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QTL analysis of the developmental response to L-glutamate in Arabidopsis roots and its genotype-by-environment interactions

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Abstract

Primary root growth in Arabidopsis and a number of other species has previously been shown to be remarkably sensitive to the presence of external glutamate, with glutamate signalling eliciting major changes in root architecture. Using two recombinant inbred lines from reciprocal crosses between Arabidopsis accessions C24 and Col-0, we have identified one large-effect quantitative trait locus (QTL), GluS1, and two minor QTLs, GluS2 and GluS3, which together accounted for 41% of the phenotypic variance in glutamate sensitivity. The presence of the GluS1 locus on chromosome 3 was confirmed using a set of C24/Col-0 isogenic lines. GluS1 was mapped to an interval between genes At3g44830–At3g46880. When QTL mapping was repeated under a range of environmental conditions, including temperature, shading and nitrate supply, a strong genotype-by-environment interaction in the controls for the glutamate response was identified. Major differences in the loci controlling this trait were found under different environmental conditions. Here we present evidence for the existence of loci on chromosomes 1 and 5 epistatically controlling the response of the GluS1 locus to variations in ambient temperature, between 20°C and 26°C. In addition, a locus on the long arm of chromosome 1 was found to play a major role in controlling the ability of external nitrate signals to antagonize the glutamate effect. We conclude that there are multiple loci controlling natural variation in glutamate sensitivity in Arabidopsis roots and that epistatic interactions play an important role in modulating glutamate sensitivity in response to changes in environmental conditions.

Key words: Environmental interactions, epistatic effects, glutamate, natural variation, nitrate, QTL mapping, root architecture, root growth, temperature sensitivity.

Introduction

Root growth and branching are highly responsive to a wide range of biotic and abiotic stimuli that play a major role in shaping the development of the root system. Amongst the most important abiotic factors affecting root development are the availability and spatial distribution of nutrients within the soil. The enhancement of root growth within fertile soil patches was reported 150 years ago (Nobbe, 1862) and a century later Wiersum (Wiersum, 1958) demonstrated that nitrate-rich zones of soil stimulated root branching. Drew (1975) reported that localized supplies of ammonium...
and phosphate, but not potassium, were also able to trigger a similar localised response as that elicited by localised nitrate. These responses are considered to be examples of foraging responses because of the way that they enable more precise placement of roots within those soil zones where the greatest benefit will be obtained (Hutchings and de Kroon, 1994). More recently, it was reported that different, but equally dramatic, changes in root architecture could be elicited when roots of a number of species, including Arabidopsis, are exposed to the amino acid L-glutamate (Glu) (Walch-Liu et al., 2006). In Arabidopsis, the response to Glu consisted of a slowing or cessation of primary root (PR) growth, with apical meristem activity being the primary target, and a stimulation of lateral root outgrowth behind the PR tip. Lateral roots also became sensitive to Glu but the response was developmentally delayed until they were >5–10 mm long (Walch-Liu et al., 2006).

Amino acids, including Glu, represent a significant proportion of the soluble N pool in unfertilized soils (Christou et al., 2005). Roots are known to possess a set of high affinity amino acid transport systems capable of absorbing these amino acids from the rhizosphere at naturally occurring concentrations (Svennerstam et al., 2011). Although the extent to which plants are able to utilise organic forms of soil N is still unclear (Jones et al., 2005; Moran-Zuloaga et al., 2015), it has been suggested that a localised source of Glu in the soil could act as a cue to trigger increased root branching within an organic N-rich patch, enhancing the plant’s ability to compete with microbes and other plants for uptake of the available amino acids within that patch (Forde and Lea, 2007). According to this hypothesis the root response to Glu represents another kind of foraging response, serving to enhance the precision of root placement within the soil with respect to heterogeneously distributed sources of organic N.

There are a number of lines of evidence that the root response to Glu involves a signalling effect, rather than a nutritional one. These include the finding that the effect is not elicited by glutamine or other related amino acids, the relatively low concentrations of Glu (50 μM) that are needed to trigger the response and the requirement for the PR tip to be in direct contact with the external Glu (Walch-Liu et al., 2006; Forde, 2014). The case for a signalling role for Glu was further strengthened by the finding that MEKK1, a MAP kinase kinase kinase best known for its role in defence signalling, has a key role in eliciting the root’s response to Glu (Forde et al., 2013).

A particularly striking aspect of the Glu response is the degree of natural variation in Glu sensitivity amongst Arabidopsis accessions, with C24 being the most sensitive of those tested and Col-0 being one of the least sensitive (Walch-Liu et al., 2006). Natural variation has proved to be a powerful tool for the genetic analysis and dissection of complex traits in Arabidopsis (Weigel, 2012). Previous studies have found that combining naturally occurring genetic variation with multiple environmental treatments can increase the power of this approach (Tonsor et al., 2005). This paper describes the results of a multi-environment study aimed at using natural variation in Arabidopsis to investigate the genetic control of the Glu response and its interactions with nitrate and other environmental factors. The results reveal a remarkable level of complexity in the genetic control of Glu sensitivity in Arabidopsis roots and in the genotype-by-environment interactions that modulate this sensitivity.

**Materials and methods**

**Plant material**

*Arabidopsis thaliana* L. (Heynh.) ecotypes were originally sourced from Lehle Seeds (Round Rock, TX, USA). The F$_{1}$ recombinant inbred lines (RILs) were previously developed from reciprocal crosses between the Arabidopsis accessions Col-0 and C24 and genotyped using a set of 110 framework single nucleotide polymorphism (SNP) markers (Törjék et al., 2006). Reciprocal sets of introgression (ILs) between Col-0 and C24 were generated and mapped as described previously (Törjék et al., 2008).

**Plant growth and phenotyping**

Seedlings were cultured vertically on 1% Phytagel plates containing a dilute basal medium at pH 5.7 (see supplementary Table S1 at JXB online) with 0.5% sucrose and 0.5 mM glutamine as the background N source (Walch-Liu and Forde, 2008). After sowing on 90 mm Petri dishes, sterilized seeds were stratified in the dark for 2 d at 4°C. The plates were then transferred to a growth room with a light intensity of ~100 μmol m$^{-2}$ s$^{-1}$ or a growth cabinet (Snijders, Tilburg, The Netherlands) with a light intensity of 330 μmol m$^{-2}$ s$^{-1}$, at the required temperature and for a 16/8 h photoperiod. After 4 d, seedlings were transferred either to 90 mm diameter Petri dishes, with 3 seedlings per plate, or 120 × 120 mm square plates, with 6 seedlings per plate, onto which either 50 μM K glutamate or 50 μM KCl had been added. When nitrate treatments were applied, KNO$_{3}$ was added to a concentration of 5 mM and additional KCl was added to control treatments to maintain a uniform K$^{+}$ concentration. After transfer, the positions of the PR tips were marked on the base of the plates and growth was continued under the same conditions. After a further 5 d, roots were imaged using a flatbed digital scanner and root growth analysed using Optimas Image Analysis software (Version 6.1, Media Cybernetics Inc., Silver Spring, MD, USA).

