

## CHAPTER 2

# FROM QTLs TO GENES CONTROLLING ROOT TRAITS IN MAIZE

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**Abstract.** In maize, two major quantitative-trait loci (QTLs) on chromosome bins 1.06 and 2.04 have been shown to affect root architecture and a number of agronomic traits, including grain yield. The QTL on bin 2.04 (*root-ABAI*) also affects root lodging and ABA concentration in the leaf. To evaluate the effects of *root-ABAI* better, near-isogenic lines (NILs) have been produced and evaluated *per se* and in testcross combinations under different water regimes. Additionally, the NILs have been crossed to obtain large mapping populations suitable for the fine-mapping of *root-ABAI* and, eventually, its map-based cloning. The identification of the sequence responsible for a target QTL can be facilitated by the candidate-gene approach coupled with a comparative *in silico* analysis based on sequence information of model species and other crops. Genomics, when appropriately integrated with other relevant disciplines, will positively impact our understanding of root growth and functions.

## INTRODUCTION

The main challenge faced by plant scientists during the 21st century will be to increase crop productivity per unit area while enhancing the sustainability of agricultural practices and preserving the remaining biodiversity (European Plant Science Organization 2005). Of all the factors that presently limit crop yield, irrigation water and fertilizers play an increasingly important role, due to their escalating costs and diminished availability. Clearly, a better knowledge of the developmental processes that impart tolerance to drought and low nutrients will allow for a more effective identification of target traits for boosting yield potential while optimizing water and nutrient use efficiency.

Among the morphological factors that affect tolerance to drought and low nutrients, root traits play a major role. In a landmark review, Ludlow and Muchow (1990) listed a number of traits and their orders of priority for improving drought

resistance in both intermittent and terminal stress environments and under conditions of either subsistence or modern agriculture. In three of these four situations, rooting depth and density were considered to be of primary importance. Roots show a high level of developmental plasticity in response to external cues, an essential feature for the adaptation of plants to different environmental conditions and for optimizing the utilization of water and nutrients. Among the multiple factors that regulate root plasticity in maize, abscisic acid (ABA) has shown a positive role for sustaining root elongation under conditions of water deficit that inhibit shoot elongation (Sharp et al. 2004).

From a breeding standpoint, although extensive genetic variation for root architecture has been reported (O'Toole and Bland 1987), root traits have seldom been considered as selection criteria to improve yield, mainly due to the difficulty in their measurement and their quantitative mode of inheritance. As compared to conventional breeding approaches, the identification of quantitative-trait loci (QTLs) that may concurrently affect root traits and grain yield under varying water and nutrient regimes would allow for more targeted selection approaches, such as marker-assisted selection (Ribaut and Hoisington 1998). Additionally, cloning the sequences underlying such QTLs (Salvi and Tuberosa 2005) and exploring allelic variability through EcoTILLING (Comai et al. 2004) or association mapping (Yu and Buckler 2006) pave the way for the identification of agronomically superior alleles at these loci. Therefore, the advent of genomics has ushered in novel opportunities to elucidate the genetic and functional basis of root growth and to manipulate allelic diversity more effectively at relevant loci affecting yield under low-input conditions (Tuberosa et al. 2002a; 2005; Sharp et al. 2004; Zhu et al. 2005a; 2005b). In this context, the objectives of this review are to (i) summarize briefly the information available on two major QTLs for root traits in maize and (ii) describe the procedures required for their cloning based also on the exploitation of model species.

### QTLs FOR ROOT TRAITS IN MAIZE

When QTL data become available from two or more populations of the same species, it is possible to compare their position by using common anchor markers (e.g., RFLPs and SSRs) and/or through the use of a reference map (Tuberosa et al. 2002b; Tuberosa and Salvi 2004). In maize, the comparative analysis of QTLs from different populations is facilitated by the subdivision of the reference map into 100 sectors (bins) of comparable size (Davis et al. 1999). Based on the bin framework of the UMC reference map, a comprehensive survey on QTLs for root traits in maize was recently presented (Tuberosa et al. 2003). Here, we summarize the main results for two major QTLs (on bins 2.04 and 1.06) shown to affect root architecture and other traits, including grain yield.

