Quantitative trait loci (QTLs) linked with root growth in lettuce (*Lactuca sativa*) seedlings

by Roberts, J., Broadley, M.R., Pink, D., Hand, P., Lynn, J. and Monaghan, J.M.

DOI: https://doi.org/10.1007/s11032-019-1089-x

Quantitative trait loci (QTLs) linked with root growth in lettuce (*Lactuca sativa*) seedlings.

J. Roberts[1], M.R. Broadley[2], D. Pink[1], P. Hand[1], J. Lynn[3] & J.M. Monaghan[1*]

[1]Fresh Produce Research Centre, Crop and Environment Department, Harper Adams University, UK.

[2]Plant and Crop Sciences Division, School of Biosciences, University of Nottingham, UK.

[3]Applied Statistical Solutions Ltd, Leamington Spa, UK.

[*]Corresponding author (jmmonaghan@harper-adams.ac.uk).

Abstract

In-field variation of transplanted lettuce (*Lactuca sativa* L.) due to variable soil and environmental conditions is one of the major restrictions in the optimization of production and yield. Marker assisted breeding for lettuce varieties with a more rapid rooting phenotype has the potential to improve the performance of lettuce transplants. This study aimed to identify traits linked with increased primary root length, lateral root length and lateral root emergence in 14 d *L. sativa* seedlings from an intra-specific cross (Saladin x

Iceberg). In total 16 significant quantitative trait loci (QTLs) were associated with increased root growth traits that would allow direct introgression of the traits. Six of the QTLs were associated with increased primary root growth, accounting for 60.2 % of the genetic variation for the trait. Three QTLs were associated with lateral root growth (38.6 % of genetic variation); two QTLs were associated with lateral root length density (27.6 % of genetic variation) and three with root number density (33.4 % of genetic variation) and two QTLs were associated with mean lateral root length (21.1 % of genetic variation). The statistical QTLs were located across 9 different linkage groups (LGs) representing loci on 7 of the 9 *L. sativa* chromosomes. A combination of restriction fragment length polymorphism (RFLPs) and Kompetitive allele specific PCR (KASPs) markers linked to these rooting traits were identified, which could allow breeders to select for a rapid establishment phenotype.

Key words: *Lactuca sativa*, Rapid rooting, Establishment, Mapping population, Transplant, Root traits, Quantitative trait loci.

Author contribution statement

J.R; Carried out the main body of the research and main author of the paper.

J.M; Principle research and paper advisory.

M.R.B; Secondary research and paper advisory.

D.P; Secondary research and paper advisory.

P.H; principle advisory for QTL analysis and secondary paper advisory.

J.L; Principle advisory for the statistical analysis of trait data.

Key message

The study has identified genotypic variation for root growth traits within cultivated lettuce that will allow direct introgression of these traits into commercial cultivars for improved uniformity and establishment.

Acknowledgements

The research undertaken in this study was conducted primarily at Harper Adams University. We would like to thank our collaborative funding bodies; Syngenta, Harper Adams University and the University of Nottingham and the Vegetable Genetic Improvement Network for providing the plant materials to use in this study. A special thank you goes to Catherine Thomas who helped with the initial modification of the high-throughput platform for the use with lettuce seedlings, Rory Hayden for delivery of the equipment to Harper Adams University and Lolita Wilson for all her support at The University of Nottingham. Gratitude also goes to Shelley Roberts for her help with placement of the seedlings within the platform.

Introduction.

In Europe and North America, lettuce (*Lactuca sativa* L.) seedlings are typically grown during the early stages of production in glasshouses prior to transplanting out into the field. This removes issues associated with direct drilled seed such as, germination, crop uniformity and avoidance of early weed infestation, while optimizing growth and yield (Sharma et al. 2005; Maltais et al. 2008). Transplant establishment requires the regeneration of new roots and resumption of shoot growth in the field following transplanting (Orzolek 1991). Transplanted crops

differ morphologically from direct drilled crops with loss of the tap root resulting in the development of a larger number of lateral roots (NeSmith & Duvall 1998).

Each lettuce plant within a crop needs to achieve similar establishment to give as uniform a crop as possible for the optimization of production. Lettuce is still manually harvested and growers will only carry out ‘once-over’ harvest therefore crop uniformity is essential for profit. Transplant establishment can be negatively impacted by many factors within a field. For example, the variability of soil parameters, such as pH can reduce nutrient availability and root growth (Orzolez 1991). Compaction and poorly tilled soil result in poor root penetration (Grassbaugh & Bennett 1998). Soil moisture can be too high or low for adequate root development (Grassbaugh & Bennett 1998). Transplant shock, which describes the sudden transient stresses at transplanting (Kerbiriou et al. 2013), such as temperature change can also impact establishment. Better establishment would improve crop uniformity by minimising the variation between plants caused by abiotic stress at the time of transplanting through the rapid establishment of young plants and the associated access to nutrient and water (Johnson et al. 2000).

