × 42 cm each) and soil contained in a rhizobox. Two seedlings were placed on each sheet of paper attached to Canon CanoScan 5600F flatbed scanners using 30 × 20 cm clear-Perspex plates (Adu 2014; Adu et al. 2014, 2015). Scanners were fixed in near-vertical positions, 5 cm above 40 L of nutrient solution contained in tanks (scanner banks) constructed using transparent poly-vinylchloride plates. The tanks measured 100 × 60 × 24 cm and had a tap for drainage. The tanks were covered with black plastic sheets to limit exposure to light. Each tank housed eight scanners that were held vertically above the nutrient solution using supports built in the tanks. Approximately 10 cm of the bottom end of the sheets of paper was submerged in the nutrient solution. Nutrient solution was supplied via the sheets of papers held between a transparent film and the scanners. Nutrient solution was prepared with 40 L deionised water with the macronutrients [in mM] KH_2PO_4 [0.25], $MgSO_4{\cdot}7H_2O$ [0.75], FeNa EDTA [0.1], Ca $(NO_3)_2{\cdot}4H_2O$ [2] and NH_4NO_3 [2], and the micro-nutrients [in μM] H_3BO_3 [30], $MnSO_4{\cdot}4H_2O$ [10], $ZnSO_4{\cdot}7H_2O$ [1], $CuSO_4{\cdot}5H_2O$ [3], and $Na_2MO_4{\cdot}2H_2O$ [0.5]. The pH of the nutrient solution was maintained around 6 using H_2SO_4.

For the soil media, the soil in each rhizobox was watered once with deionised water to 80% field capacity on a weight basis just before planting the pre-germinated seeds. Rhizoboxes were oriented in landscape format with two gaps, each approximately 3 cm long, provided along the top surface to allow gas exchange with the surrounding atmosphere and unimpeded shoot growth. Two seedlings were placed on each rhizobox and the boxes were fixed on scanners using duct tape and with the transparent wall aligned with the scanning glass.

The experiment was conducted under controlled conditions in a growth room at the James Hutton Institute Invergowrie, Dundee, UK (56°27′23.368″ N, 3°4′9.879″ W). Temperature in the growth room was kept constant at 20 °C. Light intensity during the day was maintained at 100 μmol m^{-2} s^{-1} at plant height. Relative humidity was approximately 60% (Adu et al. 2014). Two independent experiments were run and data were pooled. The treatment factors were experimental run, scanner, genotype and rooting media. Two seedlings were monitored on each scanner and there were six replicates per genotype, per rooting medium and per experiment. Rooting media treatments were randomised within scanner banks.

Monitoring of root growth

Images of root growth were captured daily from 1 to 10 days after transfer (DAT) using flatbed scanners. Twenty-four flatbed scanners were employed for the experiment and three scanner banks were used. The frequency of image acquisition, scanning resolution and file format was controlled by three computers using in-house software (ArchiScan; Adu 2014; Adu et al. 2014, 2015). Time-lapse images were taken at 12-hour intervals using the fixed flatbed scanners abutting the plates and rhizoboxes. All plants were harvested 10 DAT to scanners. Roots were excised from the shoot base and fresh weight (FW) of roots and shoots was recorded. Shoot and root samples were dried at 60 °C for 72 h and dry weight (DW) was determined.

Extraction of geometrical features

Root traits were extracted with ImageJ software (http://rsb.info.nih.gov/ij/) from each image. Traits extracted included elongation rate, total root length (TRL), primary root length (PRL), total lateral root length (TLRL) and root-to-shoot ratio (R:S), the quotient of root dry weight and shoot dry weight (RDW/SDW). Time-lapse emergence of lateral roots (LRs) was also extracted from images by counting the number of LRs that emerged each day. Daily root emergence and growth were measured by tracing the new growth increments on the images. Here, time-lapse root images were imported into ImageJ and point selections were placed manually on the tip of each root axis in successive images using mouse clicks. Root tip displacement (Δu) was recorded and calculated as:

$$\Delta u = u_f - u_o \quad (1)$$

where u_f denotes the original x coordinate and u_o denotes the x coordinate of the point selection in the next image in the sequence. Vertical patch (Δv) displacement was calculated as:

$$\Delta v = v_f - v_o \quad (2)$$

where v_f denotes the original y coordinate and v_o denotes the y coordinate of the point selection in the next image of the sequence.