In maize, QTLs for root architecture were first reported by Lebreton et al. (1995) using 81 F<sub>2</sub> plants derived from the cross between two lines (Polj17 and F-2) known to differ for a number of root traits, including root pulling force (RPF), and for ABA concentration in the leaf and xylem sap. For all QTLs but one, the signs of the

additive effects for ABA concentration and RPF were similar. As to nodal root number (NRN), the comparative analysis of QTL effects revealed a striking correlation between the QTL effects of NRN and ABA concentration in the xylem sap ( $r = 0.84$ ) and suggested that variation in xylem ABA content is largely determined by variation in NRN. The QTL region with the strongest concurrent effect on root traits and leaf ABA concentration (L-ABA) was mapped near *csu133* on bin 2.04. A strong effect of this region on L-ABA was also reported in the Os420  $\times$  IABO78 background (Tuberosa et al. 1998). It should be noted that none of the major mutants impaired in ABA biosynthesis mapped to this region, a result that led the authors to hypothesize that the effect of the QTL on L-ABA may actually be due to differences in the water status of the plant consequent to a primary effect of the QTL on root size/architecture. More recently, the meta-analysis conducted by Sawkins et al. (2004) has validated the key role of bin 2.04 on yield in water stressed maize.

QTLs for root traits were also investigated by Tuberosa et al. (2002c) using 171  $F_3$  families derived from the cross Lo964  $\times$  Lo1016. In an experiment conducted in hydroponics, 11, 7, 9 and 10 QTLs influenced primary-root length (R1L), primary-root diameter (R1D), primary-root weight (R1W) and the weight of the adventitious seminal roots (R2W), respectively. The high LOD values ( $> 5.0$ ) of 10 QTLs and their sizeable  $R^2$  values (from 14.7 to 32.6%) suggested the presence of highly significant QTLs. The most significant QTLs (LOD values of 14.7, 6.4 and 8.3 for R1D, R1L and R2W, respectively) were mapped on bin 1.06. In order to verify to what extent the QTLs influencing root growth in hydroponics may also modulate root growth in the field, a random sample of 118 (Lo964  $\times$  Lo1016)  $F_3$  families was tested for RPF in replicated field trials (Landi et al. 2002). Among the 30 bins with QTLs for RPF and/or number of brace roots, 15 (including 1.06) also harboured QTLs for root traits in hydroponics. QTLs for root traits on bin 1.06 have also been reported in Polj17  $\times$  F-2 (Lebreton et al. 1995) and B73  $\times$  Mo17 (Kaeppler et al. 2000). Additionally, Hirel et al. (2001) reported a major QTL for nitrogen use efficiency and grain yield (GY) on bin 1.06, a finding that underlines the importance of this region for GY in conditions not only of limited water availability but also of limited N supply. Field trials conducted during two seasons to measure GY under well-watered (GY-WW) and water-stressed (GY-WS) conditions with the Lo964  $\times$  Lo1016  $F_3$  families revealed a number of QTLs whose peaks overlapped with those for root traits measured in hydroponics (Tuberosa et al. 2002c) and/or in the field (Landi et al. 2002). In particular, QTLs for R2W co-localized with QTLs for GY-WW and/or GY-WS in bins 1.03, 1.06, 1.08, 7.02, 10.04 and 10.07. In five of these six regions, an increase in root weight was associated with a higher GY. Of all regions that concomitantly influenced root traits and GY, the strongest and most consistent effects were revealed by a 10-cM interval on bin 1.06 that influenced root traits and GY in both years and under both water regimes.

In order to evaluate the effects of the QTLs on bins 2.04 and 1.06 on root traits and grain yield more accurately, near-isogenic lines (NILs) differing for the parental segment at these QTL regions have been developed (Landi et al. 2005, S. Salvi et al. unpublished).

*Isogenization of QTLs for root traits*

The isogenization of a QTL is an essential prerequisite for its fine-mapping and to investigate in greater detail its direct effects on the target trait as well as the associated effects on other traits (Shen et al. 2001). Near-isogenic lines (NILs) contrasted for the parental chromosome regions at the target QTL can be obtained through repeated selfings (at least 5–6) of one or more individuals heterozygous at the QTL region followed by the identification of the homozygotes for each one of the two parental segments. Alternatively, each parental line of the original mapping population evaluated for discovering the QTL can be used as recurrent parent in a backcross scheme in which a single plant heterozygous at the QTL in question is utilized as donor of the alternative QTL regions; in this case, the congenic lines are identified as backcrossed derived lines (BDLs). For a cross-pollinated species such as maize, the evaluation of the effects of a particular QTL on yield or other highly heterotic traits should preferably be carried out using near-isogenic hybrids (NIHs), which can be obtained by crossing BDLs at the same target region introgressed in different genetic backgrounds. Depending on the BDLs used as parents, NIHs are either homozygous or heterozygous at the target QTL region, while being heterozygous for most of the remaining portion of the genome.