As for most crops, lettuce breeding has to date been focused on yield, leaf/head traits and pest and disease resistance with little or no direct attention given to the root system. A root breeding strategy in lettuce would be to identify quantitative trait loci (QTLs) linked to beneficial root growth traits and introduce these into crop varieties through marker assisted selection breeding programmes to develop lettuce cultivars capable of rapid establishment under variable soil conditions. The introduction of root trait QTLs has been previously shown to be successful in upland rice (*Oryza sativa*), where root traits for longer and broader roots were introduced into a new variety which improved yields (Steele et al. 2006;

95 Steele et al. 2013). Identifying genetic resources that allow lettuce cultivars to
96 achieve uniform establishment will be of great importance as future more
97 'sustainable' crop production will most likely be carried out under conditions of lower
98 fertilizer and water use (Zhu et al. 2011) and increased fertilizer prices as nutrients
99 such as phosphorus diminish (Le Marié et al. 2014).

100 Previously, QTLs based on segregating root traits have been identified in two
101 studies on lettuce. Both studies used an inter-specific cross between cv. Salinas and
102 the wild relative *Lactuca serriola* (Johnson et al. 2000; Wei et al. 2014). The first
103 study analysed drought tolerance through deep soil water exploitation and identified
104 QTLs involved with root growth and biomass (Johnson et al. 2000). The second
105 study analysed salt tolerance in seedlings through changes to root system
106 architecture (Wei et al., 2014). Both studies demonstrated that a number of *Lactuca*
107 species root traits are under genetic control in seedling assays. However, it is not
108 known whether these traits are related to a rapid rooting phenotype. The study
109 reported here utilised a high-throughput growth pouch assay to analyse root growth
110 traits in an intra-specific cross mapping population with the aim of identifying QTLs
111 associated with an increased root growth phenotype in 14 d old seedlings that may
112 then be used for marker assisted breeding for the improvement of lettuce transplant
113 establishment.

114 **Methods.**

115 **Plant material.**

116 A mapping population was previously produced from an intra-specific cross
117 between the crisphead *L. sativa* cv Saladin (syn Salinas) bred in the US and the
118 Batavian *L. sativa* cv Iceberg, bred in France (Atkinson et al. 2013). The mapping

119 population used in this study for QTL analysis consists of 125 F_8 recombinant inbred
120 lines (RILs) that were selected as the most genetically informative subset from 254
121 F_5 genotyped individuals (Atkinson et al. 2013).

122 **Seed germination.**

123 Germination paper (SD7640; Anchor Paper Company, St Paul, MN, USA)
124 was placed in petri dishes with 10 numbered sections marked out with a pen (Fig
125 1a). The germination papers were pre-soaked with 7 ml of tap water for imbibition of
126 the seed. Once the seeds had been placed on the sections they were placed in a
127 310 x 340 mm lidded opaque plastic tray and held in a cold store (14-16˚C) with 24 h
128 low irradiance lighting (1.5 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR)).
129 The seeds were left for up to 48 h to reach a pre-determined stage of germination,
130 which was defined as the presence of a radicle 1- 5 mm long and initial root hairs
131 that formed an arrowhead-like appearance (Fig 1b). This assured all seedlings were
132 placed on any given assay at the same growth stage, removing any variation due to
133 germination time.

134 **High through-put growth pouch assay.**

135 A high through-put growth pouch assay (Atkinson et al. 2015; Thomas et al.
136 2016) was constructed as described by Thomas et al. (2016) but modified for use
137 with lettuce by the inclusion of two sheets of porous tissue paper (TFM Farm and
138 Country Superstore Ltd, Shropshire, UK), which increased water availability to the
139 seedlings. Germinated seeds were placed at the top of the growth pouch with the
140 radicle orientated towards the bottom of the paper (Fig 1c), with 2 seeds on each
141 side of the pouch at approximately 15 cm spacing (Fig 1d). The growth pouches
142 were suspended over drip trays supported within an aluminium frame as described

143 by Atkinson et al. (2015). Each drip tray had 2 L of tap water containing 15% (0.24 g
144 L^{-1}) Hoagland's solution (Hoagland's No. 2 Basal Salt Mixture, Sigma Aldrich, Dorset
145 UK) added. Above each tank were six 550 mm strip white light emitting diode (LED)
146 lights (Leyton Lighting, Essex, UK) providing a mean PAR of 90.1 µmol m^{-2} s^{-1},
147 ranging from 68.5-113.4 µmol m^{-2} s^{-1}.

148 **Seedling growth.**

149 Following germination six replicate growth pouches of each genotype were
150 allocated to positions in the support frames using a one-way design with no blocking
151 (GenStat 17th edition, VSN International Ltd, Hemel Hempstead, UK). The seedlings
152 were grown across two frames for a 14 d period with a 20 h photoperiod. The
153 temperature and relative humidity (RH) was recorded every 2 hours with a data
154 logger (TinyTag Plus2, Gemini Data Loggers Ltd, Chichester, UK). The mean
155 temperature was 13.8°C and ranged between 13.6°C and 18°C. The mean RH was
156 99.2 % with a minimum of 78.7 % and a maximum of 100%. Following 14 d growth
157 the pouches were removed from the system for imaging.

158 **Image analysis.**

159 The growth pouches were removed from the frame and dismantled to expose
160 the root system. The root system was then imaged with a digital camera (Lumix -
161 DMC-FP2, Panasonic, Berkshire, UK) at fixed distance of 200 mm. The images were
162 analysed using ImageJ (Abràmoff et al. 2004; Schneider et al. 2012) and
163 measurements for primary root length, total lateral length and number of laterals
164 were recorded and analysed.