The daily root elongation rate (cm d^{-1}) of each root axis was calculated using movement in both x and y coordinates as:

$$y = \frac{\sqrt{(\Delta u)^2 + (\Delta v)^2}}{px} \quad (3)$$

where y denotes growth rate (cm d^{-1}) and px denotes the scale factor calibration value (pixels cm^{-1}).

The number of root tips on each seedling was counted to calculate root topological indices. The topological index (TI) was calculated as the slope of the linear regression between log(altitude, α = number of branching points from the base to the extreme root tip) and log(magnitude, μ = the number of root tips) (Fitter 1987; Fitter et al. 1991; Glimskar 2000). The derived parameters α and μ, and hence the TI, express the branching pattern of the root system. Root systems with TI values approaching one are described as simple herringbone root systems with unbranched laterals

off a primary root (PR) axis. Lower values of TI indicate dichotomous systems with increasing orders of lateral roots (Crush et al. 2005). The relative rates of extension (RRE) of total, primary and total LRs, the relative multiplication rate (RMR) of LRs and the mean extension rate (MER) of seedlings grown with the different rooting media were calculated as described by May et al. (1965) and Tennant (1976):

$$\mathrm{RMR} = \frac{\log_e n_2 - \log_e n_1}{t_2 - t_1} \approx \frac{n_2 - n_1}{t_2 - t_1} \approx \frac{d_n}{d_t} \quad (4)$$

$$\mathrm{RRE} = \frac{\log_e l_2 - \log_e l_1}{t_2 - t_1} \approx \frac{l_2 - l_1}{t_2 - t_1} \approx \mathrm{MER} \quad (5)$$

where n, l and t for each parameter refer to root number, root length and time (measured in DAT) the image was acquired. The data were analysed with GenStat 15th Edition (VSN International, Hemel Hempstead) using residual maximum likelihood (REML). Experimental run, scanner, genotype, rooting medium and their interactions were initially employed as the sources of variation in the analyses. Experimental run and scanner effects were not significant and so subsequently a simpler model was employed:

$$y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ij} \quad (6)$$

where y_{ij} is the trait value of the ith genotype ($i = 1,2$) grown on the jth medium ($j = 1,\ldots,4$), α is the main effect of the genotype, β is the main effect of the rooting medium, $\alpha\beta$ is the genotype × rooting medium interaction, and ε_{ij} is the residual.

Results

Effect of rooting medium on seedling biomass and root topological indices

All four growth media provided good colour contrasts with roots and were ideal for imaging root structures of both *B. rapa* genotypes (Figure 1). The construction paper was, however, vulnerable to discolouration, which then affected root colour (Figure 1). The R500 genotype attained a greater biomass and root system size than the IMB211 genotype. Shoot biomass of R500 on the blue paper was greater than those of the other rooting media (Table 1).

There was no significant difference between shoot biomass of R500 on the blue paper and the shoot biomass of R500 recorded on soil (Table 1). With IMB211, shoot biomass was significantly higher in soil than on the other rooting media ($p < 0.001$). Root dry matter was highest for genotypes on the blue paper. However, the higher root biomass on the blue paper did not appear to be a consequence of root length as the greatest root length was not recorded on blue paper. The R:S ratio was higher on construction paper for both genotypes. There was a significant genotype × rooting medium interaction for root biomass and R:S, but no such interaction for shoot biomass (Table 1).

There was a significant difference between genotypes, rooting media and genotype × rooting medium interaction for TI values derived from the data (Table 1, Supplementary Figure S1a and b). When TI indices were plotted for the two *B. rapa* genotypes grown on the four rooting media, there were strong linear relationships and the TI values obtained were comparable and higher (Supplementary Figure S1a and b). Mean TI values of 0.93, 0.95, 1.0 and 1.0 were recorded for the IMB211 genotype grown on construction, blue, brown and soil media, respectively. Mean TI values of 0.82, 1.0, 0.97 and 0.99 were recorded for the R500 genotype grown on construction, blue, brown and soil media, respectively (Table 1, Supplementary Figure S1a and b).