The major drawback to the utilization of NILs is the long time required for their production. This problem can be partially overcome by producing a library of introgression lines (ILs), namely a collection of NILs obtained by backcross and differing only for a small portion (usually ca. 10–30 cM) of the donor genome. In maize, an adequate coverage of the genome requires ca. 80 lines. Once the ILs are made available, the fine-mapping of any major QTL segregating in the original cross can be readily pursued. We have developed a library of ILs derived from B73 (recurrent parent)  $\times$  Gaspé Flint (donor parent) to identify major QTLs for root growth and architecture. The preliminary results are quite encouraging as to the possibility of using this approach to identify major QTLs for root traits.

*Root-ABA1 affects root traits and ABA concentration in maize*

The isogenization of the region near *csu133* on bin 2.04 in the Os420  $\times$  IABO78 background allowed Landi et al. (2005) to obtain pairs of BDLs contrasted for the parental chromosome segments at this region, herein identified as (+/+) and (-/-) for their effects on L-ABA. Field testing of the BDLs under both water-stressed (WS) and well-watered (WW) regimes confirmed the effect of the QTL on L-ABA. Subsequently, NIHs for the QTL near *csu133* were developed and field-tested for two years under WW and WS conditions. Differences among NIHs for L-ABA and other morpho-physiological traits were not affected by water regimes (Giuliani et al. 2005b). Interestingly, the (+) QTL allele for high L-ABA markedly reduced root lodging. To further elucidate the effects of the QTL on root architecture and L-ABA, root traits of two pairs of BDLs were measured in plants grown in soil columns at three water regimes. On average, the QTL confirmed its effect on L-ABA and showed a significant, concurrent effect on the angle, branching, number, diameter and dry weight of the roots. Based on these results, Giuliani et al. (2005b) suggested

that the QTL affects root lodging through a constitutive effect on root architecture and size which, in turn, affects L-ABA. Consequently, the QTL has been identified as *root-ABAI*.

The effects of *root-ABAI* on grain yield were also evaluated in various genetic backgrounds. For this purpose, the (+/+) and (-/-) BDLs were factorially crossed with five and 13 inbred lines of different origin, thus producing two sets of test crosses (TCs) that were tested in Italy and China, respectively, under both WW and WS conditions (Landi et al. in press). In Italy, the TCs derived from (+/+) BDLs were less susceptible to root lodging across both water regimes than the TCs derived from (-/-) BDLs (28.0 vs. 52.5%), but were also less productive under WS conditions (4.88 vs. 6.27 Mg ha<sup>-1</sup>). The TCs derived from (+/+) BDLs were also less productive in China (6.83 vs. 7.49 Mg ha<sup>-1</sup>; average of WW and WS conditions). In both sites, the lower grain yield of the TCs derived from (+/+) BDLs was prevalently due to a lower number of both ears/plant and kernels/plant. These results indicate that the (+) *root-ABAI* allele confers a lower susceptibility to root lodging but also a lower grain yield, especially when root lodging does not occur. The yield loss associated with the (+) *root-ABAI* allele has tentatively been ascribed to a negative effect of an excessive accumulation of ABA on reproductive fertility (Landi et al. in press). An alternative explanation might be that *root-ABAI* affects biomass production in response to sensing of drought stress. The fine-mapping of *root-ABAI* has now been undertaken as a preliminary step to its positional cloning. In order to investigate the effects of *root-ABAI* on the transcriptome and identify functional markers tightly linked to the QTL, microarray analysis has been used to profile the transcripts of the contrasting BDLs (Giuliani et al. 2005a). This study has led to the identification of several genes preferentially expressed in only one of the two BDLs; among these genes, those that map within the supporting interval of *root-ABAI* may provide useful clues as to the functional polymorphisms associated with its effects.