165

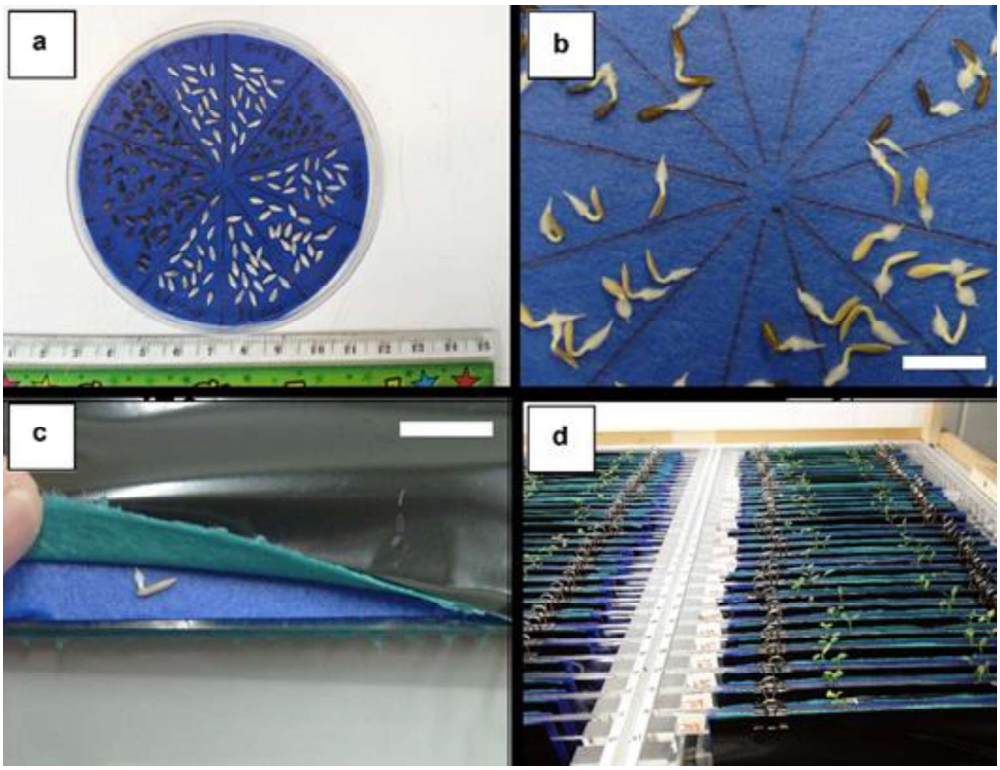


Fig. 1 Seed germination, position and growth in the pouch assay.

The imbibed seed on the germination paper in a petri dish (a). The predetermined stage at which the germinated seedlings were placed in the growth pouches, scale bar =1 cm; (b). A germinated seed of the parent Iceberg at the position placed in the growth pouch with the radicle orientated towards the bottom of the paper, scale bar = 1 cm; (c). Some of the seedlings 10 d from the date they were placed in the growth pouch (d).

Data analysis.

An increase in root growth resulted in an increase in the variance of the residuals indicating that the data had non-constant variance and was not normally

178 distributed. The raw data for the RILs and the parental lines were therefore
179 transformed to square root and the mean calculated to normalise the distribution of
180 the data for statistical analysis.

181 The transformed data were analysed using restricted maximum likelihood
182 (REML) variance component analysis which accounted for variation, such as light
183 level or edge effect that occurred within the frames. The resultant predicted means
184 for all lines were then analysed to determine significant differences between
185 genotypes. From the three measured phenotypes; primary root length (PRL), total
186 lateral root length (TLL) and total number of lateral roots (TNL) three further ratios
187 were produced, which were lateral root length density ($LRLD = TLL/PRL$), lateral
188 root number density ($LRND = TNL/PRL$) and the mean lateral root length ($MLRL =$
189 TLL/TNL). Broad sense heritability (H^2) for each trait was calculated from the
190 variance component analysis ($VG/(VE + VR)$ where VG is the genotypic variance,
191 VE the sum of the component variance and VR is the residual variance). All
192 statistical analysis of the mapping population data was done using GenStat 17th
193 edition (VSN international Ltd, Hemel Hempstead, UK).

194 **QTL analysis.**

195 Restriction fragment length polymorphism (RFLP) and Kompetitive allele
196 specific PCR (KASP) markers were used to genotype both the parents and the RIL
197 population. The RFLP markers were previously published for the Saladin x Iceberg
198 linkage map (Atkinson et al. 2013). Additional KASP markers were derived from
199 single nucleotide polymorphisms (SNPs) between the genomic sequences of the
200 parent accessions. To identify SNPs, Oligo(dT) selection of mRNA was performed
201 twice from total RNA extracts from each parental line of the RIL population using