In the multi-environment experiment, the shading treatment was achieved by suspending a steel plate containing an array of 5 mm square holes above the plates, reducing the light intensity reaching the seedlings from 330 μmol m$^{-2}$ s$^{-1}$ to 130 μmol m$^{-2}$ s$^{-1}$.

**QTL analysis**

Composite Interval Mapping was performed using PLABQTL software (Utz and Melchinger, 1996) using the mean values of PBT and PAT$_{Glu}$ for each RIL. Cofactors used for calculation were automatically chosen by the PLABQTL program by forward selection. Permutation analysis, using 1000 permutations, was performed to calculate the critical log of the odds (LOD) score ($\alpha$ = 0.05). Genotypic data used for the analysis were as previously determined (Törjék et al., 2003).

**Fine mapping of ILs**

Total DNA was extracted from leaves of selected ILs using the Qiagen Plant DNeasy minikit according to the manufacturer’s instructions. SNPs between Col-0 and C24 in the regions of interest were identified using the Arabidopsis GEBrowser (http://signal.salk.edu/atg1001/3.0/gerowser.php) and dCAPS primers designed with the aid of dCAPS Finder 2.0 software (Neff et al., 2002). MarkerTracker software (http://bbc.botany.utoronto.ca/markertracker/index.spy) was used to identify suitable PCR primers for the SNP loci.
CAPS (Cleaved Amplified Polymorphism) markers (Konieczny and Ausubel, 1993). PCR reactions were performed for 35 cycles using DreamTaq DNA polymerase (ThermoFisher Scientific) according to the manufacturer’s instructions. After digestion with the appropriate restriction enzyme, PCR reactions were electrophoresed on 1.5% agarose gels and stained with ethidium bromide to visualise the DNA fragments. Details of the SNPs, primers and restriction enzymes used in mapping are in Supplementary Table S2.

Results

Using RILs to map major QTLs controlling the Glu response

Strong differences previously observed between C24 and Col-0 in their sensitivity to low concentrations of Glu (Walch-Liu et al., 2006) led us to choose a C24/Col-0 population of RILs (Törjek et al., 2006) to map QTLs controlling the Glu response. A preliminary experiment was performed to establish the suitability of the C24/Col-0 RIL population for this analysis and to identify the most appropriate parameter for assaying the Glu response. A set of 28 RILs was grown on medium with and without 50 µM Glu, a concentration chosen because it was previously shown to strongly inhibit PR growth in C24 but to have almost no effect on Col-0 (Walch-Liu et al., 2006). Amongst the RILs, a high degree of variation in the responsiveness to Glu was observed, values for % inhibition ranging from 0 to 46%, with a mean of 16.7% and a coefficient of variation of 0.80. When the increase in PR length in the 5 d after transfer to Glu (PATGlu) was plotted for each RIL against PR growth in the control, no Glu, plates (Fig. 1A), there was only a weak correlation (r² = 0.045). This showed that growth in the presence of Glu was largely determined by the root’s response to Glu and that intrinsic genetically determined differences in growth rate played little part. In agreement with this, when percentage inhibition was plotted against PATGlu (Fig. 1B), there was a strong negative correlation (r² = 0.73). On this basis PATGlu rather than percentage inhibition was chosen as the parameter to use when analysing larger numbers of RILs for their Glu sensitivity, since it avoided the need for a control treatment for each line and therefore allowed more lines and/or treatments to be analysed in each experiment.

Using this approach, the root response to Glu was assayed using two populations of RILs, one comprising 193 lines derived from a Col-0 × C24 cross and the other comprising 175 lines derived from the reciprocal C24 × Col-0 cross (Törjek et al., 2006). The growth of the PRs in the 4 d period before transfer (PBT) and their growth in the 5 d period after transfer to plates containing Glu (PATGlu) were measured for each RIL and the frequency distributions for each trait are shown separately for the two populations in Fig. 2A and B. Both traits display a high degree of phenotypic variation with an essentially normal distribution, indicating the involvement of multiple genes, with only a small amount of transgressive segregation with respect to the parental lines. The ranked means of PBT and PATGlu for all lines used in this analysis, together with standard errors, have been plotted in Supplementary Figs S1 and S2, respectively. Since it was these mean values that were used for the QTL analysis, it is important to note the extent to which differences between the means are statistically significant across the two populations of RILs. The broad-sense heritabilities for the two traits were high in both RIL populations (H² = 0.80 and 0.71 for PBT; H² = 0.85 and 0.81 for PATGlu), indicating their suitability for QTL mapping.

QTL analysis by composite interval mapping was performed using PLABQTL software. LOD score plots for the PBT and PATGlu traits are shown in Fig. 3. Three QTLs for PATGlu were identified whose LOD scores exceeded the threshold in both RIL populations (Fig. 3B). The strongest of these, Glu Sensitivity 1 (GluS1), accounted for an average of 22.5% of the phenotypic variance for this trait in the two populations and mapped in the vicinity of MASC01171 on chromosome 3 with peak LOD scores at 58 and 62 cM, respectively. The other QTLs were located in the vicinity of MASC02788 on chromosome 3, GluS2 with peak LOD scores at 78 and 80 cM, and
In preliminary experiments it was noted that the root response to Glu in Col-0 was surprisingly sensitive to relatively small differences in the growth temperature. To investigate this further and to determine its genotype dependence, the Glu sensitivities of five Arabidopsis accessions were compared at 20°C and 26°C (Fig. 4). At 20°C, PR growth in all five accessions was significantly inhibited by Glu, although to varying extents, consistent with previous evidence for the strong variation between accessions in their Glu sensitivity measured at 22°C (Walch-Liu et al., 2006). However, in three of the five accessions the sensitivity to Glu declined markedly at 26°C. Col-0 was the most temperature-sensitive, almost completely losing its responsiveness to either 0.5 mM or 1 mM Glu at the higher temperature, while C24 was the least temperature-sensitive, its roots being strongly inhibited by Glu at both temperatures. Dijon-G and Ler had an intermediate phenotype in which the Glu response at 26°C was partially reduced and Nd-0 showed the opposite effect, being slightly more responsive to Glu at the higher temperature. Thus, like the Glu sensitivity trait itself, the temperature sensitivity of the Glu response is highly dependent on genotype and therefore, as described below, is a potential target for QTL mapping the responsible loci.