Effect of rooting medium on root proliferation or multiplication

Lateral roots emerged generally from 3 DAT. The lowest LR number was recorded on soil media at almost all time points for both genotypes (Figure 2a). Emergence of LRs for the R500 genotype was greater on the blue paper, whereas that of the IMB211 genotype was greater on the construction paper (Figure 2b). The derived RMR, however, showed that emergence of LRs on the black and brown papers were higher for the R500 genotype. Relative rates of increase in LR number were highest initially and decreased with time for both genotypes and for each rooting medium. The decrease in RMR approached constant levels and fell to zero during the experimental period, particularly for R500 (Figure 2b).

Effect of rooting medium on root growth

Type of rooting media significantly affected TRL and PRL ($p < 0.05$). Generally, seedlings grown on the construction paper showed faster root growth (Figure 2c and d). The effect of medium on root length was evident from 6 DAT (Figure 3a–d). On all rooting media, the highest relative rates of increase occurred over the first 6 DAT and subsequently declined until nearly constant, mainly with the PRs (Figure 2d). On all media, RRE values during the periods of constant relative increase were higher for LRs than for PRs and the total root system. Soil medium, however, seemed to favour PR growth than LR growth, as RRE of PR on soils was generally greater (Figure 2d). Paper rooting media, particularly the construction and brown papers, seemed to favour LR growth.

There was significant variation ($p < 0.05$) between treatments in MER as a function of time for LRs (Figure 4a and b). For R500 (Figure 4a), MER increased with time to a peak at 6–7 DAT after which it declined, but the decline was sharper on the construction paper. For IMB211 (Figure 4b), MER appeared to be constant with time and was significantly higher on the blue paper. In both genotypes, MER recorded on soil medium was generally the lowest.

Discussion

Roots are hidden in the soil and do not lend easily to empirical methods to quantitatively describe root growth and architecture. Methods of phenotyping root systems currently do not have the same degree of sophistication or throughput as genomes or processes more proximate to the genome and frequently limit functional genomic studies. Efforts to understand interactions of roots and their environments therefore are limited in many instances, leading to the use of simple and instantaneous variables, which might not be accurate, to characterise root systems. Laboratory-based root phenotyping processes must be improved in simplicity, accessibility, reproducibility