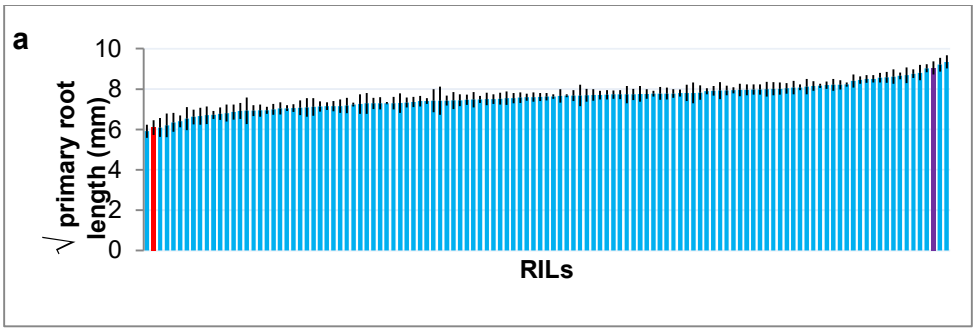
Dynal magnetic beads (Invitrogen-ThermoFisher Scientific, Massachusetts, USA) according to the manufacturer's instructions. Sequencing libraries were prepared using mRNA-TruSeq sample prep kit v5 (Illumina Inc., San Diego, USA) according to the manufacturer's protocol (15018818 revA). These libraries were sequenced using Illumina's GAIIx sequencing system. Using a CASAVA pipeline, 70 base paired-end sequence reads were base-called and scored for read quality.

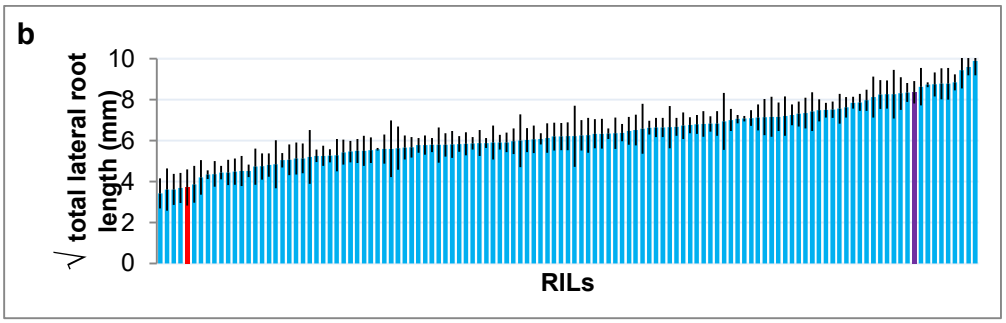
The linkage maps were constructed using JoinMap4 (Kyazma B.V, Wageningen, The Netherlands). Following REML transformation of the data the predicted mean values for all traits were analysed using MapQTL6 (Kyazma B.V, Wageningen, The Netherlands). Initially the data were analysed using interval mapping to identify putative QTLs (Zeng 1994) before further analysis was done using multiple QTL model (MQM) mapping, adding significant cofactor markers to eliminate genetic variation (background noise) caused by QTLs located elsewhere on the genome (Jansen & Stam 1994). The statistical logarithm of odds (LOD) score was calculated for a genome wide and chromosome wide significance of P<0.05 ($1 - \alpha c = \sqrt[n]{(1 - \alpha g)}$, where αc is the chromosomal significance threshold, αg is the genome wide significance threshold and n is the number of chromosomes) (van Ooijen 1999).

Results.

Some individual seedlings did not emerge, had severely inhibited primary root growth or browning of the root tissue. These seedlings were not included in the data analysis (99 seedlings from a total of 726). In total 42 lines had one data point missing, 19 lines had 2 data points missing, 5 lines had 3 data points missing and 1 RIL (RIL 36) had 4 data points missing.

There was very high significant variation (P<0.001) between lines of the mapping population, including the parents, for all six root traits; primary root length (SEM=0.041, Fig 2a); total lateral root length (SEM=0.116, Fig 2b); total number of lateral roots (SEM=0.029); total lateral root, length/primary root length (SEM 0.013 Fig 2c); number of laterals/primary root length (SEM=0.003) and mean lateral root length (SEM=0.025).





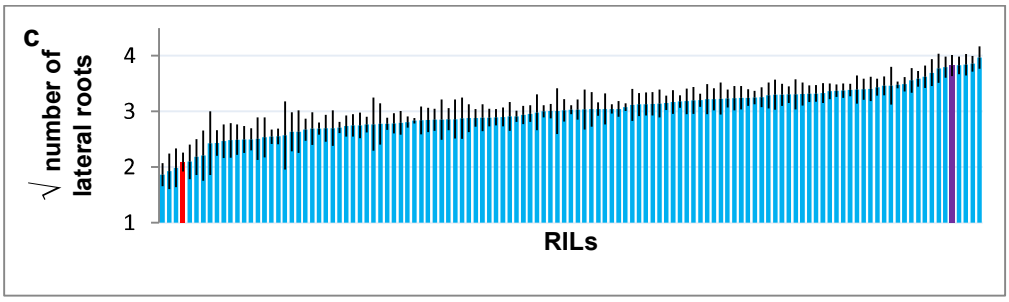


Fig. 2 Segregation of 14 d seedlings of the 125 RILs of the Saladin X Iceberg mapping population and the parents for the measured traits; a) Primary root length, b) total lateral root length and c) total number of lateral roots. Red bars are the Saladin parent. Purple bars are the Iceberg parent and blue bars are the RILs; Error bars are SEM.