**QTL mapping of Glu sensitivity loci under multiple environmental conditions**

The exceptional environmental sensitivity of the Glu response is illustrated both by its temperature dependence (Fig. 4) and by the loss of Glu sensitivity that has previously been observed in the presence of excess nitrate (Walch-Liu and Forde, 2008). To investigate the genetic control of this environmental sensitivity, a series of experiments was performed in which 88 of the C24/Col-0 RILs were cultivated in growth cabinets under five different environmental conditions: minus nitrate at 20°C, minus nitrate at 24°C; minus nitrate at 26°C; minus nitrate at 24°C with shading, and plus nitrate at 24°C. The nitrate concentration used, 0.5 mM, was previously shown to strongly antagonise the root response to 50 µM Glu in C24 (Walch-Liu and Forde, 2007). An overlapping series of experiments was initiated at intervals over a period of 4 weeks and each environmental treatment was repeated once, resulting a total of 10 experiments. The frequency distribution plots (Supplementary Fig. S3), which are based on the combined data for all experiments, confirm the high degree of phenotypic variation for PR growth both PATGlu and under all five conditions. For the most part the environmental conditions had only a minor effect on PR growth before the Glu treatment (PBT) in either the RIL population or the parental lines (Supplementary Fig. S3A). However, at 20°C the average PR length was markedly reduced compared to 24°C, and there was also a slight positive effect of the nitrate treatment. The data for PATGlu (Supplementary Fig. S3B) reveal a similar picture, except that the phenotypic variance in the RIL population was much greater than for PBT under all conditions. In addition, the positive effect of nitrate was much stronger on PATGlu than on PBT in both the C24 and RIL populations, reflecting the antagonistic effect of nitrate on Glu sensitivity in C24. Note that because Col-0 is not significantly inhibited by this low concentration of Glu, the

**Temperature sensitivity of the Glu response and its genotype dependence**

In preliminary experiments it was noted that the root response to Glu in Col-0 was surprisingly sensitive to relatively small
antagonistic effect of nitrate on its Glu sensitivity was not detectable here. Transgressive segregation for the $\text{PAT}_{\text{Glu}}$ trait was also particularly evident in the presence of nitrate, suggesting that alleles from both parents were contributing to nitrate antagonism of the Glu response.

The LOD score plots obtained from composite interval mapping of the PBT and $\text{PAT}_{\text{Glu}}$ traits in the multi-environment experiment are shown in Supplementary Fig. S4 and Fig. 5, respectively. Two complete datasets representing all five environmental conditions were analysed using...

**Table 1. Summary of the QTLs controlling Glu sensitivity in the Col-0/C24 RIL population based on CIM mapping of PR growth in the presence of Glu ($\text{PAT}_{\text{Glu}}$)**

| Environment | Exp't | QTL | Peak | SI | Left marker | LOD | Allelic effect | $pR^2$ (%) | $R^2$ (%) | SD |
|-------------|-------|-----|------|---|-------------|-----|---------------|------------|----------|----|
| Temperature (°C) |       |     |      |   |             |     |               |            |          |     |
|              |       |     |      |   |             |     |               |            |          |     |
| 21 Low light | Col-0x C24 | GluS1 | 3/62 | 58–64 | MASC01171 | 19.81 | $-6.138$ | 30.76 | 66.5 | 3.9 |
|              | GluS2  | 3/78 | 74–82 | MASC03218 | 5.24 | $-2.771$ | 10.78 |
|              | GluS3  | 5/78 | 72–80 | MASC04394 | 3.52 | $-1.532$ | 3.16 |
|              | C24x   | GluS1 | 3/58 | 56–62 | MASC01171 | 14.54 | $-3.967$ | 14.28 | 47.9 | 5.5 |
|              | GluS2  | 3/80 | 78–84 | MASC02788 | 3.84 | $-1.927$ | 4.29 |
|              | GluS3  | 5/72 | 68–74 | MASC04591 | 13.31 | $-4.252$ | 21.43 |
| 20 High light | Rep.1  | GluS1 | 3/58 | 54–60 | MASC01171 | 12.03 | $-4.214$ | 32.05 | 43.8 | 7.9 |
|              | Rep.2  | GluS1 | 3/56 | 54–62 | MASC04819 | 6.18 | $-5.095$ | 32.32 | 40.4 | 8.1 |
| 24 High light | Rep.1  | GluS1 | 3/52 | 50–56 | MASC05045 | 6.55 | $-3.917$ | 8.41 | 43.9 | 7.9 |
|              | Rep.2  | GluS1 | 3/58 | 54–62 | MASC01171 | 4.84 | $-6.770$ | 13.63 | 18.6 | 7.5 |
|              | Rep.2  | GluS4 | 5/14 | 12–18 | MASC05127 | 7.95 | $-7.331$ | 22.37 | 22.4 | 7.8 |
|              | Rep.2  | GluS4 | 5/14 | 10–18 | MASC05217 | 4.76 | $-6.815$ | 16.03 | 29.5 | 8.2 |
| 26 High light | Rep.1  | GluS5 | 4/6  | 2–8  | MASC04725 | 6.15 | $-5.935$ | 22.67 | 44.0 | 7.9 |
|              | Rep.2  | GluS5 | 4/2  | 0–6  | MASC04123 | 4.21 | $-4.866$ | 14.67 | 28.4 | 8.1 |

a Only those QTLs whose LOD score exceeded the threshold in the populations from both reciprocal crosses, or in both of the replicate experiments that were performed at each environmental condition in the multi-environment experiment, are shown. (Note that no reproducible QTLs were detected in the $24°C +$ shade treatment).

b Position of the highest LOD score in the QTL region (as chromosome/cM).

c The support interval (SI) within a LOD decrease of 1.0 from the QTL peak.

d The physical and genetical positions of the MASC framework markers are listed in Supplementary Table S5.

e Contribution of individual QTLs to the phenotypic variation.

f Effect of carrying the C24 allele at the respective position.

g $R^2$ and SD give the explained phenotypic variation and associated standard deviation obtained from the final simultaneous fit of all putative QTLs in PLABQTL.
the PLABQTL software, each comprising data from one of the two sets of replicates. The results indicate a strong genotype-by-environment interaction in the controls for the Glu response, with major differences in the loci controlling this trait under different environmental conditions. The major QTL on chromosome 3 that was identified at 21°C in the first experiment, GluS1, was confirmed in these experiments at both 20°C and 24°C. In each case it was the only QTL to be detected in both replicate experiments, contributing an average of 32% and 13.5% of the phenotypic variance for PATGlu at 20°C and 24°C, respectively. New QTLs were observed at 26°C, GluS4 with both peak LOD scores at 14 cM on chromosome 5, and at 24°C plus nitrate, GluS5 with peak LOD scores at 2 and 6 cM on chromosome 4. Again GluS4 and GluS5 were the only QTLs detected in both replicate experiments under these conditions, accounting for an average of 19% of the phenotypic variance in each case. No consistent QTL peaks for PATGlu were detected in the 24°C with shade treatment, but in one of the replicates there were peaks above the LOD threshold that colocalised with GluS1 and GluS3 on chromosomes 3 and 5, respectively (Fig. 3). As noted above for the QTLs detected in the first experiment, all of the newly detected QTL in this series of experiments were attributable to C24 alleles conferring enhanced sensitivity to Glu. Table 1 summarizes the QTLs whose LOD score exceeded the 5% threshold value in both replicates.