### CLONING QTLS FOR ROOT GROWTH

Different options are available to proceed from a supporting interval delimiting a target QTL to the actual gene(s) responsible for the QTL effect. According to the mapping accuracy and the magnitude of the QTL effect, the support interval of the QTL may span several hundreds of genes. Additionally, non-coding regions may also be responsible for QTLs through a cis-acting effect on the promoter region of nearby genes. Clearly, identifying the right ‘needle’ in the ‘genome haystack’ is a daunting undertaking, although one well-worth pursuing for the possible applications and for elucidating the genetic basis of quantitative traits.

The positional cloning of a major QTL (reviewed in Salvi and Tuberosa 2005) requires the availability of (i) a large mapping population (> 1,500 plants) derived from the cross of two NILs for the target QTL, (ii) a contiged genomic (BACs or YACs) library spanning the QTL region and (iii) a system for validating the identity and testing the effects of candidate genes. Only a few of the root QTLs so far described are amenable to a positional-cloning approach, particularly in view of the

large amount of resources required to measure roots accurately in the hundreds of plants that are phenotyped in any QTL-cloning project. Additionally, positional cloning in maize is made more complex by its large genome size and redundancy.

### *The candidate-gene approach*

At its simplest, the candidate-gene approach exploits information on the role and function of a particular coding sequence and verifies whether it may represent a feasible candidate for the QTL in question (Pflieger et al. 2001). Therefore, candidate genes can also be identified in species other than the one being directly investigated. If a plausible cause–effect relationship can be hypothesized between a QTL and a candidate gene mapping nearby, then validation of its role could be attempted through genetic engineering and/or the screening of knockout mutants, avoiding the tedious procedures of the positional-cloning approach. The identification of suitable candidate genes and the elucidation of their function can be facilitated by combining different approaches and high-throughput platforms (Schnable et al. 2004; Giuliani et al. 2005a; Guo et al. 2005; Salvi and Tuberosa 2005). The recent progress in the high-throughput profiling of the proteome (Hochholdinger et al. 2005; Wen et al. 2005; Sauer et al. 2006) and metabolome (Steuer et al. 2003) provides additional leads to analyse the changes in the concerted expression of the genes involved in root growth and their response to environmental cues. However, it should be appreciated that, at present, proteomics and metabolomics can indirectly report changes occurring in only a fraction of the genome; additionally, proteomics is often unable to detect the changes in gene products (e.g., transcription factors) that, despite their low level, are more likely to play a pivotal role in root growth. A detailed study on proteome profiling is in progress to ascertain the role of cell-wall proteins (CWPs) in the elongation of the primary root in maize (Zhu et al. 2006). Although many of the CWPs identified in this study have previously been shown to be involved in cell-wall metabolism and cell elongation, a number of CWPs (e.g., endo-1,3;1,4- $\beta$ -D-glucanase and  $\alpha$ -L-arabinofuranosidase) were not described in previous cell-wall proteomic studies.

From a technical standpoint, it should be noted that the combination of the ‘omics’ platforms with laser-capture microdissection allows for unprecedented levels of functional resolution at the anatomical level. In maize, a combination of laser-capture microdissection and subsequent microarray analyses applied to the root pericycle of wild-type and *rum1* mutant allowed Woll et al. (2005) to identify 19 genes involved in signal transduction, transcription and the cell cycle that are active before lateral-root initiation; these findings will contribute to the identification of the developmental checkpoints involved in lateral-root formation in maize downstream of *rum1*.

### *Arabidopsis as a model*

Although root development in *Arabidopsis* and rice differs from maize in both overall architecture and the anatomy of individual roots, genes cloned in

*Arabidopsis* and rice may in some cases provide interesting leads for QTL cloning in maize, particularly for those functional/morphological features of root development which from a phylogenetic standpoint may have been conserved to a greater extent across species (e.g., signalling cascades, cell elongation, growth and density of root hairs, etc.). The power of combining QTL analysis for root morphology and target metabolites with fine-mapping and mutant analysis, in order to elucidate the genetic and functional basis of root growth, was recently shown in *Arabidopsis* (Sergeeva et al. 2006) where the possible role in root elongation of the sucrose-splitting enzymes sucrose synthase and invertase was tested. Several QTLs affected both invertase activity and root length. The fine-mapping of a major QTL for root length revealed consistent co-location with the locus for invertase activity containing a gene coding for a vacuolar invertase. The role of this invertase gene in root elongation was confirmed by the analysis of a functional knockout line.