The chromosomal wide and genome wide significance at $P<0.05$ was 0.994. This value when interpolated into the table by van Ooijen (1999) corresponding to the average chromosomal map length of 116 cM gave a LOD score of 3.1 for statistically significant QTLs ($P<0.05$) for the size and type of population used. The permutation test using the MapQTL software gave a LOD score of 3.2 and using this more conservative value a total of 16 statistical QTLs were identified (see Table 1 & Fig. 3).

Six significant QTLs were found for primary root length on linkage group (LG) 2c, 4b, 5b, 7b, 8 and 9, which were labelled PRL-01 through to PRL-06 and 60.2 % of the genotypic variance can be explained by these QTLs. Variance components analysis showed that the primary root length trait had a H^2 score of 0.37. For total lateral root length three statistical QTLs, labelled TLL-01 through to TLL-03 were identified on LG 3, 5b and 9b and 38.6 % of the phenotypic variance was explained by these QTLs. The H^2 score was 0.35 for the total lateral root length trait. No statistical QTLs were discovered for total number of lateral roots. The H^2 score for total number of lateral roots was 0.28 (Table 1; Fig. 3).

The first of the three ratios, LRLD had two statistical QTLs on LG 4 and 9b and were labelled LRLD-01 and LRLD-02. The H^2 for the LRLD trait was 0.29. These

two QTLs explained 27.6 % of the phenotypic variance for this trait. Three statistical QTLs were found for LRND. These QTLs were on LG 7b, 8b and 9, explaining 33.4 % of the phenotypic variation for the trait and were labelled LRND-01, LRND-02 & LRND-03. The H^2 for the ratio LRND was 0.24. For MLRL two statistical QTLs were identified on LG 8 and 9b and these QTLs were labelled MLRL-01 and MLRL-02. A total of 21.1 % of the phenotypic variance of the MLRL trait can be explained by these two QTLs and the H^2 score for MLRL was 0.24 (Table 1; Fig. 3).

Table 1 Statistical QTLs (P<0.05) for root traits and their genetic positions in 14 d old seedlings of the Saladin x Iceberg mapping population.

QTL (P<0.05)	LOD score	Linkage Group	Position (cM)	Associated markers	Allelic contribution	% phenotype explained
PRL-01	5.82	7b	33.5 – 35.5	7_LS1_750 ;39	Iceberg	17.1
PRL-02	3.84	9a	10.0 - 10.5	AQYG-OP3 9_LS1_319 ;53	Iceberg	11.1
PRL-03	3.38	5b	0.0 – 1.0	E35M47_191i E45M60_160i	Saladin	8.4
PRL-04	3.27	8a	27.4 – 24.4	ARRK-OP4 AKDB-OP4 BVTF-OP4 E35M61_280s	Iceberg	7.8
PRL-05	3.26	4b	14.9 – 15.9	4_LS1_324 ;23 AVZB-OP4	Iceberg	7.5
PRL-06	3.22	2c	35.3 - 35.3	2_LS1_664 ;11	Iceberg	8.3
TLL-01	8.83	9b	12.8 – 12.8	9_LS1_694 ;52	Saladin	23.8
TLL-02	3.24	5b	0.0 – 1.0	E35M47_191i E45M60_160i	Saladin	7.2
TLL-03	3.2	3a	13.7 - 13.7	AVSI-OP3 3_LS1_14 ;15	Saladin	7.6
LRLD-01	6.78	9b	8.6 – 12.8	9_LS1_392 ;52	Saladin	19.0

LRLD-02	3.33	4a	5.1 - 5.1	BSCC-OP3-1	Saladin	8.6
LRND-01	4.13	8b	10.0 – 12.0	E45M59_265i AKQB-OP4	Saladin	10.4
LRND-02	4.02	7b	31.7 – 31.7	E35M47_244i	Iceberg	8.7
LRND-03	3.97	9a	10.5 – 11.2	9_LS1_470 ;53 9_LS1_496 ;53	Saladin	14.3
MLRL-01	4.38	9b	7.4 – 7.4	BEMX-OP4	Saladin	10.8
MLRL-02	4.18	8a	1.0 – 3.1	8_LS1_591 ;48 8_LS1_58 ;49 8_LS1_229 ;49	Saladin	10.3

Trait abbreviations are PRL (primary root length), TLL (total lateral root length), LRLD (lateral root length density), LRND (lateral root number density) and MLRL (mean lateral root length).

Four linkage groups contained regions with clustered QTLs, namely LG 5a, 7b, 9a and 9b (Table 1, Figure 3) highlighting regions of interest.