The environmental conditions also had strong effects on the QTLs identified for the PBT trait (Supplementary Fig. S4 and Table S3). The peak (PBT1) observed near the top of chromosome 1 in the initial experiment (Fig. 3) was only seen at 24°C plus nitrate in these experiments, with both peak LOD scores at 20 cM on chromosome 1. Three other QTLs for the PBT trait were observed in both replicate experiments: PBT2 with peak LOD scores at 10 and 12 cM on chromosome 4 at 24°C only, PBT3 with peak LOD scores at 24 and 26 cM on chromosome 5 at 24°C plus nitrate only and PBT4 with peak LOD scores at 48 and 52 cM on chromosome 4 at 24°C with shade. Note that here we cannot distinguish between genotypic effects on germination time and effects on root elongation rate, since both will potentially affect the length of the PR at the time of transfer. Importantly, however, none of the QTLs identified for the PBT trait corresponded to those seen for PATGlu.

Confirmation and mapping of a major QTL for glutamate sensitivity using a set of Col-0/C24 ILs

We employed a set of reciprocal Col-0/C24 ILs [(Törjek et al., 2008) and Supplementary Table S4] to confirm and more accurately map GluS1, the major QTL for Glu sensitivity on chromosome 3. Glu sensitivity of PR growth was assayed at 20°C in seedlings of nine Col-0 ILs carrying introgressions from C24 in the relevant region of chromosome 3 and nine C24 ILs with Col-0 introgressions in the same region. The map positions of the introgressions and the results of the Glu sensitivity assay are shown in Fig. 6. Six of the ILs in the Col-0 background resembled Col-0 in being almost insensitive to 50 µM Glu. However the other three, N52/2, N21/3/14 and N67/5, showed greatly increased sensitivity. Amongst the C24 ILs, seven lines were similar to the C24 parent, but two lines, M34/5/1 and M34/7/1, had almost completely lost Glu sensitivity and one line, M34/5/8, had intermediate sensitivity. The map locations of the introgressions in these 18 ILs define the region of chromosome 3 in C24 responsible for conferring Glu sensitivity as located between framework markers MASC01171 and MASC09224 (Fig. 6C). These markers are located at 57.1 and 67.7 cM on chromosome 3, respectively, which corresponds well with the location of GluS1 close to MASC01171 as determined by several QTL mapping experiments (Figs 3 and 5, summarized in Table 1)

Six of the ILs, N52/2, N21/3/14, M61/7/3, M34/5/1, M34/5/1 and M34/5/8, were of particular interest because their introgressions begin or end within the region of interest. The images in Supplementary Fig. S5 show the extent to which these introgressions affected how the roots responded to Glu. Supplementary Fig. S6 shows how more detailed mapping of these lines allowed the GluS1 locus to be located to the interval between the At3g44830 and At3g46880 genes.

Using ILs to locate loci conferring temperature sensitivity on the root response to Glu

To investigate the genetic control of temperature sensitivity of the Glu response in more detail, a set of 32 ILs in the C24 background were assayed for their sensitivity to 50 µM Glu at three temperatures: 20°C, 24°C and 26°C. These 32 ILs carry introgressions from Col-0 that collectively cover around 80–90% of the genome (Törjek et al., 2006). The data are plotted in Supplementary Fig. S7 in the form of reaction norms. The ILs have been divided into four groups according to their Glu sensitivity at 20°C and the responsiveness of this trait to
temperature. Group 1 (Supplementary Fig. S7A) is the largest group and contains 13 ILs that resembled the C24 parent in being both hypersensitive to Glu and having low sensitivity to the increase in temperature, with 64–85% inhibition by Glu at 26°C. The nine ILs in Group 2 (Supplementary Fig. S7B) are similarly hypersensitive to Glu but show a moderate degree of temperature sensitivity, with 43–61% inhibition by Glu at 26°C. The six ILs in Group 3 (Supplementary Fig. S7C) are characterized by an already diminished sensitivity to Glu at 20°C combined with only minimal responsiveness to temperature. Group 1 (Supplementary Fig. S7A) is the largest group and contains 13 ILs that resembled the C24 parent in being both hypersensitive to Glu and having low sensitivity to the increase in temperature, with 64–85% inhibition by Glu at 26°C. The nine ILs in Group 2 (Supplementary Fig. S7B) are similarly hypersensitive to Glu but show a moderate degree of temperature sensitivity, with 43–61% inhibition by Glu at 26°C. The six ILs in Group 3 (Supplementary Fig. S7C) are characterized by an already diminished sensitivity to Glu at 20°C combined with only minimal responsiveness.
to temperature. The four ILs in Group 4, M100/2/9/5, M31/8, M48/5/1 and M97/1/6, stand out as having acquired strong temperature sensitivity while maintaining the Glu hypersensitivity of the C24 background, showing >80% inhibition at 20°C and just 4–37% inhibition at 26°C (Supplementary Fig. S7D). Only two chromosomal regions are represented in these four ILs, one at the top of chromosome 1 and the other on the long arm of chromosome 5. M100/2/9/5 carries an introgression on chromosome 1 that includes framework markers MASC03771–MASC09203. However the lack of clear temperature sensitivity in two other ILs, M82/1/2 and M65/6/6, with introgressions at framework markers MASC03771 and MASC03758–MASC05303, respectively, indicates that the relevant locus that we designate \( TSI \) lies between markers MASC03771 and MASC03758 (genes At1g01471–At1g07520). The second temperature sensitivity locus (\( TS2 \)), is defined by the introgressions in M31/8 (MASC04983–MASC04350), M48/5/1 (MASC04591–MASC04576) and M97/1/6 (MASC03559 plus MASC01545–MASC04350), as being between markers MASC02675 and MASC09211 (genes At5g49680–At5g63920). This locus overlaps with the Glu sensitivity QTL GluS3.