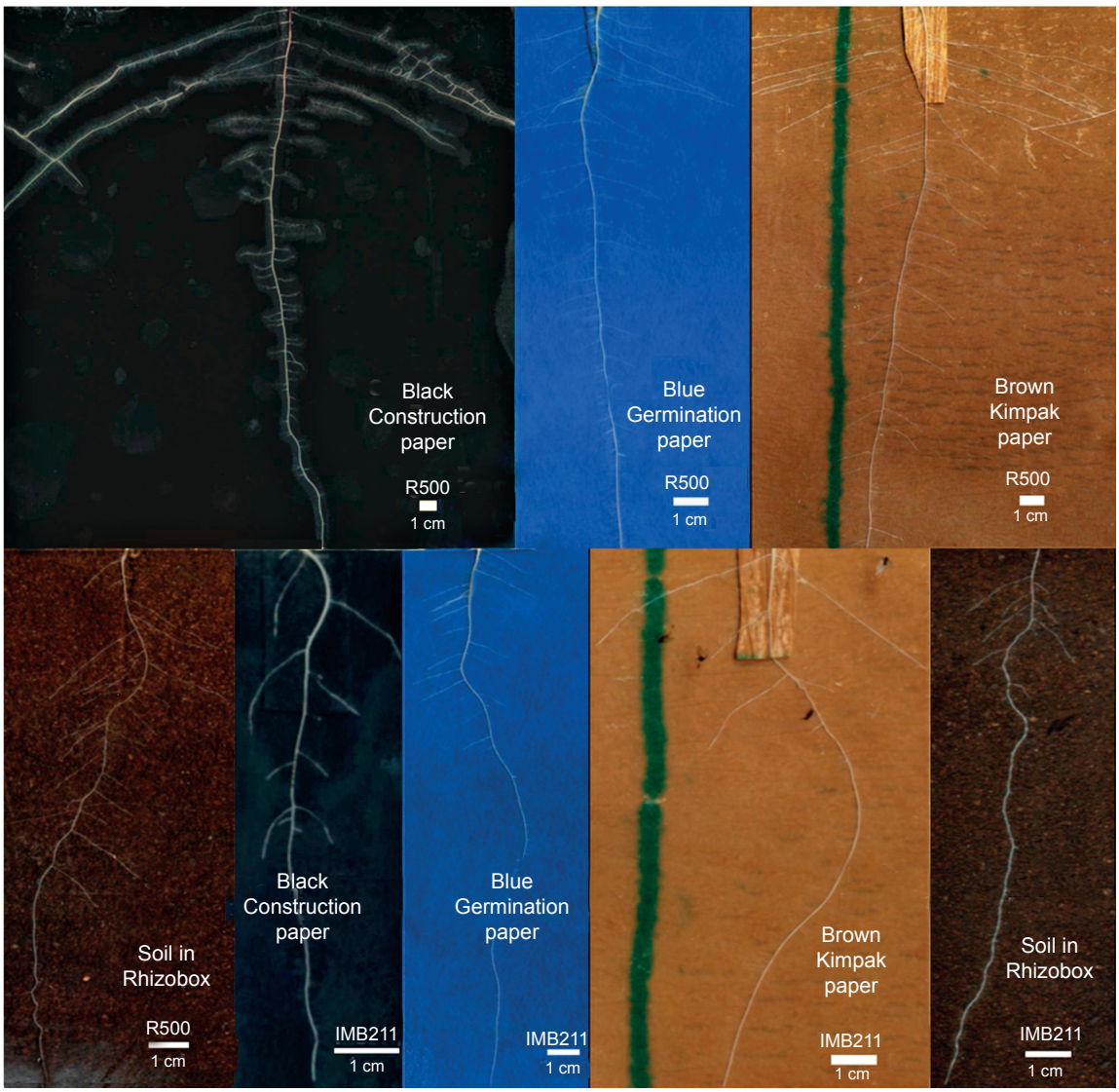

Figure 1: Root system architecture of *Brassica rapa* genotypes R500 and IMB211 seedlings at 10 days after transfer grown on four media: Black construction paper, Steel blue germination paper, Brown Kimpak (or Versapack) paper and a soil-filled rhizobox

Table 1: Treatment mean values for shoot and root biomass, root:shoot ratio and topological index for 12 replicates of *Brassica rapa* genotypes R500 and IMB211 grown for 10 days on Steel blue germination paper, Brown Kimpak (or Versapack) paper, Black construction paper and a soil-filled rhizobox. The topological index (TI) was calculated as the slope of the linear regression between log(altitude, α) and log(magnitude, μ). Probability levels for treatment main effects and interactions from REML. The R^2 value for the linear regression between α and μ is indicated in parentheses after the respective TI value (±SE)

		Shoot dry weight (mg)	Root dry weight (mg)	Root:shoot ratio	Topological index
Genotype effect (SED)		$P < 0.001$ (0.965)	$P < 0.001$ (0.136)	$P < 0.001$	$P < 0.001$
Medium type effect (SED)		$P < 0.05$ (1.322)	$P < 0.001$ (0.187)	$P < 0.001$	$P < 0.001$
Genotype × medium type effect (SED)		ns	$P < 0.001$ (0.264)	ns	$P < 0.001$
IMB211	Black Construction paper	2.16	0.72	0.34	0.93 ± 0.008 ($R^2 = 0.94$)
	Blue Germination paper	2.47	0.73	0.30	0.95 ± 0.005 ($R^2 = 0.95$)
	Brown Kimpak paper	1.87	0.44	0.23	1.00 ± 0.006 ($R^2 = 0.98$)
	Soil-filled rhizobox	6.88	0.35	0.09	1.0 ± 0.011 ($R^2 = 0.98$)
R500	Black Construction paper	6.57	2.57	0.39	0.82 ± 0.004 ($R^2 = 0.68$)
	Blue Germination paper	10.07	3.77	0.37	1.0 ± 0.006 ($R^2 = 0.97$)
	Brown Kimpak paper	6.97	2.41	0.35	0.9 ± 0.005 ($R^2 = 0.89$)
	Soil-filled rhizobox	9.97	1.75	0.24	0.99 ± 0.003 ($R^2 = 0.96$)