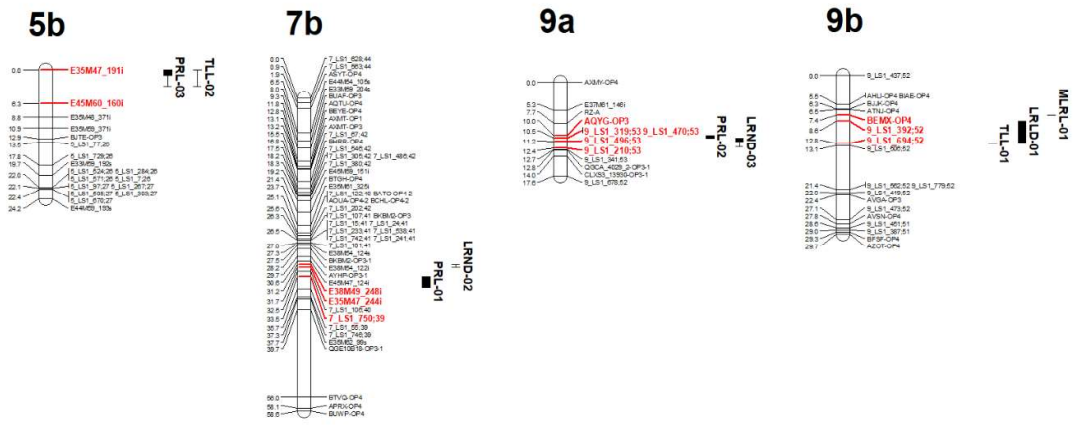


Fig 3. Clustered QTL positions and associated markers for the root traits on the linkage map of the Saladin x Iceberg mapping population.

Statistical (black bars) QTL positions in centimorgans (cM) on the Saladin x Iceberg linkage map. The solid blocks are the 1-LOD threshold (LOD score of 3.2), the outer intervals are the 2-LOD threshold. The markers in bold red are those associated with the significant LOD of the QTLs. Abbreviations of traits are PRL (primary root length), TLL (total lateral root length), TNL (total number of lateral roots), LRLD (lateral root length density), LRND (lateral root number density) and MLRL (mean lateral root length).

Discussion

The study identified 16 statistical QTLs associated with early stage rapid root development in an intra-specific *L. sativa* mapping population. The markers associated with these traits could be used for marker assisted selection in breeding programmes in the future should an increase in root growth prove to be associated with better establishment in field grown lettuce transplants.

The study has identified genetic potential, within the intra-specific cross in a 2D assay that could be utilised within a breeding programme. Further studies would need to be undertaken however, to better understand what interaction the environment has on these traits in field conditions. The 2D high-throughput assay used in this study allows the rapid analysis of root traits in seedlings in a time, cost and labour efficient manner compared with other techniques, such as computed tomography (CT) 3D analysis (Mooney et al., 2012). Over 762 germinated seedlings were sown in <6 h, covering an area <1.5 m^2 at a cost of <£0.50 per seedling. This technique offers greater efficiency than sand or soil pot grown root analysis, which

301 increases area use, labour and time costs dramatically as the roots need to be
302 washed and separated before imaging/measuring can be accomplished.

303 In directly seeded crops the ability to produce a longer tap root early may be
304 advantageous. Greater primary root length observed in 14 d old seedlings using the
305 pouch system has been positively correlated with root emergence, faster
306 establishment and increased seed yield in field grown *Brassica napus* (Thomas et al.
307 2016). Increased primary root length in seedlings potentially allows root access to
308 deeper water resources (Johnson et al. 2000). Cultivated lettuce was described by
309 Jackson (1995) as having a short tap root compared to its wild progenitor *L. serriola.*
310 This study has observed significant difference within a *L. sativa* intra-specific cross
311 for primary root length that may allow the ability to explore deeper soil layers and
312 allow faster establishment and emergence in field grown lettuce. Six QTLs were
313 identified for increased primary root length of which one was contributed by the
314 Saladin parent on LG 5b (PRL-03) while the others were contributed by the Iceberg
315 parent and were located on LG 7b (PRL-01), 9 (PRL-02), 8 (PRL-04), 4b (PRL-05)
316 and 2c (PRL-06). Further work would be needed to identify if the RIL lines with a
317 greater primary root length trait in 14 d seedlings emerge and establish faster and
318 develop a longer, deeper tap root at maturity in the field.

319 In transplanted lettuce where mechanical pruning of the primary root often
320 occurs (Kerbiriou et al. 2013), recovery of the root:shoot ratio may be governed by
321 the plants ability to rapidly replace lost root mass through lateral root growth.
322 Establishment is also dependent on the crops ability to regenerate lateral roots
323 during establishment (Orzolek 1991) allowing early capture of the resources
324 available to further optimise shoot growth. Longer total root length of wheat seedlings
325 in a growth pouch assay has been associated with increased yield and shoot

biomass in the field (Xie et al. 2017). Five statistical QTLs were found that were linked with total lateral root length. Two QTLs were located along LG 9b (TLL-01 and LRLD-01) overlapping the same region and probably represent a single locus. The further three QTLs were located on LG 5b (TLL-02), 3 (TLL-03) and 4 (LRLD-02).

Decapitation of the root tip from primary lateral roots in lettuce seedlings has been shown to slow and even cease the emergence of any further secondary or tertiary lateral roots along the length of the decapitated root (Biddington & Dearman 1984). The pruning of the lateral roots often occurs as a consequence of the mechanical separation of adjacent peat blocks in the process of transplanting lettuce. Hence, breeding for cultivars that can regenerate greater numbers of primary lateral roots more efficiently may be a desirable trait that helps plants establish more rapidly. There were three individual statistical QTLs linked to the total number of lateral roots. The QTLs were for the lateral root number density trait and were located on LG 8b, (LRND-01), 7b (LRND-02) and 9 (LRND-03). LRND-01 and LRND-03 were contributed by the Saladin parent, while LRND-02 was contributed by the Iceberg parent.