It is striking that all five C24 ILs in which the region of chromosome 3 containing the \( GluS1^{C24} \) allele has been replaced by the equivalent region from Col-0 (M34/7/1, M28/11/2, M34/5/8, M34/5/1, M28/11/1) fell into Group 3 i.e. they showed the same low degree of temperature sensitivity as C24 itself (Supplementary Fig. S7C). This indicates that the \( GluS1^{Col-0} \) allele is not intrinsically temperature sensitive and that epistatic interactions with other loci in the Col-0 genome are probably responsible for its temperature-sensitive phenotype. This was confirmed by the reciprocal introgressions, where the region containing the \( GluS1^{Col-0} \) allele was replaced by the \( GluS1^{C24} \) allele in two Col-0 ILs, N52/2 and N213/14. These lines were, as expected, much more sensitive to Glu than Col-0 at 20°C but this sensitivity was lost at 24°C and 26°C (Fig. 7B), indicating that temperature sensitivity had been conferred on the \( GluS1^{C24} \) allele by its presence in the Col-0 background. It seems likely that the Col-0 alleles at the \( TSI \) and \( TS2 \) loci, on chromosomes 1 and 5, respectively, are at least partly responsible for these epistatic effects.

### Using ILs to locate loci conferring nitrate sensitivity on the root response to glutamate

The C24/Col-0 ILs were also used to identify loci responsible for controlling nitrate antagonism of the Glu response. When 32 C24 ILs were grown on medium with and without Glu in the presence and absence of nitrate there was a wide and continuous variation in the degree to which nitrate was able to overcome the Glu effect. This is illustrated by percentage inhibition by Glu in the absence of nitrate...
plotted against percentage inhibition in the presence of nitrate (Supplementary Fig. S8). This indicates that there are probably multiple genes of small effect contributing to this response to nitrate. However, there were several ILs that were notable for being as sensitive as C24 to Glu in the absence of nitrate but, unlike C24, lost little of this sensitivity in the presence of nitrate (Supplementary Fig. S8). The line that showed the least sensitivity to nitrate was M37/7/1/6 and the images in Fig. 8A illustrate the striking difference in root system architecture between it and C24 when seedlings of each genotype were grown on Glu in the presence of nitrate.

Another of the lines showing greatly diminished sensitivity to nitrate was M16/6/1/4, which carries an introgression on chromosome 1 that overlaps with the one in M37/7/1/6 (Supplementary Table S4). Both these lines were therefore analyzed in more detail, alongside an additional IL with an introgression in the same region, M16/6/4/4/3, by growing them on medium with and without Glu and with nitrate concentrations of 0 mM, 1 mM or 5 mM. The data in Fig. 8B confirm the low sensitivity of all three lines to 5 mM nitrate compared to C24. Only the chromosomal segment carrying markers MASC03631–MASC03684 is common to all three ILs, indicating that the introgressed locus responsible for the reduction in nitrate sensitivity, designated NS1, is located between markers MASC03447 and MASC03930 (genes At1g64680–At1g74045). However, another C24 IL with an introgression spanning markers MASC03631 and MASC03684, M37/7/8/6, showed normal sensitivity to nitrate (Supplementary Fig. S8), indicating that within this region of interest we are able to exclude the genes located in the interval between genes At1g67350 and At1g70220. M63/9/3, which carries an introgression at the top of chromosome 4 spanning markers MASC04123–MASC04685, showed a loss of nitrate sensitivity similar to M16/6/1/4 and M37/7/1/6 (Supplementary Fig. S8). However this locus was not investigated in more detail.

**Discussion**

**Identification of a major QTL for Glu sensitivity on chromosome 3**

It was previously established that there are marked differences between Arabidopsis accessions in the Glu sensitivity of their PR growth, the most sensitive of those tested being C24 and one of the least sensitive being Col-0 (Walch-Liu *et al.*, 2006). Using a combination of RILs and ILs from a Col-0/C24 population it has now been possible to identify several QTLs that significantly contribute to the Glu hypersensitivity of C24. The RIL mapping experiments (Figs 3 and 5) identified one large-effect QTL, GluS1, and two less prominent QTLs, GluS2 and GluS3, which together accounted for up to 42% of the phenotypic variance depending on the experimental conditions. In all three QTLs, Glu sensitivity was conferred by the C24 genome, suggesting a history of strong selection for these alleles in this accession.

The contribution of GluS1 to Glu sensitivity was dependent on environmental conditions (Fig. 5), but in most experiments it was either the sole or the major QTL detected, contributing up to 32% of the variation in Glu sensitivity in the RIL population (Table 1). ILs in the C24 background carrying Col-0 introgressions in the region of MASC01171 and reciprocal Col-0 ILs carrying C24 introgressions in the same region, were used to demonstrate that this C24 locus,
indicated that it is a component of a MAP kinase signaling cascade downstream of MAP kinase kinase 9 (MKK9), which is involved in regulating the physiological response to low phosphate (Lei et al., 2014). Mutants at the MPK3 locus had shorter PRs and higher lateral root densities than Col-0 under both low and high phosphate conditions, indicating effects on root growth and development that are independent of phosphate status (Lei et al., 2014). A previous study revealed an important positive role for the MEKK1 MAP kinase kinase kinase in controlling the root’s sensitivity to Glu (Forde et al., 2013). Despite earlier evidence for a role of MEKK1 in a signaling cascade leading to activation of MPK3 (Asai et al., 2002), it is no longer clear whether MEKK1 is required for activation of this pathway, at least in the case of the flg22-elicited defence response (Suarez-Rodriguez et al., 2007). Nevertheless it remains possible that MEKK1 and MPK3 are part of the same signaling cascade by which Glu inhibits root growth. Note that MEKK1 (At4g08500) maps to ∼10 cm on chromosome 4 (Supplementary Table S5) and therefore does not co-locate with any QTLs identified in this study.

Potential candidates for a role in Glu-elicited effects on root growth include the family of 20 glutamate receptor-like (GLR) genes that encode amino acid activated Ca2+ channels (Dietrich et al., 2010). A defect in a rice GLR gene led to loss of meristematic activity in the root tip, suggesting a role in maintaining the integrity of the root apical meristem (Li et al., 2006). Of the QTLs identified in this study, only one maps close to any members of the Arabidopsis GLR (AtGLR) family. The marker most closely linked to GluS4, which was only detected at 26°C, is MASC05127. It is located within At5g13260, so GluS4 overlaps with the nearby AtGLR2.5 (At5g11210) and AtGLR2.6 (At5g11180) genes. Like all other GLR genes, both AtGLR2.5 and AtGLR2.6 are expressed in roots (Chiu et al., 2002; Roy et al., 2008), but their function is unknown.