ns = not significant

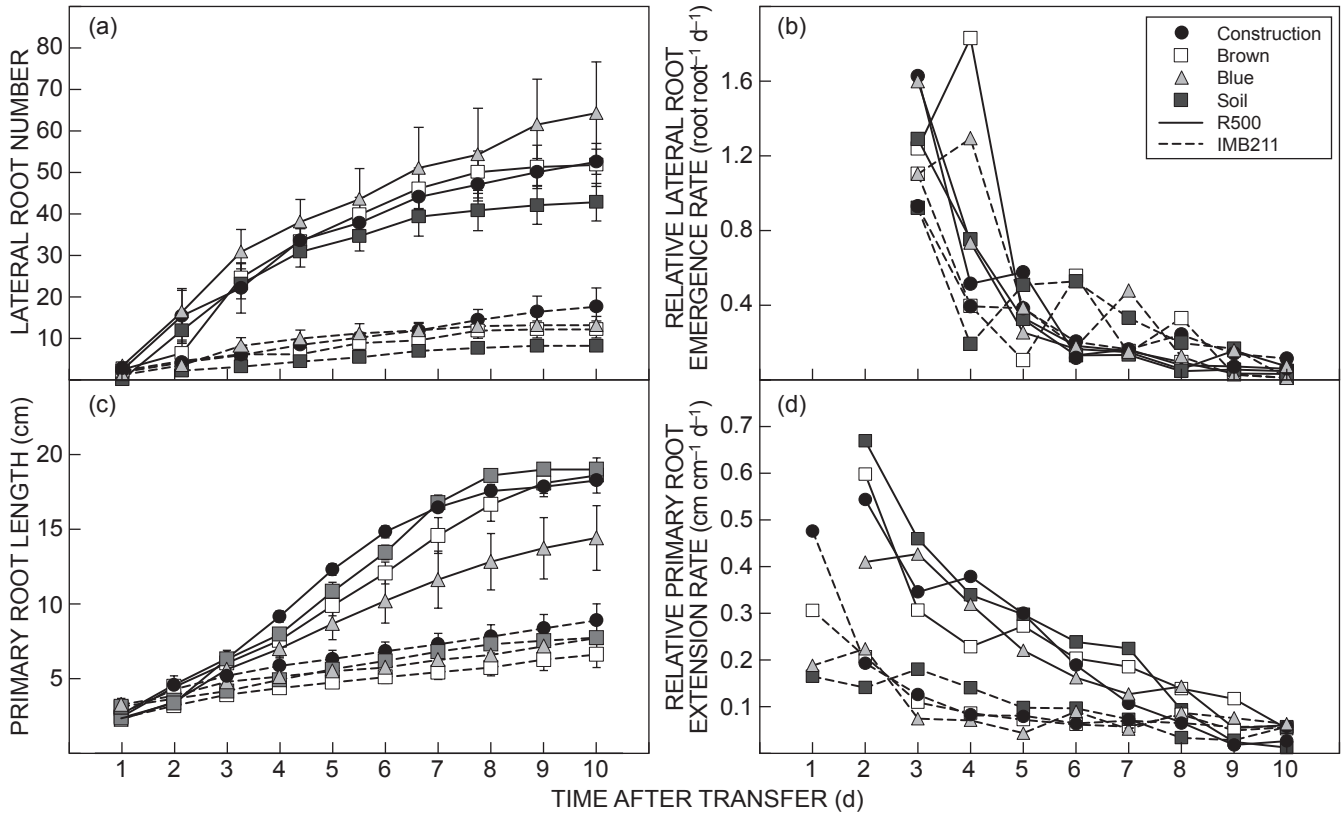

Figure 2: Lateral root number (a), relative lateral root emergence rate (b), primary root length (c) and relative primary root extension rate (d) during the first 10 d growth of *Brassica rapa* genotypes R500 and IMB211 on four rooting media