The ability of a lettuce transplant to produce fewer longer lateral roots (greater MLRL) may be advantageous. Fitter et al. (1991) suggested exploitation efficiency (amount of soil exploited per carbon unit cost of root) may be beneficial to crops. If lettuce transplants were able to produce fewer longer lateral roots with less branching following transplanting, then the plant would be able to utilise the resources captured mainly on shoot growth. There were two statistical QTLs identified for MLRL in this study that may be beneficial to exploitation efficiency in lettuce transplants. The first was located on LG 9b (MLRL-01) and the second was on LG 8 (MLRL-02).

351 The overlying region on LG 9b between 8.6 and 12.8 cM for both the total
352 lateral root length and lateral root length density traits (3 QTLs), but not total number
353 of lateral roots, lateral root number density and mean lateral root length suggests
354 that this region is genetically involved with increased individual lateral root length or
355 decreased branching/topology, which would indicate this region could be exploited to
356 increase the root exploration potential (Fitter & Stickland 1991) in lettuce transplants.

357 Only one of the six statistical QTLs identified in this paper for primary root
358 length (i.e. tap root length) was located on LG 2-(LG 2c), where QTLs for the trait
359 were identified by Johnson et al. (2000), however, the study cannot identify if the loci
360 are the same. One of the QTL in this study located to the region towards the end of
361 linkage group 2c (35.3 cM) close to the area on LG 2 where Johnson et al. (2000)
362 had mapped a QTL associated with tap root length contributed by the wild parent. A
363 further QTL identified in this study (TLL-03) mapped to LG 3 (13.7 cM), which is in
364 proximity to the QTL identified by Johnson et al. (2000) associated with number of
365 lateral roots.

366 The two QTLs identified on the LG 5 group (5b), PRL-03 and TLL-02 locate to
367 the same LG as a QTL linked to lateral root length and lateral root number observed
368 by Wei et al. (2014). Johnson et al. (2000) also located a QTL on LG 5 that was
369 linked to lateral root number in the lower soil profile contributed by the wild relative *L.*
370 *serriola*. This region is therefore strongly linked to lateral root emergence and growth
371 in both cultivated and wild parents. The QTLs PRL-02, TLL-01, LRLD-01, LRND-03
372 and MLRL-01 located on the same LG (LG 9 and 9b) to the QTLs identified by Wei et
373 al. (2014) linked to general root growth. Our study identifies this region as being
374 linked with all the root growth traits – primary root growth, lateral root growth and
375 lateral root emergence.

376 Of the population, RIL 87 and RIL 114 had the highest and lowest scores
377 respectively for the three measured traits primary root length, total lateral root length
378 and total number of lateral roots indicating that these lines would be the best
379 candidates to use in a gene expression study to identify the genes underlying the
380 QTLs and others that are involved with increased root growth rate traits. Increased
381 root growth traits could reduce the period of the recovery phase, caused by
382 transplant shock (van Iersel 1998), by quickly restoring the root:shoot ratio and
383 therefore increasing crop uniformity by reducing transplant establishment time.
384 However; certain negative possibilities could occur. In a rapid rooting line, the
385 increase in growth could mean more lateral roots are pruned leading to an enhanced
386 transplant shock, meaning no benefit to establishment would apply. These concerns
387 would need further studies to be resolved.

388

389 **Conclusion**

390 A rapid rooting phenotype may be beneficial to the establishment of lettuce
391 transplants in commercial field production. Such a phenotype could reduce
392 transplant shock and alleviate reduction in shoot growth due to mild abiotic stresses
393 that occur in the field. The use of a high throughput rooting growth pouch assay
394 revealed significant genetic variation in a Saladin X Iceberg cross RIL population to
395 identify QTLs linked to the traits associated with a rapid rooting phenotype in 14 d old
396 seedlings. A total of 16 statistical QTLs were identified. The statistical QTLs were
397 located across 9 different LGs representing loci on 7 of the 9 *L. sativa* chromosomes.
398 DNA markers linked to these rooting traits were identified, which could allow
399 breeders to select for a rapid establishment phenotype.

The linked markers could also be directly applied in lettuce breeding programmes and may be of more direct utility compared to markers from inter-specific crosses, which contains genetic material from the wild *L. serriola* parent (Atkinson et al. 2013).

Conflicts of interest

The authors are not aware of any conflict of interest in this study via funding or any affiliations.

References

Abràmoff.M.D, Magalhães.P.J & Ram.S.J (2004) Image processing with ImageJ. Biophotomics International 11(7):36-42.

Atkinson.J.A, Wingen.L.U, Griffiths.M, Pound.M.P, Gaju.O, Foulkes.M.J, Le Gouis.J, Griffiths.S, Bennett.M.J, King.J & Wells.D.M (2015) Phenotyping pipeline reveals major seedling root growth QTL in hexaploid wheat. Journal of Experimental Botany 66:2283-2292.