QTLs for primary root growth in the absence of glutamate were distinct from the GluS loci

Four QTLs for PR growth in the absence of Glu i.e. in the period before Glu treatment started, were identified (PBT1-4; Supplementary Table S3). Like the GluS QTLs, all the PBT QTLs were sensitive to the environment, none being found consistently across all conditions tested. Importantly, however, none of the PBT loci co-locate with any of the GluS loci, showing that the QTLs responsible for differences in PR growth in the absence of Glu are distinct from those responsible for variations in growth rate in the presence of Glu.

PBT1 is in the same region of chromosome 1 as LPR1, a QTL for the PR growth response to low phosphate that was originally mapped in a Bay-0 × Shahdara population (Reymond et al., 2006). The PBT1 peak was located between 16 and 22 cm (Supplementary Table S3), while LPR1 was mapped at ∼18 cm on chromosome 1 and has been identified as At1g23010, which encodes a multicopper oxidase (Svistoonoff et al., 2007). The low phosphate concentration of 22 µM in our growth medium would be consistent with detection of this QTL. In addition, PBT4, mapped at ∼50 cm on chromosome 4, which

**Fig. 8.** Identification of a QTL on chromosome 1 controlling nitrate sensitivity of the Glu effect. (A) Four d-old seedlings of introgression line M37/7/1/6 and its C24 parental line were transferred to agar plates containing medium with or without 5 mM KNO3 in the presence or absence of 50 µM Glu. Seedlings were imaged after a further 5 d. White lines mark the positions of the PR tips at the time of transfer. Note that where PRs were strongly inhibited by Glu it was sometimes necessary to move adjacent lateral roots aside to reveal the PR tip (arrowed) before imaging. (B) Seedlings of C24 and three ILs carrying overlapping introgressions on the long arm of chromosome 1 were transferred to agar plates containing 0 mM, 1 mM or 5 mM KNO3 in combination with either no Glu or 50 µM Glu. For each nitrate concentration, percentage inhibition of PR growth in the 5 d after transfer to Glu was calculated relative to roots treated with the same nitrate concentration without Glu (± SE; n = 6). Different letters indicate statistically significant differences between groups based on one-way ANOVA, Tukey post-hoc, P = 0.05.

GluS1C24, was not only required for the Glu hypersensitivity phenotype in C24 but was also sufficient to confer Glu hypersensitivity when introgressed into Col-0 (Fig. 6).

Based on the six RIL mapping experiments in which the QTL was detected (summarised in Table 1), the mean location of the GluS1 peak on chromosome 3 was at 57.3 cM, placing it very close to MASC01171 located at 57.1 cM. This was confirmed from analysis of the Glu sensitivities of a series of available ILs with introgressions in this region (Fig. 6 and Supplementary Fig. S6), which showed that the GluS1 locus is located much closer to MASC01171 than to MASC09224 and, more specifically, that it lies between genes At3g44830 and A3g46880. Of the 200 or so genes in this interval, one noteworthy candidate is At3g45640, which encodes MAP kinase 3 (MPK3). MPK3 is known to be involved in defence signaling (Pitzschke et al., 2009). However a recent report

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was detected only in plants grown in low light conditions at 24°C, maps in the same region of chromosome 4 as LPR3 at 50.8 cm. LPR3 is a QTL for root growth in the same population whose effect was independent of the phosphate supply (Reymond et al., 2006). LPR3 also co-locates with PRL3 at 52.1 cm, a QTL for PR growth mapped in the same set of Bay-0 × Shahdara RILs (Loudet et al., 2005).

Temperature sensitivity of the Glu effect is conferred epistatically by at least two separate loci

The environmental sensitivity of the Glu effect was previously demonstrated by the ability of excess nitrate to suppress it (Walch-Liu and Forde, 2008). This paper supports these findings through the identification of Arabidopsis accessions where Glu sensitivity was strongly influenced by the prevailing temperature, even over a relatively narrow temperature range of 20°C to 26°C (Fig. 4). Why there should be such a strong interaction between temperature and Glu sensitivity is unknown, but it may be significant that C24, the accession that was least sensitive to temperature, is also the one that originates from the most southerly latitude of 40.2°N in Portugal, whereas Col-0, Ler-0, Dijon-G and Nd-0 are all from Central Europe with latitudes in the range of 47–52°N (Schmid et al., 2006). A study of the temperature sensitivity of petiole hyponasty found that C24 and other accessions from nearer the equator were less responsive to high temperatures than accessions from more northern latitudes, such as Ler and Col-0 (van Zanten et al., 2009), although the temperatures involved were much higher and exposure much briefer than here i.e. 7 h at 38°C.

Further evidence of the environmental sensitivity of the Glu effect can be seen in how the environmental conditions of temperature, light and nitrate, affected which GluS QTLs were detected, with none being common to all conditions (Fig. 5 and Table 1). Similar interactions between the environment and QTL effects have previously been reported for flowering time variation in Arabidopsis (Stratton, 1998; Weinig et al., 2002).

The contrasting temperature sensitivities of C24 and Col-0 prompted us to use the Col-0/C24 ILs to investigate the genetic control of this phenomenon. Five C24 ILs carried introgressions that replaced the region of chromosome 3 containing the major Glu sensitivity locus, GluS1, with the corresponding region from Col-0. In each case their temperature sensitivity was found to resemble that of C24 rather than Col-0 (Supplementary Fig. S7C). Furthermore, the reciprocal lines where the GluS1C24 allele was introduced into the Col-0 background showed Col-0-like temperature-sensitivity (Fig. 7B). Thus it appears that temperature sensitivity is conferred on the GluS1 locus by genes elsewhere in the Col-0 background. Two candidate epistatic loci, TS1 and TS2, were identified on the basis that Col-0 introgressions from these regions in the C24 background conferred strong temperature sensitivity (Supplementary Fig. S7D). TS1 was provisionally mapped to the region between genes At1g01471 and At1g10560, and TS2 to the region between At5g49680 and At5g63920 overlapping with GluS3. Ambient temperature signaling has received less attention than signaling related to extremes of heat and cold. Nevertheless, a number of mechanisms for sensing smaller shifts in temperature have been proposed, including RNA folding, protein-protein interactions,
epigenetic effects and changes in membrane fluidity (Lee et al., 2008). One candidate gene located within the TSI interval is LONG HYPOCOTYL IN FAR-RED LIGHT1 (HFR1; At1g20340), which encodes a bHLH protein that has been identified as a component of a regulatory circuit, along with PHYTOCHROME INTERACTING FACTOR4 (PIF4), which is responsible for maintaining plant growth at high ambient temperatures of 25°C (Foreman et al., 2011).