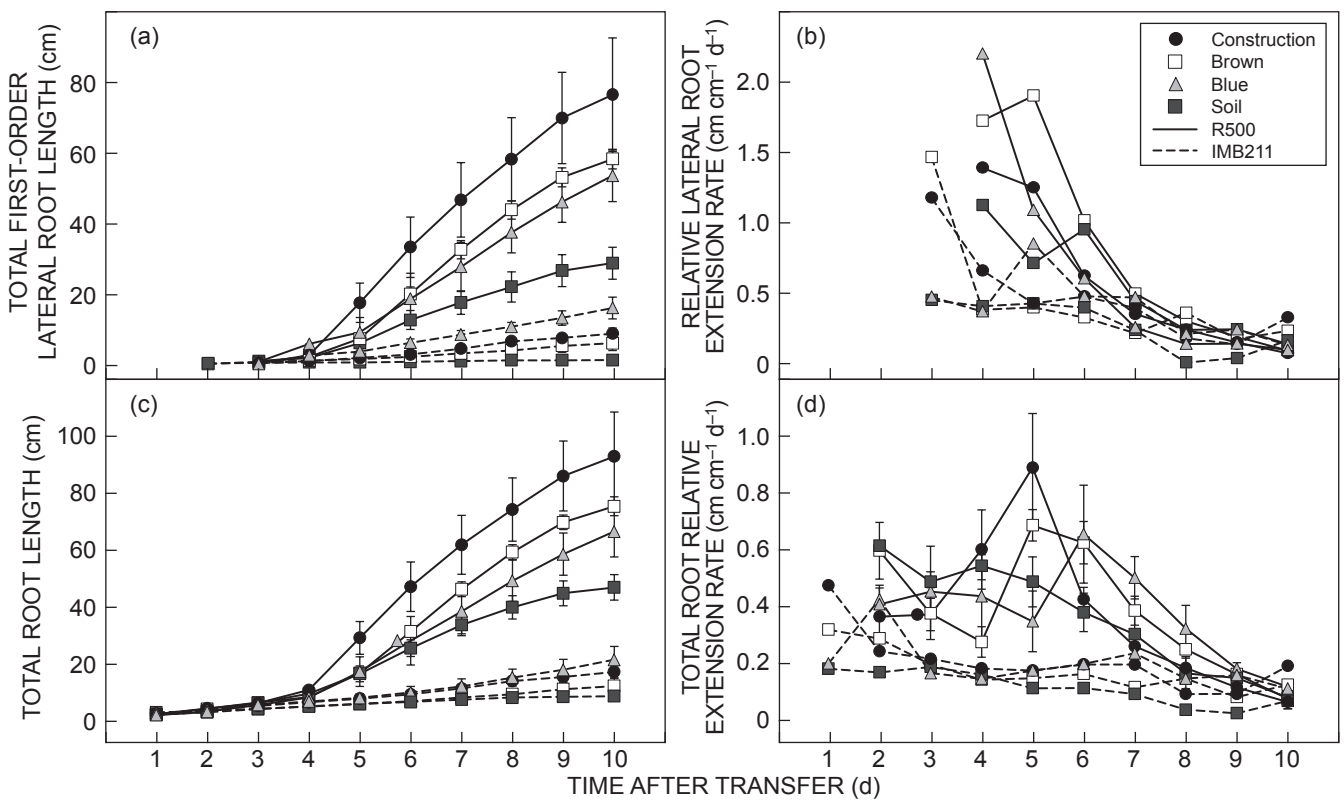

Figure 3: Total lateral root length (a), relative extension rate of lateral lroots (b), length of the total root system (c) and relative extension rate of the total root system (d) during the first 10 d growth of *Brassica rapa* genotypes R500 and IMB211 on four rooting media