Atkinson.L.D, McHale.L.K, Truco.M.J, Hilton.H.W, Lynn.J, schut.J.W, Michelmore.R.W, Hand.P & Pink.D.A.C (2013) An intra-specific linkage map of lettuce (*Lactuca sativa*) and genetic analysis of postharvest discolouration traits. Theor. Appl. Genet. 126(11):2737-2752.

Biddington.N.L & Dearman.A.S (1984) Shoot and root growth of lettuce seedlings following root pruning. Annals of Botany 53:663-668.

Fitter.A.H, Stickland.T.R, Harvey.M.L & Wilson.G.W (1991) Architectural analysis of plant root systems 1. Architectural correlates of exploitation efficiency. New Phytologist 118:375-382.

Fitter.A.H & Stickland.T.R (1991) Architectural analysis of plant root systems 2. Influence of nutrient supply on architecture in contrasting plant species. New phytologist 118:383-389.

Grassbaugh.E.M & Bennett.M.A (1998) Factors affecting vegetable stand establishment. Sci. Agric. (Piracicaba, Braz.) 55:116-120.

Jackson (1995) Root architecture in cultivated and wild lettuce (*Lactuca* spp.). Plant, Cell and Environment 18(8):885-894.

Jansen.R.C & Stam.P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136:1447-1455.

441 Johnson.W.C, Jackson.L.E, Ochoa.O, van Wijk.R, Peleman.J, St.Clair.D.A, &
442 Michelmore.R.W (2000) Lettuce, a shallow-rooted crop, and *Lactuca serriola*, its wild
443 progenitor, differ at QTL determining root architecture and deep soil water
444 exploitation. Theor. Appl. Genet. 101:1066-1073.

445

446 Kerbiriou.P.J, Stomph.T.J, Lammerts van Bueren.E.T & Struik.P.C (2013)
447 Influence of transplant size on the above- and below-ground performance of four
448 contrasting field-grown lettuce cultivars. Frontiers in Plant Science 4(379):1-16.

449

450 Le Marié.C, Kirchgessner.N, Marschall.D, Walter.A & Hund.A (2014)
451 Rhizoslides: paper-based growth system for non-destructive, high throughput
452 phenotyping of root development by means of image analysis. Plant Methods
453 10(13):1-16.

454

455 Maltais.A, Gosselin.A, Tremblay.N & van Winden (2008) Effects of
456 temperature and fertigation on lettuce seedling production using peat blocks. Acta
457 Horticulturae 782: 367-374.

458

459 Mooney.S.J, Pridmore.T.P, Helliwell.J & Bennett.M.J (2012) Developing X-ray
460 computed tomography to non-invasivley image 3-D root systems architechture in
461 soil. Plant Soil 352:1-22.

462

463 Nesmith.D.S & Duval.J.R (1998) The effect of container size. HortTechnology
464 8(4):495-498.

465

466 Orzolek.M.D (1991) Establishment of vegetables in the field. HortTechnology
467 1(1):78-81.

468

469 Schneider.C.A, Rasband.W.S & Eliceiri.K.W (2012) NIH image to ImageJ: 25
470 years of image analysis. Nature Methods 9(7):676-682.

471

472 Sharma.N, Abrams.S.R & Waterer.D.R (2005) Abscisic acid analogs reduce
473 transplant shock in tomato seedlings. Journal of Vegetable Science 11(3):41-56.

474

475 Steele.K.A, Price.A.H, Shashidhar.H.E & Witcombe.J.R (2006) Marker-
476 assisted selection to introgress rice QTLs controlling root traits into an Indian upland
477 rice variety. Theor. Appl. Genet. 112:208-221.

478

479 Steele.K.A, Price.A.H, Witcombe.J.R, Shrestha.R, Singh.B.N, Gibbons.J.M &
480 Virk.D.S (2013) QTLs associated with root traits increase yield in upland rice when
481 transferred through marker-assisted selection. Theor. Appl. Genet. 126(1):101-108.

Thomas.C.L, Graham.N.S, Hayden.R, Meacham.M.C, Neugebaur.K, Nightingale.M, Dupuy.L.X, Hammond.J.P, White.P.J & Broadley.M.R (2016) High-throughput phenotyping(HTP) identifies seedling root traits linked to variation in seed yield and nutrient capture in field-grown oilseed rape (*Brassica napus* L.). Annals of Botany 118:655-665.

Van Iersel.M (1998) Auxins affect posttransplant shoot and root growth of Vinca seedlings. HortScience 33(7):1210-1214.

Van Ooijen.J.W (1999) LOD significance thresholds for QTL analysis in experimental populations of diploid species. Heredity 83:613-624.

Wei.Z, Julkowska.M.M, Laloë.J-O, Hartman.Y, de Boer.G-J, Michelmore.R.W, van Tienderen.P.H, Testerink.C & Schranz.M.E (2014) A mixed-model QTL analysis for salt tolerance in seedlings of crop-wild hybrids of lettuce. Molecular Breeding 34(3): 1389-1400.

Zeng.Z-B (1994) Precision mapping of quantitative trait loci. Genetics 136:1457-1468.

503 Zhu.J, Ingram.P.A, Benfey.P.N & Elich.T (2011) From lab to field, new
504 approaches to phenotyping root system architecture. Current Opinion in Plant
505 Biology 14:310-317.