Identification of a QTL controlling nitrate sensitivity of the Glu effect

It has been found that there are highly specific interactions between nitrate and Glu signalling at the Arabidopsis root tip. If the PR tip is simultaneously exposed to excess nitrate, but not other forms of N, then the inhibitory effect of Glu on root growth can be completely suppressed (Walch-Liu and Forde, 2008). The antagonistic effect of the NO3− ion was shown to be dependent on the functionality of the NPF6.3 (NRT1.1/CHLI) gene, probably in its role as a nitrate sensor (Walch-Liu and Forde, 2008). It has been suggested that the antagonistic interactions between nitrate and Glu signalling at the root tip could enable plants to modify their root architecture in response to changes in the relative abundance of the two forms of N, acting as cues for the inorganic and organic N pools in the soil (Walch-Liu and Forde, 2008).

Using the C24/Col-0 ILs to further investigate the genetic control of nitrate’s effect on Glu signalling, we found evidence for multiple loci affecting nitrate sensitivity. One locus found on the long arm of chromosome 1 had a particularly strong effect (Fig. 8). This region includes a cluster of four genes belonging to the same family as the NRT1 nitrate transporter: At1g72120 (NRT1.15/AtNPF5.14), At1g72125 (NRT1.16/AtNPF5.13), At1g72130 (AtNPF5.11), At1g72140 (AtNPF5.12). The NPF family in Arabidopsis has 53 members belonging to 8 subfamilies and between them they transport a diverse range of substrates, including peptides and hormones, in addition to nitrate (Léran et al., 2014). AtNPF5.13 and AtNPF5.14 are both reported to act as nitrate transporters when expressed in Xenopus oocytes (Tsay et al., 2007). However, none of the four closely related genes in this cluster belongs to the same NPF subfamily as NRT1.1 and none has previously been implicated in nitrate sensing.

Conclusions

The high degree of natural variation in Glu sensitivity in Arabidopsis roots, together with the extent to which it is affected by the environmental factors of temperature, light and nitrate availability, has made it possible to use QTL analysis to gain new insights into the genetic control of these traits. Fig. 9 summarises the results of our mapping experiments in which RILs and ILs generated from Col-0 × C24 crosses were cultivated under a range of environmental conditions. These studies have led to the identification of three QTL, GluS1, GluS2 and GluS3, which in combination can account for up to 42% of the phenotypic variance for Glu sensitivity in the RIL population, but whose contribution is strongly dependent on the environment. One QTL, GluS1, is particularly notable for its ability to confer Glu hyper-sensitivity when introgressed into a background with low Glu sensitivity. Other QTL were identified that are involved in conferring either temperature sensitivity (TS1 and TS2) or nitrate sensitivity (NS1) on the Glu response. Thus we have obtained a first picture of the multiplicity of loci responsible for modulating the Glu sensitivity trait and their epistatic interactions. Further research will be needed to establish the identity of the genes that underlie these QTLs, beginning with the candidate genes identified above, and to understand the physiological relevance of the remarkable environmental sensitivity of the root’s response to Glu.

Supplementary data

Supplementary data are available at JXB online.

Table S1. Composition of basal medium.

Table S2. CAPS and dCAPS primers used for fine mapping the GluS1 locus.

Table S3. Summary of the QTL controlling the PBT trait.

Table S4. Map locations of introgressions in the ILs used in the present study.

Table S5. Physical and genetic map positions of framework SNP markers.

Fig. S1. Plots of ranked means and standard errors for the PBT data from the Col-0xC24 and C24xCol-0 RIL populations.

Fig. S2. Plots of ranked means and standard errors for the PATGlu data from the Col-0xC24 and C24xCol-0 RIL populations.

Fig. S3. Frequency distribution plots for PR growth of a RIL population in a multi-environment experiment ± Glu.

Fig. S4. QTL analysis of the PBT trait from a RIL population grown under a range of environmental conditions.

Fig. S5. Effect of a series of introgressions in the vicinity of the GluS1 locus on Glu sensitivity.

Fig. S6. Fine mapping of the introgressions in six ILs used to define the position of the GluS1 locus.

Fig. S7. Reaction norm plots for the effect of temperature on the Glu sensitivity of a set of C24 ILs with introgressions from Col-0.

Fig. S8. Scatter plot showing the effect of nitrate on the Glu sensitivity of a set of ILs in the C24 background.

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References

Asal T, Tena G, Plotnikova J, Willmann MR, Chiu WL, Gomez-Gomez L, Bolier T, Ausubel FM, Sheen J. 2002, MAP kinase signalling cascade in Arabidopsis innate immunity. Nature 415, 977–983.
Chiu JC, Brenner ED, DeSalle R, Nitabach MN, Holmes TC, Coruzzi GM. 2002. Phylogenetic and expression analysis of the glutamate receptor-like gene family in Arabidopsis thaliana. Molecular Biology and Evolution 19, 1066–1082.

Christou M, Avramides EJ, Roberts JP, Jones DL. 2005. Dissolved organic nitrogen in contrasting agricultural ecosystems. Soil Biology & Biochemistry 37, 1560–1563.

Dietrich P, Anschütz U, Kugler A, Becker D. 2010. Physiology and biophysics of plant ligand-gated ion channels. Plant Biology 12(4 Suppl 1), 80–93.

Drew MC. 1975. Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. New Phytologist 75, 479–490.

Fan SC, Lin CS, Hsu PK, Lin SH, Tsay YF. 2009. The Arabidopsis nitrate transporter NRT1.7, expressed in phloem, is responsible for source-to-sink remobilization of nitrate. The Plant Cell 21, 2750–2761.

Forde BG. 2014. Glutamate signalling in roots. Journal of Experimental Botany 65, 779–787.

Forde BG, Cutler SR, Zaman N, Krysan PJ. 2013. Glutamate signalling via a MEKK1 kinase-dependent pathway induces changes in Arabidopsis root architecture. The Plant Journal 75, 1–10.

Forde BG, Lea PJ. 2007. Glutamate in plants: metabolism, regulation, and signalling. Journal of Experimental Botany 58, 2339–2358.

Foreman J, Johansson H, Hornitschek P, Josse EM, Fankhauser C, Halliday KJ. 2011. Light receptor action is critical for maintaining plant biomass at warm ambient temperatures. The Plant Journal 65, 441–452.

Gifford ML, Dean A, Gutierrez RA, Coruzzi GM, Birnbaum KD. 1999. Cloning and functional characterization of Arabidopsis glutamate receptor-like gene that encodes a constitutive component of low-affinity uptake. The Plant Cell 11, 1381–1392.