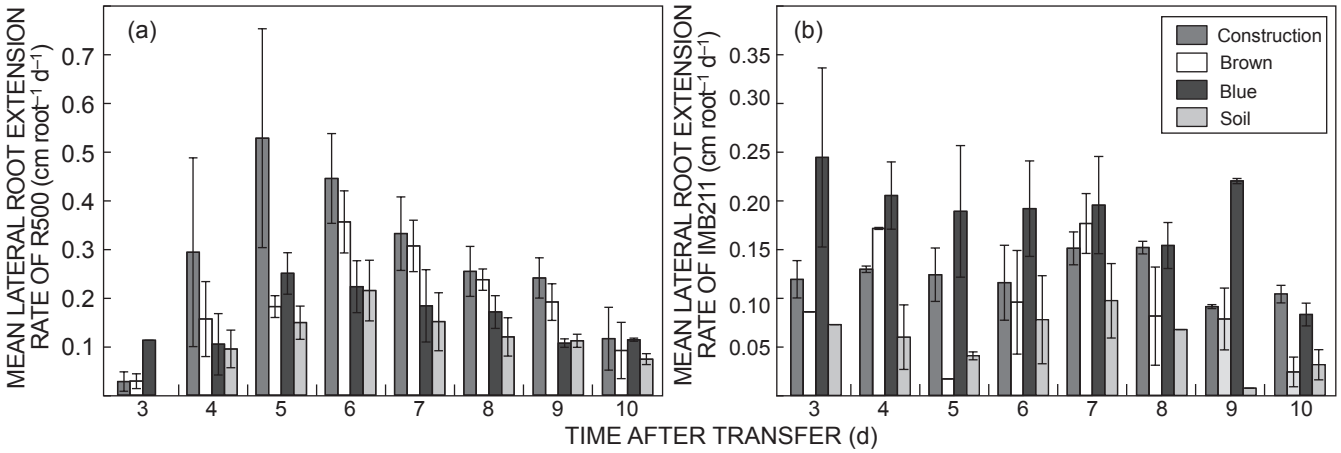

Figure 4: Mean extension rates of lateral roots during 10 d growth of *Brassica rapa* genotypes (a) R500 and (b) IMB211 on four rooting media

and scalability to field data, in order to cater for complex dynamic root traits. This would give confidence that any phenotypes observed in the laboratory are the result of the imposed treatments rather than environmental factors, such as growth medium, temperature, light and aeration. It would also facilitate the extrapolation of data across both spatial and temporal scales.

Effect of rooting medium on root topological indices

In the present study, mean TI values indicated that root medium had little effect on the typical herringbone pattern of *B. rapa* seedlings. Relatively lower TI values, however, were recorded on the construction paper. The responses observed here may be explained by root systems reacting to inadequate water or perhaps increased innate minerals originating from the dye or the raw material of production. The construction paper was lighter and less hydrophilic and the plants may have been subjected to drought. Akhtar et al. (2009) reported that *Brassica* cultivars grown in rhizoboxes showed similar TI values to those reported here but the TI values increased when cultivars were constrained with limited external phosphorus supply. The results here indicated that simple indicators, such as topological indices of root systems, can be used to characterise variations in seedling root traits. It is worth noting that the present results are based on growth media of different physiochemical properties and thus may have different water and nutrient retention properties.

Effect of rooting medium on root growth

Growth medium effects were observed on primary and total root length and their corresponding relative extension rates. On all rooting media, rates of extension for both the PR and total root system began to approach constant following initial higher extension rates. It has been suggested that total root system and PR growth follow a similar growth pattern with time but differ in their absolute growth rate (Adu 2014; Adu et al. 2014). For first-order LRs, the relationship between the growth rate and day of emergence follows a quadratic function but LRs that emerged first generally have faster elongation rates than those that emerge later (Adu 2014; Adu et al. 2014). In the present study, experiments lasted only 10 DAT but the constant root growth in the later stages irrespective of the rooting medium suggests that the rate of root growth may be under stable internal control, and perhaps large responses to environmental variation occur only during the early stages of growth. This confirms the suggestion that the responsiveness of plants to environmental variation decreases with the age of the plant (May et al. 1965, 1967).

As expected, LR number and length increased with time to a point. There were consistent patterns of root number and length increase in the *B. rapa* seedlings. This trend has long been established in many crops, including barley (May et al. 1965; Rahman et al. 1975) and wheat (Tennant 1975). An interesting feature, however, is the observation here that number and RMR of first-order LRs was altered by the nature of the medium in which the roots were growing and that a medium effect on RMR was evident between 2 and 5 DAT for R500 and between 2 and 8 DAT for IMB211. Irrespective of the genotype, the brown and construction paper media induced the greatest number of LRs and the mechanism operated within 8 d following germination. As indicated earlier, these paper types were relatively lighter in weight and may have suboptimal capillarity and water retention capacities. Nutrition and water retention status of these media types therefore could be implicated in the increased lateral rooting (Malamy and Ryan 2001).