An area worthy of exploration relates to the mechanisms regulating the level of gene expression. Also in this case, the model species *Arabidopsis* has provided useful insights. Although several plant microRNAs (miRNAs) have been shown to play a role in plant development, a study in *Arabidopsis* has shown for the first time an effect on the root phenotype due to a reduced expression of a miRNA (Guo et al. 2005). *Arabidopsis thaliana* miR164 was predicted to target five NAM/ATAF/CUC (NAC) domain-encoding mRNAs, including NAC1, which transduces auxin signals for lateral-root emergence. Cleavage of endogenous and transgenic NAC1 mRNA by miR164 was shown to be blocked by NAC1 mutations that disrupt base-pairing with miR164. Compared with wild-type plants, *Arabidopsis* miR164 mutants expressed less miR164 and more NAC1 mRNA and produced more lateral roots. The results of this landmark study indicate that auxin induction of miR164 provides a homeostatic mechanism to clear NAC1 mRNA to down-regulate auxin signals and clearly show the value of using *Arabidopsis* as a model for elucidating the complex molecular mechanisms regulating root growth. Further insights on the role of auxins on root growth were provided by the study of Okushima et al. (2005): their data suggest that the ARF7 (Auxin Response Factor 7) and ARF19 proteins play essential roles in auxin-mediated growth of lateral roots by regulating both unique and partially overlapping sets of target genes.

Recently, the screening of nine *Arabidopsis* accessions grown under rigorously controlled conditions revealed that one accession was unaffected by water deficit in terms of root growth (Granier et al. 2006). A mapping population including this accession as one of the parents might facilitate the identification of QTLs modulating the response of roots to decreasing soil moisture.

## CONCLUSIONS AND PERSPECTIVES

The sequencing of the maize genome is now well underway ([http://www.maizegdb.org/sequencing\\_project.php](http://www.maizegdb.org/sequencing_project.php)). Once the annotated sequence is released, additional opportunities will become available for identifying the genes controlling root traits. The genomics approach, when appropriately intersected and integrated with other relevant disciplines (e.g., soil science, agronomy, crop

physiology, biochemistry, etc.) will positively impact our understanding of root growth and development. As shown in this article, QTL analysis and genomics are powerful tools to disentangle the genetic complexity governing root growth and its plasticity. In a limited number of cases, such genetic complexity has been 'Mendelized' as a prerequisite to QTL cloning, now a reality, albeit applicable only to a few major QTLs. QTL cloning will shed light on the mechanisms regulating the quantitative expression of root traits. In this respect, modulation of gene expression is likely to play a pivotal role, and new insights will derive from a better understanding of the role of miRNAs. On the molecular side, extensive EST databases and unigene sets derived from cDNA libraries of root tissues provide valuable markers to construct functional maps that will facilitate the identification of QTL candidates. High-throughput genomic profiling based on the detection of single-nucleotide polymorphisms (SNPs) and other enabling platforms (Kilian 2005; Woll et al. 2005) will vastly improve our capacity for QTL cloning and/or allele mining. From an applicative standpoint, the challenge faced by plant scientists is how to integrate best and most effectively into extant breeding programmes the deluge of information generated through the 'omics' platforms. Despite the spectacular progress on the molecular side, our capacity to phenotype roots accurately on the massive scale that is often required by genomics studies remains the major limiting factor. A partial solution to the shortcomings of phenotyping is provided by an appropriate use of modelling, an approach which expands our capacity to predict the effects that specific environmental (e.g., water availability) and genetic (e.g., QTL effects, Tardieu 2003) variables might have on plant growth and final yield. Clearly, integrative and interdisciplinary approaches will be instrumental to further our understanding of root growth and, eventually, enhance our ability in tailoring root architecture in order to improve water and nutrient use efficiency of crops.

#### ACKNOWLEDGEMENTS

We acknowledge the collaboration of Massimo Bellotti, Sergio Conti, Simona Corneti, Silvia Giuliani, Pierangelo Landi, Marco Maccaferri, Francesca Rotondo, Maria Corinna Sanguineti and Sandra Stefanelli.

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