Hutchings MJ, de Kroon H. 1994. Foraging in plants: the role of morphological plasticity in resource acquisition. Advances in Ecological Research 25, 159–238.

Jones DL, Healey JR, Willett VB, Farrar JF, Hodge A. 2005. Dissolved organic nitrogen uptake by plants - an important N uptake pathway? Soil Biology & Biochemistry 37, 413–423.

Konieczny A, Ausubel FM. 1993. A procedure for mapping Arabidopsis mutations using co-dominant ecotype-specific PCR-based markers. The Plant Journal 4, 403–410.

Lee JH, Lee JS, Ahn JH. 2008. Ambient temperature signaling in plants: an emerging field in the regulation of flowering time. Journal of Plant Biology 51, 321–326.

Lei L, Li Y, Wang Q, Xu J, Chen Y, Yang H, Ren D. 2014. Activation of MKK9-MPK3/MPK6 enhances phosphate acquisition in Arabidopsis. New Phytologist 203, 1146–1160.

Léran S, Varala K, Boyer JC, et al. 2014. A unified nomenclature of NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER family members in plants. Trends in Plant Science 19, 5–9.

Li J, Zhu S, Song X, et al. 2005. A rice glutamate receptor-like gene is critical for the division and survival of individual cells in the root apical meristem. The Plant Cell 18, 340–349.

Loudet O, Gaudon V, Trubuil A, Daniel-Vedele F. 2005. Quantitative trait loci controlling root growth and architecture in Arabidopsis thaliana confirmed by heterogeneous inbred family. Theoretical and Applied Genetics 110, 742–753.

Moran-Zuloaga D, Dippold M, Glaser B, Kuzyakov Y. 2015. Organic nitrogen uptake by plants: reevaluation by position-specific labeling of amino acids. Biogeochemistry 125, 359–374.

Neef MM, Turk E, Kalishman M. 2002. Web-based primer design for single nucleotide polymorphism analysis. Trends in Genetics 18, 613–615.

Nobbe F. 1862. Über die feinere Verästelung der Pflanzenwurzeln. Landwirtschaftlichen Versuchs-Stationen 4, 212–224.

Pitzschke A, Schikora A, Hirt H. 2009. MAPK cascade signaling networks in plant defence. Current Opinion in Plant Biology 12, 421–426.

Reymond M, Svistoonoff S, Loudet O, Nussaume L, Desnos T. 2006. Identification of QTL controlling root growth response to phosphate starvation in Arabidopsis thaliana. Plant, Cell & Environment 29, 115–125.

Roy SJ, Gilliham M, Berger B, et al. 2008. Investigating glutamate receptor-like gene co-expression in Arabidopsis thaliana. Plant, Cell & Environment 31, 861–871.

Schmid KJ, Törjék O, Meyer R, Schmuths H, Hoffmann MH, Altmann T. 2006. Evidence for a large-scale population structure of Arabidopsis thaliana from genome-wide single nucleotide polymorphism markers. Theoretical and Applied Genetics 112, 1104–1114.

Stratton DA. 1998. Reaction norm functions and QTL-environment interactions for flowering time in Arabidopsis thaliana. Heredity 81 (Pt 2), 145–155.

Suarez-Rodriguez MC, Adams-Phillips L, Liu Y, Wang H, Su SH, Jester PJ, Zhang S, Bent AF, Krysan PJ. 2007. MEKK1 is required for fig22-induced MPK4 activation in Arabidopsis plants. Plant Physiology 143, 661–669.

Svennerstam H, Jämtgård S, Ahmad I, Huss-Danell K, Näsholm T, Ganeteg U. 2011. Transporters in Arabidopsis roots mediating uptake of amino acids at naturally occurring concentrations. New Phytologist 191, 459–467.

Svistoonoff S, Creff A, Reymond M, Sigoillot-Claude C, Ricaud L, Blanchet A, Nussaume L, Desnos T. 2007. Root tip contact with low-phosphate media reprograms plant root architecture. Nature Genetics 39, 792–796.

Tonsor SJ, Alonso-Blanco C, Koornneef M. 2005. Gene function beyond the single trait: natural variation, gene effects, and evolutionary ecology in Arabidopsis thaliana. Plant Cell Environment 28, 2–20.

Törjék O, Berger D, Meyer RC, Müssig C, Schmid KJ, Rosleff Sörensen T, Weisshaar B, Mitchell-Olds T, Altmann T. 2003. Establishment of a high-efficiency SNP-based framework marker set for Arabidopsis. The Plant Journal 36, 122–140.

Törjék O, Meyer RC, Zehndorf M, Teltow M, Strompen G, Witucka-Wall H, Blacha A, Altmann T. 2008. Construction and analysis of 2 reciprocal Arabidopsis introgression line populations. The Journal of Heredity 99, 396–406.

Törjék O, Witucka-Wall H, Meyer RC, von Korff M, Kusterer B, Rautengarten C, Altmann T. 2006. Segregation distortion in Arabidopsis C24/Col-0 and Col-0/C24 recombinant inbred line populations is due to reduced fertility caused by epistatic interaction of two loci. Theoretical and Applied Genetics 113, 1551–1561.

Tsay YF, Chiu CC, Tsai CB, Ho CH, Hsu PK. 2007. Nitrate transporters and peptide transporters. FEBS Letters 581, 2290–2300.

Utzh J, Melchinger A. 1996. PLABQTL: a program for composite interval mapping of QTL. J Agric Genom 2, 1–5.

van Zanten M, Voesenek LA, Peeters AJ, Millenaar FF. 2009. Hormone- and light-mediated regulation of heat-induced differential petiole growth in Arabidopsis. Plant Physiology 151, 1446–1458.

Walch-Liu P, Forde BG. 2007. L-glutamate as a novel modifier of root growth and branching: What’s the sensor? Plant Signaling & Behavior 2, 284–286.

Walch-Liu P, Forde BG. 2008. Nitrate signalling mediated by the NRT1.1 nitrate transporter antagonises L-glutamate-induced differential petiole growth in Arabidopsis. Plant Physiology 151, 1446–1458.

Weigel D. 2012. Natural variation in Arabidopsis: from molecular genetics to ecological genomics. Plant Physiology 158, 2–22.

Weinig C, Ungerer MC, Dorn LA, Kane NC, Toyonaga Y, Halldorsdottir SS, Mackay TF, Parurangan MD, Schmuths H, Weigel D. 2011. Novel loci control variation in reproductive timing in Arabidopsis thaliana in natural environments. Genetics 162, 1875–1884.

Wiersum LK. 1958. Density of root branching as affected by substrate and separate ions. Acta Botanica Neerlandica 7, 174–190.