When lateral root number was compared with total lateral length, it was seen that during early growth (up to 10 DAT), the number of first-order LRs was higher than the total length of LRs, expressed in centimetres, on the blue paper culture and in the soil but not on the construction and brown paper cultures. This is consistent with the findings of Wahbi and Gregory (1995) and May et al. (1965) for barley seedlings, where there was rapid increase in the length of laterals in comparison with number such that mean length increased. It is thus possible that during the early growth of *Brassica* roots, there is increased emergence of first-order LRs, which extend relatively faster than the production of new LRs of the same order, so that relative rates of extension decreased with time but this trend is subject to suitable environmental conditions. Furthermore, differences in MER with respect to time give evidence of changing rate of cell division in LR meristems resulting in a MER that at first increases, and then declines, with time.

This observation suggests that assigning a single value of elongation rate to LRs of different ages may be impractical. In general, the differences between roots grown in soil and on blue paper culture were relatively small. Consequently, it would be advantageous to use the blue paper culture as a proxy for soil in screening *Brassica* seedlings for RSA and root growth traits, especially in cases when the physical effect of a solid root medium on roots was important.

Root growth medium × genotype interaction

It was expected that root growth dynamics and RSA would differ depending on the root growth medium used, especially when a diverse range of substrates from construction paper to soil was employed. An evaluation of substrate × genotype interaction was thus important in the present study. Our preliminary analyses indicated that experimental run and scanner had no effects on the parameters measured (data not shown). The effects of genotype and the effects of interactions between genotype × rooting medium accounted for most of the experimental variation. This was consistent with a similar study where little variation in the traits assayed was attributed directly to run or scanner (Adu et al. 2014).

Genotype × substrate interactions observed here for root biomass and TI may be explained exclusively by difference in the extent of response by the two genotypes. For example, IMB211, the genotype with a smaller root system, was relatively less responsive to a change in rooting medium than R500, which has longer and more highly branched roots. Our results seem to agree with the suggestion of Crush et al. (2005) that genotype × root medium interactions are likely to occur more frequently in highly branched genotypes with older and therefore most probably bigger root systems. In these circumstances choice of root medium is more critical, especially in empirical studies targeting the phenotypic selection of exploratory root systems characterised by increased LR emergence and growth. Ideally, the application of scanner-based, high-resolution root phenotyping of mature plants grown in soil must be sought to facilitate the development of crop varieties that are better adapted to future environmental conditions (Adu et al. 2014). However, in the absence of soil-based and/or field-based root phenotyping protocols, substrate proxies that yield data closest to those produced in soil-based substrates and/or those obtained from field conditions must be employed in assaying plants for root system traits.

Conclusion

Root traits that improve soil resource acquisition are extremely sensitive to their environment and require robust screening approaches to be measured accurately. Filter paper screens and image analysis protocols offer cheap and accessible options to screen genetic-mapping populations *in situ* for root growth, morphology and RSA traits in young seedlings to identify optimal phenotypes for efficient soil resource acquisition. The study shows that germination papers, some of them treated as inert media and used in screening plants for variations in root system characteristics, could have significant effects on the root system data acquired. There were growth medium effects on topological indices (TI), attributable to roots branching more on construction paper. Root extension rates and multiplication of lateral roots were responsive to rooting medium. Mean extension rates of LRs varied with time of emergence, suggesting that it would be more rewarding if root system analysis procedures could account for this time-dependent variability rather than assigning single extension rate values to LRs of the same order but with different time of emergence. From the results, non-conventional substrates such as craft paper, albeit economical and easily accessible, do not offer ideal solutions for screening seedling root traits. It is also essential that potential confounding effects of using filter paper screens are identified and accounted for in any experimental design. This will ensure that erroneous interpretations are not drawn from nutrition studies when different paper pouches or germination papers have been used. It would also ensure that data from such artificial rooting media can be compared with data on the root systems of plants grown in other proxy rooting media such as in pots or tubes, in agar or gels, or in other paper pouches and ultimately to plants grown in soil under field conditions.

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